D1.14 Summary Progress Report Year 4

WP1 Coordination

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1 Glossary

AH: Animal Health
ASM: Annual Scientific Meeting
AWP: Annual Work Plan
CaM: Communication workshop and Media training
CPD: Continuing Professional Development
CT: Coordination Team
ESAB: External Scientific Advisory Board
FS: Food Safety
JIP: Joint Integrative Projects
JRP: Joint Research Project
MS: Member State
OHEJP: One Health European Joint Programme
PH: Public Health
PMC: Programme Managers Committee
PMT: Project Management Team
POC: Programme Owners Committee
REA: Research Executive Agency
SPR: Summary Progress Report
SS: Summer School
SSB: Scientific Steering Board
ST: Support Team
STM: Short Term Mission
WS: Workshop

2 Publishable summary

2.1 Summary of the context and overall objectives of the project

2.1.1 What is the problem/issue being addressed?

The One Health EJP is a policy driven research network addressing issues related to needs identified in the food safety area.

- Need to strengthen the links between human health, animal health and environmental aspects: One Health approach
- Need to further integrate surveillance and response capacities, preventive approaches, detection systems as well as preparedness and response to disease outbreaks
- Need of collaboration in Joint Research and Joint Integrative Projects, as well as Training and Education activities throughout a consortium of national public mission organisations
- Need to foster interaction between European, national authorities and stakeholders
- Need to update policy makers on these achievements and, built on this knowledge, to take appropriate action

2.1.2 Why is it important for society?

The integrated health approach, known as 'One Health', is based on strengthening collaboration between human health, animal health and environmental management. It focuses on developing surveillance and response capacities, strengthening early-warning and detection systems; reinforcing the capacities of public health and veterinary authorities as regards prevention, preparedness and

response to disease outbreaks; evaluating the social and economic impact of diseases; promoting inter-sector collaboration for the health of the livestock, wildlife and ecosystems concerned; research on the conditions under which diseases emerge and spread. Thus coordination between the different health systems, which are generally run separately, must enable economies of scale by encouraging synergies, and guarantee improved health security. Particular attention is paid to the communication of risks at all levels of action.

2.1.3 What are the overall objectives?

The overall objective of the One Health EJP is to develop a European network of research institutes, mainly with reference laboratory functions, integrating medical, veterinary and food scientists in the field of food and feed safety in order to improve research on antimicrobial resistance and on the prevention and control of mainly foodborne zoonoses, while taking into account the public health concerns of consumers and other stakeholders throughout the food chain.

2.2 Work performed during the reporting period (M37-M45) and main results achieved

2.2.1 WP1

The Coordination Team continues with the management of the One Health EJP by having weekly conference calls for the day-to-day management of the project. Regular videoconferences with PMT were organised (5 February, 6 April and 2 June), as well as one videoconference with SSB (19 March) and another planned for September 2021). The planned PMC and POC meeting will be jointly organised on 24 November 2021.

The Project Management Team welcome the 6 new organisations that have joined the consortium in a welcome meeting held on the 3rd of March. Inclusion of these new partners into the existing activities and allocation of corresponding budget was performed.

The Support Team has managed finances and the financial report of year 3 as well as the third amendment to the Grant Agreement. The Coordination Team together with the Project Management Team has prepared the periodic report and submitted it to REA on 1 April 2021. In addition, the CT and the PMT have implemented together the OHEJP Consortium enlargement procedure and prepared the procedure to finalise the request for a no-costs extension of the OHEJP.

As for the follow-up of possible ethical issues, the Ethics Advisors evaluated both the ongoing first round projects, the projects that started in January 2020 and all the PhD studies. The ethics report (D1.24) was delivered as planned.

The Communication Team has successfully managed to create a strong impact by developing relationships between the One Health EJP and the identified audiences and by creating content for a strong, cohesive brand and new dissemination material to highlight our outcomes and demonstrate impact.

2.2.2 WP2

In WP2, relevant scientific developments were monitored by screening the output of OHEJP projects as well as relevant information from external sources. A gap analysis of the priority topics selected in the second internal call has been conducted to identify gaps in One Health research and/or integrative areas. Also, an expert elicitation has been conducted to update the priority research topics. In addition, the repository of EU projects/initiatives has been regularly updated on the OHEJP website. Strategic interactions with a number of related EU projects and initiatives were established. WP2 has collaborated with WP4 to select the projects/initiatives to organize the cogwheel workshops during

2021. WP2 has also worked closely with WP7 towards the elaboration of the OHEJP Strategic Research and Innovative Agenda.

2.2.3 WP3

A main task of WP3 is the monitoring of ongoing Joint Research Projects (JRP), both those that started in January 2018 (first call) and those launched in January 2020 (second call). A full report (D3.15) on the progress made by all JRP was delivered on time in spring 2021. It describes the full details of the five projects that were finalized in December 2020 (i.e. ARDIG, RADAR, METASTAVA, AirSample and MedVetKlebs), and the progress of all other 18 ongoing JRP. The process regarding the evaluation of the final reports by external experts was launched and completed. The evaluation reports were inserted in deliverable D3.17 (First report on the evaluation of finalised JRPs), which is due by the end of December 2021.

The preparation of the third Annual Scientific Meeting (ASM), a hybrid (in person-online) event that took place from 9 to 11 June 2021, ran without any major issue. About 500 delegates took part in the conference, on site and online (hybrid).

2.2.4 WP4

The year has been very productive. Six JIPs have run in parallel, among which one started during the year and two will be completed. ORION has successfully delivered according to the original project proposal and among the achievements there are many valuable contributions to the harmonisation and intepretation of surveillance data by providing procedures and tools for data analyses and standardised data formats and ontologies. The other successful JIP to be completed is COHESIVE, facilitating the communication of surveillance data by developing common signalling and reporting procedures. In March the sixth JIP, COVRIN, started. This project focuses on One Health aspects of SARS-CoV2.

Despite the pandemic most activities in the JIPs have been effectuated, even if some have been delayed. Both ORION and COHESIVE were approved an extension of 6 and 12 months, respectively. The remaining JIPs have all applied for an extension period of up to 6 months.

The WP4 Team successfully organised a Cogwheel Workshop in February and another one in September. A very well attended Thematic Integrative Meeting, on the theme "*EU surveillance frameworks and infrastructures in a harmonized One Health perspective*", was arranged during May. The trend that these integrative activities have more participants now, when everyone is used to digital meetings, is obvious.

The organisation of an OHEJP Simulation Exercise has started and the exercise is planned to be executed in May-July 2022. During spring there has also been an intense work to attract the OHEJP new partner institutes to join the JIPs. All JIPs have provided options for the new partners to join or take part of results from the projects.

2.2.5 WP5

In Y4, the purpose of the activities was to consolidate the dialogue with ECDC and EFSA as the Key EU stakeholders of the One Health EJP, as well as and with other European (EEA, EMA) and global (FAO, OIE, WHO-Euro) stakeholders. The seventh Stakeholder Committee meeting was held in connection to ASM 2021 as a hybrid in person/on online meeting, and saw participation from ECDC, EFSA, FAO, OIE, and WHO-EURO. The eight will take place in November 2021 the day before the joint POC/PMT meeting in Berlin.

Stakeholders' needs were collected by the regular "scanning of stakeholders' documents" activity, an activity continuously adjusted to keep track of evolving stakeholders' priorities, as well as by direct

contacts with organisations' representatives. The summary reports were presented on the consortium webpage accessible for all consortium members and stakeholders.
The dissemination activities of WP5 were further developed to disseminate new scientific data and results to the different stakeholder groups in a targeted manner. These included regular targeted reports to Key EU stakeholders, thematic reports, planning of dissemination workshops (mainly targeted at national stakeholders), direct contact, and other ways of targeted dissemination.
The One Health Outcome Inventory (OHOI) was deployed and content updated to support interaction and collaboration between consortium members and stakeholders. In synergy with the OHOI, as an additional way to disseminate projects' metadata, the Data Management Plan (DMP) Reader has been set up and made publicly accessible.

Additional efforts were put at targeting national stakeholders, and at mapping the situation of bilateral contacts between projects and national stakeholders, to survey the impact that projects are having at the national level

2.2.6 WP6

In Y4, several WP6 training events and educational activities and the collaborative interactions planned were impacted because of the continued travel restrictions associated with the COVID-19 pandemic.

Short Term Missions (STMs) have been most significantly impacted. The four STMs funded to take place in 2021 have been postponed until travel resumes. Two STMs funded to take place in 2020 were also postponed until 2021, however, these have now been further postponed until 2022.

The scientific research being carried out in the doctoral programme has progressed and planned outputs have continued to be produced despite the practical challenges. WP6 monitors the progress of the PhD projects through the submission of project deliverables and publications produced by the 17 PhD projects (WP6 and WP7). In total, in M37-M45, 15 deliverables were submitted (5 confidential deliverables, 10 public deliverables) and one more publication was produced in addition to two previous publications in M35 and M36. Further details of the progress of each PhD project are discussed in more detail in the individual PhD reports in this deliverable.

The second Continuing Professional Development (CPD) module was a 5-day event (theme- Digital Innovations for One health Practitioners) and was organised by the German Federal Institute for Risk Assessment and took place successfully online in M38. The second CPD deliverable report D6.9 was submitted in M40.

The ASM Satellite workshop 2021 (theme- Online Software Fair) was also organised by the German Federal Institute for Risk Assessment and took place successfully as a hybrid event in M42. The ASM 2021 deliverable report D6.11 will be submitted in M48 as planned.

The Summer School 2021 (theme- Environmental Issues in One Health- from risk assessment to surveillance) was organised by the Italian National Institute of Health (Istituto Superiore di Sanità, ISS) and took place successfully as a two-week online event over M43 and M44. The Summer School 2021 deliverable report D6.12 will be submitted in M48 as planned.

The final WP6 calls for the summer school, CPD module, satellite workshop and STMs to take place in Y5 were successfully launched in M37. Six STM applications were selected to be co-funded through the STM 2022 call. One application was received to organise the ASM Satelite Workshop 2022 from University of Surrey and NUIGalway. This application was reviewed by PMT who provided their validation and feedback to the applicants who adapted the programme to ensure these points were addressed. There were no applications received to organise the third or fourth CPD modules or the fourth summer school. These calls were extended until M47, and in addition to promoting these calls via the education and training monthly bulletin and newsletters, WP6 made specific requests to PMT, SSB and new consortium partners to forward to contacts in their respective institutes.

2.2.7 WP7

WP7 has build-up important steps in order to achieve the OHEJP long-term sustainability, namely

- Two modules on AMR and OH have been organized in order to gather inputs and expectations of stakeholders and exploit data analysis. The AMR module has already started, in collaboration with the core group of the future AMR partnership in Horizon Europe, the OH module is ready to be launched
- An outline of SRIA has been prepared and is being circulated to PMT for comments
- The PhD project SUSTAIN is investigating limits and opportunities for OH institutionalization, using as models Italy and Sweden. Published peer-reviewed papers document the progress achieved until now.

2.3 Progress beyond the state of the art, expected results until the end of the project and potential impacts

Consistent with the "Prevent-Detect-Respond" concept, integrative activities will feed the approach of evidence based risk assessment and therefore risk management by the competent authorities.

Intensive collaboration between the most relevant partners in Europe in the field of foodborne zoonoses and antimicrobial resistance contribute to help to reduce unnecessarily duplication of work on these topics.

It is of importance to efficiently organize knowledge dissemination to the appropriate stakeholders (ECDC, EFSA, DG AGRI, DG Santé, the national authorities and beyond); these tasks were and will be taken forward by WP2 (Strategic Research Agenda), WP5 (Science to Policy) and WP6 (Education & Training).

The EJP aims at enhancing harmonization, alignment and integration of activities in these domains, but this process may not be finalised at the end of the 5-year programme. To make sure that the integrative activities will last beyond the lifespan of the One Health EJP, a specific WP (WP7) is dedicated to create a significant long-term capacity building and alignment among all EJP partners.

3 WP1 - Coordination and Management

3.1 Work carried out to date

The One Health EJP has entered its last phase, commenced the descent with main objective of a smooth and successful landing. Upon validation by the REA, the initial ending date of the project will be postponed to 9 months later, i.e. end of September 2023. Among other objectives, that will notably allow to dedicate most of the extension period to dissemination of result and demonstration of impact provided by the scientific production of the OHEJP towards national, EU and Int'l stakeholders. A clear plan of dissemination activities, allowing coordinating all efforts from all protagonists in charge of dissemination and impact, namely the PMT and the Communications Team, is currently being reflected and drafted. The major goal for this last phase of the project is to optimise dissemination of outcomes and production of impact. To this end, from September 2020 on, all governance meetings will now be turned to contributing to disseminate outcomes and prove impact. Indeed, the governance meetings of the OHEJP gathers Scientific and General Directors of the participating institutes, national stakeholders such as the line Ministries, European stakeholders namely ECDC, EFSA, EMA, EEA, EC-DGs Health & Consummers, Agri and RTD, international stakeholders namely WHO, FAO and OIE. This is a perfect forum where to address dissemination of results and demonstration/promotion/creation of impact.

3.1.1 Task 1.1: Management of EC contractual obligations

Regarding contractual procedures, the OHEJP Support Team (ST) has ensured a strict monitoring of the deliverables and milestones, which has allowed the submission of the deliverables due in the period as well as the notification of the milestones achieved. The WP1 deliverables (Summary Progress Report Y4 - D1.14, Annual Work Plan Y5 - D1.15 and Ethical review report for year 3 – D1.25) have been prepared by the Coordination Team in order to report to the REA. The ST has also prepared the third amendment to the Grant Agreement with the purpose to modify the composition of the EJP One Health Consortium by addition of 6 new beneficiaries and to update the Annexes 1 and 2 of the GA in order to reflect any modification occurred in 2020 (Y3) in the action planned. The third Amendment to the Grant Agreement (AMD-773830-46) was approved by the European Commission on 16 February 2021.

3.1.2 Task 1.2: Project management

The Coordination Team (CT), consisting of the Coordinator, Scientific Coordinator and Support Team, provided effective management support to ensure the quality of the work both in terms of results and timing and to manage the relationships between partners and to ensure an effective internal communication. The CT has frequently organised videoconferences to monitor the project's progress and to ensure the timely implementation of the AWP year 4. When any important issue has arisen, the Coordination team has liaised with the Research Executive Agency (REA) to inform the Project Officers (PO) in the first place and request a delay in the submission of deliverables or a change of content of Annex 1 of the Grant Agreement whenever needed and relevant.
The CT and the Project Management Team (PMT) held regular videoconferences to monitor the progress of the activities per work package (WP). The PMT reviewed, commented and provided relevant guidance and input on important WP documents, and validated the deliverables, which have been prepared and submitted during this period.

3.1.2.1 *Carrying out the enlargement procedure*

The enlargement campaign was initiated in June 2020 and finalised in March 2021 through validation of the 3rd amendment to the GA (AMD-773830-46)

During the enlargement process, all the organisations identified as relevant to either achieve the twining PH/AH in already participating EU Member States, or to bring into the Consortium the Institutes from the missing EU Member States, were invited to join the Consortium. The steps undertaken resolved with the successful inclusion of the following 6 new Beneficiaries in the OHEJP consortium:

- P42-NMVRVI-National Food and Veterinary Risk Assessment Institute, Lithuania (VET/Food or Animal Health/food Safety), allowing Lithuania to be part of the OHEJP consortium as a new EU Member State.
- P43-ISCIII-Institute of Health Carlos III (SP) (MED or Public Health), allowing to achieve the twinning PH/AH in Spain.
- P44-BIOR-Institute of Food safety, Animal Health and Environment, Latvia (VET/Food or Animal Health/food Safety), allowing Latvia to be part of the OHEJP consortium as a new EU Member State.
- P45-RUOKA-Finnish Food Authority, Finland (VET/Food or Animal Health/food Safety) and P46-THL-Finnish Institute for Health and Welfare, Finland (MED or Public Health), allowing Finland to be part of the OHEJP consortium as a new EU Member State and to achieve the twinning PH/AH
- P47-NEBIH- National Food Chain Safety Office, Hungary (VET/Food or Animal Health/food Safety), allowing to achieve the twinning PH/AH in Hungary.

The OHEJP Consortium is now brought to 44 members from 22 countries out of which 20 EU Member States and 2 EU countries, namely Norway and the UK. Three new EU Member States are now involved: Finland, Latvia and Lithuania.

The official kick-off of the newly enlarged OHEJP Consortium was given during an online welcome meeting organised on 3 March 2021 and gathering the representatives of the new partners and the OHEJP PMT, CT and Communications team.

The effective integration into OHEJP activities was implemented from March to June, when the new members were encouraged to join the ongoing activities, in particular the integrative projects of WP4 and the education and training activities proposed by WP6.
The integration of the research teams into the JIPs was carried out by WP4, which coordinated the discussions between the scientific teams to identify the integrative activities relevant for the new members. The Support Team, for its part, has allocated the necessary budget to the new members participating to these integrative activities. A financial meeting for new members where internal rules of financial management were introduced, was organised on 20 May 2021.

3.1.2.2 *Preparation of the request for a no-cost extension of the OHEJP*

Last year at the SSB meeting in September 2020, the possibility of requesting a no-cost extension request for the OHEJP that would cope making up delays encountered in implementation of the work due to the COVID-19 pandemic and the lockdowns endured all over European countries, was mentioned. The duration of such extension was presented at that time as having to be 9 months, i.e. the project would last no more up until December 2022 but up until end of September 2023.
The rationale behind asking a 9 months extension is:

- having more time to terminate the scientific activities by giving them 6 more months to achieve their work plan and thus make up delays due by the COVID-19 pandemic, in no more ending 6 months before the end of the OHEJP, i.e. at the end of June 2022, but 9 months before the extended end of the OHEJP, i.e. at the end of December 2022.

- being able to optimize all the dissemination of outcomes over the extended period in 2023 and then maximise impact, which will be the main parameter onto which the success of the OHEJP will be assessed by the EU participating countries and the EC.

A pre-requisite of requesting such no cost extension for a definite duration is ensuring:

- that there would be enough unspent budget at the end of the initial ending date of the project, allowing to cover the budget needs over the extended period,
- that the cofunding rate at the end of the OHEJP resulting from the proportion of the 100% EU funded costs declared in relation to the total costs declared (100% EU funded costs + cofunded costs), remains acceptable by the consortium i.e. +/- 44%.

The Coordination Team, in collaboration with the efficient support of the Support Team, undertook this analysis. A detailed document presenting the methodology, findings and conclusions of this financial analysis has been elaborated. The conclusions have been presented to the Project Management Team, which endorsed them and to the Scientific Steering Board, during its meeting of 15 September 2021, which did not provide any objection to the choice of requesting a 9 months extension of the project. The final approval of the 9 months extension request is expected to be delivered at the next joint PMC/POC meeting of 24 November 2021.

3.1.3 Task 1.3: Organisation of EJP management and governance meetings

All consortium governance bodies (Coordination Team, Project Management Team, Scientific Steering Board, Programme Managers Committee) have actively cooperated in supporting the efficient implementation of the One Health EJP activities. Due to Covid-19 context, all the management and governance meetings have been implemented as online meetings.
The Scientific Steering Board (SSB) holds two meetings per year. The first 2021 SSB meeting was held online on 18 March. Due to Covid-19 pandemics, it was implemented as an online meeting that successfully meets its objectives including:

- review and validation of the periodic technical and financial report of year 3 (2020) and discussion on the options of OHEJP's duration extension.
- presentation of the outcomes of the Consortium enlargement campaign and welcome of the new SSB members issued from the new partner Institutes.
- Presentation of the new Integrative activities to be launched in 2021/2022 (JIP COVRIN and Simulation exercise).
- discussion on the progress of the JRPs and JIPs and on the sustainability of the OHEJP, notably within the future EU Partnerships.
- Discussion to request an extension of the project over 2023, which exact duration has still to be decided according to the expected remaining budget.

The second 2021 SSB meeting took place on 15 September as an online event. At this meeting, the Consortium has succeeded in implementing an effective process to disseminate the outcomes of the EJP One Health and to demonstrate its impact at the national levels. This exercise, implemented with an active participation of the scientific projects leaders and SSB members, allowed fostering the outcomes implementation efforts within the OHEJP Consortium. This effort to collectively aim to an effective translation of science to policy will be pursued and encouraged at the next Consortium meetings. In addition, the meeting allowed to validate the OHEJP Annual Workplan Y5 and the Summary Progress Report Y4, to discuss the implementation of the simulation exercise to be

conducted in Y5 and to provide the SSB members with a detailed analysis of the existing options to implement the 9M extension of the OHEJP duration during 2023.

Similar to last year, the PMT agreed to organize a joint meeting of the Programme Manager Committee (PMC), the Governing Board of the OHEJP and the Programme Owner Committee (POC), composed of the Beneficiaries' line Ministries Representatives. It will be delivered as an online meeting on 24 November 2021. The objective pursued is to update the attendees with the available outcomes of the One Health EJP activities, specifically related to the work in the relevant reference centres and laboratories, as well as related to risk assessment and management. In addition, further initiatives for collaborations and perspectives for the future of the EJP will be discussed. Therefore, all One Health EJP stakeholders (EEA, EMA, EFSA, ECDC, FAO, OIE and WHO-Europe) and relevant EC DGs representatives (REA and the steering Group members of DG RTD, H&C and Agri) will be invited to attend as well.

Furthermore, an online meeting with the External Scientific Advisory Board (ESAB) was held on 21 June 2021 and discussed the progress One Health EJP made since the last ESAB meeting in June 2020. Questions were raised about the ability of the One Health EJP to timely react on upcoming urgencies, like the COVID-19 crisis. ESAB members requested more information about the training and education activities. The main discussion in the ESAB meeting was about the possible incremental benefit that the One Health EJP has on One Health issues, as described in the NEOH handbook on Integrated approaches to health. Although PMT members are convinced of the potential cross-sector cooperation that is facilitated by the EJP, it is difficult to attribute, after 3,5 years, some unequivocal impact the project has on One Health in Europe. The challenge for the upcoming months will be to detail what the One Health definition of the EJP is, and to demonstrate how outputs and outcomes have an impact on the cooperation and collaboration between local, regional, national or international authorities across animal health, public health and food safety in Europe.

3.1.4 Task 1.4: Communication tools

- The Communications Team have strengthened the One Health EJP brand and communications outputs for One Health EJP events, including the Summer School 2021, ASM 2021, CPD 2020 & 2021, ASM Satellite Workshop 2021.
- The Communications Team have supported all OHEJP events. This included the promotional social media campaign, posting on the website, providing videoconference links and logos to local event organisers. The Communications Team provides an extensive list of support tools before, during and after each event providing delegates with a branded and professional experience.
- The Communications Team have worked to improve the dissemination of outcomes, including overarching One Health EJP deliverables, Publications, WP6 outcomes etc.
    - Communication with WP3 and 4 has been strengthened, in addition to improving relationships and building trust with the Project Leaders. This has improved the ability to collection information from the One Health EJP projects, in addition to supporting the Project Leaders in disseminating their outcomes.
    - The Communication Team have also worked with WP5 to ensure that the One Health EJP outcome inventory was widely disseminated to relevant audiences.
    - The Communications Team have supported events such as the Cogwheel Workshops and Thematic Integrative Missions to create flyers and disseminate meeting details to ensure relevant members of the consortium were invited.
- The website has been further expanded to accommodate new One Health EJP brochures such as the Scientific Activities Document, Annual Report 2021, OHEJP Overview brochure, One Health Outcome Inventory and Data Management plan.

- The website now includes a One Health EJP blog which is utilised to highlight outcomes and news for the consortium is a less formal way to appeal to wider audiences such as non-scientists.
- The One Health EJP social media channels have been a key hub of information for our online audiences and continued to grow and develop in the fourth year. The Communications Team focussed attention to supporting stakeholders spread their messages to support sustainability, sharing key One Health EJP news and information and using WP5's scanning documents to increases the number of social media posts.
    - Supporting key stakeholder's social media campaigns and sharing their policy documents is a key factor in social media planning.
- The deliverable D1.12: Annual report on the internal and external newsletters produced during the third year was completed and disseminated in M37.
- The deliverable D1.13: annual report for stakeholders n°3 has been completed in M42 and widely disseminated to all OHEJP audiences, including the general public. It details the key objectives of the OHEJP and the progress made by all WPs, JRPs, JIPs and other activities, including but not limited to dissemination and communications.
- The Communications Team have taken an active role in the DMP Committee to ensure that communication aspects of the data management plan are taken into consideration and information about the One Health EJP DMP is disseminated.
- Creating and updating a "Dissemination Information Pack" (DIP) was an important task of the fourth year. This year was the first year that the DIP has been updated and therefore supporting WP3 and WP4 with updating the Dissemination Procedure, Zenodo User Manual and Publication Policy have been key tasks. Additionally, the effective dissemination of the DIP to the consortium was of paramount importance to ensure that consortium members are supported.
- An Overview Presentation for the One Health EJP was created. This presentation will act as a template for consortium members when they present the One Health EJP to internal or external audiences allowing communication of consistent messaging. This presentation was shared with PMT on SharePoint and also disseminated to consortium members including, SSB, CCPs, Project Leaders and PhD students.
- Consortium newsletters have been sent on a quarterly basis in M39, M42 and M47. These newsletters are targeted to consortium members only and were disseminated to internal OHEJP mailing lists. These newsletters were also uploaded to the 'Newsletters' page on the OHEJP website.
- External Newsletters, which were published in M38 and M45, were significantly improved in terms of its design and content. This improvement aimed to improve engagement from external audiences and elevate the branding and impact of the One Health EJP news. These newsletters are targeted to those outside of the OHEJP consortium and those who have signed up to the OHEJP mailing list (819 subscribers). The newsletter is sent to the external mailing list, internal mailing lists, shared on social media and is uploaded to the OHEJP website on the 'Newsletters' page.
- Support for creating and editing videos for the WP6 2022 calls was provided to improve communications surrounding the funding calls for the 2022 events organised by WP6. Furthermore, videos were created and embedded in a presentation for the ASM to highlight the activities of the One Health EJP.
- A substantial role in dissemination of deliverables, outcomes and data has been undertaken by the Communications Team this year. The Communications Team have worked closely with the METASTAVA project to create an interactive pdf document which highlights the key outcomes of the project. Work has also started to create an interactive pdf for the IMPART project which will be disseminated before the end of year 4. These documents will be created for additional One Health EJP projects in year 4 and 5.

- During the One Health EJP Summer School 2021 the Communications Team organised a Communication Workshop which addressed topics such as social media, branding and event communications.
- Presenting at the governance meetings to update and demonstrate what the Communications Team have achieved and plan to achieve for the upcoming year, in addition to highlighting the impact of OHEJP projects.

3.1.5 Task 1.5 Ethics

The Ethical review report for Y3 (D1.25) was delivered as expected and has not identified any significant issues. In their report, the ethics advisors recommended that additional training activities related to ethics oversight, research ethics review and wider ethics in research would be useful to both young and experienced researchers. For that reason, the ethics advisors were invited to give a keynote speech on ethics on the third day of the ASM2021 meeting in Copenhagen.

3.2 Deliverables and Milestones

3.2.1 Deliverables

Del. Ref.	Deliverable title	Est. Del. Month	Notification
D1.12	Annual report on the internal and external newsletter produced during the third year	M37	Submitted timely
D1.13	Complete version of annual report for stakeholders n°3	M41	Submitted timely
D1.14	Summary progress report year 4	M45	Submitted with a one week delay
D1.15	Annual work plan for the fifth year	M45	Submitted with a one week delay
D1.25	Ethical review report for Y3	M38	Submitted timely

3.2.2 Milestones

Mil. Ref.	Milestone title	Est. Del./ Achievement Month	Notification
MS7	SSB Meeting n°6	M38	Acheived
MS8	SSB Meeting n°7	M44	Acheived
MS15	PMC/POC/ESAB & SC Annual meeting n°4	M45	In preparation, to be held on 24 Nov 2021

4 WP2 – Integrative strategic research agenda

4.1 Work carried out to date

4.1.1 Task 2.1: Development of the SRA

At the start of OHEJP, in January 2018, a provisional Strategic Research Agenda (SRA) has been delivered. In 2018, the SRA was updated and in the first month of 2019 the updated SRA was delivered (D2.7). The SRA is the product of a structured prioritisation process in which priority research and integrative topics have been identified. In this process both research and integrative needs of the EU member states participating in OHEJP as well as EU stakeholders (ECDC and EFSA) have been taken into account. The lists and descriptions of the priority topics have provided the basis for the internal calls for Joint Research Projects (WP3), Joint Integrative Projects (WP4) and PhD projects (WP6). In addition to the updated SRA, which was delivered as a confidential document (D2.7), a more concise, public version of the SRA was developed to be used for external dissemination purposes. This public SRA was delivered as an extra deliverable (D2.10). Furthermore, in task 2.1 relevant scientific developments, including the emergence of zoonotic threats and opportunities for scientific innovations are monitored. For this, the output from OHEJP projects (e.g. deliverables, scientific publications) is screened, as well as relevant information from external sources (e.g. conferences, scientific meetings, published papers, etc.). In 2021, a gap analysis of the priority topics selected in the second internal call for project proposals has been conducted to identify gaps in One Health research and/or integrative areas that have not been (sufficiently) covered by the OHEJP, as well as key outputs/unique selling points of OHEJP (e.g. scientific approaches and innovations, guidelines, databases developed, etc.) that would benefit future partnerships. Also, an experts elicitation has been conducted among the partner organizations, including partners that recently joined the OHEJP, to update the priority research topics. This information will be used as input for the development of the strategic research and innovation agenda (SRIA) in WP7.

4.1.2 Task 2.2: Strategic interactions with EU projects and initiatives

The main objective of this task is to foster strategic interactions with related EU projects and initiatives in order to avoid duplicity and strengthen collaboration between projects working in the same thematic areas. For this, an analysis of the relevant EU-projects/initiatives and potential strategic interactions is made. The information available in the CORDIS (Community Research and Development Information Service) database and the OHEJP partners regarding relevant EU-projects/initiatives has been compiled and centralized in a repository of EU projects/initiatives that is available to all partners through the OHEJP website. This inventory (Milestone 22) and the reports on the identification of relevant EU-projects and initiatives and the procedure to identify potential strategic interactions (D2.3) and potential strategic interactions with EU projects and initiatives (D2.4) was delivered during year 1. During the following years, as defined in the deliverables, the repository has been updated and uploaded on the website. During 2021 new relevant EU-projects/initiatives in the three main domains of foodborne zoonoses, antimicrobial resistance (i.e. IMPACT) and emerging threats (i.e. PhagoPROD) have been included. Regarding potential strategic interactions, contact has been established through e-mails, videoconferences or in congresses/meetings to look for synergies with other projects and initiatives. All WP and project leaders have also participated actively in looking for interactions with other relevant EU projects to avoid duplications. WP2 has been in contact with WP4 for the selection of EU projects (VEO and MOOD) for the organization of the Cogwheel workshops of 2021.

4.2 Deliverables and Milestones

4.2.1 Deliverables

Del. Ref.	Deliverable title	Est. Del. Month	Notification
D2.8	Report on scientific developments	M48	The initial date of submission in M42 has been postponed to M48 to allow the finalisation of the task. Finally, the D2.8 will be submitted in early Oct 2021 (M46).
D2.9	Report on strategic links established and strategic developments	M48	The initial date of submission in M42 has been postponed to M48 (new date will be requested I the next amendment to the GA) to allow the finalisation of the task.

4.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS23	Inventory of scientific developments	M42	A gap analysis of the priority topics selected in the second internal call has been conducted as well as an expert elicitation to update the priority research topics.
MS24	List of strategic synergies with EU projects and strategic developments	M42	Detailed list of strategic synergies with other EU projects working on the same thematic areas as the OHEJP.

5 WP3 - Joint research projects

5.1 Work carried out to date

5.1.1 Task 3.1: Drawing up of guidelines for submission, selection and evaluation of JRP proposals as well as request of extension of accepted JRPs.

The activities related to this task and the corresponding deliverables have been finalised in 2019; no more new official guidelines are expected. However, due to the COVID-19 crisis and consequently the many delays encountered in most projects, there was a need to allow more time to the researchers to accomplish their tasks. This was especially pressing for the second call projects, for which the crisis takes place almost from the very start of their activities. Similar to what was done for the first round projects, guidelines were drafted that served Project Leaders from the second call to ask for a no cost extension until the end of December 2022. In the procedure a template was included to streamline the requests.

5.1.2 Task 3.2: Supervision of the JRP in the first round of projects

In December 2020, five first call Joint Research Projects were finalized, i.e. IMPART, RADAR, METASTAVA, AirSample and MedVetKlebs. MAD-Vir had already come to an end a year before, in December 2019. In June of year 4, the final first-call JRP were terminated, i.e. ARDIG, TOX-Detect, NOVA, ListAdapt and MoMIR-PPR. The final reports were submitted in two steps: a first provisional version by mid-June and a final version at the end of August 2021.

The monitoring of projects of the first and second round is described further under “Detailed follow up of Joint Research Projects”.

In the spring of 2021, the project evaluation process was started for the five JRP that submitted their final report. The evaluation procedure D.9 (Guidelines for WP3 on the evaluation of final reports) was clarified to describe the evaluation by two scientific external experts (one of whom had previously evaluated the project proposal) and one ‘end user’, i.e. a representative of the Programme Owner Committee. This approach allowed the evaluation of both the scientific and integrative outcomes. An online registration form was developed to facilitate the final selection of the evaluators. The evaluation forms were received and were copied in the evaluation report D3.17 that will be completed in the autumn of 2021 when the evaluation reports of the last JRP will become available.

5.1.3 Task 3.3: Organisation of a second round of projects and their supervision.

A full report (D3.15) on the progress made by all JRP was delivered on time in spring 2021. It describes the full details of the five projects that were finalized in December 2020 (i.e. ARDIG, RADAR, METASTAVA, AirSample and MedVetKlebs), and the progress of all other 18 ongoing JRP. The process regarding the evaluation of the final reports by external experts was launched and completed. The evaluation reports were inserted in deliverable D3.17 (First report on the evaluation of finalised JRPs), which is due by the end of December 2021.

5.1.4 Detailed follow up of JRP

5.1.4.1 *Milestones and Deliverables of JRP*

Deliverables

88% of the due deliverables are available.

The delays were mainly due to Covid-19 situation or to the confidentiality (the deliverable will be disclosed after the approval of the publications).

PROJECT	DELIVERABLES DUE FOR SEPTEMBER 2021	ACHIEVED DELIVERABLES	% OF ACHIEVED DELIVERABLES
JRP02-ARDIG	17	15	88%
JRP05-TOXDETECT	20	17	85%
JRP06-NOVA	34	31	91%
JRP07-LISTADAPT	23	21	91%
JRP10-MOMIRPPC	32	30	94%
JRP12-FARMED	1	1	100%
JRP13-WORLDCOM	6	4	67%
JRP14-FULLFORCE	20	20	100%
JRP15-FEDAMR	12	12	100%
JRP16-TELEVIR	6	5	83%
JRP17-IDEMBRU	4	4	100%
JRP18-MEME	5	5	100%
JRP19-PARADISE	7	7	100%
JRP20-DISCOVER	3	1	33%
JRP21-BIOPIGEE	9	6	67%
JRP22-TOXOSOURCES	5	5	100%
JRP23-ADONIS	6	2	33%
JRP24-BEONE	4	3	75%
TOTAL	**214**	**189**	**88%**

Milestones

PROJECT	MILESTONES DUE FOR SEPTEMBER 2021	ACHIEVED MILESTONES	% OF ACHIEVED MILESTONES
JRP02-ARDIG	16	15	94%
JRP05-TOXDETECT	25	19	76%
JRP06-NOVA	29	29	100%
JRP07-LISTADAPT	24	23	96%
JRP10-MOMIRPPC	38	33	87%
JRP11-MEDVETKLEBS	5	0	0%
JRP12-FARMED	2	1	50%
JRP13-WORLDCOM	18	11	61%
JRP14-FULLFORCE	28	20	71%
JRP15-FEDAMR	4	2	50%
JRP16-TELEVIR	17	14	82%
JRP17-IDEMBRU	17	12	71%
JRP18-MEME	16	7	44%
JRP19-PARADISE	22	16	73%
JRP20-DISCOVER	20	12	60%
JRP21-BIOPIGEE	18	18	100%
JRP22-TOXOSOURCES	14	10	71%
JRP23-ADONIS	22	8	36%
JRP24-BEONE	16	15	94%

PROJECT	MILESTONES DUE FOR SEPTEMBER 2021	ACHIEVED MILESTONES	% OF ACHIEVED MILESTONES
TOTAL	**335**	**250**	**75%**

5.1.4.2 *Publications of JRP*

5.1.4.2.1 Scientific Publications

In 2021, there were 30 JRP publications. (Please note that the following table does not take into account the publications of the first round projects that came to an end in 2020. The publications of Impart, Radar, Madvir, Metastava, Airsample and MedVetKlebs were reported in the previous OHEJP SPR (2020)).

PROJECT	2018	2019	2020	2021	TOTAL
JRP02-ARDIG	0	2	9	5	16
JRP05-TOXDETECT	0	2	1	3	6
JRP06-NOVA	1	3	5	1	10
JRP07-LISTADAPT	0	0	2	3	5
JRP10-MOMIRPPC	0	1	5	0	6
JRP12-FARMED	0	0	0	0	0
JRP13-WORLDCOM	0	0	0	1	1
JRP14-FULLFORCE	0	0	1	1	2
JRP15-FEDAMR	0	0	0	0	0
JRP16-TELEVIR	0	0	1	0	1
JRP17-IDEMBRU	0	0	0	0	0
JRP18-MEME	0	0	7	3	10
JRP19-PARADISE	0	0	2	2	4
JRP20-DISCOVER	0	0	0	1	1
JRP21-BIOPIGEE	0	0	0	0	0
JRP22-TOXOSOURCES	0	0	4	10	14
JRP23-ADONIS	0	0	0	0	0
JRP24-BEONE	0	0	1	0	1
TOTAL	1	8	38	**30**	77

JRP02-ARDIG

2019

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Schares, G.; Globokar Vrhovec, M.; Tuschy, M.; Joeres, M.; Bärwald, A.; Koudela, B.; Dubey, J. P.; Maksimov, P.; Conraths, F. J. **A Real-Time Quantitative Polymerase Chain Reaction for the Specific**

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https://zenodo.org/record/4647840#.YGNQk68zaUk

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https://zenodo.org/record/5516907#.YUg8qOxxe70
Verkola, M.; Järvelä, T.; Järvinen, A.; Jokelainen, P.; Virtala, A.; Kinnunen, P. M.; Heikinheimo, A**. Infection Prevention and Control Practices of Ambulatory Veterinarians: A Questionnaire Study in Finland**. *Vet Med Sci* **2021**, vms3.464. https://doi.org/10.1002/vms3.464.
https://zenodo.org/record/4647937#.YGNfea8zaUk

Schares, G.; Joeres, M.; Rachel, F.; Tuschy, M.; Czirják, G. Á.; Maksimov, P.; Conraths, F. J.; Wachter, B. **Molecular Analysis Suggests That Namibian Cheetahs (Acinonyx Jubatus) Are Definitive Hosts of a so Far Undescribed Besnoitia Species.** *Parasites Vectors* **2021**, *14* (1), 201. https://doi.org/10.1186/s13071-021-04697-3.
https://zenodo.org/record/4694200#.YHg5Z-gzaUk

JRP24-BEONE

2020
Dahl, L. G.; Joensen, K. G.; Østerlund, M. T.; Kiil, K.; Nielsen, E. M. **Prediction of Antimicrobial Resistance in Clinical Campylobacter Jejuni Isolates from Whole-Genome Sequencing Data.** *Eur J Clin Microbiol Infect Dis* **2020**. https://doi.org/10.1007/s10096-020-04043-y.
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5.1.4.2.2 Oral presentation

JRP02-ARDIG

Katharina Juraschek, Maria Borowiak, Simon H. Tausch, Burkhard Malorny, Annemarie Käsbohrer, Saria Otani, Stefan Schwarz, Diana Meemken, Carlus Deneke and Jens Andre Hammerl (2021). DIFFERENT SEQUENCING AND ASSEMBLY APPROACHES INFLUENCING THE DETECTION OF PLASMIDS AND ANTIMICROBIAL RESISTANCE GENES IN COMMENSAL ESCHERICHIA COLI. In: OHEJP ASM. Oral presentation. https://zenodo.org/record/4957118

JRP06-NOVA

STORY MAP: https://arcg.is/1zHSub. Summary of main outputs from WP4, available online.

JRP10-MOMIRPPC

RRNA MICROBIAL COMMUNITY ANALYSIS AND RELATIONSHIP WITH SALMONELLA SUPER SHEDDER STATUS IN PIGS Guido Cordoni One Health EJP Annual Scientific Meeting 2021

Albena Dimitrova, Gergana Mateva, Gergana Krumova-Valcheva, Eva Gyurova, Mihail Milanov, Helen Brown, Guido Cordoni, Daniel Horton, Roberto La Ragione, Hristo Daskalov. INFLUENCE OF ORAL ADMINISTRATION OF LACTOBACILLUS REUTERI PROBIOTIC STRAINS AND GOS PREBIOTIC ON THE

PRESENCE OF SALMONELLA SPP. IN FATENNING PIGS. One Health EJP Annual Scientific meeting, Virtual, Copenhagen, 9th-11th June 2021.

Gergana Mateva, Krasen Penchev, Gergana Krumova-Valcheva, Albena Dimitrova, Eva Gyurova, Mihail Milanov, Helen Brown, Guido Cordoni, Jade Passey, Daniel Horton, Roberto La Ragione, Hristo Daskalov. INFLUENCE OF ORAL ADMINISTRATION OF LACTOBACILLUS REUTERI PROBIOTIC STRAINS AND GOS PREBIOTIC ON THE PRESENCE OF SALMONELLA SPP. IN BROILERS. One Health EJP Annual Scientific meeting, Virtual, Copenhagen, 9th-11th June 2021.06.28

JRP17-IDEMBRU

Brucella canis workshop, 10.5281/zenodo.4748926, Brucella canis workshop | Zenodo

JRP18-MEME

Presentation of MEME project to the One Health EJP Annual Scientific meeting, held online. 9-11 June 2021. Copenhagen, Denmark.

JRP22-TOXOSOURCES

Oral presentations at TOXO-21 webinar, 2021,
by Rafael Calero-Bernal and Pikka Jokelainen

Oral presentations at ApicoWplexa virtual meeting series, February 18, 2021:

Selection, validation and SOP development of a molecular detection method for identification of Toxoplasma gondii oocysts in leafy-green vegetables

Iva Slana1, Nadja Bier2, Borbara Bartosova1, Gianluca Marucci3, Anne Mayer-Scholl2, Pikka Jokelainen4, Marco Lalle3

Oral presentation at ApicoWplexa virtual meeting series, February 18, 2021: (audience n 120, scientists)
A comparative study of the most widely used serological tests in the diagnosis of Toxoplasma gondii infection in small ruminants
López-Ureña, Nadia María1; Calero-Bernal, Rafael1; Pazmiño-Bonilla, Elvis Daniel1; Vázquez-Calvo, Ángela 2; Sánchez-Sánchez, Roberto1; Ortega-Mora, Luis Miguel1; Álvarez-García, Gema1

Invited oral presentation at RSU conference, virtually in Riga, Latvia
Pikka Jokelainen
Poster presentation at CSBSP
Pikka Jokelainen

Presentation at internal dissemination event 'OHEJP at SSI' 4.5.2021 (audience n=55, scientists)
Pikka Jokelainen

OHEJPASM 2021, June 9-11, 2021 (audience n= 530, scientist, policy makers)
Two oral presentations and several posters. Details will be added to final 9M report.

5.1.4.2.3 Poster

JRP02-ARDIG

Mesa-Varona O, Tenhagen B-A. Comparison of antimicrobial use and resistance data on clinical and non-clinical isolates from livestock in four countries. In: Junior Scientist Zoonoses Meeting 2021. Virtual. Poster. https://zenodo.org/record/4905583#.YL3gqvkzbY0

Mesa-Varona O, Kaspar H, Grobbel M, Tenhagen B-A. The influence of the antimicrobial use in the resistance data on clinical and non-clinical isolates from broilers and turkeys in Germany. In: the 5th

International Conference on Responsible Use of Antibiotics in Animals. Virtual. Poster. https://zenodo.org/record/4916495

Katharina Juraschek, Carlus Deneke, Silvia Schmoger, Mirjam Grobbel, Burkhard Malorny, Annemarie Käsbohrer, Stefan Schwarz, Diana Meemken and Jens Andre Hammerl (2021). HIGH DIVERSITY OF PLASMIDS CARRYING QNR RESISTANCE GENES IN FLUOROQUINOLONE RESISTANT ESCHERICHIA COLI ISOLATED IN GERMANY IN 2017. In: OHEJP ASM. Poster. https://zenodo.org/record/4957003

JRP06-NOVA

STORY MAP: https://arcg.is/1zHSub. Summary of main outputs from WP4, available online.

JRP07-LISTADAPT

Taran Skjerdal#, Tone Fagereng, Eve Fiskebeck, Karin Lagesen, Sophie Rousse. Impact of genetic variation of 200 Listeria monocytogenes strains on the growth in presence of preservatives. OH-EJP annual scientific meeting Copenhagen, 9-11 June 2021.

Toresi M, Rinaldi A, Marzio D et al. Genomic features of two main Clonal Complex groups of Listeria monocytogenes strains isolated from European wild animal. 6th World One Health Congress; 30 October - 3 November 2020.

JRP10-MOMIRPPC

F. Kempf, R. Drumo, Anne Marie Chaussé, T. Kubasova, I. Caballero, S. Roche, P. Menanteau, E. Guitton, I. Rychlik, P. Velge-Immune response, gut microbial composition, *Salmonella* super-and low-shedder phenotypes can be modulated by the inoculation of four commensal bacteria in chicken. One Health EJP Annual Scientific meeting, Virtual, Copenhagen, 9th-11th June 2021.

Poster

Poster in OHEJP ASM 2021. Rebollada-Merino, A.; Ugarte-Ruiz, M.; Miguela-Villoldo, P.; Domínguez, L.; Rodríguez-Bertos, A. Histomorphological Changes in the Bursa of Fabrizio Associated with *Salmonella* Typhimurium Infection in Animals Feed with a Nutraceutical Derived from the Olive Oil Production

JRP12-FARMED

Poster of project "FARMED: Long-read metagenomic sequencing workflow for the identification of pathogens/AMR on-site" presented at the One Health EJP Meeting, hosted in Copenhagen and online between 9-11th June 2021.

JRP13-WORLDCOM

For WP2-T1 and WP2-T2, posters entitled ''Development of LAMP assays for the detection of key AMR targets in animal faeces and water samples'' and ''Rapid LAMP detection of key AMR targets for use as bed-side and/or pen-side diagnostics'' were presented by Marwa Hassan at the 2021 Microbiology Society conference and the OHEJP 2021 ASM, respectively.

For WP1-T3 - an abstract entitled 'Automated detection of AMR target genes using loop-mediated isothermal amplification (LAMP)' was submitted by PDRA Brian Gardner at UoS to the OHEJP Annual Scientific Meeting 2021, 9th-11th June 2021 in Copenhagen. This was selected as an e-Poster presentation.

For WP2-T2 and WP2-T3, poster entitled "Rapid detection and discrimination of closely related Enterobacteriaceae CTX-M group 1 variants, *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$, using an internally controlled multiplex loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) assay" was presented by Owen Higgins at the 2021 Microbiology Society conference, the OHEJP 2021 ASM, and is accepted for the ECCMID 2021 conference.

For WP2-T1 and WP2-T2 an abstract entitled "Whole-genome sequencing of phenotypically characterized isolates from various settings" was presented by Vera Manageiro at the OHEJP Annual Scientific Meeting 2021, 9th-11th June 2021 in Copenhagen (e-Poster).

JRP14-FULLROCE

Poster presentation at the ASM meeting (Copenhagen, June 9-11, 2021).

JRP15-FEDAMR

Accepted abstract in ECCMID 2021 (e-poster): Antimicrobial resistance and genetic relatedness of environmental bacteria across the animal-human-wildlife interface in Austria (WP2, 2-AGES).

Accepted abstract in Annual Scientific Meeting 2021 (e-poster): Diversity of bacterial communities and genes encoding AMR in different environmental compartments along the food/feed chain (WP2, 36-INSA).

Accepted abstract in Annual Scientific Meeting 2021 (e-poster): Dissemination of antimicrobial resistant *Clostridium difficile* RT078/ST11 in Austria across the human-animal-wildlife interface (WP3, 2-AGES).

Accepted abstract in Annual Scientific Meeting 2021 (e-poster): Zoonotic spread of multi-resistant *C. difficile* (WP3, 13-SSI).

Accepted abstract in Annual Scientific Meeting 2021 (e-poster): Diversity and Dynamics of *Clostridioides difficile* in a farm environment PT (WP3, 36-INSA).

Accepted abstract in Annual Scientific Meeting 2021 (e-poster): FED-AMR: Determination of selection pressures for AMR in environmental samples (WP4).

Accepted abstract in Annual Scientific Meeting 2021 (e-poster): Ecological modelling of microbial communities subject to perturbation (WP6, 23-UoS).

JRP16-TELEVIR

ASSESSING CORONAVIRUS RISKS: FEASIBILITY OF PREDICTING TRAITS OF CLINICAL AND EPIDEMIOLOGICAL RELEVANCE FROM SEQUENCE DATA
C. Bogaardt 1, 2 • V. Borges 3 • J. P. Gomes 3 • J. Isidro 3 • J. M. Prada 2 • D. L. Horton 1

Poster presentation, OHEJP Copenhagen June 9-11th 2021

Poster presentation at One Health EJP Annual Scientific Meeting :
Fast and easy field-deployable Sars-CoV-2 virus inactivation for downstream analysis.
Anna S. Fomsgaard[1,2], Graham J. Belsham[2] , Ria M. Lassaunière[1] , Jannik Fonager[1] , Katja Spiess[1]
1. Virus Research & Development Laboratory, Statens Serum Institut , Copenhagen, Denmark

2. Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

JRP17-IDEMBRU

Natural habitats for detection of emerging Brucella species: a new strategy to identify putative threats (IDEMBRU) - One Health EJP Annual Scientific Meeting 2021
ASM2021_Posters.pdf (ohejp2021.com)

OHEJP2021_Abstractbook_A4_finalWEB.pdf

An innovative microfluidic qPCR platform for high throughput testing of Brucella sp. and Ochrobactrum in environmental samples- One Health EJP Annual Scientific Meeting 2021

ASM2021_Posters.pdf (ohejp2021.com)

OHEJP2021_Abstractbook_A4_finalWEB.pdf

JRP20-DISCOVER

Abstracts from the ASM One Health EJP 2021, 9-11 June, 2021:

Sara Perestrelo, Guido Correia Carreira, Lars Valentin, Jennie Fischer, Yvonne Pfeifer, Guido Werner, Judith Schmiedel, Linda Falgenhauer, Annemarie Käsbohrer. "Source Attribution of ESBL-producing Escherichia coli in Germany" Poster presentation at the ASM One Health EJP 2021, 9-11 June, Denmark and online.

Guido Correia Carreira, Annemarie Käsbohrer. "Mapping knowledge gaps of source attribution methods and sources for zoonoses and resistant bacteria using a rapid review approach". Oral presentation held at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.

Rebekka Sørensen, Tine Hald, Gaia Scavia, Michele Luca D'errico. "MAPPING THE CURRENT EXISTING HAZARDS' CONTROL PROGRAM AND STRATEGIES IN THE VARIOUS SECTORS AND TRACTS OF THE ANIMAL-ENVIRONMENTFOOD-HUMAN CHAIN IN EU". Oral presentation held at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.

Mónica Oleastro, Maria Leonor Lemos, Alexandra Nunes, Paulo Martins da Costa. "A PRELIMINARY STUDY OF CAMPYLOBACTER SPP. IN DOGS IN PORTUGAL – A ONE HEALTH PERSPECTIVE". Poster presentation at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.

Ana Amaro, Célia Leão, Patrícia Themudo, Lurdes Clemente. "EMERGENCE OF MULTIDRUG RESISTANT SALMONELLA INFANTIS ST32 IN THE POULTRY INDUSTRY, PORTUGAL 2016-2020". Poster presentation at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.

Leonor Silveira, Sofia Ribeiro, Iúri Lopes, Mariana Fontes, Rita Castro, Frederico Lemos, Angela Pista. "Prevalence and characterization of Salmonella spp. and pathogenic Escherichia coli in food-producing animals in Portugal". Poster presentation at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.

Pista A., Ribeiro S., Fontes M., Batista R., Coelho A., Furtado R., Lopes T., Moura I., Saraiva M., Maia C., Belo-Correia C., Lopes I., Silveira L. "Prevalence and characterization of pathogenic Escherichia coli and Salmonella spp. in wild animals in mainland Portugal". Poster presentation at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.

Other abstracts:
Sara Perestrelo, Guido Correia Carreira, Lars Valentin, Jennie Fischer, Yvonne Pfeifer, Guido Werner, Judith Schmiedel, Linda Falgenhauer, Annemarie Käsbohrer. "To which extend do humans and animals share the same reservoirs of ESBL producing Escherichia coli?" Poster presentation at the Junior Scientific Zoonosis Meeting 2021, 3-4 June, online.

Scientific reports:

Eric Evers (RIVM, The Netherlands), Annemarie Käsbohrer (BfR, Germany). "Assessing and developing source attribution approaches based on risk assessment", OHEJP Discover WP3-T7, milestone M-JRPFBZ-1-18, 10 February 2021.

JRP21-BIOPIGEE

Ivana Koláčková, Renáta Karpíšková, Tereza Gelbíčová, Zdenka Vacková, Jonaáš Vaňhara, Daniel Sperling: Salmonella spp. in pig farms – still an issue?. Poster at ESPHM. Online. 2021.

Gergana Krumova-Valcheva, Albena Dimitrova, Eva Gyurova, Gergana Mateva, Mihail Milanov, Hristo Daskalov. Hepatitis E in humans and pigs in Bulgaria (REVIEW). Poster at OHEJP ASM. Copenhagen. 2021. https://doi.org/10.5281/zenodo.4926180

Pachka Hammami, Nicolas Rose, Vladimir Grosbois,Stefan Widgren, Andrea Apolloni and Mathieu Andraud. Live animal movements - Understand trade partners choices to predict chains of contact. Poster at SVEPM. Online. 2021.

T. Niine, A. Viltrop, I. Nurmoja, R. Smith, E. Burow. Salmonellain slaughterhouse!? What would be the right questions in that situation?. Poster at SVEPM. Online. 2021

Smith, R.P., Vilar, M.J., Jones, H., Burow, E.. Preliminary description of biosecurity practices related to importing pig and semen onto European pig farms. Poster at OHEJP ASM. Copenhagen. 2021. https://zenodo.org/record/4906860

T. Niine, A. Viltrop, I. Nurmoja, R. Smith, E. Burow. Identification of the best biosecurity practices in slaughterhouse in regards to Salmonella and hepatitis E virus. Poster at OHEJP ASM. Copenhagen. 2021.

C. Oastler, M. Arvand, K. Konrat, A.M Osland, V. Pfiffer, L. Vestby, B. Gosling. Assessment of the biofilm forming capability of Salmonellaisolates sourced from pig farms in Great Britain. Poster at OHEJP ASM. Copenhagen. 2021.

N. Huber, E. L. Sassu, G. Krumova-Valcheva, M. Meester, I. Kolackova, P. Vasickova, E. Waller, G. Aprea, A. Käsbohrer, V. Zoche-Golob, E. Burow. Biosecurity measures reducing Salmonella ssp. andhepatitis E virus prevalence in pig farms: A systematic Review and Meta-analysis. Poster at OHEJP ASM. Copenhagen. 2021. https://zenodo.org/record/4905616
C. M^{c}Carthy, R. Simons, P. Hammami, M. Andraud, S. Widgren, B. Conrady (2021). BIOPIGEE: Modelling of the cost and effectiveness of biosecurity measures. Poster at OHEJP ASM. Copenhagen. 2021.

JRP22-TOXOSOURCES

Poster presentation at conference 'Responsible Use of Antibiotics in Animals'
Infection prevention and control practices among ambulatory livestock and equine veterinarians
Marie Verkola[1], Terhi Järvelä[1], Asko Järvinen[2], Pikka Jokelainen[3,4], Anna-Maija Virtala[4], Paula M. Kinnunen[4], Annamari Heikinheimo[1,5]

5.1.4.3 *Details of the JRPs activity reported in 9M reports*

5.1.4.3.1 JRP02-AMR2-ARDIG – Final report

5.1.4.3.1.1 Consortium composition

Name of the Project Coordinator and of all applicants, with full affiliations

Project Coordinator	Position	Affiliation	email	Gender
Prof. Muna F. Anjum	Senior Scientist; Bacterial Characterisation Workgroup Leader	Animal and Plant Health Agency (APHA), United Kingdom	Muna.Anjum@apha.gsi.gov.uk	Female

Applicant	Position	Affiliation	email	Gender
Dr. Martina Velasova	Senior Scientist	Animal and Plant Health Agency (APHA), United Kingdom	Martina.Velasova@apha.gsi.gov.uk	Female
Dr. Francesca Martelli	Senior Scientist	Animal and Plant Health Agency (APHA), United Kingdom	Francesca.Martelli@ apha.gsi.gov.uk	Female
Professor Roberto La Ragione	Professor of microbiology and pathology and Head of the Department of Pathology and Infectious Diseases	University of Surrey, School of Veterinary Medicine	r.laragione@surrey.ac.uk	Male
PD Dr. Bernd-Alois Tenhagen	Senior Scientist; AMR2-BfR Project leader	Federal Institute for Risk Assessment (BfR), Germany	Bernd-Alois.Tenhagen@bfr.bund.de	Male
Dr. Jens A. Hammerl	Research Scientist, AMR2-BfR Project Deputy	Federal Institute for Risk Assessment (BfR), Germany	Jens-Andre.Hammerl@bfr.bund.de	Female
Dr. Marianne Sunde	Senior researcher	Norwegian Veterinary Institute	marianne.sunde@vetinst.no	Female
Dr Philippe Glaser	Research Director; head of the EERA research Unit	Institut Pasteur, Paris, France	pglaser@pasteur.fr	Male
Prof. Bruno Gonzalez Zorn	Professor, Group Leader on AMR and Head of the	Complutense University, Veterinary	bgzorn@ucm.es	Male

Applicant	Position	Affiliation	email	Gender
	Department of Animal Health	School and VISAVET		
Jean-Yves Madec	Senior Scientist Head of the Antimicrobial Resistance Unit	ANSES, Lyon, FR	Jean-Yves.MADEC@anses.fr	Male
Marisa Haenni	Senior Scientist Deputy-Head of the Antimicrobial Resistance Unit	ANSES, Lyon, FR	Marisa.haenni@anses.fr	Female
Dr. Michael Brouwer	Postdoctoral researcher at the department of Bacteriology and Epidemiology	Wageningen Bioveterinary Research, formerly Central Veterinary Institute (CVI)	mike.brouwer@wur.nl	Male
Dr. Tim Eckmanns	Medical Epidemiologist	Robert Koch Institute, Germany	EckmannsT@rki.de	Male
Dr. Sebastian Haller	Medical Epidemiologist	Robert Koch Institute, Germany	HallerS@rki.de	Male
Prof. Neil Woodford	Head of AMRHAI	Public Health England, UK	Neil.Woodford@phe.gov.uk	Male
Dr. Matthew Ellington	Senior Scientist, AMRHAI	Public Health England, UK	Matthew.Ellington@phe.gov.uk	Male
Dr Sarah Gerver	Senior Epidemiologist	Public Health England, UK	berit.muller-pebody@phe.gov.uk	Female

Short CV of each participating key scientist (at least one per participating organization)

APHA: Muna Anjum

Prof. Muna Anjum and the APHA team have extensive expertise in characterization of AMR in Enterobacteriaceae isolated from food animals. The multidisciplinary team of molecular biologists, bioinformaticians, veterinarians, and epidemiologists conduct national surveillance, outbreak investigations as well as research activities to understand persistence and dissemination of AMR through the food chain. Muna Anjum is the Lead for Antimicrobial Resistance research and the Bacterial Characterisation Workgroup Leader in the Department of Bacteriology at the APHA. She leads a team of senior scientists, post-doctoral scientists and PhD students. She holds a visiting Professorship at the University of Surrey and is a member of the Health Protection Research Unit at the University of Oxford, John Radcliffe Hospital, in partnership with Public Health England. She is also a member of the DEFRA Antimicrobial Resistance Coordination (DARC) Group, which reviews activities of the UK government on antimicrobials. Muna has led or is the APHA lead in a number of national (VMD/DEFRA, DoH, NERC, BBSRC), EU and commercial projects.

APHA: Martina Velasova and Francesca Martelli

Two key members of the APHA team includes Dr. Martina Velasova, who is a senior epidemiologist and Dr. Francesca Martelli, who is a senior veterinarian. Some recent key publications are:

Abuoun M, 14 authors and Anjum MF. A genomic epidemiological study shows prevalence of antimicrobial resistance in Enterobacterales is associated with the livestock host, as well as antimicrobial use. Microbial Genomics, 2021.

Stubberfield, 4 authors and Anjum, MF. Use of whole genome sequencing of commensal *Escherichia coli* in pigs for antimicrobial resistance surveillance, United Kingdom, 2018. Eurosurveillance, 2019.

UoS: Roberto La Ragione

Prof. Roberto La Ragione, HoD for Pathology and Infectious Disease and Director of the Veterinary Pathology Centre at Veterinary School, UoS. In 2005 Roberto La Ragione was appointed head of pathogenesis and control at the AHVLA and in 2010 he was appointed Professor of Veterinary Microbiology and Pathology at the University of Surrey. Roberto gained the FRCPath in 2010 and in 2012 was appointed the Associate Dean for Veterinary Strategy in the new School of Veterinary Medicine at the University of Surrey. Roberto has published over 125 peer reviewed publications in the area of host-microbe interaction with a particular emphasis on foodborne pathogens. His current research interests focus on AMR and the pathogenesis of food-borne pathogens with a particular interest in the development of rapid diagnostics and intervention strategies including vaccination, pre and probiotics for the control of bacterial pathogens in animals. He has led a number of commercial, BBSRC, EPSRC, Defra and EU projects. Roberto is the current MedVet-Net Association president. Some important recent publications include:

Freire Martín I, Thomas CM, Laing E, AbuOun M, La Ragione RM, Woodward MJ. 2016. Curing vector for IncI1 plasmids and its use to provide evidence for a metabolic burden of IncI1 CTX-M-1 plasmid pIFM3791 on *Klebsiella pneumoniae*. J Med Microbiol.

Freire Martín I, AbuOun M, Reichel R, La Ragione RM, Woodward MJ. 2014. Sequence analysis of a CTX-M-1 IncI1 plasmid found in *Salmonella* 4,5,12:i:-, *Escherichia coli* and *Klebsiella pneumoniae* on a UK pig farm. J Antimicrob Chemother. 69(8):2098-101.

BfR: Bernd-Alois Tenhagen

PD Dr. Bernd-Alois Tenhagen is a veterinarian with a specialisation in food animal medicine and epidemiology. Since 2007 he is working at the Federal Institute for Risk Assessment in Berlin, Germany in the Department Biological Safety. He is head of the unit “Epidemiology, Zoonoses and Antimicrobial Resistance” that also houses the National Reference Laboratory for Antimicrobial Resistance. His works focus is on the epidemiology of resistant bacteria and resistance determinants in the food chain and the associated risks for public health. He has published more than 100 papers in peer reviewed journals and was involved in national and international research projects on AMR. He has been a member of several working groups of EFSA on topics related to the monitoring of AMR.

BfR: Jens A. Hammerl

Jens A. Hammerl obtained his Ph.D. from the Humboldt University of Berlin in 2012 working on the regulation of genetic switch in linear plasmid prophages. He works now in the National Reference Laboratory for Antimicrobial Resistance at the German Federal Institute for Risk Assessment on microbiological and molecular tracing of food-borne pathogens in food chains to protect consumer´s health. Further research interests are focused on the biology and genetics of antimicrobial resistances, mobile genetic elements and therapeutic applications of phages from environmental, food-borne and highly pathogenic bacteria.

Relevant papers from BfR include:

Irrgang A, N Roschanski, BA Tenhagen, M Grobbel, T Skladnikiewicz-Ziemer, K Thomas, U Roesler, and

A Käsbohrer. 2016. Prevalence of mcr-1 in E. coli from Livestock and Food in Germany, 2010-2015. PLoS One. 25;11(7):e0159863.

Fromm S, Beißwanger E, Käsbohrer A, Tenhagen BA. Risk factors for MRSA in fattening pig herds - a meta-analysis using pooled data. Prev Vet Med. 2014 Nov 1;117(1):180-8. doi: 10.1016/j.prevetmed.2014.08.014. Epub 2014 Sep 2.

NVI: Marianne Sunde

Dr. Marianne Sunde is a senior researcher (DVM, PhD, Professor equivalent) in veterinary microbiology at the Norwegian Veterinary Institute. Her main research activity has been on antimicrobial resistance. Dr. Sunde also holds a position at the Norwegian Institute of Public Health were the main research area is antimicrobial resistance in a "One Health" perspective. She has long-time experience in research on antimicrobial resistance and has been a key person in bacteriological diagnostics as well as monitoring of resistance in the veterinary sector in Norway. She has been project leader for national and Scandinavian research projects, has supervised Master and PhD students, has experience as a reviewer for scientific journals and is Assistant Editor for BMC Vet Res. Recent publications are:

Grøntvedt CA/P Elstrøm, M Stegger, RL Skov, PS Andersen, KW Larssen, AM Urdahl, Ø Angen, J Larsen, S Åmdal, SM Løtvedt and M Sunde/JV Bjørnholt. 2016. MRSA CC398 in humans and pigs in Norway: A "One Health" perspective on introduction and transmission. Clin Infect Dis.

Mo SS, JS Slettemeås, ES Berg, M Norström and M Sunde. 2016. Plasmid and Host Strain Characteristics of Escherichia coli Resistant to Extended-Spectrum Cephalosporins in the Norwegian Broiler Production. PLOSone 0154019

CVI: Michael Brouwer

In 2006 Michael graduated from the University of Amsterdam in Biomedical sciences, followed by a MSc degree in Immunology in 2008. His PhD research focussed on studying the mobile genetic elements of the nosocomial pathogen *Clostridium difficile* in the group of Prof. Peter Mullany at University College London. During his first postdoctoral appointment in the group of Dr. Adam Roberts in 2012, also at UCL, Michael started the study of resistance conferring plasmids in Gram-positive bacteria. In 2013 he switched to studying the plasmids of Gram-negative bacteria in the group of Prof. Dik Mevius at the Central Veterinary Institute. This research focusses on the molecular mechanisms employed by plasmids for conjugation, plasmid genomics and testing and development of novel strategies for the detection of plasmids and resistance genes. Some important publications include:

Veldman K, van Essen-Zandbergen A, Rapallini M, Wit B, Heymans R, van Pelt W, Mevius D. Location of colistin resistance gene *mcr-1* in Enterobacteriaceae from livestock and meat. J Antimicrob Chemother. 2016 May 30. pii: dkw181. PMID: 27246233

Brouwer MS, Tagg KA, Mevius DJ, Iredell JR, Bossers A, Smith HE, Partridge SR. IncI shufflons: Assembly issues in the next-generation sequencing era. Plasmid. 2015 Jul;80:111-7. doi: 10.1016/j.plasmid.2015.04.009. Epub 2015 May 4. PMID: 25952328

Institut Pasteur: Philippe Glaser

Philippe Glaser is co-director with Thierry Naas of the Ecology and Evolution of Antibiotic Resistance Unit, a joint unit between the Institut Pasteur and the Bicêtre hospital. It hosts the F-NRC laboratory for CPE. Philippe Glaser is an internationally recognized expert in bacterial genomics and evolution. He has a broad experience in analysing large sets of WGS data. He has recently shown by the analysis of 230 Group B Streptococcus (GBS) genomes that the massive use of tetracycline has been responsible for the emergence of neonatal infections in the 1960s. He also compared population structures of humans and bovine GBS. Thierry Naas director of the NRC is a pioneer in the field of carbapenemase genes and of their mobility. The Institut Pasteur Biomics group will provide all the equipment and the expertise in genomics. Some key publications are:

Glaser P, et al. Demography and Intercontinental Spread of the USA300 Community-Acquired Methicillin-Resistant Staphylococcus aureus Lineage. MBio. 2016, 7(1).

Da Cunha V,28 authors & Glaser P (2014) Streptococcus agalactiae clones infecting humans were selected and fixed through the extensive use of tetracycline. Nature communications, 2014, 5:4544.

UCM: Bruno Gonzalez-Zorn

Prof. Bruno Gonzalez-Zorn is Professor at the Complutense University in Madrid. He gained his DVM in 1996 and his european PhD in 2001. After his Postdoc at the Pasteur Institute in Paris he received a Ramon y Cajal tenure-track contract from the Spanish Ministry ofScience to return to Spain. Currently he leads a group working on molecular microbiology and the ecology of antimicrobial resistance in Madrid. He is currently Head of the Animal health Department. His research interests focus on the role and function of small plasmids in antimicrobial resistance, the bacterial SOS-response and the 16S rRNA methyltranferases in pathogenic bacteria. In 2011 he was awarded the bianual Jaime Ferran Award from the Spanish Society for Microbiology. He paricipates in several International Commitees and Projects on AMR, including the SAB of the JPIAMR and EFFORT. Some key publications include:

San Millan A, Santos-Lopez A, Ortega-Huedo R, Bernabe-Balas C, Kennedy SP, Gonzalez-Zorn B. Small plasmid-mediated antibiotic resistance in Haemophilus influenzae is enhanced by increases in plasmid copy number and bacterial fitness. Antimicrob Agents Chemother. 2015 Mar 30. Pii: AAC.00235-15.

Delgado-Blas JF, Ovejero CM, Abadia Patiño L, Gonzalez-Zorn B. Coexistence of mcr-1 and blaNDM-1 in Escherichia coli from Venezuela. Antimicrob. Agents Chemother. 2016 Oct (in press).

ANSES: Jean-Yves Madec

Dr. Jean-Yves Madec (ANSES)(DVM, PhD) at the French Agency for Food, Environmental and Occupational Health Safety (Anses) is involved in National Surveillance Programs of AMR in veterinary medicine in France and has recognized expertise in molecular genetics and epidemiology of resistance in Gram negative and Gram positive bacteria of animal origin (commensal, pathogenic, zoonotic) and issues regarding the animal-human transfer of AMR. He is member of several expert groups and committees on antimicrobial resistance and antibiotics use in animals and humans in France and Europe and is an active participant/leader in recent European scientific projects. A key recent publication:

Haenni M. et al Madec JY (2016) Co-occurrence of Extended-Spectrum β Lactamase and MCR-1 encoding genes on plasmids. Lancet Infectious Diseases, January 7, 2016. http://dx.doi.org/10.1016/S1473-3099(16)00007-4).

RKI: Tim Eckmann and Sebastian Haller

Dr. Tim Eckmanns, Dr. Sebastian Haller are medical epidemiologists based at the German national public health institute with extensive research experience on AMR, conducting surveillance and analysing transmission of resistant bacteria from infections in humans as well as on antibiotic prescription at national level in Germany. Key recent publications are:

Haller S, Eller C, Hermes J, Kaase M, Steglich M, Radonić A, Dabrowski PW, Nitsche A, Pfeifer Y, Werner G, Wunderle W, Velasco E, Abu Sin M, Eckmanns T, Nübel U. What caused the outbreak of ESBL-producing *Klebsiella pneumoniae* in a neonatal intensive care unit, Germany 2009 to 2012? Reconstructing transmission with epidemiological analysis and whole-genome sequencing. BMJ Open. 2015; 5 (5).

Walter J, Haller S, Blank HP, Eckmanns T, Abu Sin M, Hermes J. Incidence of invasive meticillin-resistant Staphylococcus aureus infections in Germany, 2010 to 2014. Euro Surveill. 2015;20(46).

PHE: Neil Woodford Prof Neil Woodford, is head of PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) unit. He has worked on antibiotic resistance for over 25 years and is an internationally recognized authority on the molecular epidemiology of resistance with more than

250 published papers on resistance mechanisms and epidemiology, he has edited three books on the subject and is a Fellow of the Royal College of Pathologists on the basis of his published works. Prof Woodford has served for 10 years as an Editor of the Journal of Antimicrobial Chemotherapy, and is on the Editorial Board of Microbial Drug Resistance. Prof Woodford is a member of two Health Protection Units examining antimicrobial resistance, one with Imperial College and the other with the University of Oxford in partnership with APHA. As a committee member of several groups advising government on research and policy relating to antimicrobial resistance in humans and animals he has the insight and necessary understanding of the complex, multi-factorial problem of resistance epidemiology to advise on policy and research in relation to antimicrobial resistance in humans and animals.

PHE: Matthew Ellington and Berit Muller-Pebody

The PHE team will also include Dr. Matthew Ellington who is a senior scientist in AMRHAI and Dr. Sarah Gerver who is the lead epidemiologist and Head of Antimicrobial Resistance and Prescribing Section in the department of Healthcare associated infections at PHE and has been part of the team producing the reports on "English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR)" from Public Health England and other agencies including the Veterinary Medicines Directorate (https://www.gov.uk/government/publications/english-surveillance-programme-antimicrobial-utilisation-and-resistance-espaur-report)

Key recent publications are:

Day MJ, Doumith M, Abernethy J, Hope R, Reynolds R, Wain J, Livermore DM, Woodford N. Population structure of Escherichia coli causing bacteraemia in the UK and Ireland between 2001 and 2010. J Antimicrob Chemother. 2016 May 5. pii: dkw145. [Epub ahead of print] PubMed PMID: 27150395.

Doumith M, Godbole G, Ashton P, Larkin L, Dallman T, Day M, Day M, Muller-Pebody B, Ellington MJ, de Pinna E, Johnson AP, Hopkins KL, Woodford N. Detection of the plasmid-mediated mcr-1 gene conferring colistin resistance in human and food isolates of Salmonella enterica and Escherichia coli in England and Wales. J Antimicrob Chemother. 2016 Apr 18. pii: dkw093.

5.1.4.3.1.2 Summary of the work carried out in the Project

The ARDIG project has continued to progress, and after 42 months there have been substantial achievements made by partners, including numerous peer-reviewed publication of papers from the ARDIG consortium. Several project-wide, as well as work package specific, meetings have also taken place by either meeting physically or by tele/videoconference, and there have been regular communication by email. Both oral and poster presentations made by ARDIG partners were well received at the OH-EJP Annual Scientific Meetings held during this project.

WP1 **(Comparison of AMR and antibiotic sales/usage data collected through existing national surveillance and research programs and assessment of risk factors).** The ARDIG project has identified that there is a lack of harmonisation on AMU and AMR in the livestock sector and on AMR in the human sector, although there is some overlap between national and international systems. A One-Health approach for AMU and AMR requires harmonisation in various aspects between systems in the human, animal and food sector; suggestions are provided within ARDIG to address this. Differences in AMR between clinical and non-clinical isolates were identified within and between countries for different animal populations. Higher resistance levels in clinical isolates than in non-clinical isolates were found for calves, while the opposite was found in isolates from broilers and turkeys. Decreasing resistance was found in animal isolates between 2014 and 2017. This suggests that measures carried out against AMR including the reduction in AMU in each country have effective results.

A workshop was held with experts in the field, including those from EFSA, EMA and ECDC and other European projects, to make recommendations for improved "One Health" surveillance strategies.

WP2 (**Longitudinal studies of AMR persistence).** Both Med and Vet partners have been collecting *E. coli* isolates from retrospective as well as prospective longitudinal studies, including *E. coli* isolated from urinary tract infections from local hospitals, as well as livestock (cattle, pigs and poultry). Several partners have also characterised *E. coli* isolated through EU harmonised surveillance for AMR. In addition, all partners who participated in a whole genome sequencing (WGS) AMR workshop have submitted ~50 WGS each (~450 in total) for analysis by five pipelines: APHA SeqFinder/Abricate, PHE GeneFinder, WBVR, Ariba, ResFinder/PointFinder. The AMR genotypes were compared with the corresponding MIC phenotypes for these isolates. WGS of >3000 isolates collected from longitudinal, as well as national surveillance by partners were also compared, and show human isolates generally to be more similar to each other than those from animals, and vice versa.

WP3 (**AMR characterization, transmission of plasmids and fitness of MDR isolates**). All WP3 partners participated in the AMR pipeline comparison work to help harmonise *in silico* AMR gene prediction and to assess the impact of methodology on AMR gene prediction; a manuscript is currently being prepared for this work. In addition, all WP3 partners have continued AMR gene, plasmid and mobile genetic element characterization of isolates collected in WP2 by WGS (short and long reads), as well as other molecular techniques. Phylogenetic analysis and AMR profiling performed by partners on their dataset, as well as across ARDIG, have helped identify possible transmission events or epidemiological links between different compartments and countries. Also, plasmid characterisation between and across partners, has provided an overview of the types of AMR plasmids commonly circulating which belong to the following types: IncI, IncF and IncX.

5.1.4.3.1.3 Work carried out in the JRP, scientific results and integrative outcomes

WP1. Comparison of antimicrobial resistance (AMR) and antibiotic sales/usage (AMU) data collected through existing surveillance, monitoring and research programs and assessment of risk factors.

This WP was led by BfR, with major contributions from APHA, ANSES, NVI, RKI and PHE; other members of the consortium also contributed as and when appropriate. Details of the work performed in WP1 by the consortium is provided below.

WP1-T1 - Exploration and collection of data available on AMR, AMU and potential risk factors

Institut Pasteur

Monitoring of antibiotic consumption and of antibiotic resistance of bacteria responsible for infections has evolved at the start of the project in France with new actors. Monitoring is now under the responsibility of Santé Publque France (SPF) by the CPias nouvelle Aquitaine with a new tool called Consores for AMU, and by the CPias Pays de la Loire, with also a new tool called Medqual for AMR. In the past, AMR was monitored by ONERBA (Observatoire National de l'Epidémiologie de la Résistance Bactérienne aux Antibiotiques) which is a private association, collecting information on the voluntary basis. Given this situation, despite much efforts we have not been able to collect additional data on E. coli infections beyond what is transmitted by the French authorities to the ECDC (ESAC-net and EARS-net).

WP1-T1 - Exploration and collection of data available on AMR, AMU and potential risk factors

Data were collected from six countries represented in ARDIG (UK, NL, DE, FR, NO, ES) and collated in a database. This included data available in the respective institutions involved in ARDIG but also other National and regional systems in animal husbandry and the medical field. A detailed description of current (2019) surveillance and monitoring systems in the animal and the human sector was prepared, submitted as a deliverable and subsequently published in a peer reviewed paper (Mesa-Varona et al. 2020). A substantial diversity of systems and data types was encountered and comparability of data was largely hampered by this diversity. Metadata collected in the surveillance and monitoring systems were typically limited which further limited the scope of the analyses in task 2.

WP1-T2- Investigation of trends, associations and risk factors

The available data were first descriptively analysed (see deliverable 1.2) and subsequently a framework was developed to compare AMR in *E. coli* from different animal and human populations. A focus was on the comparison of clinical and non-clinical isolates from animals that showed substantial differences which, however, were observed in both directions, depending on the animal population and the antimicrobial considered. One main obstacle to this analysis were differences in the substances included in AMR-testing between countries and populations (clinical vs. non-clinical) that limited the range of substances with comparable data. Another obstacle, the use of different laboratory methods could be partly overcome by the development of harmonized cut offs. The approach used the same methodology to determine the cut offs for different laboratory methods (i.e. broth microdilution vs. disk diffusion).

Analysis of clinical *E. coli* isolates was carried out to describe and compare trends in resistant *E. coli* isolates originating from clinical diagnostic submissions from three livestock species in three European countries. Associations between antimicrobial resistance and year of sampling, species and country were also investigated. The number of isolates tested increased over years 2014 to 2016 in all three countries and decreased in 2017. Similar patterns in resistance were observed between the countries, with cattle isolates having significantly higher odds of being resistant compared to both pigs and chicken isolates. The level of resistance decreased with age in cattle and chicken, younger animals had significantly higher resistance compared to older animals.

Similarly to the analysis of clinical vs. non-clinical isolates, also analysis of clinical isolates from different livestock species and countries faced the same challenges. Although each country monitors resistance levels of a large number of antimicrobials, only a small number of antimicrobials overlapped between different countries and livestock species, highlighting a need for a better harmonization between different countries and production systems. The use of different animal age categories by different countries also had a limiting impact on the scope of analysis. A further finding was that despite the short observation period (2014 -2017, i.e. four years) for several antimicrobials and animal populations a decrease in resistance was observed in the *E. coli* tested. This indicates that efforts taken by countries to control AMR mainly by reducing AMU did foster some progress, although – given the short time period – this was limited. First results have been published in two peer reviewed papers comparing

clinical and non-clinical isolates of *E. coli* from three animal populations (broilers, turkeys and calves) (Mesa Varona et al. 2020b, 2021). Two further papers are in preparation that compare clinical isolates from animal populations between countries and isolates from different pig populations and humans in Germany.

WP1-T3- Develop recommendations for improved "One Health" surveillance strategies

Based on the results of tasks 1 and 2 and experiences of other research groups, namely the Aacting consortium and the EU-JAMRAI project, recommendations were drafted and presented to experts in the field in a workshop on March 1 and 2 of 2021. The workshop included experts from the countries involved in ARDIG and the European Agencies (EFSA, ECDC, EMA). One day of the workshop was dedicated to reporting from the different projects (i.e. ARDIG, Aacting and EU-JAMRAI), from EMA and from Norway that has some experiences in presenting AMU and AMR data in a one health approach. The second day was devoted to three discussion groups working on pre-defined questions on surveillance of AMU (Group 1), AMR (Group 2) and the contribution of molecular methods to routine surveillance (Group 3). The workshop gave evidence of different attitudes to the questions that were partly driven by the different roles of the participants. In the AMU workshop the data collection systems currently developed under the new EU-veterinary medical legislation were extensively discussed and EMA clearly elaborated their needs based on the legislation, which are limited to certain tasks. This of course does not constrain the member states in setting up more ambitious collection systems provided that those systems are also fit to cover EMAs needs. While some participants, including members of the ARDIG consortium, underlined the usefulness of a harmonization process across the systems, others questioned this need with reference to the diversity of the underlying systems of animal production in the different countries.

A report on the workshop is currently in progress and is meant to be shared and discussed with the workshop participants. It will presumably be published after the consultation phase in early autumn 2021.

Main conclusions and recommendations from ARDIG WP1

On AMR

Collection systems on AMR often adopt specific standards and their corresponding evaluation criteria that do not cover all drug and bug combinations. Therefore, different standards and/or evaluation criteria are sometimes used for different drug and bug combinations in the same system. These systems show a lack of harmonisation within and between countries across the human and animal sectors.

Data collecting systems on AMR should collect data:

- applying the same standard (e.g. EUCAST and CLSI)
- using the same evaluation criteria (i.e. epidemiological vs. clinical approach)
- from both isolate types (i.e. clinical and non-clinical isolates)
- applying a similar antimicrobial panel
- from the same sample type
- addressing the same animal categories

These requirements in the same isolate type should be complied in order to compare, evaluate and analyse AMR. Otherwise, data comparisons could not be in most cases directly addressed.

The NRI approach is a statistical method that can be applied, to overcome the lack of harmonisation on laboratory methods and methodologies. These statistical methods require quantitative data across a substantial range of values for the interpretation of data. Therefore, reports on AMR should provide

quantitative values rather than data on the SIR level.

Significant dissimilarities were found between data on clinical and non-clinical isolates within animal categories and countries. Higher resistance proportions were mainly encountered in non-clinical isolates of broilers and turkeys and in clinical isolates of calves. Results in calves confirmed previous descriptive data from other studies and were in line with expectations. However, results in poultry were surprising and require further targeted investigations. The results underline that AMR in clinical and non-clinical isolates needs to be evaluated separately and preferably both should be collected.

The lack of AMR harmonisation on further issues such as the lack of harmonisation on antimicrobial panels and animal categories remains. It is necessary to define a harmonised antimicrobial panel in clinical isolates and between clinical and non-clinical isolates.

Our analyses were applied in *E. coli* isolates of livestock between 2014 and 2017. However, this work could be extended in the future to include other bacteria and for longer periods.

On AMU

EU agreed units are not always provided in reports that show data from regional and national monitoring and surveillance systems on antimicrobial consumption in Europe. These units should be always applied.

ESVAC collected sales data in Europe using the agreed unit mg/PCU. However, this measurement only provides a general overview. Farm-level data are required for further analyses. However, there is no AMUsage agreed unit in the animal sector. To overcome this issue reusing the already collected data, two different approaches have been suggested:

- A harmonised AMUsage unit
- Numerator/denominator data to transform one unit into another:
    a. The name of the active ingredient
    b. The amount of active ingredient
    c. The number of treated animals
    d. The population at risk
    e. The weight of treated animals
    f. The time under treatment
    g. The duration of the therapeutic effect of the active ingredient in the body

The implementation of the Regulation (EU) No. 6/2019 on veterinary products will greatly improve the comparability of AMUsage data in a large number of animal categories.

AMU data collection should be provided by drug, animal species (e.g. broilers and turkeys instead of poultry), type of production (e.g. broiler or laying hens instead of chicken), age categories when the animal production cycle is long (e.g. weaning or fattening pigs instead of pigs) and the mixture of age category and production (e.g. calves instead of cattle).

Reports on AMU should always report on the same drug and antimicrobial drug categories for the same animal category that should likewise be defined in a harmonised way.

It is significantly important to understand the data collection of the health care systems implemented in each country since the data collection procedure may vary. Therefore, data comparisons should be done with care.

On AMU and AMR

Availability of harmonised usage data for animals will allow enhancing the analyses on the association of use and resistance across countries.

In the field of non-clinical AMR, EFSA drove the harmonisation of EU data-collection. However, there is no incentive to harmonise AMU and AMR systems. In the absence of harmonisation, there is no requirement to report to the EU. A large number of AMU and AMR collecting systems were found per country and sector. Some overlap between monitoring and surveillance systems on AMU and AMR was encountered. This overlap may be convenient for system and data validation. However, a better use of the available sources could save resources.

Overlap between reports has been encountered as a logical consequence of the overlap presence between systems. AMU and AMR reports are published in different languages and time ranges. They should be provided annually in one international language to ease published data access.

Antimicrobial use was identified as an influencing factor on AMR. However, AMR prevalence might not necessarily decrease in some drugs addressing only the reduction of AMU due to phenomena such as co-resistance and multidrug resistance. AMR crisis must be addressed from all available angles (e.g. hygienic measures, vaccination, support of scientific studies and reduction of AMU) and as a collaborative action between countries.

During the funding period of ARDIG, the three agencies, EFSA, EMA and ECDC have jointly prepared the JIACRA report based on reported data from all EU-MS and some other EEA countries. As the WP leader of WP1 was member of the JIACRA working group on behalf of EFSA, he was able to consider the perspective of both projects in his work. The JIACRA report is solely based on non-clinical animal isolates as opposed to clinical animal isolates. The JIACRA report demonstrates that where there is a certain degree of harmonization, analysis can be run, keeping in mind the limitations to these analysis. For example the consumption data in animals in the JIACRA report are based on general sales data which limits the analytical potential. In ARDIG we tried to include population based data on AMU as derived from the national systems, but as pointed out above, this was hampered by differences in the definition of populations and the units used in the description of use. Therefore, both, our work and the work in JIACRA underline the need for more harmonized data. Moreover, in ARDIG we focused on one bacterial species, including data on clinical and non clinical isolates. The differences we observed between clinical and non clinical isolates may indicate that analysis of clinical animal isolates might provide an additional perspective in one health analyses. ARDIG results show that, provided more harmonized data are available, additional analyses can be run. Currently, a lot of collected data have only limited use, as they are difficult to compare with data from other populations/compartments and countries.

WP2. Longitudinal studies of persistence ESBL/AmpC/carbapenem/mcr-1/PMQR producing Enterobacteriaceae on farms or hospitals

WP2-T1- Assessment and selection of longitudinal data from historical studies

UCM

Data from national surveillance projects carried out between 2014 and 2019 focused on antimicrobial resistance surveillance in different animal production sectors have been arranged to be added to the consortium common data for further analyses. From the EFFORT (Ecology from Farm to Fork Of microbial drug Resistance and Transmission) project, up to 10 *E. coli* isolates were collected from faecal samples of 5 pig farms and 5 poultry farms located in different Spanish regions between 2014 and 2015, obtaining a total of 100 *E. coli* isolates. These isolates were not selected under antibiotic pressure and were further characterized by a standard panel of different antimicrobial compounds in order to assess Minimal Inhibitory Concentrations (MICs). All of them were sequenced by Illumina technology. Random-selected *E. coli* isolates (38 from pig samples and 35 from poultry samples) were included in further analyses for AMR detection pipeline comparisons and phylogenetic studies.

From the national surveillance program of antimicrobial resistance carried out by the VISAVET Health

Surveillance Centre and the Spanish Ministry of Agriculture, Fisheries and Food (MAPA), a total of 200 *E. coli* isolates were collected from faecal samples: 100 isolates from swine production in 2017 and 100 isolates from poultry production in 2016 (50 isolates) and 2018 (50 isolates). These isolates were not selected under antibiotic pressure. All isolates were phenotypically characterized according to their antimicrobial resistance profiles to a standardized panel of antimicrobial compounds. Then, all of them were sequenced by Illumina technology and included in posterior analyses for AMR and plasmid characterization and phylogenetic studies. The results of these analyses are described in AMR characterization section.

Institut Pasteur.

In the course of the ARDIG project we have analyzed two set of data from historical studies.

- ESBL producing *E. coli* collected in the course of the i-bird project: The i-bird project coordinated by D. Guillemot (IP) was based on a 4-month study which took place in 2009 in a rehabilitation hospital. It combined weekly rectal swabs and human-human proximities recorded from wireless captors. 3500 rectal swabs have been collected from 329 patients. A total of 4882 bacterial isolates have been analyzed including 425 *E. coli*. Antibiotic susceptibility testing was performed for 30 antibiotics. 604 ESBL-Enterobacterales were isolated from 84 patients including 332 *E. coli* from 75 patients. One to 25 isolates were collected per patient, including patient colonized by ESBL isolates during the whole study. The main aim of the study was to analyze ESBL-bacteria transmission and plasmid acquisition. We selected 204 isolates for WGS (WP3). Selection was based on the patient to sequence at least one isolate per patient and on the week of sampling and taking into account negative samples (one isolate before and after each negative sample). By comparing over time within and between patient genomic diversity of ESBL-producing bacteria together with contact data, we aim to infer transmission routes and the possibility of an environmental reservoir.
- Carbapenemase producing *E. coli* (CP*Ec*) isolates collected by the French National Reference laboratory for carbapenemase producing enterobacteriaceae (Fr-CNR). CP*Ec* represents a major public health threat for two main reasons. First only few therapeutic options remain to treat bacterial infections due to MDR CPEc and second given the ubiquitous nature of *E. coli,* these bacteria contribute to the dissemination of carbapenemase genes and of plasmids carrying those resistance genes. In Europe CPEc from animal origins are extremely rare and none isolates showing this resistance were isolated in the course of the project. However, we have shown that the acquisition of MDR and of carbapenemase genes is a multistep process. A still open question is the contribution of the animal sector in this process. We have focused our study on the four first years of activity of the Fr-NRC from 2012 (year of creation of the NRC) to 2015. During this period, a steady increase of the number of isolates received by the NRC was observed (44 in 2012, 94 in 2013, 250 in 2014 and 388 in 2015). It is likely a consequence of different factors: (i) an increased circulation of CP*Ec* in France; the increased screening of potential carbapenemase carriers at their admission at hospital, as the number of disease associated isolates was found to increase more slowly than the number of samples from screening and (iii) to a raising awareness of hospital and private laboratories to send CP*Ec* isolates to the Fr-NRC. In order to characterize the diversity of these isolates and of the carbapenemase gene, changes during the four years and their evolutionary trajectory related to the acquisition of the carbapenemase gene, we selected for WGS 713 isolates including 22 isolates received by the Bicêtre Hospital laboratory before 2012 (WP3)

NVI

Isolates from a previous study focusing on cephalosporin resistant Enterobacteriacecae have been characterized. All broiler flocks raised on ten broiler farms were sampled during the period from May to October in 2016 and a total of 43 positive isolates were obtained (one isolate per flock). These isolates have been sequenced with Illumina technology in order to study a possible on-farm persistence/transmission between batches of animals on the same farm or broiler house. In total, 11 different *E. coli* Sequence Types were identified. A possible clonal persistence of ESC-resistant *E. coli* at house level was shown for only a minor proportion of the included houses. Isolates from the same house belonging to the same ST could differ by a considerable number of SNPs, shown for ST38 isolates found in three different houses at one farm from several flocks throughout the sampling period. Similar plasmids were detected in different STs, suggesting possible horizontal transfer and/or persistence of plasmids. Seven ESC-resistant *E. coli* of different STs originating from two farms were selected for ONT sequencing and in-depth characterization of bla_{CMY-2}/IncK2 plasmids. We performed hybrid assemblies and SNP analysis. On one farm, highly similar plasmids of approximately 85 kb size were present in three different STs. The plasmids differed by 17 SNPs. On the other farm, two plasmids of 116-117 kb, harboured additional resistance to sulfamethoxazole, tetracycline and aminoglycosides. When compared using Snippy, 0 SNPs were present between these plasmids. The results indicate that highly similar/identical plasmids can exist in *E. coli* of different STs, and possibly persist on broiler farms. Our data show variation at both gene, plasmid and ST level when ESC resistant E. coli from consecutive flocks were characterized. It is not possible to determine whether different *E. coli* variants and/or ESC resistance genotypes were present simultaneously in a flock, as only a single isolate was characterized per sample. A manuscript is in preparation and will be submitted later this year.

PHE

PHE collected carbapenemase producing Enterobacterales, including *E. coli*, isolated from colonisation and infections by hospital laboratories and referred to the PHE reference national reference lab. The isolates were received by the PHE National Reference laboratory in 2014 and 2016 and have been analysed for their genomic and resistance gene diversity (collaborative manuscript with Katie Hopkins submitted).

ANSES

Extended-Spectrum-Cephalosporins (ESC)-resistant Enterobacteriaceae have been isolated from veal calves in France have been included to be studied within ARDIG. Two studies were set up to investigate the trends in ESBL/AmpC prevalence and antimicrobial usages (AMU) in veal calves during the fattening process.

In a first study, ten fattening farms were selected and visited twice. A total of 50 animals per farm were sampled for ESC-R carriage and other AMR phenotypes upon arrival and 5-6 months later before slaughter.

A second study was then set up to get further insights into the dynamic of ESBL/AmpC spread over the fattening period. Three farms out of the ten from the first study were visited 11 or 12 times at regular intervals of 15 days. A total of 15 calves per farm were sampled and processed as for the first study. In the two studies, the number and types of treatments during fattening were collected.

WP2-T2- Isolation of resistant Enterobactericeae on farms

UoS

Task completed. See second annual report, 2019.

UCM

A total of 110 *E. coli* isolates recovered from urinary tract infections were collected from March 2019 to November 2020 in collaboration with a reference hospital located in Madrid. Out of the 110 isolates,

77 were collected from GP patients (community-related infections) and 33 were obtained from inpatient samples (hospital-related infections). We performed the phenotypic characterization according to the antimicrobial resistance profile of all isolates following standard surveillance antimicrobial panels. We sequenced all collected isolates by Illumina technology to carry out the genomic characterization. Illumina data were applied for subsequent AMR, plasmid and phylogenetic analyses.

WBVR

In 2019 and 2020, a group of approximately 700 veal calves in the Netherlands was individually followed from birth to slaughter on 5 to 6 sampling moments. The animals were born on 13 dairy farms spread throughout the country and transported between 14 and 28 days of age to 8 veal farms for fattening. Rectal swabs were taken at each sampling moment for selective culturing on cefotaxime containing media to determine the prevalence of ESBL/AmpC producing E. coli. At the dairy farms the prevalence of E. coli ranged from 0-86% (average 26.4%). At all veal farms the prevalence of ESBL/AmpC producing E. coli amongst the animals went up to >50% at least one sampling moment. In 6 farms, prevalence significantly decreased over time.

Extensive records were recorded on farm management, hygiene, antimicrobial usage and health parameters of the animals and analysed using a linear mixed and multivariant regression model to determine risk factors associated with ESBL/AmpC carriership. No association between age of transportation, sex, breed, and presence of ESC-R *E. coli* at the dairy farm was found. At the dairy farm, different management practices for veal calves destined for fattening or replacement heifers, as well as the presence of pets (cats and dogs) and other livestock (horses, sheep and goats) were positively associated with presence of ESBL/AmpC producing *E. coli*. The presence of ESBL/AmpC producing *E. coli* at the veal farm was positively associated with presence of these bacteria at consecutive time points. Antibiotic batch treatment of the animals is positively associated with presence of ESBL/AmpC *E. coli* while individual treatment was not. A manuscript describing these results has been prepared and is currently awaiting approval by collaborators before submission.

Short-read WGS data has been generated of > 300 isolates and confirms extensive clonal spread of ESBL/AmpC producing *E. coli*, both on dairy farms through the year and on veal farms during a single production cycle. Nonetheless, multiple variants are spread through each farm and animals are colonised by different *E. coli* variants over time. In order to determine the role plasmid spread in the transmission of ESBLs within the farms, ~70 isolates were selected for long-read using Oxford Nanopore Technologies in collaboration with the OH-EJP Full-Force project which is currently under way.

ANSES

In the two studies, ESBL-producing *E. coli* were collected from MacConkey agar for the culture of the dominant flora and onto selective ChromID ESBL agar (bioMérieux) for the specific selection of ESC-resistant isolates from the subdominant flora. After incubation at 37°C for 24 h, one presumptive *E. coli* colony was arbitrary selected from each plate and isolates were identified using MALDI-TOF.

In the first study, ESBL-producing *E. coli* rates have significantly decreased in all 10 farms (arrival: 67.7%; departure: 20.4%)(Gay et al, Frontiers in Microbiology 2019). Feeding milk containing antimicrobial residues to veal calves is hypothesized to explain the high ESBL loads in animals at the entrance on farms. In the dominant flora, proportions of resistances to amoxicillin, tetracyclines, streptomycin and sulfonamides were very high (>60%) at arrival of animals in the farm and had significantly increased at departure. Proportions of resistances to other beta-lactams than amoxicillin were overall low and significantly decreased during the fattening process. Resistance to quinolones also significantly decreased from arrival to departure. A total of 11 isolates were resistant to colistin (MICs ranging between 6 and 16 mg/L) of which 9 were detected in animals upon arrival (originating from 7 different farms), and 2 in animals at departure (both originating from the same farm). The

proportion of multi-resistant isolates significantly increased from 60.2% upon arrival to 67.2% at departure of animals. The proportion of isolates susceptible to the seven selected antibiotics was 23.3% upon arrival and 7.3% at departure. Only two isolates displayed co-resistances to all seven antibiotics.

The second study was performed in three veal farms (A, B and C) located in the region of Brittany, France (Massot *et al*, manuscript submitted). Rectal swabs from 15 calves per farm were collected every 15 days until departure to slaughterhouse and screened for intestinal carriage of ESBL-producing *E. coli.* A decrease in ESBL prevalence in calves was observed over the fattening period, although each farm displayed a specific dynamic. In conclusion, we showed that the diffusion of ESBL-encoding genes in calves is a combination of two scenarios encompassing bacterial clone and/or plasmid spread, which highly rely on local features and contexts, including the types and number of antibiotic treatments per farm.

In this second study, we also took the opportunity of the same sampling to characterize the dynamics of the calves' microbiota subjected to antimicrobial pressure (Massot et al., Animal Microbiome, 2020). This observational study showed early convergence of the developing microbiota between veal calves and demonstrated associations between the dose of milk powder and composition of the microbiota. It suggested that group treatments of antibiotics resulted in a reduction of microbial diversity and size of the *E. coli* population in the gut and highlighted the need for additional work to fully understand the impact of antibiotherapy in the veal industry.

Finally, we used the three veal calf fattening farms of the second study as a model to investigate and compare the effectiveness of possible interventions to reduce the AMR burden in this sector (Bastardet al, One Health 2021). Longitudinal data of ESBL-EC carriage and antimicrobial use (AMU) were used to develop agent-based mechanistic models to assess different hypotheses regarding the main drivers of ESBL-EC dynamics in calves. The models were independently fitted to the longitudinal data using Markov Chain Monte Carlo and the best model was selected. Within-farm transmission between individuals and sporadic events of contamination were found to drive ESBL-EC dynamics on farms. In the absence of AMU, the median carriage duration of ESBL-EC was estimated to be 19.6 days (95% credible interval: [12.7; 33.3]). In the best model, AMU was found to influence ESBL-EC dynamics, by affecting ESBL-EC clearance rather than acquisition. This effect of AMU was estimated to decrease gradually after the end of exposure and to disappear after 62.5 days [50.0; 76.9]. Moreover, using a simulation study, we quantified the efficacy of ESBL-EC mitigation strategies. Decreasing ESBL-EC prevalence by 50% on arrival at the fattening farm reduced prevalence at slaughter age by 33.3%. Completely eliminating the use of selective antibiotics on arrival had a strong effect on average ESBL-EC prevalence (relative reduction of 77.0%), but the effect was mild if this use was only decreased by 50% compared to baseline (relative reduction of 3.3%).

APHA

We have completed the collection of samples for a longitudinal study which focused on two sites of the same UK pig farm which are separated geographically; a non-clinical farm site that houses five age classes of healthy pigs and has ceased group antimicrobial treatments for at least five years, and a clinical farm site that is comprised of three age classes of pigs sent from healthy sites following disease, that have subsequently undergone group and individual antimicrobial treatment. Faecal samples were obtained from both sites from pigs at four time-points at 6 month intervals over 18 months, alongside seagull faecal samples from two time points. Representative *E. coli* were purified from all time points from non-selective and antibiotic selective agar plates (cefotaxime and ciprofloxacin), followed by Illumina whole-genome sequencing (WGS). The WGS data was analysed by reconstructing phylogeny of the *E. coli* isolates, determining presence of AMR genes, plasmid replicon types, in silico Multilocus Sequence Type and mobile genetic elements (WP3).

MIC determination for the isolates collected during visits 4 and 5 has also been completed. An overall analysis of the MIC data across 5 visits has been carried out. Temporal trends were evaluated, as well

as a comparison between antibiotic treated and non-antibiotic treated groups of pigs was carried out. A draft publication is in in preparation to report these results.

WP2-T3-Isolation of resistant Enterobacteriaceae in hospitals and care facilities

UoS

Task completed. See third annual report, 2020.

Institut Pasteur

The ARDIG project focused on *E. coli*. Following within the consortium discussion, it was decided to focus on *E. coli* isolates responsible for Urinary Tract Infections (UTI) as those isolates are representative of extra intestinal pathogenic *E. coli* and are collected in different countries in a similar way. In addition, it was decided in this prospective study to collect both isolates of community or hospital origins. Community isolates were either from city microbiology laboratories or from outpatients (emergency) in the hospital. Hospital isolates were from hospital clinical microbiology laboratories. As the objective of the study was to analyze the diversity of the isolates, no data on patient were collected. Several studies have been published on isolate collections following selection for either resistance to third generation cephalosporin (3CG) or producing ESBL and we expected, following such selection to retrieve a majority of ST131 isolates, we decided to have a more agnostic approach and not to select for a specific antimicrobial resistance phenotype. In total, we analyzed 1067 isolates collected in 2019 by five partners:

- Institut Pasteur together with the team of Thierry Naas at the Bicêtre hospital collected a total of 251 isolates, including 123 from emergencies (community) and 128 from the hospital wards. These 251 isolates were submitted to WGS (WP3)

NVI

E. coli isolates from humans with UTI in a large centrally located hospital in Norway, and from GP in the same area were collected (the 20 first isolates of each category, from each month in 2019). The isolates have been sequenced (Illumina short read) at NVI and MIC determination have been carried out. The dominating sequence types were ST131, ST73 and ST69. Resistance genes were uncommonly present in ST73 isolates, in contrast to ST131 isolates that usually were resistant to several antimicrobial agents. No particular ST dominated among hospitalized patients. WGS data are included in analyses in WP3.

PHE

PHE have collaborated with UoS (Rob LaRagione, Maria Getino) to short-read WGS sequence a collection of E. coli isolated from human urinary tract infections occurring in the community and a hospital setting. Quality checked sequences have been shared for collaborative analysis at IP.

WP2-T4- Data analysis of collected resistant Enterobactericeae on national levels

UCM

Within the framework of the ARDIG project, we have characterized and studied the dissemination dynamics of multi-resistant bacterial clonal groups and the genetic determinants that they carry in different settings, including animal production and hospital environments. The isolates were sequenced by Illumina technology and the resulting data were analyzed for AMR and plasmid characterization and phylogenetic studies. The epidemiological metadata and phenotypic resistance profiles of the isolates were integrated with the genotypic data. Thanks to the studies developed during the project, we have identified risky *E. coli* STs that are disseminated in different production types and environments, such as ST1196 and ST10, as well as genes of resistance against antibiotics of clinical importance, such as *mcr* genes and determinants of resistance to cephalosporins, in both pig and poultry farms. Some of them were selected and sequenced by Nanopore technology, based on

Illumina genomic analysis and genotypic resistance profiles, in order to study particular antimicrobial resistance gene dynamics. We are working on the manuscript to public the results obtained at national level, but also, we have collaborated with other partners of the project that have similar results in order to analyse international trends and epidemiological dynamics of specific resistance genes and/or STs.

BfR

On request, the BfR had provided sequencing data on *mcr*-1- and *qnr*-carrying isolates for comparative analysis.

APHA

Whole genome sequencing of ESBL/AmpC *E. coli* that have been collected by APHA from national surveillance of livestock (pig and poultry) since 2013 have been analysed for their AMR gene content as well as plasmid diversity. A paper has been published in Science Reports on AMR analysis in *E. coli* from pigs collected between 2013-2017.

WP2-T5: Comparative analysis of collected isolates on a Europe-wide level (M30-M36)

APHA, ANSES, BfR, IP, NVI, PHE, UCM, UoS, WBVR

ARDIG partners have submitted WGS of up to 50 Escherichia coli isolates per institute to a repository and five partners (APHA, WBVR, PHE, UCM, and NVI), representing the diversity of pipelines (APHA SeqFinder/Abricate, PHE GeneFinder, WBVR, Ariba, ResFinder/PointFinder), have analysed ~450 WGS data through the pipelines. All partners have performed MICs on the EFSA panel of antimicrobials on their isolates from this panel of 450 isolates, so phenotypic data could be obtained for comparison with the AMR genotypes resulting from each of the 5 bioinformatic pipelines.

UoS

The UoS has carried out a comparative analysis of swine E. coli isolates collected from four different European countries. A total of 1223 WGS assemblies, including 916 from the APHA (UK), 117 from the UoS (UK), 122 from the UCM (Spain), 50 from the BfR (Germany) and 18 from the WBVR (Netherlands) were included in the analysis. Phylogroups and sequence types were determined using ClermonTyping and MLST bioinformatic tools, respectively. Half of the isolates belonged to phylogroup A, 25% to B1 and 13% to C, whereas the common human phylogroup B2 only represented 1% of the swine isolates, reflecting a low degree of transmission between these hosts. At sequence type level, the most common MLST groups found among swine isolates were ST10 (11%), ST744 (11%) and ST88 (7%).

ResFinder and PlasmidFinder databases were used to characterise the acquired AMR genes and plasmid replicons harboured by the isolates. While the most common plasmid types were IncFIB (48%) and IncI1 (36%), the most abundant AMR genes were tetA (41%), aph(6)-Id (35%), blaTEM-1B (34%), sul2 (30%) and blaCTX-M1 (30%). In addition, point mutations were determined using PointFinder, phylogenetic trees were produced with ParSNP, genes were annotated with Prokka and a pangenome analysis was produced with Roary. Out of a total 33797 genes included in the pangenome analysis, 3006 were core genes with an identity between 99 and 100%. An initial representation of the swine collection of isolates based on core genome phylogeny, gene content and country of isolation, showed no specific pattern, possibly due to the bias of the collection towards UK isolates. However, a complete analysis of over 3,000 E. coli isolates from humans and different animal species that is being performed by the different ARDIG partners may provide additional insights into host- and country-specific determinants that may be important for AMR transmission.

UCM

We provided the phenotypic resistance data and genomic sequencing data of 50 *E. coli* isolates from poultry and swine farms in order to compare and analyse our results from AMR detection to those obtained by other partners with different pipelines and AMR databases. We performed the AMR

genetic detection over the total 450 *E. coli* isolates collected from all partners using the ARIBA (*Antimicrobial Resistance Identification By Assembly*) pipeline and applying the ResFinder database. Regarding the comparative study of certain clonal groups of epidemiological importance, as well as genetic determinants of resistance and plasmid types involved in their dissemination, our group participated with different partners. We provided sequencing data and metadata of *E. coli* isolates belonging to specific STs requested by partners for in depth studies (ST744 and ST38, among others). Likewise, we have focused on the epidemiological study of ST1196 *E. coli* in diverse environments from different countries, for which other partners provided metadata and sequencing data from their project databases. We have integrated these data to perform epidemiological analysis of clonal dissemination at European level. We cooperated with other partners in the study of the epidemiological dynamics of *mcr* genes across Europe, focusing on the mobile genetic elements involved in their dissemination, by providing Illumina-Nanopore hybrid assemblies of *E. coli* isolates carrying these resistance genes from national surveillance studies. The same way, we have facilitated hybrid assemblies and metadata of multiple *E. coli* isolates harbouring resistance plasmids belonging to specific incompatibility groups (IncFII, IncI1 and IncX) to partners of the project that are developing AI tools focused on the rapid prediction and accurate identification of these AMR genetic platforms.

PHE

PHE has shared short read data with partners for *E. coli* belonging to the ST744 and ST1196 lineages in addition to any *E. coli* harbouring CTX-M-1 and IncI1 sequences in order to facilitate the comparisons of isolates across countries and One Health compartments.

APHA

APHA has collected WGS from *E. coli* ST744 isolates from partners in the consortium for further characterisation. Work is progressing to compare the WGS data by reconstructing phylogeny of the *E. coli* isolates, determine presence of AMR genes and mobile genetic elements present in the isolates.

WBVR

Colistin resistance due to mcr-encoding E. coli have been described in many countries but most reports concern limited numbers of isolates due to the relatively low prevalence in most countries compared to ESBL/AmpC producing *E. coli*. WBVR have collected mcr-encoding *E. coli* from livestock, meat and humans from the Netherlands between 2010 and 2020 and sequenced these using Illumina short read sequencing and a selection of isolates also with Long-read sequencing to complete plasmid sequences. Furthermore, partners from the UK, Germany and Spain have submitted WGS data of mcr-encoding *E. coli* from livestock in their collections in order to perform a Europe-wide analysis of > 250 isolates. Analysis of this dataset is currently still ongoing.

WP3. AMR characterization, transmission of plasmids and fitness of MDR isolates.

WP3-T1 - Detailed molecular characterisation of AMR genes present in human, animal, food and environment isolates from WP1 and WP2.

Five partners (APHA/PHE/WVBR/NVI/UCM) have run WGS data from 450 E. coli isolates submitted by ARDIG partners (APHA, ANSES, BfR, IP, NVI, PHE, UCM, UoS, WBVR) through their pipelines (APHA SeqFinder/Abricate, PHE GeneFinder, WBVR, Ariba, ResFinder/PointFinder) to assess the impact of similarities and differences in methodologies commonly used for AMR genotyping within European Institutes on AMR gene predictions. Results from the pipelines analysis have been shared with the consortium, and partners have compared the AMR genotype prediction for each isolate with the corresponding antimicrobial tested by MIC (EFSA panel), using the database/gene catalogue available to them through their Institute. The results of the genotype/ phenotype comparison have been compiled by APHA and analysed further to identify agreements and discrepancies between each approach, as well as the sensitivity and specificity values to aim for between genotype and phenotype correlations. A manuscript of this work is in its final stages and will include recommendations for future use of WGS for surveillance activities. Such comparisons are of extreme importance to EFSA and ECDC

as they are moving to reporting of AMR data by genotyping, and we expect our study to make a valuable contribution to harmonisation of current approaches between animal and human sectors in this context of One Health.

UoS

A total of 730 *E. coli* isolated from four different host species have been characterised by whole genome sequencing. Out of 9 sets of isolates, 4 were collected during longitudinal studies over 12-month periods, including 255 from human urinary tract infections, 108 from human bloodstream infections, 85 from healthy poultry faeces and 117 healthy swine faeces. The other 5 collections of isolates are composed of 94 ESBL-harbouring *E. coli* from human urinary infections, 15 *E. coli* from healthy human faeces, 16 collected from the caeca of healthy poultry, 26 from avian colibacillosis samples and 14 from healthy cattle faeces.

The genome assemblies, available metadata and molecular characterization results (including assembly quality, MSLT, phylogroups, AMR genes, AMR point mutations and plasmid replicons detected in these isolates) have been uploaded to the ARDIG shared folder for further analysis.

PHE

Analysis of Enterobacterales producing carbapenemase enzymes revealed that, in addition to isolates producing the OXA-48 carbapenemase associated with a pandemic of self-transferable IncL/M incompatibility group plasmids, other plasmids belonging to this incompatibility grouping have emerged with the NDM carbapenemase. Analysis revealed the NDM gene had been acquired by IncL/M plasmids on multiple occasions occurring in geographically widespread isolates (manuscript submitted to the Journal of Antimicrobial Chemotherapy). To undertake a risk assessment for the threat posed by these emergent NDM encoding plasmids, examples of the most notable 'type' have been shared for fitness comparisons vs. the pandemic OXA-48 plasmid, at the UoS.

APHA

The farm isolates were characterised further by analysis of the WGS data. Levels of AMR genes present within indicator E. coli from non-selective media varied significantly between sites, with 84% identified as multi-drug resistant (3 or more AMR genes) on the clinical site in comparison to 4% on the non-clinical, with a corresponding difference in Sequence Types (ST) identified. In contrast, E. coli isolated on both sites from antibiotic selective media were mostly identical STs, with ST744 being the dominant E. coli isolated from ciprofloxacin containing media and ST88 the dominant from cefotaxime media. Persistence of ST744 clones with <10 SNP differences were identified across time-points, age classes of pigs and seagull samples in both sites. Both STs have previously been reported from animals and humans globally.

The presence of E. coli of the same ST with few SNP differences across time points, pigs and gulls indicates persistence and transmission of E. coli subtypes on and between sites. Further work is planned to identify factors that may be selecting these clones on site and maintaining AMR in the absence/low use of antimicrobials. A manuscript of this work is currently under review.

BfR

Within the work package 3 of the ARDIG project, we characterized *Escherichia* (*E.*) *coli* isolates recovered from livestock and food, provided by the National Reference Laboratory (NRL) for Antimicrobial Resistance (AR) in depth. Selection of isolates for detailed molecular characterization was based on the prevailing phenotypic characteristics (esp. MIC values, resistance profiles) of the (fluoro)quinolone-resistant *E. coli*. By focusing on nalidixic acid (NAL) and ciprofloxacin (CIP)-resistant *E. coli*, all isolates with a MIC of ≥8 mg/L for NAL and/or a MIC of ≥0.25 mg/L for CIP were further characterized. In total, 452 *E. coli* from the NRL-AR collection of 2017 fulfill the requirements for further investigation. These isolates were screened for six different *qnr*-variants, namely: *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS* and *qnrVC*. The most frequent *qnr*-variant was *qnrS* with 16.1 % among all investigated

isolates. Despite of *qnrS,* the occurrence of other *qnr* genes in the isolate collection was rather low. For screening of genomic relation of *qnr*-positive *E. coli*, we conducted XbaI macrorestriction analysis (XbaI-PFGE). The resulting phylogenetic tree revealed certain relations between a few isolates, but overall a broad diversity of XbaI-patterns was observed. For plasmid prediction, the isolates were subjected to S1-nuclease PFGE. This investigation in combination with DNA-DNA hybridization against *qnr*-genes allowed us to derive plasmidal associations with *qnr*-determinants. One-hundred and three *E. coli* were further subjected to Illumina NextSeq sequencing and were analyzed in detail for their genetic types (i.e. cgMLST, MLST, serotype etc.) and specific features (i.e. AMR, virulence, biocides etc.). The sequencing data were further used for predicting associations between the occurrence of *qnr* and isolate- or plasmid-specific features.

UCM

A total of 383 *E. coli* isolates collected and selected in the WP2 of the project were sequenced using Illumina technology, comprising samples from diverse sources: human hospital settings (110 isolates) and swine and poultry production farms (138 and 135 isolates, respectively). All isolates were obtained from non-supplemented culture media, in order to have an extensive and representative *E. coli* population according to AMR profiles. Phenotypic resistance profiles to different antibiotic groups by MIC evaluations were performed, showing a general high prevalence of fluoroquinolone and tetracycline resistance among all collected isolates from animal production sources. With Illumina data we studied the resistance gene content to all antibiotic families, comparing our results to those obtained by other partners with different AMR detection pipelines over the same isolates. This allowed us to identify the similarities and discrepancies between different bioinformatic tools and databases, which will be extremely useful for implementation of national and global AMR surveillance programs. We assembled the reads obtained by Illumina sequencing to perform the detection of antimicrobial resistance genes following different approaches and to identify the different *E. coli* sequence types involved in the dissemination of specific resistance genes at national and international levels. From 100 analyzed isolates from swine and poultry farms, a total of 45 different STs were identified, highlighting a high clonal diversity in the animal production area. However, one of this STs, ST10, was comprised by 23 isolates from both production types, representing the 23% of the isolates, which shows the broad distribution of this clonal group. Other ST, ST1196, also showed a high dissemination in different sources (including human, animal and environmental samples), not only in Spain, but in other several countries around the world. Thus, our group is focused on the epidemiological study of this clonal complex and other ARDIG partners provided sequencing data and metadata of isolates belonging to ST1196, so we are finishing phylogenetic analysis to put in context the epidemiological links of all multi-drug resistant isolates found. We are working on the publication of these results. In addition, one of our main goals was the study of the dissemination dynamics of *mcr* colistin resistance genes on different mobile genetic elements and *E. coli* clones, such as ST1196 and plasmids belonging to IncI and IncX types. We have collaborated with other partners to combine our *mcr*-carrying isolates and we have performed Nanopore WGS to resolve the genomic structure of these isolates and the plasmids involved in the dissemination of these clinically relevant resistance genes. We provided to the project the assemblies and analyses of Illumina data from all human, swine and poultry *E. coli* isolates in order to establish their clonal relationship and common genetic and plasmid contents.

ANSES

In the first study, the ESBL phenotype was largely due to the presence of CTX-M group 1 enzymes, which were identified in 71.5% of the animals upon arrival, and in 61.2% upon departure to the slaughterhouse (Gay et al, Frontiers in Microbiology 2019). Of the 241 *bla*CTX-M-group1-carrying *E. coli* upon arrival, 200 harbored $bla_{\text{CTX-M-1}}$ (200/241, 83.0%), 23 $bla_{\text{CTX-M15}}$ (23/241, 9.5%), 12 $bla_{\text{CTX-M-32}}$ (12/241, 5.0%); 4 $bla_{\text{CTX-M-55}}$ (4/241, 1.7%) and 2 $bla_{\text{CTX-M-3}}$ (2/241, 0.8%). PFGE profiles performed on a subset of 10 ESBL-producing isolates per farm upon arrival showed a wide variability without any clustering. At departure, $bla_{\text{CTX-M-1}}$ was also the most frequently identified gene (51/60, 85%) followed by $bla_{\text{CTX-M-55}}$ (4/60, 6.7%), $bla_{\text{CTX-M-15}}$ (3/60, 5.0%), and $bla_{\text{CTX-M-3}}$ (2/60, 3.3 %). At departure, the PFGE

profiles were much more similar than upon arrival so that a high degree of clonality was observed inside each farm. As an example, the PFGE distribution in farm E at departure, where three distinct PFGE profiles were observed, highlights the epidemiological success of certain ESBL *E. coli* clones more than others during the fattening process. Nonetheless, since different CTX-M enzymes were also produced by the same clone, not only a clonal but also a plasmid dissemination has likely occurred, which illustrates the complexity of ESBL spread at farm level. Similarly, the emergence of CTX-M-2 enzymes before slaughter was most likely due to the dissemination of a single clone within farm C since all but one CTX-M-2 enzymes were identified in this farm. Altogether, depending on the farm presenting ESBL-positive isolates, from 1 (farm H, 1 ESBL producing *E. coli* isolate) to 7 (farm B, 26 ESBL-producing *E. coli* isolates), distinct PFGE profiles were observed at the end of the fattening process. Of note, none of the successful clones identified at departure for the slaughterhouse was shared between farms, proving a specific and local evolution. *E. coli* belonged to phylogroups A (n = 134, 39.8%), B1 (n = 78, 23.1%), B2 (n = 9, 2.7%), and D (n = 116, 34.4%) upon arrival, and to phylogroups A (n = 50, 51.0%), B1 (n = 15, 15.3%), B2 (n = 1, 1.0%), and D (n = 32, 32.7%) at departure to slaughterhouse. The *mcr-1* gene was identified in 18 isolates, while one isolate carried both the *mcr-1* and *mcr-3* genes. At departure for slaughterhouse, only 4 animals from 2 different farms still carried a colistin-resistant *E. coli* (MICs ranging between 2 and 4 mg/L). The *mcr-3* gene was detected in all four isolates and was co-harbored with the $bla_{\text{CTX-M-55}}$ gene.

In the second study, one colony was isolated from the 173 positive samples for further characterization (Massot *et al*, manuscript submitted). The ESBL-producing *E. coli* isolates carried either $bla_{\text{CTX-M-1}}$ (112/173 isolates, 64.7%), $bla_{\text{CTX-M -14}}$ (58/173, 33.5%) or $bla_{\text{CTX-M-15}}$ (3/173, 1.8%). All of them were resistant to at least two antimicrobials other than ß-lactams, the most common resistances being against tetracyclines, sulfonamides, trimethoprim and aminoglycosides. The median number of co-resistances was four.

Institut Pasteur

Study 1. Analysis of CP*Ec* isolates received by the Fr-NRC. We have sequenced 713 isolates received by the Fr-NRC until 2015 by using the Illumina technology. As ST38 represented the most frequent ST among those isolates, we sequenced to completion by using the PacBio technology 10 CP*Ec* ST38 isolates. Furthermore, in order to quantify the fitness cost of plasmids carrying carbapenemase genes we similarly fully sequenced six isolates from ST38, ST410 and ST131 closely related to CP*Ec* isolates, but devoid of any carbapenemase genes. We performed whole genome phylogeny of the 713 CP*Ec* isolates together with reference *E. coli* isolates. For each isolate, we determined the ARG content and the presence of mutations known to contribute to decrease susceptibility in *gyrA*, *parC* and parE QRDR regions (fluoroquinolones), in the porin genes *ompC* and *ompF* and in the penicillin binding proteins 3 (PBP3) gene *ftsI* (ß-lactams). The most frequent carbapenemase genes were $bla_{\text{OXA-48}}$, (65%), $bla_{\text{OXA-181}}$ (15%), $bla_{\text{NDM-5}}$ (7%) and $bla_{\text{NDM-1}}$ (6%). We observed a great diversity of CP*Ec* as the 713 isolates belong to 166 STs from all phylogroups. Nevertheless 57% of the isolates belong to 14 STs with at least 10 isolates. In particular we identified 3 dominant lineages: clonal complex (CC) 10 (including ST10, ST167 and ST617), CC23 including ST410 and ST38 which together gather 50% of the isolates (Figure X). In terms of relation with animal isolates, two CC23 lineages need to retain further attention: ST410 for which closely related ESBL producing bovine isolates have been characterized and ST88 (15 isolates), which was the most frequent lineages among 540 3GC-R *E. coli* isolates from diarrheic veal in Belgium we have analyzed.

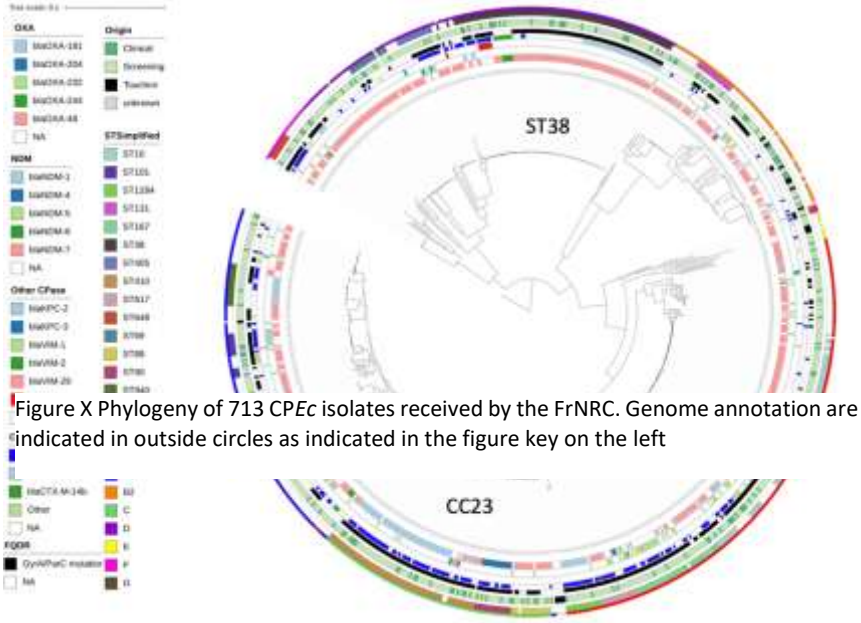

Figure X Phylogeny of 713 CP*Ec* isolates received by the FrNRC. Genome annotation are indicated in outside circles as indicated in the figure key on the left

Phylogenetic analysis (Figure X) revealed a combination of disperse isolates and of lineages enriched in CP*Ec* isolates. We first focused our analysis on an ST410 clade broadly disseminated and producing OXA181. $bla_{\text{OXA-181}}$ is carried by a incX3 plasmid. Analysis of mutations in the branch leading to the ST410 OXA-410 clade revealed candidate loci which might have contributed to the dissemination of this clone. Among those mutations, we focused our analysis on mutation in *ompC*, *ompF* and *ftsI* which lead to to decrease susceptibility to some ß-lactams including ertapenem. We first extended this analysis to the whole species by retrieving CP*Ec* genome from the NCBI and Enterobase web sites. We combined phylogeny and association study to define three evolutionary trajectories: (i) broadly disseminated MDR lineages mutated in these three genes before the acquisition of the carbapenemase gene and contributing to the "success" of these clones, (II) ST38 which shows specific properties including an intrinsic reduced susceptibility to certain ß-lactams, and (iiii) diverse isolates which have likely recently acquired a carbapenemase gene with no sign of dissemination. This last class included isolated from the pandemic lineage ST131 (Patino Navarrete et al. 2020).

We next analyzed data on Fr-NRC isolates considering these observations (Rosinski-Chupin et al. in preparation). Similarly, to what was observed on worldwide collection of isolates, Fr-NRC isolates belong to the same three categories. Sporadic cases were mainly associated with $bla_{\text{OXA-48}}$. It could be due to the high conjugation efficiency of the IncL pOXA-48 plasmid and its high prevalence in *K. pneumoniae* isolates in Frances. We also identified and confirmed three clusters of isolates

corresponding to hospital outbreaks which all belong to MDR lineages.

Finally, we focused our analysis on ST38 isolates by characterizing the disseminated lineages. Strikingly, the vast majority of isolates belong to three disseminated lineages. In these three lineages, all ARG were inserted into the chromosome and were generally devoid of any plasmid. Combined analysis with publicly available ST38 genomes showed that two of these clades were broadly disseminated, whereas one clade was mostly restricted to France with few isolates from the Netherland. In order to better characterize the insertion and their dynamic, we fully sequenced 10 isolates belonging to these lineages. In addition to the OXA-48 producing clades, we identified a small cluster of seven isolates carrying bla_{VIM-4}. In this case, the carbapenemase gene was not inserted into the chromosome but carried by a new plasmid we characterized. The bla_{VIM-4} gene is carried by an integron. Strikingly, it is virtually identical to the non-conjugative plasmid pROUEN1, a plasmid identified in an MDR *Pseudomonas putida* clinical isolate (Eleni Liapis et al. Frontiers in Microbiology 2019). pROUEN1 carries a similar integron with five resistance genes including the bla_{IMP-63}. Therefore, the only difference between the two plasmids is the gene content of the integron. This observation raised the issue of the evolution from one plasmid to the other one. Our hypothesis is that pVIM-4 derived from pROUN1 by integration (including bla_{VIM-4}) and loss of cassettes (including bla_{IMP-63}). It raised also the issue of the acquisition of this plasmid. Analysis of *E. coli* sequences from Enterobase did not reveal any related isolates carrying bla_{IMP-63} and BLAST search at the NCBI no similar plasmid. This plasmid does not seem to have disseminated.

Study 2: Analysis of the i-bird collection. The 205 isolates belong to 32 different STs revealing a broad diversity of ESBL-*Ec* carried by the patients. However, 100 belong to ST131, almost 50%. They are carriage isolates recovered from systematic screening and not clinical isolates, showing that, in this environment, ST131 is dominant even among colonizing ESBL isolates. In-depth phylogenetic analysis of the 205 isolates revealed cases of transmission or of a common source only for ST131 isolates (Fig. 1). ST131 isolates clustered in four groups (Figure 1) with exchanges between patients, as exemplified for group 3 (in blue) identified in seven patients. Furthermore, we observed a within host diversity. A representative isolate of each lineage was fully sequenced by combining long read (PacBio) and short read (Illumina) sequencing to characterize the within- and between host diversity and to infer transmission and directionality. The genomic data will be confronted with temporal and contact data collected during the i-bird project. Therefore, the prolonged carriage of ST131 ESBL-P isolates and their capacity to be transmitted from patient to patient have likely contributed to their predominance among ESBL-P *E. coli*. We also identified three cases of plasmid transmission from *K. pneumoniae* to *E. coli* but no cases of transmission from *E. coli* to *K. pneumoniae*, suggesting that in this context, E. coli act mainly as a receiver more than a donor of antibiotic resistance plasmids.

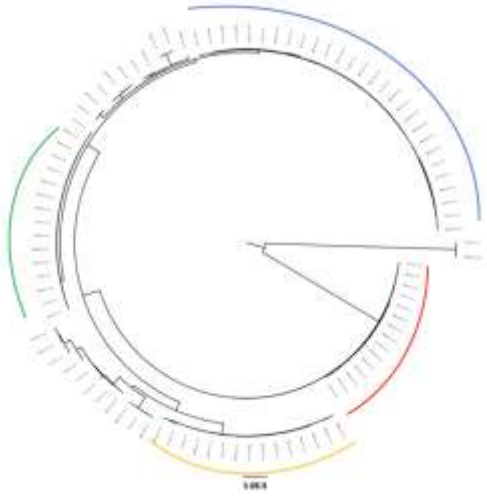

Figure X Phylogenetic tree o 100 ST131 isolates from the i-bird project. The majority of isolates clusters in four different clades that have disseminated in the hospital.

Analysis of UTI isolates from ARDIG

The 250 isolates from UTI collected by ARDIG partners (IP, UCM, NVI, UoS) have been sequenced by the Illumina technology. The isolates belong to 81 different ST revealing a broad diversity (Fig. X). The four most frequent STs are also frequently reported in other studies as responsible for UTI (ST73, 31 isolates; ST131, 20 isolates; ST69, 19 isolates and ST95, 18 isolates). Susceptibility to ß-lactams has been determined by disc diffusion assays for the first 120 isolates. MICs of 25 isolates for 14 antibiotics were determined by serial microdilution by ANSES for benchmarking pipelines for ARG identification and for genotype phenotype correlation. Antibiotic resistance genes have been detected using Resfinder and candidate mutations have been investigated by using point finder. 21% of the isolates share a mutation in the FQRD region of *gyrA* and/or *parC* and were predicted to show a reduce susceptibility to fluoroquinolons, whereas 11% express a ESBL of the CTX-M family. Only one isolate, of ST410, carries a carbapenemase gene (bla_{OXA181}). These genome sequences are combined to other ARDIG *E. coli* sequences for a global analysis.

NVI

NVI was responsible for phylogenetic analysis of *E. coli* isolates of human and animal origin collected by from partner countries (UCM, IP, NVI, UoS, WBVR, APHA, BfR). A phylogenetic reconstruction of all *E. coli* isolates from longitudinal studies and national surveillance (n = 3812) was carried out to investigate possible cross-country zoonotic transfer. Briefly, all genomes were quality controlled and checked for contamination using QUAST and Kraken2, respectively. In total, 33 genomes were removed due to low quality or contamination. Additionally, isolates from ducks, roe deer, and clinical isolates of animal origin were removed from the dataset, leaving 3745 genomes. Panaroo pangenome was used to calculate the pangenome and generate the core gene alignment. The core gene alignment was used for reconstructing the phylogeny with IQ-Tree. The program snp-dists was used to calculate the SNP distances between the genomes. After inspecting the phylogenetic tree, subclades were selected for further analysis. The selection of clades was based on the following: To show a possible transmission between food producing animals and humans (demonstrate shared strains between humans/animals), and to demonstrate closely related strains from the same animal species, but originating from different countries (which again can indicate AMR transfer via international animal trade). Each subclade was subjected to another phylogenetic pipeline, using the ALPPACA pipeline (DOI: 10.5281/zenodo.4452122). Briefly, ParSNP was used to predict the core genome, and Gubbins was used to identify recombinant areas in the alignment. Then, maskrc-svg was used to mask these regions in the alignment, and IQ-Tree reconstructed the phylogeny from that alignment.

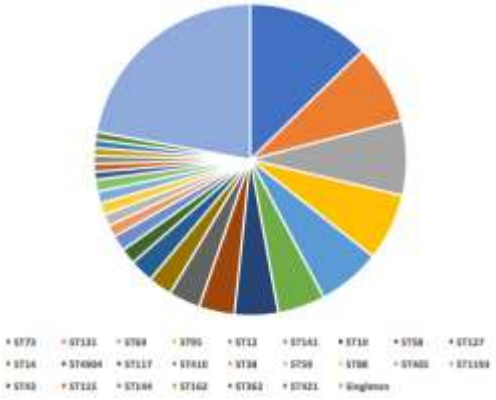

Figure X. ST distribution among the 250 UTI isolates

WBVR

WBVR have been responsible for determining the AMR gene content of the 3812 *E. coli* isolates of human and animal origin collected by from partner countries (UCM, IP, NVI, UoS, WBVR, APHA, BfR). These isolates were run through the ResFinder pipeline, which performs similar to other pipelines used by consortium members, to determine the isolate AMR genotypes. Differences between host species, geography and management systems that may impact on AMR content and distribution is currently being assessed.

WP3-T2- Characterisation of prevalent circulating plasmids and their transfer in vitro

UoS

The UoS has quantified the transfer of a representative set of broad host range plasmids carrying NDM-

1 and OXA-48 carbapenemase genes. Original host species (*E. coli, Enterobacter cloacae, Klebsiella pneumoniae, Klebsiella oxytoca and Citrobacter*) were used as donors in conjugation experiments performed for 1h on LB-agar. A broad range of frequencies was observed when original hosts were used as donors (from no detected transconjugants to high conjugation rates), while more homogeneous results were obtained when the obtained transconjugants (*E. coli* MG1655) were used as donors, reflecting the high sequence similarity between plasmids.

As a general rule, IncL OXA-48 plasmids conjugated at a higher rate than IncM NDM-1 plasmids, likely due to the inactivation of the fertility inhibition gene *tir* in IncL plasmids, and possibly causing the more extensive global spread of OXA-48 plasmids.

UCM

We determined the plasmid content of *E. coli* isolates from WP2, identifying all different plasmid incompatibility groups according to plasmidic replication structures using short-read sequencing data. We focused on the study of dissemination dynamics of *mcr* genes, among others. Out of 35 *mcr-1*-carrying *E. coli* isolates identified in both swine and poultry production farms, we selected 20 belonging to diverse clonal complexes and sources to perform WGS using Nanopore technology. We generated Illumina-Nanopore hybrid assemblies of the *E. coli* genomes, which allowed the in-depth study of plasmids and mobile genetic elements involved in the dissemination and mobilization of *mcr* genes among these bacteria at farm, regional and national levels, as well as the association of these genes with other genetic resistance mechanisms in the same plasmidic platform. The results showed two different epidemiological routes of *mcr-1* dissemination in animal farms in Spain: i) dissemination via specific bacterial clones, which are present in different farms, even in different animal production types; ii) dissemination via specific plasmid types highly associated with *mcr* genes, which are spread and present in multiple bacterial clones. Among these plasmids, those belonging to the IncI1 type were the most prevalent ones. We are finishing conjugation experiments to assess the capacity of these plasmids to be transferred and maintained at intra and interspecies level. We are currently preparing a manuscript with the aforementioned results. Furthermore, we provided the metadata and Illumina-Nanopore sequencing data of all these *mcr-1*-postive *E. coli* isolates to other partners of the project in order to study the dissemination dynamics of *mcr* colistin resistance genes and their carrying mobile genetic elements and *E. coli* clones at the European level.

BfR

For determination of plasmid-types carrying *qnr*, we focused on *in-silico*-based finishing of the plasmid genomes. For this, the refSNPer tool was used, which elects the closest reference, by mapping the input sample to a chosen reference set and identifies the coverage by using bedtools. With this, we were able to assign several *qnr*-carrying plasmids to reference plasmids and track putative plasmid paths. Moreover, we discovered plasmids, which are yet not described to be associated with *qnr*. In this manner, we detected the most frequently detected plasmid to cluster for the groups of IncY (n=19) and IncX (n=27). It has been emphasized before, that there is a correlation between this IncX plasmids, harboring bla_{TEM} and $bla_{CTX\text{-}M\text{-}15}$ genes next to *qnrS*, resulting in ESBL-producing *E. coli*. We could confirm this observation through annotation of our sequences. IncX plasmids are regularly described as a group inhabiting the *qnrS1* gene. Therefore, we decided to determine this plasmid group in detail. We wanted to find out more about the *E. coli*, prone to inhabit this plasmid type and we wanted to describe a proper plasmid backbone. Moreover, our aim was to derive a core genome for the individual *qnr* plasmid types. With this, we wanted to understand their potential in disseminating *qnrS1* and other potential resistance genes. However, the *E. coli* inheriting the IncX-plasmid carrying the *qnrS1* were highly heterogenic. We found diverse ST-types of *E. coli* as well as different matrices, which did hold the respective IncX plasmids. From the XbaI-macrorestriction profile, we confirmed a high diversity of *E. coli*, possibly transmitting this plasmid. Thus, one can conclude that this plasmid, carrying the *qnr*S1 gene, is spreading over different *E. coli* types within multiple sources. Further, we screened for their conjugational behavior. First, we determined the conjugational behavior *in silico* with the mob-suit

tool. Thus, we found most of the *qnr*S carrying IncX plasmids to hold the respective relaxase and oriT region on the plasmid, suggesting possible transfer. This observation was then validated by laboratory experiments. However, the *in vitro* and *in silico* results for evaluation of the transmissibility of the plasmids mostly showed divergent results.

Next to the *in-silico* detection of plasmid references, we analyzed plasmid structures with long-read sequencing methods. Thus, we were able to generate scaffolds for the most prevalent *qnr*-carrying plasmid types. These basis structures assisted when reconstructing of other *qnr*-plasmid was aimed for characterization of the most prevalent plasmids.

Next to *qnrS* we set our focus on *qnrB*-carrying plasmids in ESBL producing *E. coli*. Therewith, we found some prevailing plasmid types as IncH and Col440I. Further characterization of these plasmids indicates that especially *qnrB19* did mainly exist on small, non-conjugative plasmids. Thus, the spread of these small plasmids remain a mystery. Furthermore, we detected *qnrB2* on IncH plasmid, known for spreading multi-resistance across different matrices. The presence of these *qnrB* resistance determinants in ESBL-producing *E. coli* do pose a particular risk, as it may confer resistance against a "Highest Priority Critically Important Antimicrobials" for human medicine next to the already present genes leading to ESBL producing *E. coli*.

Altogether, with those comprehensive investigations of *qnr*-positiv *E.coli* isolates a thorough and complex picture will be generated for the mobile genetic elements and their dissemination as well as their commonalities in commensal *E. coli*.

NVI

Analysis of circulating ESBL plasmids/strains from broilers in Norway have been performed by using both short and long-read sequencing (isolates with blaCTX-M-1 located on IncI1-Iγ plasmids). Our data showed that dissemination of blaCTX-M-1 in Norwegian broiler production is due to both clonal expansion and horizontal transfer of plasmids carrying blaCTX-M-1. The genetic diversity at both strain and plasmid level indicates multiple introductions to Norwegian broiler production. The study was published in Frontiers in Microbiology.

Comparison studies showed that blaCTX-M-1 plasmids circulating in Norwegian broiler production are highly similar to plasmids previously described from broiler production in other countries. Reconstruction of blaCTX-M-1/ IncI1-Iγ plasmids from broilers in Norway showed that a plasmids from ST57 isolates harboured IncI1-Iγ/IncFIB hybrid plasmids with blaCTX-M-1. Conjugation experiments showed that a non-hybrid IncI1-Iγ blaCTX-M-1 plasmid (sequence similar to the IncI1-Iγ part on the co-integrated IncI1-Iγ/IncFIB plasmid) was transferred into more recipient strains, indictating that that the non-hybrid plasmid exhibited a greater promiscuity in terms of acceptance and maintenance by various E. coli strains.

ANSES

The $bla_{CTX\text{-}M\text{-}1}$ gene was carried by five plasmid types disseminated in 16 clones (encompassing 112/173 isolates, 64.7%), while the $bla_{CTX\text{-}M\text{-}14}$ gene was carried by five plasmid types disseminated by 15 clones (encompassing 58/173 isolates, 33.5%) (Massot *et al*, manuscript submitted). The $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}14}$ genes were carried by distinct plasmids and clones. Of note, the $bla_{CTX\text{-}M\text{-}15}$ gene was detected on the chromosome of the same clone in farms B and C. Four ESBL plasmids were identified in several farms: IncI1/ST312 in farms A and B, IncK in farms A and C, IncF/F2:A-:B- in all three farms, and IncF/F2:A-:B42 in farms A and C. IncF/F2:A-:B42 plasmid was spread by the same *E. coli* clone in different farms, and the others by distinct *E. coli* clones in the different farms. While present in two farms, IncK and IncF/F2:A-:B42 plasmids were detected only in one clone per farm, suggesting a limited spread. On the opposite, IncI1/ST312 and IncF/F2:A-:B- have spread in several clones in each farm.

APHA

To characterize prevalent plasmids and their dissemination between different systems (D-3.3 and D-

3.4), including different host types and geography, APHA has been evaluating a machine learning approach where by presence of some of the most common AMR plasmid types such as those harbouring Inc-I1, Inc-F and Inc-X can be determined. Validation of a test set of isolates harbouring these Inc-types from various partners (APHA, NVI, UCM, and WBVR) with long and short read WGS available, has indicated clearly that the machine learning approach can identify isolates from each Inc-type, as well as separate AMR from non-AMR harbouring plasmids. As part of the next step we are testing all 3812 *E. coli* isolates of human and animal origin collected by partner countries (UCM, IP, NVI, UoS, WBVR, APHA, BfR) to determine what plasmids may be common and disseminating across these regions and host-types.

WP3-T3- Fitness cost of AMR and stability of plasmids in different host strain background

UoS

The UoS has performed *in vitro* growth curves in different media to determine the fitness cost of IncL/M plasmids carrying NDM-1 and OXA-48 genes in *E. coli* MG1655 and other Enterobacterial hosts. In most cases, the tested plasmids had an associated fitness cost to the bacterial host when compared to the growth rate of the same bacteria with no plasmid. In addition, the fitness cost associated with OXA-48 plasmids in *E. coli* MG1655 was slightly lower than the corresponding cost of NDM-1 plasmids, which could also contribute to the increased success of OXA-48 plasmids.

UCM

Fitness and stability experiments of most prevalent *mcr-1*-carrying plasmids from swine and poultry Spanish farms are programmed after completing conjugation assays. Experiments are scheduled to be performed with a broad range of bacterial hosts, including diverse *Enterobacterales* species.

BfR

In this task, selected plasmid types (n=12) were further chosen for fitness and stability tests. Therefore, the *qnr*-carrying isolates were introduced into *E. coli* J53 by transformation or conjugation. The growth performance of the *E. coli* with and without the different *qnr*-plasmid types showed no significant differences. We had also not observed a significant difference in the stability of the plasmids in *E. coli* J53 under selective and non-selective conditions over a time of >150 bacteria generation. Thus, we suppose that the selected *qnr*-carrying plasmids probably had not an influence on the growth of the *E. coli* and will persist without any significant loss over time within its host bacterium. However, up to now we cannot predict any potential influence of the different plasmid types on the fitness and their stability in other Enterobacteriaceae. Based on the results of the conjugation, the majority of the plasmids could be also successfully transferred, but with lower efficacy to other *Enterobacteriaceae* (i.e. *Klebsiella*, *Morganella*). Further fitness and stability tests need to be done to develop reliable data on this issue.

NVI

Fitness cost and competitive growth of IncI plasmids with ESBL/pAmpC genes from Norwegian broiler production have been performed. We have also investigated the rearrangement of IncI1 shufflons which segment B was interrupted by the transposition unit ISEcp1-blaCTX-M-1-orf477. The study included two IncI1 plasmids (pMLST CC3) harbouring shufflon interrupted by the transposition unit, and a plasmid harbouring uninterrupted, complete shufflon. Plasmids with interrupted shufflons were present in their original hosts and conjugated into an *E. coli* ST162 strain. Liquid single-strain cultures were sampled at four time points during bacterial growth and the shufflon region was PCR amplified and amplicons were subjected ONT sequencing. The interrupted shufflons generated a lower number of shufflon variants in comparison to the uninterrupted shufflon. Segment-loss frequency of the interrupted shufflons was distinctive in different plasmid hosts. In both uninterrupted and interrupted shufflons, segment A was more favoured to complete partial *pilV* ORF. The study was published in Plasmid.

WP3-T4- Measuring AMR plasmid dissemination in vivo in mouse and Galleria, and chicken and pig in-vitro gut models

UoS

Representative examples of OXA-48 and NDM-1 IncL/M plasmids transferred to *E. coli* MG1655 were used as donors in *Galleria mellonella* conjugation experiments. These studies were performed over a 4h period following injection of 10^7 CFU of donors and recipients into the *Galleria*, separately. Although the differences in transfer rates between both plasmids were not statistically significant due to high variability rates, the frequencies of the OXA-48 plasmid were slightly higher, reinforcing previous findings *in vitro*. Interestingly, in each case, both transfer rates *in vitro* (1 h in LB-agar) and *in vivo* (4h in *Galleria*) were very similar.

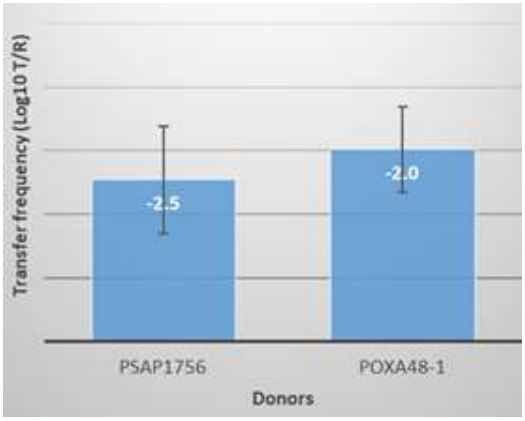

Figure X. *In vivo* conjugation frequency of IncL/M plasmids harbouring NDM-1 (PSAP1756) and OXA-48 (POXA48-1). 10^7 CFU of donors (*E. coli* K12 carrying the plasmid) and 10^7 CFU of recipients (*E. coli* K12) were injected into *Galleria mellonella* larvae. Inoculated larvae were incubated for 4 h at 37 °C, euthanised and homogenised. Donor, recipient and transconjugant CFU/ml were selected in plates containing the corresponding antibiotic combinations. Transfer frequency was calculated as the logarithm of transconjugants per recipient.

The *in vivo* mouse work and *in-vitro* gut models could not be performed due to continued restrictions from Covid19 severely impacting the amount of laboratory activities that could be undertaken for this workpackage; therefore Milestone M-AMR2.ARDIG.16 could not be delivered. The WGS pipeline comparison and analysis of isolates collected from both national and longitudinal farm studies by phylogenetics were additional *in silico*, and the laboratory component (i.e. isolation of *E. coli* and WGS) had already been delivered.

WP4. Project coordination and management.

WP4-T1- Steering committee quarterly meeting

Regular tele- and video-conference meetings, and updates by email, have been made to all members in the steering committee within ARDIG.

WP4-T2- Consortium members annual meeting

Due to COVID19 posing restrictions on travel the ARDIG consortium was unable to meet physically for an annual ARDIG meeting in both 2020 and in 2021, where at least one member from each partner organization was expecting to attend. However, an online meeting via Zoom or MS Teams, was successfully held between partners. It provided an opportunity for partners from all WPs to interact and discuss the work being performed in ARDIG.

Previous to 2020, the ARDIG consortium met physically during the OH-EJP ASMs to discuss and review the project, its objectives and deliverables.

ARDIG partners have used telecommunications and video conferencing facilities, to remain in touch. Regular meeting have been held over the past 42 months of this project to ensure the project is

progressing well, and to capture any delay which may have resulted, especially due to the impact of COVID19.

WP4-T3- Reporting and communication

Several work package associated subgroup meetings have been held online to provide time for more in-depth discussion between partners.

All ARDIG 9M and 12M reports were submitted in full and in a timely manner. There have been a number of publications, presentation (both oral and poster) from ARDIG partners which has included work performed within ARDIG.

5.1.4.3.1.4 Project self-assessment

The ARDIG project has been highly successful; it has been extremely collaborative, completed all major Milestones and Objectives, and partners have delivered a large number of publications, with several still in preparation or under review, despite the setbacks of COVID19. Assessment of each WP individually is given below.

WP1: Most objectives of ARDIG WP1 were achieved. However, we had hoped to collect and analyse a larger range of variables regarding AMR and AMU. Analysis was substantially hampered by a lack of harmonization across systems and within systems across regions and countries. Based on the collection efforts we are now able to better characterize the shortcomings of the current systems from a one health and transnational perspective. This may inform future efforts for more harmonization and support current efforts such as the EARS-Vet proposal.

WP2: The large number of isolates with WGS data available from both retrospective and prospective studies from partner collections has made it possible to perform AMR trend analysis both within and between countries and compartments. This was a key deliverable for ARDIG and such comparisons will help understand how mobile genetic elements may be disseminating. A possible short fall was that not all “vet” partners were able to access the same livestock farm-type or visit farms at the same frequency, which has made it somewhat challenging to compare across the ARDIG dataset. This shortfall was mainly due to budget constraints which meant partners had to incorporate ARDIG Objectives within existing national and/or research studies, in addition to leveraging national co-funding. Nevertheless, comparison of WGS data collected from both human clinical and veterinary settings, has been performed and analysed, as described in Section 2.

WP3: In general, the main objectives of the WP3 were achieved. The performed analyses had also supported the development of routine sequencing in the NRL-AR and bioinformatics analysis for assessment of AMR dynamics in commensal E. coli. However, sequence comparisons with different interpretation pipelines showed that further harmonization would be necessary to obtain comparable results at the EU level. Furthermore, the interpretation of in silico data without any additional phenotypic or genotypic data can also lead to misinterpretation. In general, the analyses confirmed the suitability of WGS for resistance prediction, but further efforts are necessary to improve interpretation of the observed genes and the genomic association (plasmid vs. chromosome). Studies on the dynamics of plasmids will need further efforts in the future, because of the broad diversity of transmissible plasmid types, which sometimes also differ from region to region and from country to country. Due to the remit of ARDIG being so broad, long read was performed on only a small proportion of isolates with short read data available, so most plasmid genomes remained unresolved. We have therefore developed a machine learning approach to utilise the short read data for plasmid characterisation, which require further testing in future.

5.1.4.3.1.5 Progress of the project: milestones and deliverables

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
02	D-1.1	A report of AMR and AMU data (and data collection activities) in livestock and humans in the seven participating countries, and with indication to its quality, comparability and purpose.	M12			Available on OHEJP Zenodo:Uploaded A publication resulting from this work is available and has already been uploaded to Zenodo: https://zenodo.org/record/997236#.XvyQfSgzZM0	
02	D-1.2	Description of the specified AMR prevalence/frequency and AMU at population/country/regional level.	24	25		Available on OHEJP Zenodo: Uploaded https://zenodo.org/record/5336876#.YURcObgza70	Report; 9
02	D-1.3	A list of the regions identified for in-depth analysis, and a report including the assessments of parallel trends and estimates of potential associations between AMR	24	25		Available on OHEJP Zenodo: Uploaded Two publicatiosn resulting from this work is available and has already been uploaded to Zenodo: https://zenodo.org/record/4328124#.YKS5sagzbY0 https://zenodo.org/record/4704208#.YKS6KKgzbY	Report; 9

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
		and AMU.				0	
02	D-1.4	A report of the assessment of risk factors for AMR and AMU	42	42		Available on OHEJP Zenodo: Uploaded https://zenodo.org/record/5336902#.YURcfLgza70	
02	D-1.5	Recommendations and target zones for improved "One Health" surveillance	42	42		Available on OHEJP Zenodo: Uploaded https://zenodo.org/record/5416606#.YURcqLgza70	
02	D-JRP2-2.1	Assessment of criteria for inclusion of retrospective and prospective longitudinal studies.	36	42		A questionnaire has been completed by all partners to assess the criteria for inclusion. For both the animal and human isolates that were used from retrospective and prospective studies the criteria was discussed and finalized so comparisons can be made across all datasets available.	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
						For the prospective studies for the human isolates the criteria is that the first 20 E. coli isolated from urine from each hospital at each month over a year will be included. There are no criteria for the animal isolates as different livestock and management systems have been sampled. However, we are evaluating differences and similarities in AMR *E. coli* given the diverse sample types. OHEJP: available Zenodo link: https://zenodo.org/record/5236699#.YSPSruIUmM9	
02	D-2.2	Assessment of retrospective longitudinal studies and collection of necessary data.				OHEJP: available https://zenodo.org/record/5236764#.YSPU1eIUmM8	
02	D-2.3	A project isolates database accessible to all member of the consortium	24			As part of WP2 and WP3, a hub has been created at WBVR for depositing WGS data for analysis across partners, which will also be a database of isolates. The full data set of 3812 *E. coli* will become	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
						accessible on publication, but WGS of many isolates are already available through the publications listed in D-2.4 (below).	
02	D-2.4	Data analysis of all collected isolates on a national level.	42	42		Various national studies have been performed. These have been published in various manuscripts and are available also through Zenodo (see links below). They are: https://zenodo.org/record/3730637#.Xn3Pm4hKi70 https://zenodo.org/record/4249017#.YAqc_uhKjcc https://zenodo.org/record/4456741#.YAqkH-hKjcc https://zenodo.org/record/4456718#.YAqfnuhKjcc https://zenodo.org/record/4981248 https://zenodo.org/record/4981351	
02	D-2.5	Comparative analysis of results from the various national studies.	42	42		A manuscript is currently being prepared which compares the individual/country level WGS of isolates from the national studies (D-2.4), as detailed in WP3 T1 and T2. Confidential	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
02	D-2.6	Comparative analysis of strains persistence in farms and hospital through longitudinal studies	42	42		This task has been completed and phylogenetic analysis has been performed for comparison across partners or geography, and different compartments (humans/livestock) to detect possible clonal transmission. Details of the work performed by partners for D-2.6 is given in WP2, and in WP3-T1 and T2. Confidential	
02	D-3.1	Prevalent AMR genes and platforms in enterobacteria from humans, animals, food and environment.	20	42		All partners have assessed and are reporting on the AMR gene content of their isolates, especially those already collected in prospective studies and from historical collections. Following a workshop in 2019 to harmonise AMR gene analysis within ARDIG, we have used the ResFinder pipeline to look at AMR content across sectors, although partners have reported used their preferred pipeline on collections from their own dataset. The publications have been uploaded to Zenodo and include those already given for D-2.4:	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
						https://zenodo.org/record/3730637#.Xn3Pm4hKi70 https://zenodo.org/record/4249017#.YAqc_uhKjcc https://zenodo.org/record/4456741#.YAqkH-hKjcc https://zenodo.org/record/4456718#.YAqfnuhKjcc https://zenodo.org/record/4981248 https://zenodo.org/record/4981351	
02	D-3.2.	Circulating plasmids in humans, animals, food and environment	24	42		Partners have assessed plasmids and mobile elements harbouring AMR genes, including their fitness, as detailed in WP3-T2 and T3. This has resulted in a number of publications, which have already been uploaded to Zenodo. These are: https://zenodo.org/record/3730621#.Xn3MyYhKi70 https://zenodo.org/record/4475507#.YBKNvOhKhM0 https://zenodo.org/record/3701226#.YAqeSehKjcc	
02	D 3.3.	Predictive modelling of plasmid spread	42	42		Presence and transmission of AMR plasmids are being considered across different compartments. https://zenodo.org/record/4964345	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
						https://zenodo.org/record/4244116#.X6KBDjiWxM0 https://zenodo.org/record/5506463#.YUBKk50zaUk	
02	D-3.4	Validation of plasmid AMR threats in animal models	42	42		Presence of AMR plasmids harbouring OXA-48 and NDM-1 could increase the "threat" of AMR. This theory was tested using a *Galleria mellonella* model and details are provided in WP3-T4. A study was also performed to look at the association of gut microbiome in calves with antibiotic treatments. https://zenodo.org/record/4456718#.YAqfnuhKjcc	
02	D-4.2	Annual communication to stakeholders	24	42		The stakeholder is aware of the work being performed and will receive a copy of the final report.	
02	D-4.3	Annual communication to stakeholders	42	42		The stakeholder is aware of the work being performed and will receive a copy of the final report.	

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milesones

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
02	M-JRP2-1	Two datasets on (1) AMR and (2) AB usage of the six participating countries.	12	Yes		
02	M-JRP2-2	Assessment of retrospective longitudinal studies and collection of necessary data.	12	Yes		
02	M-JRP2-3	Preliminary molecular characterization of AMR genes from isolates collected in WP1 and WP2.	12	Yes		
02	M-AMR2.ARDIG.4	Identification of MDR isolates circulating in humans, animals, food and environment	24	Yes		An assessment has been made by partners of common MDR isolates circulating within the different regions/countries. Based on this assessment partners have agreed to look in more details at the WGS of different E. coli STs collected from partner institutes. In addition, the profiles of >3000 AMR isolates, is being analysed to identify common factors associated with MDR.

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
02	M-AMR2.ARDIG.5	Assessment of AMR genes and platforms in enterobacteria collected from humans, animals, food and environment.	24	Yes		With WP1 AMR phenotypes have been correlated with AMU at regional or country level. Details are provided in D-JRP2-1.2 and 1.3.
02	M-AMR2.ARDIG.6	Assessment of AMR genes and platforms in enterobacteria collected from humans, animals, food and environment.	18	Yes		All partners have started molecular characterisation of isolates. WP3 provides details of the work. An ARDIG workshop was undertaken and differences between AMR gene analysis identified. The five pipelines used by partners will be compared to each other using WGS data from the same set of 500 isolates.
02	M-AMR2.ARDIG.7	Identification of circulating plasmids in humans, animals, food and environment	24	Yes		An assessment has been made by partners of some of the most common circulating plasmids within the different regions/countries. Based on this assessment partners have agreed to look in more details at the genomes of a number of different E. coli ST plasmids collected from partner institutes.
02	M-AMR2.ARDIG.8	Assessment of ecological and management factors associated with AMR and	42	Yes		This work has been completed (see Task 1).

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
		Antimicrobial usage (from WP1)				
02	M-AMR2.ARDIG.9	Recommendation for improved One Health surveillance	42	Yes		This work has been completed (see WP1)
02	M-AMR2.ARDIG.10	Collecting of samples and veterinary data, phenotypical testing of resistant isolates from farms and slaughterhouses.	42	Yes		This work has been completed (see Task 1).
02	M-AMR2.ARDIG.11	Collecting of samples and clinical data, phenotypical testing of resistant isolates from hospitals and care homes.	42	Yes		This work has been completed (see WP2)
02	M-AMR2.ARDIG.12	Data analysis of all collected isolates on a national level.	42	Yes		This work has been completed (see WP2 and 3)
02	M-AMR2.ARDIG.13	Comparative analysis of results from the various national studies (WP2).	42	Yes		This work has been completed (see WP2)
02	M-AMR2.ARDIG.14	Identification of plasmid and host chromosome factors that determine plasmid stability, spread, and adaptation to new bacterial hosts and conditions.	42	Yes		This work has been completed (see WP3)

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
02	M-AMR2.ARDIG.15	Identification the host factors that influence plasmid-host adaptation and conjugation rates using animal models	42	Yes		This work has been completed (see WP3)
02	M-AMR2.ARDIG.16	Identification of plasmid-modifications and effects on the resident microbiota through WGS.	42	No		This work was to be completed in WP3 T5 but as laboratory experiments were severely impacted by COVID19 in 2020 and 2021, when this work was planned.

5.1.4.3.1.6 Follow-up of the recommendations and comments by the Ethics Advisors

Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020	Comments of Ethics Advisors, October 2020	Comments of Project Leaders, January 2021
As mentioned p10 'Patient information such … will be provided once the appropriate ethics approval is in place (in the process of submission to IRAS)'. Copy of the approval must be presented.	No reply provided	Question proposed again	No reply provided	This issue must be addressed	Isolates of human origin are collected from reference laboratories in hospitals, therefore ethical approval is not required. However, where ethical approval has been required, the individual partner has gained it (e.g. Univ of Surrey, see HRA approval letter).	The beneficiaries must still confirm that the laboratories hospital received adequate ethics clearance to collect the Human samples.	Ethics to receive unidentifiable patient data (antibiotic use, gender, age, region, etc) – For Univ. Of Surrey HRA approval 23/12/2018, REC reference: 19/HRA/0552 For the I-bird cohort at IP, all authorizations were obtained in accordance with French regulations regarding medical research and information processing. All French IRB-equivalent agencies accorded the i-Bird program official approval (CPP 08061; Afssaps 2008-A01284-51; CCTIRS 08.533; CNIL AT/YPA/SV/SN/GDP/AR091118 N°909036). Signed consent by patients and staff was not required according to the French Ethics Committee to which the project was submitted.
The applicants must confirm that ethics approvals for the use of biological samples have	Ethics approval is not required as the animal samples will be collected from the farm environment rather than	The appropriate ethics approval is in place (in the process of submission to IRAS, the Integrated	No comment	Again the Ethics Advisors ask: Is the appropriate ethics approval is in place (in the process of submission to	Isolates of human origin are collected from reference laboratories in hospitals, therefore	The beneficiaries must still confirm that the laboratories hospital received adequate ethics clearance to	Ethics to receive unidentifiable patient data (antibiotic use, gender, age, region, etc.) – For Univ. Of Surrey HRA approval HRA approval 23/12/2018, REC reference: 19/HRA/0552.

Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020	Comments of Ethics Advisors, October 2020	Comments of Project Leaders, January 2021
been sought	animals themselves or national surveillance activities. Human samples will be collected from hospital reference laboratories.	Research Approval System, for ethics and approvals for health and social care / community care research in the UK)		IRAS, the Integrated Research Approval System, for ethics and approvals for health and social care / community care research in the UK)?	ethical approval is not required. However, where ethical approval has been required, the individual partner has gained it (e.g. Univ of Surrey, see HRA approval letter).	collect the Human samples.	For the I-bird cohort at IP, all authorizations were obtained in accordance with French regulations regarding medical research and information processing. All French IRB-equivalent agencies accorded the i-Bird program official approval (CPP 08061; Afssaps 2008-A01284-51; CCTIRS 08.533; CNIL AT/YPA/SV/SN/GDP/AR091118 N°909036). Signed consent by patients and staff was not required according to the French Ethics Committee to which the project was submitted.
The applicants must confirm the application of 3Rs and the ethical approvals (approval letters, etc) for animal work at a national / institutional level level. The applicants must	The applicants can confirm the application of 3Rs. Partners who will undertake any animal work eg in vivo mouse work, will do so in a justifiable way with full ethical approval, applying the principles of 3Rs.	No details are given on how the 3Rs are applied. The team was asked to provide the licence approval number / ethical approval code. This is not provided. This information should also	PL has requested additional information within the consortium	Information still pending	No vivo work has been performed to date. This work has been delayed and may be hampered due to the impact of COVID19.	In case, animal experiments will be conducted later on, the earlier requirements should apply.	It is unlikely that we will be able to perform animal experiments given the continued restrictions from Covid, especially as only 5 months are remaining of this project.

Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020	Comments of Ethics Advisors, October 2020	Comments of Project Leaders, January 2021
confirm the process for the application of the 3Rs across the whole programme of work to ensure 3Rs coordination across the programme (eg in-vivo mouse work, etc). Please elaborate.		include the name of the approving body (eg site and name of the AWERB). This can be done through the provision of the approval letter. Limited response, more information needed					

5.1.4.3.1.7 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Monitoring Antimicrobial Resistance and Drug Usage in the Human and Livestock Sector and Foodborne Antimicrobial Resistance in Six European Countries. 10.2147/IDR.S237038 https://zenodo.org/record/997236#.XvyQfSgzZM0	Yes	Gold	2590 €
Stepwise evolution and convergent recombination underlie the global dissemination of carbapenemase-producing Escherichia coli. https://doi.org/10.1186/s13073-019-0699-6 https://zenodo.org/record/3730637#.Xn3Pm4hKi70	Yes	Gold	2656 €
Extensive antimicrobial resistance mobilization via Multicopy Plasmid Encapsidation mediated by temperate phages. https://doi.org/10.1093/jac/dkaa311 https://zenodo.org/record/4244116#.X6KBDjiWxM0	Yes	Gold	2590 €
The shufflon of IncI1 plasmids is rearranged constantly during different growth conditions 10.1016/j.plasmid.2019.03.003 https://zenodo.org/record/3730621#.Xn3MyYhKi70	Yes	Gold	2590 €

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
The importance of using whole genome sequencing and extended spectrum beta-lactamase selective media when monitoring antimicrobial resistance. https://www.nature.com/articles/s41598-020-76877-7 https://zenodo.org/record/4456741#.YAqkH-hKjcc	Yes	Gold	1844 €
Antimicrobial Usages and Antimicrobial Resistance in Commensal *Escherichia coli* From Veal Calves in France: Evolution During the Fattening Process https://doi.org/10.3389/fmicb.2019.00792 https://zenodo.org/record/4249017#.YAqc_uhKjcc	Yes	Gold	2590 €
Temporal dynamics of the fecal microbiota in veal calves in a 6-month field trial https://doi.org/10.1186/s42523-020-00052-6 https://zenodo.org/record/4456718#.YAqfnuhKjcc	Yes	Gold	1390 €
Mobile colistin resistance gene mcr-1 detected on an IncI1 plasmid in Escherichia coli from meat https://doi.org/10.1016/j.jgar.2020.08.018 https://zenodo.org/record/4475507#.YBKNvOhKhM0	Yes	Gold	1660 €
blaCTX–M–1/IncI1-Iγ Plasmids Circulating in Escherichia coli From Norwegian Broiler Production Are Related, but Distinguishable	Yes	Gold	2400 €

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
https://doi.org/10.3389/fmicb.2020.00333 https://zenodo.org/record/3701226#.YAqeSehKjcc			
Phenotypical antimicrobial resistance data of clinical and non-clinical Escherichia coli from poultry in Germany between 2014 and 2017 https://doi.org/10.1371/journal.pone.0243772 https://zenodo.org/record/4328124#.YKS5sagzbY0	Yes	Gold	1695 €
Comparison of Phenotypical Antimicrobial Resistance between Clinical and Non-Clinical E. coli Isolates from Broilers, Turkeys and Calves in Four European Countries 10.3390/microorganisms9040678 https://zenodo.org/record/4704208#.YKS6KKgzbY0	Yes	Gold	1446 €
Clinically Relevant Escherichia coli Isolates from Process Waters and Wastewater of Poultry and Pig Slaughterhouses in Germany. https://doi.org/10.3390/microorganisms9040698 https://zenodo.org/record/4981248	Yes	Gold	2000 €
Outcome of Different Sequencing and Assembly Approaches on the Detection of Plasmids and	Yes	Gold	2000 €

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Localization of Antimicrobial Resistance Genes in Commensal Escherichia coli https://doi.org/10.3390/microorganisms9030598 https://zenodo.org/record/4964345			
Colistin-Resistant Enterobacteriaceae Isolated From Process Waters and Wastewater From German Poultry and Pig Slaughterhouses https://doi.org/10.3389/fmicb.2020.575391 https://zenodo.org/record/4981351	Yes	Gold	2400 €
Phenotypic and Genotypic Properties of Fluoroquinolone-Resistant, *qnr*-Carrying *Escherichia coli* Isolated from the German Food Chain in 2017 https://doi.org/10.3390/microorganisms9061308 https://zenodo.org/record/5501918	Yes	Gold	2000 €
Horsing around: Escherichia coli ST1250 of equine origin harbouring epidemic IncHI1/ST9 plasmid with bla CTX-M-1 and an operon for short-chain fructooligosaccharides metabolism DOI: https://doi.org/10.1128/AAC.02556-20 https://zenodo.org/record/5506463#.YUBKk50zaUk	Yes	Gold	2396 €

Additional output

2018

Posters

- Juraschek, Katharina; Malorny, Burkhard; Kaesbohrer, Annemarie; Hammerl, Jens A (2018). Influence of mobile genetic elements on the dissemination of important resistance determinants in commensal *Escherichia coli*. In: Doktorandensymposium der FU Berlin. Poster. https://zenodo.org/record/4956225
- Juraschek, Katharina; Shamoun, Dina; Schmoger, Silvia; Irrgang, Alexandra; Grobbel, Mirja; Käsbohrer, Annemarie, Tenhagen, Bernd-Alois; Hammerl, Jens A (2018). A predominant ColE-plasmid prototype is associated with dissemination of the *mcr-4* resistance gene in German *E. coli* isolates from food and livestock. National Symposium on Zoonoses Research. Poster. https://zenodo.org/record/4956247

Oral presentations

- K. Juraschek, B.Malorny, A. Käsbohrer und J. A. Hammerl (2018). Influence of mobile genetic elements on the dissemination of important resistance determinants in commensal *Escherichia coli*.In: Pre-Doc Symposium des BfR. Poster. https://zenodo.org/record/4956287

2019

Posters

- **Mesa-Varona O. and Tenhagen B-A (2019): Surveillance and monitoring systems for antimicrobial usage in livestock animals in six European countries. In: Quantification, Benchmarking and Stewardship of Veterinary Antimicrobial Usage (AACTING) 2nd International Conference, Bern, Switzerland. Poster.** https://zenodo.org/record/4785050
- **Mesa-Varona O. and Tenhagen B-A (2019): Evidences of overlapping between antimicrobial resistance and drug usage surveillance and monitoring systems in the Human, Animal and Food Sectors in European countries. In: Zoonosen symposium, Berlin, Germany. Poster.** https://zenodo.org/record/4785061
- K. Juraschek, M. Borowiak, D. Shamoun, S. Schmoger, A. Irrgang, M. Grobbel, A. Käsbohrer, B. Malorny und J.A. Hammerl (2019). Isolation and characterization of a novel *mcr-5* carrying *Escherichia coli* plasmid from chicken feces in Germany. In: 29th European Congress of Clinical Microbiology & Infectious Disease (ECCMID). Poster. https://zenodo.org/record/4956484
- K. Juraschek, C. Jäckel, C. Banyai, K. Nöckler, J. Beutlich, A. Käsbohrer und J.A. Hammerl (2019). Characterization of *qnr*-plasmids from *M. morganii* isolates of various sources. In: 29th European Congress of Clinical Microbiology & Infectious Disease (ECCMID). Poster. https://zenodo.org/record/4956584
- K. Juraschek, M. Grobbel, A. Käsbohrer, B.-A. Tenhagen und J.A. Hammerl. Occurrence and commonalities of plasmid-mediated quinolone resistance in *Escherichia coli* isolates recovered from livestock and food in Germany. In: 29th European Congress of Clinical Microbiology & Infectious Disease (ECCMID). Poster. https://zenodo.org/record/4956370

Oral presentations

- **Mesa-Varona O. and Tenhagen B-A (2019): Reviewing antimicrobial resistance and drug usage surveillance and monitoring systems in the Human, Animal and Food Sector in European countries. In: The 1st One Health EJP Annual Scientific Meeting (Dublin, Ireland). Oral communication.** https://zenodo.org/record/4786827
- **Mesa-Varona O. and Tenhagen B-A (2019): Reviewing antimicrobial resistance and drug usage surveillance and monitoring systems in the Human, Animal and Food Sector in**

European countries. In: The Predoc symposium (Berlin, Germany). Oral presentation. https://zenodo.org/record/4944270

- K. Juraschek, S. Krekow, N. Pauly, M. Grobbel, A. Käsbohrer und J.A. Hammerl (2019). Comparison of Plasmid-Mediated Quinolone Resistance in Escherichia coli Isolates from Bovine and Swine Origin Recovered from Livestock and Food in Germany. In: 1st Annual Scientific Meeting of the One Health European Joint Programme on Food-Borne Zoonoses, Antimicrobial Resistance and Emerging Threats. Oral presentation. https://zenodo.org/record/4956663
- K. Juraschek, B. Malorny, D. Meemken, S. Schwarz, A. Kaesbohrer, J.A. Hammerl (2019). Influence of mobile genetic elements on the dissemination of important resistance determinants in commensale *Escherichia coli*. In: Junior Scientist Zoonose Meeting Oral presentation. https://zenodo.org/record/4956734
- K. Juraschek, M. Grobbel, A. Käsbohrer, B.-A. Tenhagen, J.A. Hammerl (2019). Occurrence and commonalities of plasmid-mediated quinolone resistance in *Escherichia coli* isolates recovered from livestock and food in Germany. In: DRS Doktorandensymposium. Oral presentation. https://zenodo.org/record/4956773
- K. Juraschek, C. Jäckel, C. Banyai, S. Schmoger, T. Skladnikiewicz-Ziemer, B. Lesniewsky, K. Nöckler, J. Beutlich, A. Käsbohrer and J.A. Hammerl (2019). Dissection of a New *qnrD2 M. morganii* Plasmid Isolated from Systematically Diseased Cold-Blooded Amphibians. In: The International Symposium on Zoonoses Research. Poster. https://zenodo.org/record/4956847
- Katharina Juraschek (2019). WGS mit der PB SMRT Technologie. In: Abteilungskolloquium. Oral presentation. https://zenodo.org/record/4956855
- K. Juraschek, S. Krekow, N. Pauly, M. Grobbel, A. Käsbohrer, J.A. Hammerl (2019). Comparison of Plasmid-Mediated Quinolone Resistance in *Escherichia coli* Isolates from Livestock and Food in Germany. In: BfR-PreDoc Symposium. Oral presentation. https://zenodo.org/record/4956899

2020

Posters

Oral presentations

- Mesa-Varona O., Mader, R. Jarrige N., Granier S-A., Perrin A., Jouy E., Chauvin C., Kaspar H., Anjum M., Grobbel M., Velasova M. and Tenhagen B-A (2020): Phenotypic antimicrobial resistance in Escherichia coli strains on clinical and non-clinical isolates from broilers in Germany, France and United Kingdom. In: The 2nd One Health EJP Annual Scientific Meeting (Prague, Czech Republic). Virtual. Oral communication. **https://zenodo.org/deposit/4787487**
- Velasova M., Smith R., Chaintarli K., Mesa-Varona O., Tenhagen B-A., Kaspar H., Mader R., Amat J-P., Madec J-Y. and Anjum M-A (2020): Antimicrobial resistance of Escherichia coli isolates originating from diagnostic submissions from veterinary scanning surveillance in UK, Germany and France from 2014 to 2017. In: The 2nd One Health EJP Annual Scientific Meeting (Prague, Czech Republic). Virtual. Oral communication. **https://zenodo.org/record/4786343**
- Mesa-Varona O., Mader, R. Granier S-A., Perrin A., Jouy E., Madec J-Y., Kaspar H., Anjum M., Grobbel M., Velasova M. and Tenhagen B-A (2020): Comparison of antibiotic resistance in Escherichia coli from clinical diagnostic submissions and isolates of healthy broilers, turkeys and calves from surveillance and monitoring systems in Germany and France. In: the Tenth International Conference on Antimicrobial Agents in Veterinary Medicine (AAVM), Israel. Virtual. Oral communication. **https://zenodo.org/record/4787383**
- **Mesa-Varona O. and Tenhagen B-A (2020): Main outputs of the work package 1 from ARDIG (**Antibiotic Resistance Dynamics: the influence of geographic origin and management systems on resistance gene flows within humans, animals and the environment) **project. In: The**

workshop on surveillance/monitoring of AMR and AMU in animals and humans, Berlin. **Virtual. Oral communication.** https://zenodo.org/record/4944311

- K. Juraschek, M. Grobbel, A. Käsbohrer, B.-A. Tenhagen, J.A. Hammerl (2020). High heterogeneity of plasmid-mediated quinolone resistance in *Escherichia coli* isolates recovered from livestock and food in Germany. In: 6th Joint Conference of the DGHM & VAAM. Oral presentation. https://zenodo.org/record/4956927

2021
Poster

- **Mesa-Varona O, Tenhagen B-A. Comparison of antimicrobial use and resistance data on clinical and non-clinical isolates from livestock in four countries. In: Junior Scientist Zoonoses Meeting 2021. Virtual. Poster.** https://zenodo.org/record/4905583#.YL3gqvkzbY0
- **Mesa-Varona O, Kaspar H, Grobbel M, Tenhagen B-A. The influence of the antimicrobial use in the resistance data on clinical and non-clinical isolates from broilers and turkeys in Germany. In: the 5th International Conference on Responsible Use of Antibiotics in Animals. Virtual. Poster.** https://zenodo.org/record/4916495

Oral presentation

- **Mesa-Varona O., Mader, R. Jarrige N., Granier S-A., Perrin A., Jouy E., Chauvin C., Kaspar H., Anjum M., Grobbel M., Velasova M. and Tenhagen B-A (2020): Comparison of antibiotic resistance in Escherichia coli from clinical diagnostic submissions and isolates of healthy broilers, turkeys and calves from surveillance and monitoring systems in Germany and France. In: The Predoc symposium (Berlin, Germany). Oral communication.** https://zenodo.org/record/4944150

- Katharina Juraschek, Carlus Deneke, Silvia Schmoger, Mirjam Grobbel, Burkhard Malorny, Annemarie Käsbohrer, Stefan Schwarz, Diana Meemken and Jens Andre Hammerl (2021). HIGH DIVERSITY OF PLASMIDS CARRYING QNR RESISTANCE GENES IN FLUOROQUINOLONE RESISTANT ESCHERICHIA COLI ISOLATED IN GERMANY IN 2017. In: OHEJP ASM. Poster. https://zenodo.org/record/4957003
- Katharina Juraschek, Maria Borowiak, Simon H. Tausch, Burkhard Malorny, Annemarie Käsbohrer, Saria Otani, Stefan Schwarz, Diana Meemken, Carlus Deneke and Jens Andre Hammerl (2021). DIFFERENT SEQUENCING AND ASSEMBLY APPROACHES INFLUENCING THE DETECTION OF PLASMIDS AND ANTIMICROBIAL RESISTANCE GENES IN COMMENSAL ESCHERICHIA COLI. In: OHEJP ASM. Oral presentation. https://zenodo.org/record/4957118

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

It has been evidenced a lack of harmonisation on AMU and AMR across countries, within animals and, therefore, between humans and animal (10.2147/IDR.S237038). Higher resistance levels were found in clinical isolates of calves as compared to non-clinical isolates. The opposite was shown in isolates of broilers and turkeys (https://doi.org/10.1371/journal.pone.0243772; https://doi.org/10.3390/microorganisms9040678). It is required incentives to harmonise AMU and AMR systems as EFSA had driving the harmonisation of EU data-collection in non-clinical isolates. Otherwise, the One Health approach is not straightforwardly achievable.

If sequencing technologies are used for AMR surveillance, it will provide added value by use of AMR gene mechanisms, transmission of clones and the plasmids that contain the AMR genes. Markers of AMR genes can also be looked at and compared to review prevalence over time, effectively following in the footsteps of Enterobase but expanding upon this. Methods harmonisation of AMR predictions should be considered, as ARDIG has shown some of the common AMR WGS pipelines can vary in their outcomes. Any recommendations made on WGS pipelines should be assessed through the use of EURL.

When performing WGS, ideally all commensal as well as those *E coli* from antibiotic selective plates should be sequenced. However due to the expenses of this work, realistically there needs to be more selective approach which could be that all isolates from antibiotic plates and a subset of commensals are sequenced.

Are there any outcomes of this project that are already discussed or even implemented and in use at any institute of the project consortium, at stakeholders' organisations (ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), or at the level of national authorities?

ARDIG WP1

Sales data are collected in Europe providing a general overview on AMU. However, farm-level data are required for further analyses. Some projects, namely ARDIG and AACTING, have provided some suggestions to overcome this issue.

The implementation of the Regulation (EU) No. 6/2019 on veterinary products will allow collecting usage data in a large number of animal categories. This will greatly improve the comparability of data and populations.

Data on non-clinical isolates from livestock is harmonised by the decision 2020/1729/EU. However, except for zoonotic bacteria, there is no harmonisation on clinical isolates. Therefore, there is a lack of harmonisation between clinical and non-clinical isolates from livestock in different aspects. The EU JAMRAI project has proposed to create a European harmonised system on AMR in clinical isolates of animals called EARS-Vet. This project and authors have also suggested antimicrobials panels in clinical isolates.

The outcomes of ARDIG were presented at a workshop (1 and 2 of March 2021) that included experts in the field from EFSA, EMA, ECDC and national governments. Particularly the debate on the data collection systems for antimicrobial use in animals has been supported by the work done in ARDIG.

Bernd-Alois Tenhagen was involved in the preparation of the "3rd Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report" by EFSA, EMA and ECDC and included the findings from ARDIG in his contributions to the report which materialized in ARDIG papers being cited in the report.

Findings were also considered when advising the German Ministry of Food and Agriculture (BMEL) on changes in the antibiotics minimization strategy for Germany and when commenting on the foreseen changes in the EU-legislation with respect to the collection of AMU-data in the European Union.

5.1.4.3.1.8 One Health impact

- The outcomes of ARDIG were presented at a workshop (1 and 2 of March 2021) that included experts in the field from EFSA, EMA, ECDC and national governments. Particularly the debate on the data collection systems for antimicrobial use in animals has been supported by the work done in ARDIG.
- A member of the consortium was involved in the preparation of the "3rd Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report" by EFSA, EMA and ECDC to be published in 2021 and included the findings from ARDIG in his contributions to the report which materialized in ARDIG papers being cited in the report.
- Collaboration between the animal and medical sector in the field of AMR and AMU was fostered including interaction with other projects such as the German One Health Initiative (GOHI) involving BfR and RKI that worked in related areas. Likewise collaboration between

different institutions working on AMR in animals in Germany (BfR and BVL) was further strengthened by the exchange of data and the collaborative analysis of the data.

- Recommendations on surveillance of antimicrobial use are also fed into the current German national debate on the further development of the antimicrobial minimization concept.
- ARDIG has strengthened the collaboration between institutes involved in the consortium and the mutual understanding of the challenges each institute is facing. Further, an important collaboration has been set up between ARDIG and EU-JAMRAI in discussions on the EARS-Vet project and was also manifested in a collaborative paper (Mesa-Varona et al 2021).
- The project forces the development and optimization of WGS techniques in the NRL-AR for routine, which will be further used for *in silico*-based evaluation of the status of isolates from the ESBL-monitoring. Furthermore, the NRL-AR had successfully established in house pipelines, which are focussed on the evaluation of the resistances and the prediction of their genetic localization. Some of the results were further included in an EJP project of the second round, which is focussing on plasmid finishing and assessment of evolution of mobile genetic elements. The provided sequencing data will be used as a reference for a WGS-based ring trial for harmonization of long read sequencing.

5.1.4.3.1.9 Data Management Plan

All suggestions and comments made by the DMP leader has been addressed and the final version submitted.

5.1.4.3.1.10 List of dissemination and communication activities

Name of the activity:	*International Society for Plasmid Biology Meeting*		
Date:	*5-9 August 2018*		
Place:	*Seattle - US*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	

Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	*Poster*
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***150***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	Annual ARDIG meeting
Date:	21/05/2019
Place:	Teagasc Conference Centre, Ashtown, Dublin

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop	Yes	Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	yes
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)	>10	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	>10		

Name of the activity:	WGS analysis workshop		
Date:	21-22 October 2019		
Place:	APHA		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop	yes	Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	>10		

Name of the activity:	ARAE Conference
Date:	1-3rd July 2019
Place:	France

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	Yes
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	>10	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	>10		

Name of the activity:	ASM OH-EJP
Date:	May 2020
Place:	online

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	X
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	The Tenth International Conference on Antimicrobial Agents in Veterinary Medicine (AAVM),
Date:	23-25 Nov. 2020,
Place:	online

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	x
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Quantification, Benchmarking and Stewardship of Veterinary Antimicrobial Usage (AACTING) 2nd International Conference
Date:	2-3 July 2019
Place:	Bern, Switzerland

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories. Include hyperlink, if relevant. Zenodo grants the open access, it could be used as a repository for the presentations, posters, and other dissemination materials.

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Zoonosen symposium
Date:	16-18 October, 2019
Place:	Berlin, Germany

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories. Include hyperlink, if relevant. Zenodo grants the open access, it could be used as a repository for the presentations, posters, and other dissemination materials.

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	

Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Main outputs of the work package 1 from ARDIG		
Date:	1-2 March 2021		
Place:	Online		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories. Include hyperlink, if relevant. Zenodo grants the open access, it could be used as a repository for the presentations, posters, and other dissemination materials.			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop	Yes	Participation to a Workshop	Yes
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)	40	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	3		

Name of the activity:	Junior Scientist Zoonoses Meeting 2021		
Date:	3-4 June 2021		
Place:	Online		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories. Include hyperlink, if relevant. Zenodo grants the open access, it could be used as a repository for the presentations, posters, and other dissemination materials.			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	The 5th International Conference on Responsible Use of Antibiotics in Animals		
Date:	Online		
Place:	7-9 June 2021		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories. Include hyperlink, if relevant. Zenodo grants the open access, it could be used as a repository for the presentations, posters, and other dissemination materials.			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	

Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

5.1.4.3.2 JRP05-ET1-TOXDETECT – Final Report

5.1.4.3.2.1 Consortium composition

This is the consortium composition as presented in the project proposal. Please correct if any changes occurred to the consortium during the project life-span.

Project coordinator:

Dr Jacques-Antoine HENNEKINNE (deputy: Dr Yacine NIA). P1 (Anses).

Applicants:

- **P1**: Anses, Laboratory for Food Safety (Dr J.-A. Hennekinne, Dr Y. Nia); Laboratory for Hydrology, MALDI-ToF -based technological identification platform (Dr B. Gassilloud); Laboratory of Fougères, High content cellular analysis platform (Dr V. Fessard, Dr K HOGEVEEN).

- **P4**: Sciensano (before WIP-ISP - Scientific Institute of Public Health) Organic Contaminants and Additives, unit toxins (Dr J. Masquelier, Dr M. Andjelkovic until 01-09-2018); Foodborne Pathogens- unit toxin producers and toxins (Dr T. Van Nieuwenhuysen, Dr S. Denayer until 31-12-2020)

- **P9**: BfR (German Federal Institute for Risk Assessment) (Dr S. Marino, Dr H. Frentzel).

- **P18**: INRAe (National Research Institute for Agriculture, Food and Environment), MICALIS Institute, PIMs group (Dr N. Rama Rao) and GME group (Dr M. Gohar) ; Microbiology research unit UR454 and Plate-Forme d'Exploration du Métabolisme composante protéomique [PFEMcp] (Dr M. Hébraud).

- **P19**: IP (Institut Pasteur), Structural Mass Spectrometry and Proteomics Unit (Dr J. Chamot-Rooke); Collection of Institut Pasteur (Dr D. Clermont); French National reference center for anaerobic bacteria and botulism (Dr C. Mazuet).

- **P33**: NVI (Norwegian Veterinary Institute) (Dr T. Skjerdal)

Short CV of each participating key scientist (at least one per participating organization)

P1: Anses

Jacques-Antoine HENNEKINNE (male, 45 years-old) has a PhD degree in life sciences (AgroParis Tech, Paris). At Anses since 2000, he manages the staphylococci, bacillus, clostridia and milk unit. This unit is in charge of both national and European mandates for staphylococci and milk. He conducts research activities in the field of toxigenic bacteria. He was work package leader of the FP7 EQuATox program (www.equatox.eu) dedicated to potential misuse of bacterial toxins from 2012 to 2014. He participated in the development of certified reference material containing staphylococcal enterotoxin A in close collaboration with the JRC-IRMM. He currently leads the TAG12 of the CEN Mandate M/381 dedicated to standardization of analytical methods to screen SEs content in food matrices. He authored 27 publications and 4 book chapters.

Yacine NIA (male, 42 years-old) obtained his PhD in 2011 (Aix-en-Provence University) dealing with contamination and metals' speciation in the environment. He is deputy head of unit staphylococci, bacillus and clostridia, and responsible of National Reference Laboratory for Coagulase Positive Staphylococci. In the frame of the reference activities, he was coordinator of European Inter

Laboratory Proficiency Tests. His research activities are in the field of SEs detection in food matrices by ELISA and Mass spectrometry methods.

Valérie FESSARD (female, 48 years-old) is a toxicologist who graduated in 1996 as a PhD at the University of Paris 7 and then worked for one year at the Plymouth Marine Laboratory (UK) (European Science Foundation post-doctoral fellow). Since 1997, she has been working at the French Agency for Food, Environmental and Occupational Health & Safety (Anses). She has now nearly 20 years' experience in food toxicology, dealing in particular with the detection and hazard characterization of toxins contaminating food and drinking water such as phycotoxins and cyanotoxins. As the head of the unit "Toxicology of contaminants", she has a long experience in genotoxicity with in vitro and in vivo assays. She is also involved in the development of detection methods for unknown toxins using effect-directed analysis, as well as in the improvement of the methods used for predicting human toxicity through new cell models and high throughput investigation.

Kevin HOGEVEEN (male, 47 years old) is a toxicologist in the Contaminant Toxicology Department at ANSES, Fougères. Kevin obtained his PhD in Pharmacology and Toxicology in 2003 from the University of Western Ontario (Canada). Since 2009, he has been working at the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). With over 12 years of experience in food toxicology, Kevin has significant expertise in in vitro toxicology and High Content Analysis-based approaches. Other areas of scientific interest include the hazard characterization of toxins and nanomaterials, as well as the development of new cellular models and high throughput assays for predictive toxicology.

Benoit GASSILLOUD (male, 42 years-old) is a water microbiologist who graduated in 2003 as a PhD at the University of Henry Poincaré in Nancy in the field of environmental viruses, and then worked for 1 year in the Laboratory of Environmental Chemistry, Physics and Microbiology (LCPME-CNRS-UMR7564) on the development of a concentration process for viral detection in bottled mineral water. 2004. Since 2005, he has been working at the French Agency for Food, Environmental and Occupational Health & Safety (Anses) as the head of the water microbiology department, Nancy Laboratory for Hydrology. He has now more than 11 years' experience in water microbiology, dealing in particular with the detection and characterization of water-borne pathogens (bacteria, viruses and parasites). In 2013, he took the head of the MALDI-ToF Platform which is a structure that could be use by all the laboratories of the agency for identification or typing bacteria and fungi strains using this technology.

P4: Sciensano (ex-WIV-ISP)

Julien Masquelier (male) is a senior pharmacist holding a PhD in biomedical and pharmaceutical sciences with a focus on the analyses of different compounds with LC-MS/MS. He is head of the toxins unit in the service Organic Contaminants and Additives of Sciensano. He conducts research on the myco-, phyco-, phyto- and bacterial toxins quantification in food, human and environmental matrices. He also has more than 10 years of experience with LC-MS method development and is involved in both European and Belgian research projects dealing with toxins and food safety.

Tom Van Nieuwenhuysen (male) holds a PhD in biochemistry and biotechnology and is part of the service foodborne pathogens since 2017. He heads the unit toxins and toxin producers and is responsible for the NRL for botulism, the NRL for Coagulase Positive Staphylococci and the NRC for C. botulinum, C. perfringens and C. tetani. He is involved in several national and international projects dealing with bacterial toxins.

P9: BfR

Stephen F. Marino (male): is a protein biochemist with more than 20 years of experience in basic research. He holds M.Phil. and Ph.D. degrees in Molecular Biophysics and Biochemistry from Yale University. Before coming to the BfR, he conducted independent research in multiple Institutes in Switzerland and Germany, including the Max Planck Institutes for Biophysics and Brain Research, the Max Delbrück Centrum for Molecular Medicine of the Helmholtz Gemeinschaft and the Charité

Universitätsmedizin in Berlin. His expertise includes the characterization of membrane receptors and ion channels, recombinant protein production, protein structure determination by X ray crystallography and cryo-electron microscopy and the development and characterization of therapeutic antibodies. He supports the German National Reference Laboratory for coagulase positive Staphylococcus in developing and optimizing toxin detection assays and the production of reference material.

Hendrik Frentzel (male) is head of the Laboratory for sporeformers within the "Unit Bacterial Toxins, Food Service" in the Department Biological Safety. He studied Biology with a focus on microbiology and molecular biology at the universities of Greifswald and Potsdam and received his doctoral degree from the Freie Universität Berlin in 2017. After working experience at the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research and a private microbiological diagnostic laboratory he works as a scientist in the Department Biological Safety at the BfR, since 2010. His scientific work focusses on the detection of pathogenic microorganisms (especially members of the *Bacillus cereus*-group) in food and the characterization of these organisms by phenotypic and genotypic methods. This work includes taxonomic and phylogenetic analyses as well as the detection of genetic virulence determinants and produced virulence factors.

P18: INRAE

Nalini RAMA RAO (female) is the head of the PIMs (Pathogens, Immunity and Microbiota) team. She is a cell biologist/microbiologist specialized in host-pathogen interactions. She has a double education as a Biotechnology Engineer (International biotechnology engineering) and as a researcher (PhD, Max Planck Institute, Germany). She has been working on the interaction between *B. cereus* and host cells for the last 18 years and is now a recognized expert on *B. cereus* virulence factors and host-pathogen interaction. She has published significant papers dealing with host-pathogen interaction, and specifically with the mechanism of bacterial defence against the host immune cells. She studied the role and the expression of several virulent determinants and developed models to search for markers that could differentiate pathogenic from non-pathogenic *B. cereus* strains.

Michel GOHAR (male) worked in the research departments of pharmaceutical and crop protection companies for 16 years before joining INRA in 2003 as a senior scientist. He authors 45 articles, one book chapter and 3 patents, and his H-index is 20 (Web of Science, September 2015). His scientific expertise includes the molecular mechanisms involved in *B. cereus* pathogenesis and biofilm formation. He is a member of the GME (microbial genetic and environment) team included in MICALIS, an INRA/AgroParistech Research Institute of 300 people located on the INRA campus at Jouy-en-Josas. GME is a leader, at the international level, regarding the molecular mechanisms involved in the virulence of *B. cereus* and closely related bacteria. The other GME members involved in the project are the scientists Didier Lereclus (which leads the team), Leyla Slamti (expert in virulence factors regulation), and Alexei Sorokine (expert in comparative genomics).

Michel HEBRAUD (male) is a Research Director recruited within INRA in 1989. He works in the Research Unit of Microbiology (UR454) at the INRA Centre of Auvergne-Rhône-Alpes, site of Theix. He is the scientific responsible of the proteomic component of the "platform of metabolism exploration (PFEM)". This proteomic component is dedicated to the identification and characterization of proteins and peptides by mass spectrometry. His research activities have focused on the adaptation of *Listeria monocytogenes* to food processing plants. In this context, his work concerned the molecular determinants involved in biofilm formation, the physiology of bacteria in biofilms, their resistance to cleaning disinfection treatments and the mechanisms by which some of them face to technological stresses. He has also worked on the biodiversity of *Listeria monocytogenes* strains by proteomics approaches.

P19: IP

Dominique CLERMONT (female) received a diploma of dental surgeon from University Lyon I in 1983,

then a Ph.D. in Odontology, from University Paris VII in 1993. Following post-doctoral position at the Pasteur Institut (Laboratory of Streptococci and Enterococci) focusing on antibiotic resistance on *Streptococcus anginosus*, she joined the Collection de l'Institut Pasteur (CIP) as research engineer in 1997. Since this date, her research focuses on bacterial preservation, taxonomy and phylogenetic studies as well as bacterial strain typing. She is also involved in the bacterial collection management of the CIP. At the national level, she participates in the French infrastructure BIOANQUES and is member of scientific committee of two biological resource centers (ICAReB Biobank and the International Centre of microbial ressources (INRA-CIRM). She is in the steering committee of the Rapid project ANVBIS3 funded by Direction Générale de l'Armement (DGA) (2012-2016), co-coordinator in the transversal research project between Anses and Pasteur (IdBc) (2015-2017), and work package leader in the project pathoTOP (2015-2019) funded by the French National Agency for Research. At the European level, she participates in the European Infrastructure "MIRRI" (Microbial Resource Research Infrastructure and she is scientific officer of the European Culture Collection Organization (ECCO).

Julia CHAMOT-ROOKE (female, 46 years old) is a CNRS senior scientist and head of the "Structural Mass Spectrometry and Proteomics" Lab at the Institut Pasteur in Paris, which gathers a research group and the Pasteur proteomics platform. Her research focuses on the development of new mass-spectrometry based methodologies for the analysis of proteins involved in infectious diseases. In last years, Julia Chamot-Rooke gained an international expertise in the development of top-down proteomics approaches applied to bacterial protein analysis. She published 70 papers in total in peer-reviewed journals (h-index: 21) and has been invited in 25 international conferences in the last three years. Julia Chamot-Rooke has been the coordinator of multidisciplinary projects funded by the French National Agency in 2009 and 2015. She is a past president [2008-09] of the French Society of Mass Spectrometry (SFSM) and the French representative of the International Mass Spectrometry Foundation. She is also a founding member of the International Consortium for Top-Down Proteomics. Her lab is equipped with the most recent high-resolution mass spectrometers (including 5 Orbitrap systems) and highly trained staff (15 people).

Christelle MAZUET (female, 46 years-old) is a biochemist who graduated in 1997 as a PhD at the University of Paris 11 and then worked for six years at the Pasteur Institute as a study director of the center "Allergy and Environment/Texcell". Since 2004, she has been working in the research unit "Anaerobic bacteria and toxins" headed by Michel-Robert Popoff at the Pasteur Institute. She has now 12 years' experience in anaerobic bacteria and bacterial toxins in particular with the development of new detection and characterization methods of botulinum toxins. As deputy director of the National Reference Center (NRC) "Anaerobic bacteria and Botulism" since 2008, she is also in charge of the technical and scientific activities of the team including animal and human botulism diagnosis and investigation of *Clostridium perfringens* and *Clostridium botulinum* outbreaks.

P33: NVI

Taran SKJERDAL (female) has more than twenty-year experience of research from research institutes and companies within the areas food quality, food safety, risk assessment and multidisciplinary decision support. The last 10 years she has worked at the Norwegian Veterinary Institute with food safety and bacteriology with focus on growth, survival, pathogenicity and adaptation of *Listeria* and *Staphylococcus* during food processing. The work includes research activities and support to food authorities. She has coordinated two EU funded projects DESCOD (FP4) and STARTEC (FP7) and been strongly involved in SEAFOODplus and BASELINE (FP6 and 7). Besides, she has been the supervisor for three PhD studies, one of them about incidence, toxin profiles, antibiotic resistance and genetic relatedness of Staphylococcus in Ethiopian milk and milk products. In addition, she has leaded several multidisciplinary research and innovation projects. The project has often been initiated by challenges for the food authorities and food trade and leaded to new tools, legislation or analytical methods. She was responsible for the national reference functions for coagulase positive Staphylococci (CPS) until

July 2016. She was a guest researcher at the European reference laboratory (ANSES) in 2016.

Currently, she is also a member of the Norwegian Scientific Committee for Food Safety. She has more than thirty peer reviewed papers and book chapters in addition to two patents.

5.1.4.3.2.2 Summary of the work carried out in the Project

500 words, 1 page. Please emphasise the main scientific results and pay particular attention to the impact the project may have for the One Health EJP and its stakeholders (ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), and national ministries.

Bacterial toxins produced by *Staphylococcus* spp., *Bacillus* spp. and *Clostridium* spp. are responsible for a large number of food-poisoning outbreaks (FPOs) in the European Union. The true incidence of FPOs caused by these toxigenic bacteria is underestimated due to a lack of relevant detection tools and to a common symptomatology that makes outbreak investigation challenging. The OHEJP TOX-Detect project uses three different non-NGS approaches for a better detection of *Staphylococcus* spp., *Bacillus* spp. and *Clostridium* spp and characterization of some of their toxins, including emerging threats that remain currently undetectable.

First, collections including strains from various sources (animal, environment, food and human) and different geographical locations in the EU were established under specific criteria selected by the consortium. Strains issued from this "strains collection" were used to analyze the RNAseq, PCR and cytotoxicity data and to study potential correlations between gene presence or expression with strain toxicity and virulence. The growth conditions of the strains, the cytotoxicity assays, the RNA extraction procedures and development of RNAseq assays were defined and optimized. The data were analyzed to allow gene expression and strain toxicity measurement. PCR and RNAseq methods were developed for both *B. cereus* and *C. perfringens*. The analysis allowed to propose known as well as new biomarkers to characterize the strains.

A panel of three non-NGS based methods allowing characterization of the three studied pathogens and some of their toxins were developed: (i) MALDI-ToF , (ii) LC-MS[1] and (iii) ELISA methods.

For MALDI-ToF , a new library based on the selected strains collection was developed. Therefore, 152 reference spectra (MSP) from 76 strains were selected after the spectra-processing step. Comparisons and request access with the commercialized database showed high reliable scores that allow to differentiate these three species. The difficulty to differentiate species within the *B. cereus* group was highlighted. Transfer of this database was realized to the European Union Reference network through the organization of an inter-laboratory test.

Staphylococcus enterotoxins types SEM, SEN and SEO and *B. cereus* enterotoxin CytK2, hemolysin HlyII and Sphingomyelinase were selected for methods development, as there is a lack in commercially available methods to detect these toxins. In the absence of toxins standards, procedures for recombinant toxins production and purification were optimized. Stability studies showed that standards produced for Staphylococcal enterotoxins SEO and CytK2 are unstable and, consequently, cannot be transferred between partners for method development. Thus, standards for SEN, hemolysin HlyII and Sphingomyelinase have been produced and transferred to partners in charge of LC-MS methods development. For *Bacillus* enterotoxins, ELISA and LC-MS methods were developed, optimized and transferred to TOX-Detect partners in order to evaluate their transferability. For Staphylococcal enterotoxins, LC-MS to target type SEN was developed and transferred to partners for inter-laboratory test.
Results obtained in the TOX-Detect project allowed to enhance acknowledgment on strains producing toxins. Methods developed could be used by reference laboratories (NRLs and EURL) for further development and foodborne outbreak investigation, especially when it comes to non-classical toxins. Selected reference strains for *Staphylococcus* and *Bacillus* have been included in OHEJP CARE project.

[1] Liquid Chromatography coupled to Mass Spectrometry

5.1.4.3.2.3 Work carried out in the JRP, scientific results and integrative outcomes

WP0. Coordination, management and communication

WP0-T1- Coordination, management and communication

The overall purpose of the management structure is to ensure the timely implementation of the tasks and the smooth running of the project as a whole. Its primary goal is to identify arising opportunities and detect the occurrence of obstacles as early as possible, hence maximise the outcome of the project while preventing delays in its implementation. In total, 57 meetings, web conferences and telephone conferences were organised by the coordination team over the 42 months of the project. Objectives were to manage the progress of different tasks and to discuss with partners on technical issues. Discussion and procedure of transfer of the collection of strains between TOX-Detect partners and between TOX-Detect and CARE projects were managed by coordination team. Finally, impact of Covid 19 crisis on TOX-Detect project was also managed by the coordination.

WP0-T2 to T5: Organisation of four face-to-face meetings with all partners

The TOX-Detect kick-off meeting held in Maisons-Alfort (France) from 28th of February to the 1st of March 2018 (M3). 16 participants representing all TOX-Detect partners were present during the kick-off meeting.

The aims were:

(i) to introduce all project members;

(ii) to get information on administrative and financial issues by representatives of EJP coordination team;

(iii) to give an overview of the aims of the project and provide detailed information on all work packages;

(iv) to discuss open questions on the selection of the reference strains that should be used in this project

1st meeting was organized on 20 and 21 March 2019 at Anses (France). 15 participants representing all TOX-Detect partners were present during this meeting. General discussion dealing with EJP projects took place after the presentation of Fanny Baudoin from One Health EJP general coordination. Briefly, she presented guidelines, specific rules, budget, communication tools and spoke about the possible 6-month extension. Special discussion sessions were dedicated to the coordination between WP3 and WP4 in order to transfer the produced enterotoxins in WP4 to WP3 for LC-MS development and protein characterisation.

2nd meeting was organized on 15 and 16 January 2020 at Anses (France). 23 participants representing all TOX-Detect partners were present. C. Cordevant and A. Callegari were invited by the coordination of TOX-Detect Project as SSB member and general coordination, respectively. All participants presented their activities and involvement in the TOX-Detect project. A specific discussion was dedicated to the organization of inter-laboratory tests for each technique developed in WP1, WP3 and WP4.

3rd meeting was organized as web-meeting on 25 of June 2021. All partners were present. The aim was to discuss on the final report, and to present the restitution of WP1, WP2, WP3 and WP4. Remaining tasks of WP5 (MALDI-ToF and ELISA inter-laboratory tests) were also developed.

WP0-T6: mandatory reports on network activities: interim activity report, final report

The coordination drafted and uploaded on OHEJP website the interim and mandatory reports:

Report	Submission period
9 Month report for year 1	September 2018
9 Month report for year 2	September 2019
9 Month report for year 3	September 2020
12 Months report dispatched on	February 2019
Intermediate report (36M)	January 2021
24 Months report	February 2020
36 Months report	December 2020

WP1. Constitution of a reference strain collection for S. aureus, B. cereus and C. perfringens

Criteria for selecting the strains have been defined in connection with the needs of other work-packages in order to select the most appropriate strains to be used for the development and harmonization of methods and databases. An inventory of available resources has been done and a list of reference strains with their associated data established with all partners. This collection includes strains from various sources (animal, environment, food and human samples) and different geographical locations.

WP1-T1- Constitution of S. aureus strains collection

80 *S. aureus* strains have been proposed by TOX-Detect partners, representing human and food categories. The major part was issued from food poisoning outbreaks. The aim was to develop Ab against SEN and SEO toxins. Therefore, 21 strains encoding for SEN and SEO and a negative control (CIP 53.154) have been selected. Some of these strains have been exchanged between partners under a Material Transfer Agreement.

WP1-T2- Constitution of B. cereus strains collection

90 *Bacillus* (Bc) strains have been proposed by TOX-Detect partners, representing human and food categories. The collection includes a total of 21 *B. cereus* strains. Some of these strains have been exchanged between partners under a Material Transfer Agreement.

WP1-T3- Constitution of C. perfringens strains collection

As in CPS and BC 54 *C. perfringens* (Cp) strains have been proposed by TOX-Detect partners, representing human and food categories. The collection includes a total of 34 *C. perfringens* strains. Some of these strains have been exchanged between partners under a Material Transfer Agreement.

WP1-T4- Transfer of libraries of MALDI-ToF reference spectra

The MALDI-ToF reference spectra library has been developed using all strains referenced in tasks WP1-T1, T2 and T3. Standard Operating Procedure (SOP) was drafted and uploaded on EJP site. It will be accessible to the scientific community after the publication of this new database.

MALDI-ToF mass spectrometry was performed for bacterial species identification and for verification of purity of stock cultures as well as to establish a library of reference MALDI-ToF spectra. Protocol for protein extraction has been discussed, defined and shared among partners. For each strain, reference spectra (MSP) were generated using 26-32 spectra from total protein extracts spotted onto a target plate in eight replicates, and each spot was analysed four times. Acquisition of raw spectra was done with a laser frequency of 60 Hz, an acceleration voltage of 20 KV and extraction delay of 120 ns. All raw spectra were analysed according to the established MSP protocol of the MALDI Biotyper® V1.1 to remove suboptimal spectra. 152 MSP from 76 strains were selected after the spectra-processing step.

2 MSP have been created for each strain. Comparisons with the commercialized Bruker Daltonic Database: Version 9.0.0 containing 9997 MSP (including 7 MSP for *B cereus,* 14 MSP for *Staphylococcus* and 10 MSP for *C. perfringens*) were access. The request access with the commercialized Bruker Daltonic database shown high reliable scores that allow to differentiate these three species. A misinterpretation was obtained only for one strain for which a contamination was detected. The difficulty to differentiate species within the *B. cereus* group *sensu lato* was also highlighted. Technical and data processing transfer procedure has been established with partners before organization of a dedicated PT trial (cf WP5). This database was transferred to European Union Reference Laboratory network (11 National reference Laboratories) in June 2021. Technical assistance was provided by WP1 over the inter-laboratory period.

WP2: Characterization of toxins/virulence factors

WP2-T1- Characterization of candidate toxin and/or virulence genes using toxicity tests

This task was launched on M4. The 21 *B. cereus* strains selected in WP1 were tested for cytotoxicity using classical cytotoxicity tests. Among the 40 *C. perfringens* strains selected in WP1, a subset was tested using classical cytotoxicity tests. For those strains, the growth conditions of the strains (vegetative and sporulating) as well as the cytotoxicity assays were defined and optimized.

Following obtention of the strain supernatant of *B. cereus* and *C. perfringens*, a suitable analytical method was developed for the determination of the cytotoxic potential of the strains. Potential effects of the supernatants on the pro-inflammatory response in Caco-2 cells (IL-8 secretion) was also investigated. Cellular imaging-based High Content Analysis approaches were developed and used to characterize the mechanisms of toxicity. A subset of strains were chosen based on results from classical cytotoxicity tests, and further evaluated for markers of apoptosis (active-Caspase-3), genotoxicity (γH2AX, phospho-ATM), pro-inflammatory response (nuclear translocation of NF-kB), mitochondrial membrane potential (TMRE) and cytotoxicity (Nuclear DAPI staining). The cytotoxic potential varies amongst strains, providing further insight into the complex mechanisms of *B. cereus* and *C. perfringens* pathogenicity.

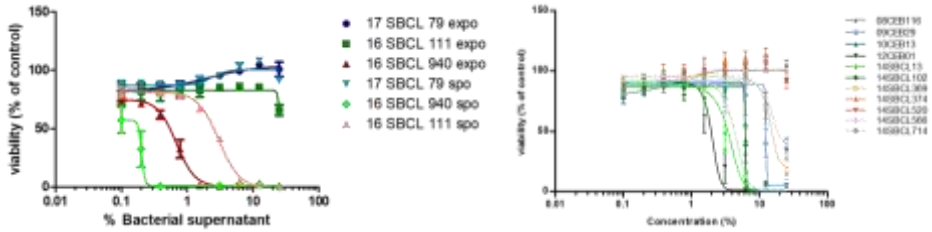

Figure 1: Cytotoxicity of bacterial culture supernatants from vegetative and sporulating cultures of *C. perfringens* (left) or *B. cereus* (right) in human intestinal Caco-2 cells.

WP2-T2- Assessment of virulence and toxin gene expression using RT-PCR and transcriptomic assays

This task was launched on M5. The aim of this task was to select genes over- or highly expressed in various strains within a panel of candidate genes suspected to play a role during *B. cereus* and *C. perfringens* pathogenesis. In addition, a whole transcriptomic assay was performed (RNAseq technology) in various growth conditions.

WP2-T2-ST1- Optimization of growth conditions to be used for gene expression analysis

For *C. perfringens* different protocols were used: vegetative and sporulating as well as co-incubation with human intestinal Caco-2 cells. Changes were made to the *C. perfringens* sporulation protocol to optimize the production of virulence factors, including CPE. For *B. cereus*, the conditions were chosen based on two parameters, the oxygen level and the pH.

WP2-T2-ST2- Development of RT-PCR assays and transcriptomic analysis

For both organisms, the protocols of bacterial growth, RNA extraction and qRT-PCR were developed

and optimized. RNAs were extracted and checked for purity and integrity. The
RNA samples were then subjected to RNAseq analysis. Analytical methods were then developed to ensure a proper analysis of the data. These analytical pipelines were then successfully used to provide heat maps and PCA analysis, generating information on up and down regulated genes for each bacterial strain.

For *B. cereus*, 15 strains grown in microaerophilia at pH 7 were sent in triplicate for RNAseq analysis. 9-15 million reads per samples were obtained, with 90% correctly paired. The biological triplicates clustered well together and showed no major differences in the profile of their transcriptomes. 3276 genes were identified in the core transcriptome, which represents approximately 65% of the genes in each strain. 264 genes were found to be differentially expressed (up or down regulated), with p-values less than 0.01.

For *C. perfringens*, 5 strains grown in vegetative and sporulation conditions were selected for the RNAseq analysis. Comparing growth conditions (vegetative *vs* sporulation), a mean of 677±290 were significantly differentially expressed, of which an average of 444±123 were upregulated and 232±169 genes were downregulated. The majority of differently expressed genes (60 % to 76 %) showed significantly upregulation in all vegetative growth condition compared to sporulation condition.

In conclusion, methods were developed as expected during this task, to 1) optimize growth conditions (subtask 2.1) and 2) develop qRT-PCR and transcriptomic analytical pipelines to successfully analyze and select genes (subtask 2.2).

This allowed to propose a panel of candidate genes to be further characterized during the task 2.3.

WP2-T3- Correlation of specific toxicity profiles with expression patterns of bacterial toxins/virulence factors

The objective of this task was to analyze the RNAseq, PCR and cytotoxicity data and study potential correlations between gene presence or expression with strain toxicity and virulence.

The growth conditions of the strains, the cytotoxicity assays, the RNA extraction procedures as well as the development of RNAseq assays were defined and optimized. The data were analyzed to allow gene expression/presence and strain toxicity and pathogenicity comparison.

First, the *B. cereus* strains were characterized by detecting by PCR the presence of ten genes involved in virulence. According to the presence/absence of the genes, the strains were assigned a genetic signature (GS). The assignment of strains according to their GS shows the genetic diversity of *B. cereus* strains.

Among the strains tested for cytotoxicity, a strong correlation could be identified between *hlyII* presence and high cytotoxicity. Similarly, a strong correlation could be measured between Nhe production and high cytotoxicity.

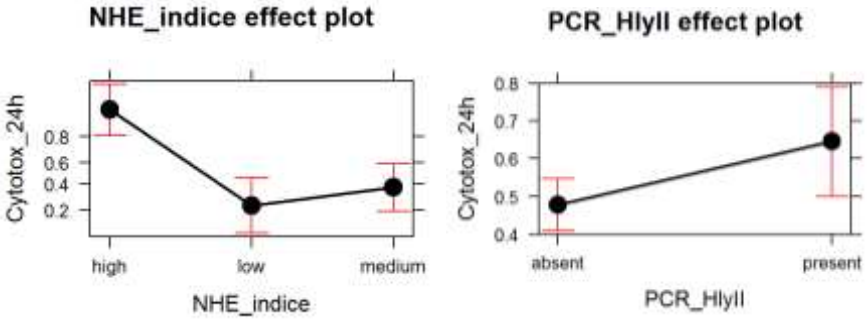

Figure 2: correlation between cytotoxicity and toxins

As such, it could be concluded that Nhe and HlyII were strong candidates for the development of further detection methods (WP3 and WP4).

For *B. cereus*, the RNAseq data were further analysed to provide correlation with strain pathogenicity. The strains were grouped according to the pathogenicity they induced (non-pathogen vs FBO or clinical strains). We used a penalized conditional logistic regression with the lasso method to select relevant genes for the prediction of pathogenic potential. By applying the prediction model to the 11,179 genes with the selected penalty constant of 0.01, 7 genes were selected as markers to differentiate the strains according to their pathogenicity.

Using this combination of genes, the best results obtained to compare non-pathogenic with FBO strains was an AUC of 0.768, the sensitivity 0.69 and the specificity 0.773.

Remarkably, regarding the clinical strains, the best results were achieved with an AUC of 0.955, sensitivity of 0.9 and specificity of 0.86. Therefore, the analysis concludes that an accurate differentiation between clinical and non-pathogenic strains can be obtained by using these biomarkers.

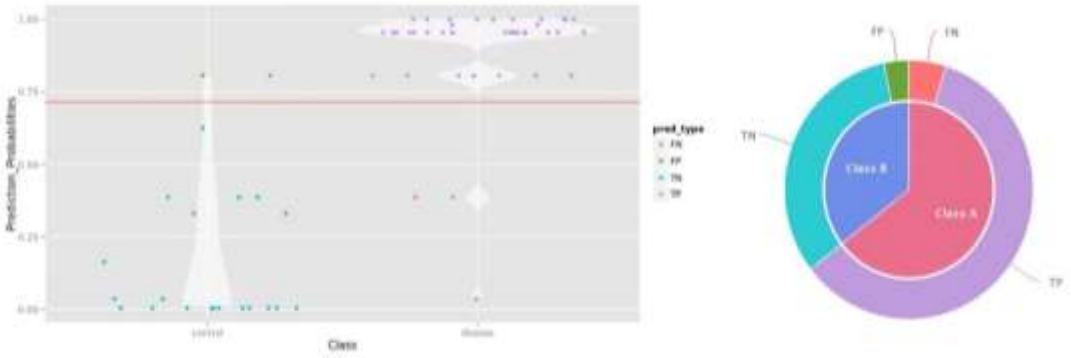

Figure 3: Best performing biomarker combination for non-pathogenic and clinical strains

Finally, the correlation of the gene expression/presence/absence with the toxicity profiles of the strains have been performed. Thus far, no correlation could be observed between the presence or the expression of the newly identified biomarkers and the cytotoxic potential of the strains.

Further analyses need to be performed to identify other biomarkers, which expression could correlate with cytotoxicity and/or HCA data.

Conclusions

The objective of this task was to analyze the RNAseq, PCR and cytotoxicity data and study potential correlations between gene presence or expression with strain toxicity and virulence.

The growth conditions of the strains, the cytotoxicity assays, the RNA extraction procedures as well as the development of RNAseq assays were defined and optimized. The data were analyzed to allow gene expression and strain toxicity measurement. PCR and RNAseq methods were developed for both *B. cereus* and *C. perfringens*. The analysis allowed to propose known as well as new biomarkers to characterize the strains. We thus successfully implemented methods to answer the initial questions, we provided accurate SOP and the deliverables were obtained.

8 SOPs were drafted in this WP2 were uploaded on the OH EJP website.

WP3: Development of Mass Spectrometry-based proteomics procedures for detection of bacterial toxins and virulence factors

WP3-T1- Development of Mass Spectrometry-based methods for the detection of new enterotoxins (eg SEG, SEH, SEI) from S. aureus

In this task, focus is laid on the development of an innovative analytical method for the quantification of three non-classical SEs (SEM, SEN and SEO) suspected to play a role in the notorious pathogenicity of *S. aureus*, specifically in food poisoning outbreaks. To this end, recombinant protein standards expressed in *E. coli* **as part of WP 4 were subjected to a quality control**, a sample preparation technique based on-filter digestion was developed with SEB pure well characterized standard (obtained from H2020 EuroBioTox project, https://eurobiotox.eu/) as a model protein and finally a targeted LC-MS² method with the on-filter digestion sample preparation was developed and validated for the quantification of SEN in cultures of *S. aureus*. To assess the quality of the recombinant SEM, SEN and SEO standards, both electrophoresis tools and mass spectrometry were used to verify the purity and amino acid sequence of each standard. The on-filter digestion sample preparation consisted of four major stages namely the filtration of the culture supernatant, the protein denaturation process, enzymatic protein digestion step and finally the elution of the obtained tryptic peptides. Each of the four steps was optimized with *S. aureus* enterotoxin B as a model protein while a standard culture of a *seb* and *sen* negative strain of *S. aureus* was used as blank matrix. Thereafter, the optimized sample preparation was applied and tested with the recombinant SEN standard for quantification by means of an UHPLC system coupled to a Orbitrap mass spectrometer. Separation of the different peptides was performed with a classical C18 column while their subsequent quantification was performed in parallel reaction monitoring mode. Subsequent validation of the obtained method was performed according to FDA guidelines for bioanalytical analyses. Thorough analysis of the three recombinant SE standards demonstrated that only SEN could be seen as a quantitative standard. Hence, only the rSEN standard was used for the development of a targeted LC-MS² method as proof of principle. Ensuing experiments showed that SEN was efficiently retained by the filter and sufficiently digested with the developed on-filter digestion technique. Due to the low expression of SEN, the method was validated in a range of 1 to 7.5 ng/mL, with 1 ng/mL as demonstrated limit of quantification (LOQ). The average recoveries calculated for the concentrations 1, 2.5, 5 and 7.5 ng/mL were respectively 114, 104, 102 and 99%. Calculated coefficients of variance for both repeatability and reproducibility were below 8%, while the calculated measurement uncertainty was below 20% for all nominal concentrations and below 30% for the established LOQ (1 ng/mL).

WP3-T2- Development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from B. cereus

Characterizing foodborne toxigenic bacteria and detecting bacterial toxins is a key goal of the project. Here we focused our work on the development of a targeted LC-MS/MS method for the identification of two foodborne *Bacillus* bacteria virulence factors: Sphingomyelinase and Hemolysin-II (HLY-II). These toxins are part of a large number of other toxins that are secreted by the bacterium and responsible for foodborne infections. They were chosen among several other ones because they are present, even if in low amount, in many described strains.

The methods we developed are based on liquid chromatography coupled to mass spectrometry (LC-MS/MS) and more specifically on targeted methods.

The first step was the selection of the strain of *Bacillus cereus*. We then first decided to set-up a Top-down proteomics approach to identify toxins from bacterial supernatant. Top-down proteomics, in contrast to the usual bottom-up approach based on protein digestion, relies on the analysis of intact proteins. It allows uniquely addressing the different forms of a protein (proteoforms) present in a sample. However, the first top-down proteomics experiments indicated a very low sensitivity of the approach since no intact toxin could be identified in the bacterial supernatant. This was due to the very low amount of toxins produced by *Bacillus cereus* strains in medium. We should note that this

information was not available in the literature. It thus quickly appeared that we had to resort to the more usual bottom-up approach to set up our analysis. To maximize the sensitivity and thus the chance to identify our toxins of interest, we decided to develop a targeted bottom-up method called PRM (Parallel Reaction Monitoring) on a Q-Exactive mass spectrometer. Even with this sensitive targeted approach, the low amount of endogenous toxins in our samples led us to spike our samples with purified toxins (obtained from WP4, INRAe partner) for both method development and sample preparation. These spiked samples were used to set-up both LC conditions and MS parameters. We used the Pinpoint 1.1 software to achieve the selection of proteotypic peptides.

The full validation of the method was therefore implemented by using a nano-HPLC easy-1200 (ThermoFisher) system coupled to Q-Exactive plus mass spectrometer (ThermoFisher). A SOP has been prepared (see document in annex) in order to allow the transfer of the method to the project partners involved in the inter-laboratories tests.

WP3-T3- Development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from C. perfringens

A nanoLC-PRM/MS method has been developed for the detection of *C. perfringens* enterotoxin CPE. Mass spectrometry allows the characterization of protein sequence and direct measurement of the toxin, leading to unambiguous identification and unequivocal evidence of its presence, with a sequence coverage up to 86% and at least 20 peptides identified. In parallel, sixteen other virulence factors as well as numerous proteins involved in the sporulation of the bacterium were present in the supernatant, confirming that the presence of CPE is dependent on sporulation. We have developed a method, focused on the detection of CPE, which is based on 6 peptides generated by its tryptic digestion, achieving a sequence coverage of 19.7%. As a protein standard is not commercially available in Europe, a strain from the TOX-Detect collection was selected for its high CPE production under culture conditions developed by ANSES to serve as reference. A homogeneity test was performed on pooled supernatants of this reference strain and 4 out of 6 peptides were found homogenous with a CV of less than 20% and the carryover in the matrix blank was estimated to be less than 0.05%. Serial dilution experiment was performed by diluting the digested reference sample in the digested matrix blank and CPE was detected for dilutions up to 128th. The full validation of the method has been implemented by using nano-HPLC (column Acclaim Pepmap 100 C18, 3 µm 75 µm x 250 mm, ThermoFischer) system coupled to Q-Exactive HF-X mass spectrometer (ThermoFischer). A SOP has been drafted (see document in annex) in order to allow the transfer of the method to the project partners involved in the Inter-Laboratories Comparison tests.

WP3-T4- Transfer of LC-MS/MS methods

The three LC-MS/MS methods, developed in the T3.1, T3.2 and T3.3 and focusing on the detection of toxins and/or virulence factors from S. aureus, B. cereus and C. perfringens, respectively, were also implemented in laboratories of several other participants.

First, the new LC-MS/MS methods were developed and in-house validated, when quantitative standard was available (e.g. SEN). Then, for each analytical method, a detailed analytical protocol (and validation report, if done) was written and these protocols were drafted as SOP (see SOPs uploaded on the OH EJP site) before the transfer to the partners for implementation. These methods were transferred to the partners of the WP3 in order to test applicability of the methods before further implementation in WP5. Some adaptations were necessary, given the differences between the instruments used by the partners involved in WP3: Liquid chromatography systems (UHPLC vs nano LC) but also mass spectrometers (ThermoScientific - Q Exactive Focus vs ThermoScientific - Q Exactive HF-X) used for development and implementation were not always the same. Finally, with some modifications, results of the transfer were quite good, with acceptable qualitative results for all the three methods (See

WP5). Inter-laboratory comparison tests and the obtained results by participants will be presented in deliverable D5.2 "Final report including results from the eight inter-lab tests".

WP4: Development of new immuno-enzymatic assays for detection of S. aureus and B. cereus toxins and virulence determinants

WP4-T1- Development of quantitative immunoassays for five known S. aureus and B. cereus toxins and virulence factors

WP4-T1-ST1- Selection of 5 target genes and construction of genetic tools for proteins overexpression

At the beginning of the project Staphylococcal Enterotoxins (SEs) G, H and I had been chosen as targets for the effort. These choices were changed in month 9 on the wish of the Coordinators to minimize overlap with contemporary projects and maximize the resources allotted to the TOX-Detect Consortium. As of October 2018, the official targets were SEN and SEO. As genetic tool for overexpression of SEs in *E. coli*, the SEN and SEO genes have been cloned in an IPTG (isopropylthiogalactoside) inducible expression vector, with additional N-terminal tags for affinity purification (His6) and protease cleavage (FLAG/Enterokinase recognition site) in place of the wt secretion signal sequences.

WP4-T1-ST2- Proteins production

The necessary SE cloning and production work at the BfR required gaining official approval from the responsible Authority for work with genetically modified organisms; this approval was granted in December 2018, subject to the fulfilling of strict safety requirements. In order to meet these requirements, the SE production procedures and the laboratory organization had to be adjusted, including the purchase of necessary equipment. Due to the need to overcome these problems, the successful production of purified recombinant SEs for both WP3 and WP4 was only possible from March 2020. Although production was successful, the enzymatic removal of the affinity tag after purification (required in order to produce a wildtype N-terminus) was surprisingly ineffective, leading to a further delay in delivering the final protein preparations. Toxin samples for the Partners in WP3 were first delivered in May 2020.

WP4-T1-ST3- Development of specific Ab (poly/monoclonal)

Due to the problems and delays outlined in the above sections, final, purified SE preparations of sufficient quality and quantity were available in June of 2020. Because the production of monoclonal antibodies requires at least 5 – 6 months, there was not sufficient time to allow for the delivery of the hybridoma cultures and characterization of the binders (all of which was necessary for the development of the immunoassays) before the end of 2020 – the original, official end of the TOX-Detect project. The Coordinators therefore requested the contracting of polyclonal sera and the antigens were delivered to the firm Covalab for the production of rabbit immune sera in June of 2020. The polyclonal sera for both SEN and SEO were delivered to the BfR in September 2020.

WP4-T1-ST4- Design of immunoenzymatic assays

The assay development for SEN and SEO began immediately upon delivery of the respective antisera and purified IgGs. While both purified IgG pools effectively and reproducibly recognized their antigens in a simple ELISA (immobilized antigens, respective pAbs and then an anti-rabbit-HRP detection antibody), only the assay for SEO was robust and reproducible in a sandwich format with toxins diluted in BHI culture medium. However, when testing culture supernatants from *S. aureus* strains having no SE genes, it became clear that the pAbs were cross-reactive against other, unknown *S. aureus* components. Although these pAbs against SEN and SEO are not alone suitable for the development of the envisioned immunoenzymatic assays, they will find application in SE affinity enrichment

procedures to support other (for example, MS-based) detection methods. In June 2021, these developed Ab were transferred to the European Union Reference Laboratory for Coagulase Positive Staphylococci for development of an LC-MS based Immuno capture extraction method for detection of enterotoxins in food matrices. Results on this application are expected by end of 2021.

WP4-T2- Development of a quantitative immunoassay on a new B cereus toxin or virulence factor

WP4-T2-ST1- Selection of 3 target genes and construction of a genetic tool for protein overexpression

Because WP2 could not deliver data on new *B. cereus* factors possibly involved in the diarrheal strains virulence, this task could not be implemented.

The development of immunoassays against *B. cereus* enterotoxins Nhe and Hbl was discarded because assays (although not quantitative) are already available against these toxins. The BCET-RPLA kit from Oxoid detects HblL2, the BDE-VIA assay from 3M-Tecra detects NheA and NheB, and the DuoPath assay from Merck detects NheB and HblL2. Therefore, in accordance with the coordinators, it was decided to develop assays against the enterotoxin CytK2, the hemolysin HlyII and the Sphingomyelinase.

WP4-T2-ST2- Protein production

For *B. cereus*, the first step was to select, for the three toxins, conserved sequences between reference diarrheal strains. Because these strains were not available at the project start, they were recovered from external laboratories and the toxins were sequenced using Micalis sequencing facilities and funds outside OHEJP TOX-Detect. Due to this preliminary task, cloning of the toxins genes started, after the sequences alignment, with a 6-months delay. The recombinant, His-tagged toxins were successfully produced in an almost pure form and could be delivered to WP3 and used for antibodies production. However, CytK2 was found to be quite unstable (half-life below 20 min.), and the design of an assay against this toxin was dropped.

WP4-T2-ST3- Development of specific Ab (poly/monoclonal)

Polyclonal rabbit antibodies against *B. cereus* toxins were also produced by the subcontractant company Covalab. Western blots with the purified toxin showed that the antibodies had a good affinity, since the serum could be used with dilutions up to 1/30000. Antibodies were purified on Protein A columns. Only the targeted toxins were recognized by the antibodies, meaning that they were quite specific.

WP4-T2-ST4- Design of the immunoenzymatic assay

Because of its ease of use, the development of a lateral flow assay (LFA) similar to the Merck DuoPath, allowing for the simultaneous detection of *B. cereus* Smase and HlyII, was initiated at first. However, despite good results, it appeared that the full design of this assay was not compatible with the project deadlines. Therefore, ST4 of WP4-T2 moved to the design of a simple sandwich ELISA, in which the capture antibody and the detection antibodies are identical. The detection antibody was HRP-tagged. The detection limit was 0.5 ng/ml for Smase and 1 ng/ml for HlyII, and the range of quantification was 0.5 ng/ml to 15 ng/ml for Smase and 1ng/ml to 40 ng/ml for HlyII. Toxins in culture supernatants could be readily quantified and spike-recover assays with these culture supernatants gave good results. SOPs are currently being written and material is being prepared for the interlaboratory trials.

WP5: Inter-laboratory ring trial scheme

The inter-lab tests were organized in order to evaluate the transferability of MALDI-ToF , LC-MS and ELISA methods developed in WP1, WP3, and WP4, respectively. These inter-lab tests have been prepared according to the regulation ISO/IEC 17043. Procedure allowing designing and implementing Inter-lab test was drafted and validated by the consortium. This document presents the objectives and a detailed plan for each ILC (Inter-lab comparison test) organization, see deliverable D5.1.

Training session dedicated to the implementation of ILC according to regulation ISO/IEC 17043, presentation of quality documents, samples preparation and establishment of inter-laboratory tests planning was organized by WP5 leader on 1st of December.

WP5-T1- Inter-lab test on MALDI-ToF for species identification

MALDI-ToF Library developed in the frame of WP1 by partner 1 was successfully transferred and implemented in the laboratory of partner 33 for the three pathogens: *Staphylococcus*, *Bacillus* and *Clostridia*.

EJP TOX-Detect and European Reference Laboratory network were invited to participate to MALDI-ToF ILC. In total 12 participants agreed and received the method for implementation. Samples used in This MALDI-ToF PT were issued from reference collection implemented in the frame of WP1 (Constitution of a reference strain collection). On 1st of June, each participant received 10 codified samples for each pathogen (30 samples in total). Results from participants were expected by end of June 2021.

WP5-T2- Inter-lab test on LC-MS/MS

Three LC-MS based methods have been developed in the WP3. SOP's were drafted and transferred to different EJP TOX-Detect partners in order to test the transferability of these developed methods.

- For *Bacillus* LC-MS method, 3 participants implemented the method, and took part to the Inter-lab exercise. In March 2021, each laboratory received standards, and 2 unknown samples. Results obtained by participants were 100% satisfactory.

- For *Clostridium* LC-MS method, 4 participants implemented the method, and participated to the Inter-lab exercise. Each laboratory received standards and several known samples for implementation of the method. In April 2021, each participant received 2 unknown samples (codified). Participants obtained 7/8 satisfactory results. The ILC report was dispatched to participants on 17th of June 2021.

- For Staphylococcal enteroxin 'type N' LC-MS method, 3 participants implemented the method, and participated to the Inter-lab exercise. In March 2021, each laboratory received standards and several known samples for implementation of the method. In April 2021, 5 unknown samples (codified) were sent to the three participants. 2/3 participants returned their results to the organizer by end of May. The last participant asked additional time due to a technical problem (device failure). The ILC final report was dispatched to participants on 23th of June 2021. Results obtained by participants were satisfactory at 100% and 93% for qualitative and quantitative results, respectively.

WP5-T3- Inter-lab test on immuno-enzymatic assays

Inter-laboratory test dedicated to ELISA method for staphylococcal enterotoxins was cancelled. See " WP4 and paragraph 3-Project self-assessment".

For Bacillus ELISA, SOPs were drafted in June 2021. Call for participation is in progress.

WP6: Dissemination, protection and exploitation of results

WP6-T1-Dissemination of information within the partners

Communication and information dissemination between partners was realized through meetings and EJP website or using e-mails. Several face-to-face and web meetings were organized within each WP and between the different WPs. The OHEJP coordination was invited to each general assembly in order to share OHEJP news, recommendations, use of the OHEJP site.

Partners were also invited to join the TOX-Detect group available on the OHEJP website. Deliverables and SOPs have been uploaded to the OHEJP website and are available to all TOX-Detect partners. The interim reports are posted on the OHEJP site and distributed by email to partners.

At OH-EJP level, three posters were presented on work implement in WP and WP2 during the three OH-EJP annual meetings.

WP6-T2- Dissemination of information to the outside.

TOX-Detect project and associated works was presented in:

- Five conferences, in 2019, 2020 and 2021, were presented in France, in Germany, in Spain and in an online meeting, for WP0, WP2 and WP3.

- Five work-shops (European Union Reference Laboratory, Italian reference laboratory, EFSA-ERAM and Astralia group).

Six articles were published in 2019, 2020 and 2021, in the journals Sensors, Food Microbiology, Clinical Microbiology and Infection, and Frontiers in Microbiology, all within the frame of WP2 and WP3.

7 more articles are expected to be published in 2021, for WP1 (1 article), WP2 (2 articles), WP3 (1 article), WP4 (2 articles) and WP5 (1 article).

Therefore, the project will be valorized by at least 10 communications and 13 scientific articles, issued from all WPs.

5.1.4.3.2.4 Project self-assessment

Please comment to what extent the initial project objectives as laid out in the proposal have been met. Any deviation with the original project plan should be commented on and justified.

WP1: The goal to constitute a reference collection strain was achieved without too much difficulty as well as libraries of MALDI-ToF reference spectra of these strains. However, the establishment of MTAs for the exchange of strains took a long time and was a source of delay in the accomplishment of the work package. Moreover, transfer of the MALDI Library was limited to all participant using a Bruker spectrometer.

During WP2, methods were set up to characterize the strain cytotoxicity and the presence/expression of toxins. Known and new virulence markers were identified. However, a specific toxin that would differentiate B. cereus FBO strains could not be identified. Thus, a complex analytical pathway had to be implemented to analyse the RNAseq data. This induced some delay in the analysis, but allowed to propose a combination of biomarkers able to accurately differentiate clinical from non-pathogenic strains. For C. perfringens, it was confirmed that CPE accounted for the strain cytotoxicity. As this WP contained laboratory work, the COVID-19-crisis led to important delays.

WP4:

- The objective to develop specific antibodies against two SEs (WP4-T1-ST3) was only partially reached and, consequently, the development of quantitative immunoassays for two SEs (WP4-T1-ST4) was not reached. The reason for this is a series of problems and delays primarily regarding the fulfilment of safety requirements for the necessary work with toxin producing GMOs. Thus, sufficient amounts of recombinant toxins were only available at a late project stage, which rendered the production of monoclonal antibodies in time impossible. Thus, polyclonal antibodies were tested, which, however, turned out to be cross-reactive.

- Task 1 had to produce three already known *B. cereus* toxins and to setup immunoenzymatic assays against them. The three toxins (CytK2, HlyII and Smase) were produced, shipped to WP3, and sandwich ELISA methods against HlyII and Smase were successfully developped. The third immunoassay development was dropped since CytK2 is highly unstable. The objectives of WP4 task 2, which were to setup immunoassays against new toxins, could not be fulfilled because WP2 did not provide toxin names in time; no toxins specific to FBO strains could be identified. This task was highly impacted by Covid crisis (animals immunisation).

For WP5, the objective was to implement inter-Laboratory tests in order to check the robustness and transferability of methods developed in WP1, WP3 and WP4. The delay observed in these 3 WPs impacted the implementation of Inter-laboratory tests. Over the 8 expected interlab tests, three were achieved (LC-MS), three were achieved end of June (three pathogens for MALDI-ToF). One interlab test achieved end of July (ELISA for *Bacillus*), and one initially dedicated to staphylococcal enterotoxins was cancelled as the method was not developed (see WP4 comment). Therefore, deliverable D5.2 (report on interlab tests) cannot be submitted on the expected time.

The coordination team faced several management issues mainly related to the changes of deputy coordinator in 2018, change of WP3 coordination twice (2019 and 2020), absence of coordinator and WP6 leader for 4 months.

For the whole project, tasks managed with personnel recruited under “short contract” were highly impacted by Covid crisis

5.1.4.3.2.5 Progress of the project: milestones and deliverables

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
05	D0.1	Report of the kick-off meeting	3			OHEJP: available Zenodo: https://zenodo.org/record/5484124#.YTed9I4zZaQ	
05	D0.2	Intermediate report	18			OHEJP: available Zenodo: https://zenodo.org/record/5516312#.YUdnGLgzZaQ	
05	D0.3	Report of the meeting 1	19			OHEJP: available Zenodo: https://zenodo.org/record/5484140#.YTefY44zZaQ	
05	D0.4	Report of the meeting 2	25			OHEJP: available Zenodo: https://zenodo.org/record/5484143#.YTef7Y4zZaQ	

05	D0.5	Report of meeting 3	42			Meeting organized on 25 June 2021 Zenodo: to be uploaded	
05	D0.6	Final report	45			In progress	
05	D1.1	List of well characterized reference strains of *S. aureus, B. cereus* and *C. perfringens*	5			OHEJP: available Zenodo: D1.1 TOX-DetectListe of well characterized strains | Zenodo	3
05	D1.2	Libraries of MALDI-ToF reference spectra	33			Confidential, this concern reference strains under MTA and will be published OHEJP: available Zenodo: D1.2 TOX-Detect Libraries of MALDI-ToF | Zenodo	3
05	D2.1	Report on results from toxicity assays (classical toxicity tests and High Content Analysis)	42			Confidential, technical data under publication OHEJP: available Zenodo: https://zenodo.org/record/5516345#.YUdrGbgzZaQ	8 Research and development activities
05	D2.2	Report on TR-PCR and RNAseq data analysis	42			Confidential, technical data under publication OHEJP: available Zenodo: https://zenodo.org/record/5516359#.YUdsV7gzZaQ	8 Research and development activities

05	D2.3	Report on correlation between RNAseq data analysis and toxicity assays (incl. toolbox for toxicity prediction)	42			Confidential, technical data under publication OHEJP: available Zenodo: https://zenodo.org/record/5516372#.YUdtRLgzZaQ	8 Research and development activities
05	D3.1	Report on MS-based methods for the detection of new enterotoxins (eg SEG, SEH, SEI) from *S. aureus*	42			Confidential, new developed method, will be published by the end of the project OHEJP: available Zenodo: https://zenodo.org/record/5516382#.YUduRLgzZaQ	8 Research and development activities
05	D3.2	Report on MS-based methods for the detection of cereulide analogs and enterotoxins from *B. cereus*	42			Confidential, new developed method, will be published by the end of the project OHEJP: available Zenodo: https://zenodo.org/record/5484152#.YTehBo4zZaQ	8 Research and development activities
05	D3.3	Report on MS-based methods for the detection of CPE and virulence factors from *C. perfringens*	42			Confidential, new developed method, will be published by the end of the project OHEJP: available Zenodo: https://zenodo.org/record/5516390#.YUdvgrgzZaQ	8 Research and development activities

05	D3.4	Report on the performance criteria for method harmonization	44			Confidential, new developed method, will be published by the end of the project OHEJP: available Zenodo: https://zenodo.org/record/5484117#.YTecDY4zZaQ	2
05	D4.1	Report on the immuno-enzymatic assays	45			Confidential, new developed method, will be published by the end of the project. OHEJP: available Zenodo: https://zenodo.org/record/5516394#.YUdxP7gzZaQ	8 Research and development activities
05	D5.1	Inter-lab tests documents according to ISO/IEC 17043 regulation, adapted to each method	45			OHEJP: available Zenodo: https://zenodo.org/record/4479096#.YBQYXuhKhM0	2
05	D5.2	Final report including results from the eight inter-lab tests	45			This report is in progress, it will be submitted on 17 of September	8 Research and development activities
05	D6.1	Dispatch of SOPs	45			OHEJP: available Zenodo: OH EJP TOX-Detect projec: Dispatch of SOPs | Zenodo	2 Research and development activities

05	D6.2	Dissemination of results (publications, conferences…)	45			OHEJP: available Zenodo: OH EJP TOX-Dtect project: Dissemination of results | Zenodo	2 Research and development activities

** Categories of Integrative activities: 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
05	MS0.1	General decision on criteria to select strains	3	Yes		
05	MS0.2	General discussion on interlaboratory trial scheme	3	Yes		
05	M-JRP5-03	Construction of the reference strains of S. aureus, B. cereus and C. perfringens	3	Yes		
05	MS1.1	List of well characterized reference strains of S. aureus, B. cereus and C. perfringens	3	Yes		
05	MS1.2	Exchange of libraries of MALDI-ToF reference spectra	24	Yes		
05	MS2.1	Culture conditions optimized	24	Yes		
05	MS3.1	Reference materials available	5	Yes		Reference strains are available for WPs depending on the availability of MTA and results of M-JRP5-04. Reference materials were transferred after MTA signature when it was requested by the host. Transfer started month 5 and achieved at the end of the project
05	MS2.2	RT-PCR assays developed	14	Yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
05	MS2.3	High content analysis methods developed	18	Yes		
05	MS2.4	RNAseq data analysed	38	Yes		
05	MS3.2	Methods developed and assessed	24	Yes		
05	MS2.5	Correlation between toxicity profiles and expression patterns assessed.	30	Yes		
05	MS3.3	Methods transferred to partners	39	Yes		
05	MS4.1	Genetic tools constructed for over-expression of known *S. aureus* and *B. cereus* toxins	9	Yes		
05	MS4.2	Antibodies produced against known *S. aureus* and *B. cereus* toxins	33	Yes		
05	MS4.3	Immuno-enzymatic assays designed for known S. aureus and B. cereus toxins	32	Yes		Milestone not achieved for S. aureus toxins (see explanation in WP4 paragraph).
05	MS4.4	Genetic tools constructed for over-expression of a	/	No		Milestone not achieved (see explanation in WP4 paragraph).

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
		new B. cereus toxin/virulence factor				
05	MS4.5	Antibodies produced against new B. cereus toxin/virulence factor overproduced	/	No		Milestone not achieved for (see explanation in WP4 paragraph).
05	MS4.6	Immuno-enzymatic assays designed for a new B. cereus toxin/virulence factor	42	Yes		
05	MS4.7	Immuno-enzymatic assays transferred to partners	42	Yes		
05	MS5.1	Dispatch of inter-lab tests documents (according to ISO/IEC 17043 regulation) adapted to each method	M25	Yes		https://zenodo.org/record/4479096#.YBQYXuhKhM0
05	MS5.2	Dispatch of samples and evaluation report to be filled by partners	M44	No	43	Done for LC-MS and MALDI-ToF Expected in M43 for ELISA Bacillus
05	MS5.3	Samples analysed by partners	M42	No	45	Done for LC-MS and MALDI-ToF Expected in M45 for ELISA Bacillus
05	MS5.4	Dispatch of final report	M45	No		Expected in M45 after receivin report on Interlab test for ELISA Bacillus

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
05	MS6.1	Final report dispatched	M45	No		

5.1.4.3.2.6 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors already accepted your comments. Therefore, this part of the report can be closed.

5.1.4.3.2.7 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Point-of-Need DNA Testing for Detection of Foodborne Pathogenic Bacteria, Vidic et al, 2019, Sensors DOI: 10.3390/s19051100 **Uploaded on Zenodo** https://zenodo.org/record/3935799#.YBQaJ-hKhM0	YES	NN	To be completed
Advanced Methods for Detection of Bacillus cereus and Its Pathogenic Factors. Nalini Ramarao, Seav-Ly Tran, Marco Marin, and Jasmina Vidic. **Uploaded on Zenodo** doi: 10.3390/s20092667	YES	NN	To be completed
Large-Scale Genomic Analyses and Toxinotyping of Clostridium perfringens Implicated in Foodborne Outbreaks in France, Abakabir Mahamat et al, Front Microbiol, 2019.	YES	NN	To be completed

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Uploaded on Zenodo https://doi.org/10.3389/fmicb.2019.00777			
The cytotoxic potential of Bacillus cereus strains of various origins, Glasset et al, Food Microbiology, 2021 DOI: https://doi.org/10.1016/j.fm.2021.103759 **Cannot be uploaded on Zenodo**	YES	No	No
New genetic biomarkers to differentiate pathogenic and clinically relevant Bacillus cereus strains, Kavanaugh et al., Clin Microbiol Infect, 2021. DOI:https://doi.org/10.1016/j.cmi.2021.05.035 **Cannot be uploaded on Zenodo**	YES	No	No
DiagnoTop: a computational pipeline for discriminating bacterial pathogens without database search. Diogo Borges Lima, Mathieu Dupré, Marlon Dias Mariano Santos, Paulo Costa Carvalho, Julia Chamot-Rooke. Journal of the American Society of Mass Spectrometry. **Uploaded on Zenodo** https://doi.org/10.1021/jasms.1c00014	YES	No	To be completed

Additional output

Other relevant dissemination outcomes can be highlighted here, e.g. non-scientific publications, proceedings, poster, video, tools, guidelines, patents, etc. If the outcome is a deliverable it should not be mentioned here. Please indicate where the item can be found, for instance on Zenodo or any open access repository. Indicate the link and check the functionality.

This project focused on virulence factors produced by some bacteria producing toxins. Tools developed are complementary with those developed in the frame of the H2020 project EuroBioTox focusing on biothreats. In this one, three partners of the One Health EJP Tox-Detect are present (Anses, Sciensano, Pasteur Insitute). Exchanges between the two consortium and overall presentations of the results will be made during the next meeting of EuroBiotox and during the final meeting of Tox-Detect which will take place in September 2021.

These two projects enhance European networks focusing on bacterial toxins in a One Health context.

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

In this paragraph you have the possibility to draw the attention to remarkable achievements that could be highlighted to illustrate the potential impact of your project.

In TOX-Detect project, deliverables D1.1 (List of well characterized reference strains of S. aureus, B. cereus and C. perfringens) and D1.2 (Libraries of MALDI-ToF reference spectra) and D5.2 task 1 (Inter-laboratory test on MALDI-ToF method) represents a complete achievement in term of implementation of new database and method from implementation of reference collection of strains until the transfer of the method to the European Union Reference Laboratory network. The **MALDI-ToF Library** will complete the commercial database with spectra obtained from strains issued from food born outbreaks occurred in European Union. After publication of the work in open access journal, scientific community can benefit from this new MALDI-ToF library to improve the MALDI-ToF method in term of discrimination potential.

WP2 deliverables and D3.3 (Report on MS-based methods for the detection of CPE and virulence factors from C. perfringens) proposes toxicity and enterotoxins data obtained for C. perfringens strains issued from food born outbreaks occurred in European Union. A set of complementary tools developed in this TOX-Detect project can be implemented and used by reference and expert laboratories for food born outbreak investigation, especially RT PCR and LC-MS techniques.

Deliverables dedicated to Staphylococcal enterotoxins study (D3.1 and D4.1) indicate that the egc enterotoxins SEM, SEN and SEO are produced at very low levels of concentration compared to classical enterotoxins. This information was presented to the EURL for coagulase Positive Staphylococci (EURL for CPS) which will use it for further study and methods development as reported in EURL working program 2019 and 2020. Also, enterotoxins and antibodies produced in WP4 were transferred to EURL for CPS in order to use them to enlarge the scope of detection methods for Staphylococcal enterotoxins.

Are there any outcomes of this project that are already discussed or even implemented and in use at any institute of the project consortium, at stakeholders' organisations (ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), or at the level of national authorities?

N/A.

5.1.4.3.2.8 One Health impact

About 500 words, 1 page – This part is extremely important, also for the One Health EJP stakeholders. Please, describe the impact of the JRP/, reflecting at least the following aspects:

- Specify any direct or indirect impact the outcome of your project may have for international stakeholders active in the domains of foodborne zoonoses, AMR and/or emerging threats (ECDC, EFSA, FAO, OIE, WHO-EU; EU reference laboratories, etc.) e.g. suggested improvements of existing /planned surveillance programmes, new relevant databases for risk assessment updates, improved methodologies for risk assessment and management, etc.; please refer to relevant documents.

- Please describe how regional or national authorities and stakeholders may implement and/or integrate any of the results from the project in their work. Which contacts, if any, have been taken between your consortium and authorities or stakeholders that may lead to such a knowledge transfer? Please precise.

- Has this project helped you building and strengthening the networking with other partners of the One Health EJP and beyond? Please describe shortly and give some examples.

In this project, we focused on bacterial producing toxins according to the One Health concept. For this purpose all partners combined isolates coming from food, environmental and clinical samples.
All the selected strains have been fully characterized by partners and were used to perform method developments.
The first outcome is the availability of this large and fully characterized collection of strains. A part of this collection has been already used to feed another OH EJP programme – CARE.
The second outcome is the development of dedicated Maldi-ToF libraries. Robustness of these MSP libraries have been evaluated by a dedicated ILS generating more than 3000 data. We highlighted that the percentage of correct identification of the three pathogens covered by ToxDetect significantly increased especially for the identification of the isolates of the B cereus group.
As results obtained were highly satisfactory, we will dispatch these libraries among partners as well in the EU networks.
Moreover, thanks to this strain collection we were able to design 15 harmonized SOPs for all the targeted virulence factors and 6 dedicated methods. All of these deliverables contributed to the tool box we would like to design at the beginning of the project to enhance characterization of bacteria producing toxins. These deliverables have been shared and could be used by partners as ILS demonstrated their robustness and efficiency.

5.1.4.3.2.9 Data Management Plan

The DMP of the project should either be uploaded on the OHEJP Data Management Plan group or the OHEJP project group site available on the OHEJP website (first call projects) or in the CDP-tool (provided to second call projects).

Describe how the FAIR (Findable, Accessible, Interoperable, Reusable) principles are fulfilled in the DMP (are all generated data/datasets described, are there contact details e.g. links, institutional addresses, personal e-mail addresses for how to access the data, do all datasets have a unique identifier etc.).

Has the final version of the DMP been submitted to the OHEJP DMP leader (geraldine.boseret@sciensano.be) (3M in advance of final project date)? Please, comment on corrections made according to the suggestions from the DMP Committee.

Two types of data were obtained in the frame of TOX-Detect project, experimental data related to method development and one MALDI-ToF database and their associated reference strains.

- For experimental data:

First data were published in 5 open access journals associated to a DOI (see section XX), the rest is expected to be published by beginning of 2022. In addition.
All methods have been developed under OH EJP ToxDetect were drafted in a harmonized SOP template (architecture of the document, scope, reagents, instructions, performance criteria,….) and dispatched to TOX-Detect partners. Deliverables were also uploaded on OH-EJP website. All these documents are fully accessible to partner consortium through the dedicated website https://onehealthejp.eu/ groupe Tox-Detect.
Once published, these documents would be shared across identified MSs and EU networks.

- For MALDI-ToF data base and their associated reference strains:

In WP1, partners designed a large strain collection for the three pathogens (21, 21, 34 isolates for S. aureus, B. cereus and C. perfringens respectively) coming from their own collections. All partners of Tox-Detect project shared these strains depending of their need. In addition, some of these characterized strains (S. aureus and B. cereus) have been already shared to the OH EJP CARE programme.
Three MALDI-ToF spectra databases (one per pathogen) were developed and optimized using these selected strains. The MALDI-ToF database have been shared among internal and external partners especially national reference laboratories to i) implement new databases in their own Maldi-ToF equipment, ii) participate to the Interlaboratory test dedicated to Maldi-ToF (D5.1).

To date, these strains are hosted by their proprietary partners. A discussion has been engaged to decide of the final location of these strains (for future dispatching) based a Material Deposit Agreement proposed by partner 19 (Pasteur institute) to collect all the strains on a same location (Pasteur Institute collection, Paris, Fr).

For MALDI-ToF spectra, the results obtained in the frame of interlaboratory tests showed that TOX-Detect library is more specific comparing with the commercialized spectra (By Brucker). A reflection is in progress in order to (i) implement a large validation study and (ii) to allow to EU laboratories a free access to this new database.

5.1.4.3.2.10 List of dissemination and communication activities

Name of the activity:	Development of a mass spectrometry-based method for detection of Clostridium perfringens enterotoxin CPE. HILLION, M., ABDELRAHIM, AM., FIRMESSE, O., VIALA, D., CHAMBON, C. and HEBRAUD, M. ProteOviedo, 1st Joint Congress of Spanish, French and Portuguese Proteomics associations, Oviedo
Date:	11-14 Novembre. 2020
Place:	Oviedo

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories. Include hyperlink, if relevant. Zenodo grants the open access, it could be used as a repository for the presentations, posters, and other dissemination materials.

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other	
Communication Campaign (e.g. Radio, TV)	No		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	?	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	OH EJP ASM 2021 Transcriptomic Profiling Analysis of *Clostridium perfringens* Isolates collected From Human and Food poisoning Outbreaks Abdelrahim ABAKABIR MAHAMAT
Date:	10-11 June 2021
Place:	Web conference

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories. Include hyperlink, if relevant. Zenodo grants the open access, it could be used as a repository for the presentations, posters, and other dissemination materials.

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	No
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other: ASM 2021	Yes
Communication Campaign (e.g. Radio, TV)	No		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	?	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	IAFP European symposium (2 oral presentations) 1) RNA-Seq analysis revealed differences in the global transcriptome of *Clostridium perfringens* isolates (Abdelrahim ABAKABIR MAHAMAT) 2) OH EJP symposia: TOX-Detect (Jacques-Antoine HENNEKINNE)
Date:	27-28/04/2021
Place:	Web conference

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories. Include hyperlink, if relevant. Zenodo grants the open access, it could be used as a repository for the presentations, posters, and other dissemination materials.

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other	
Communication Campaign (e.g. Radio, TV)	No		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	20	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	TOX-Detect Annual meeting
Date:	20-21 March 2019
Place:	ANSES, Paris

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	Yes
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	15	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	EURL for CPS annual Workshop
Date:	26-28 May 2019
Place:	Maisons-Alfort

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	yes
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	40	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Australie Group Meeting (bio threat weapons)
Date:	4 june 2019
Place:	Paris

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	yes
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	5	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	35		

Name of the activity:	Workshop EREN “Natural toxin”
Date:	20 november 2019
Place:	Anses, Maisons-Alfort

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	15	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	15		

Name of the activity:	Italian NRL annual Workshop
Date:	5 november 2019
Place:	Turin, Italy

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	yes
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	50	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	SFM conference
Date:	1 October 2019
Place:	Paris

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	40	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	OHEJP ASM poster entitled "Establishment of a shared MALDI-ToF reference spectra base, covering three pathogens of interest"
Date:	27-29 may 2020
Place:	online

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	NO	Participation to a Conference	Yes
Organisation of a Workshop	NO	Participation to a Workshop	NO
Press release	NO	Participation to an Event other than a Conference or a Workshop	NO
Non-scientific and non-peer-reviewed publication (popularised publication)	NO	Video/Film	NO
Exhibition	NO	Brokerage Event	NO
Flyer	NO	Pitch Event	NO
Training		Trade Fair	NO
Social Media	NO	Participation in activities organized jointly with other H2020 projects	Yes
Website	NO	Other	
Communication Campaign (e.g. Radio, TV)	NO		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	EURL staphylococci annual workshop: presentation of TOX-Detect project
Date:	April 2020
Place:	online

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	NO	Participation to a Conference	NO
Organisation of a Workshop	NO	Participation to a Workshop	YES
Press release	NO	Participation to an Event other than a Conference or a Workshop	NO
Non-scientific and non-peer-reviewed publication (popularised publication)	NO	Video/Film	NO
Exhibition	NO	Brokerage Event	NO
Flyer	NO	Pitch Event	NO
Training		Trade Fair	NO
Social Media	NO	Participation in activities organized jointly with other H2020 projects	NO
Website	NO	Other	
Communication Campaign (e.g. Radio, TV)	NO		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	50	Media	0
Industry	0	Investors	0
Civil Society	0	Customers	0
General Public	0	Other	0
Policy Makers	0		

Name of the activity:	OH-EJP TIM “Practical use of NGS”
Date:	April 2020
Place:	online

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	NO	Participation to a Conference	YES
Organisation of a Workshop	NO	Participation to a Workshop	NO
Press release	NO	Participation to an Event other than a Conference or a Workshop	NO
Non-scientific and non-peer-reviewed publication (popularised publication)	NO	Video/Film	NO
Exhibition	NO	Brokerage Event	NO
Flyer	NO	Pitch Event	NO
Training		Trade Fair	NO
Social Media	NO	Participation in activities organized jointly with other H2020 projects	YES
Website	NO	Other	
Communication Campaign (e.g. Radio, TV)	NO		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

5.1.4.3.3 JRP06-FBZ1-NOVA – Final Report

5.1.4.3.3.1 Consortium composition

The Project Coordinator was Jenny Frössling (SVA) and the Deputy Project Coordinator was Steen Ethelberg (SSI). The coordinators formed a coordination group together with Fernanda Dórea (SVA) and the WP leaders Maria-Eleni Filippitzi (Sciensano), Adeline Huneau (ANSES), Ana de la Torre (INIA) and Håkan Vigre (DTU).

List of project participants

Country	Institution	Name	Designated role	Remark	WP
BE	Sciensano	Maria-Eleni Filippitzi	Leader of WP1, Institute contact	Joined M3	0,1
BE	Sciencano	Valerie de Waele	Leader of WP1	Joined M0, left M21	0,1
BE	Sciencano	Sarah Welby	Leader of WP1	Left M0	1
DE	BfR	Katja Alt	Co-leader of WP2, Institute contact		2
DE	BfR	Jakub Fusiak			2
DE	RKI	Idesbald Boone			2
DE	RKI	Sebastian Haller			2
DE	RKI	Hendrik Wilking	Institute contact		2
DK	DTU	Ofosuhene Apenteng			5
DK	DTU	Håkan Vigre	Leader of WP5, Institute contact person		0,5
DK	SSI	Steen Ethelberg	Deputy project coordinator, Leader of WP2, Institute contact		0,2
DK	SSI	Frederik Møller Trier			2
DK	SSI	Helle Daugaard Larsen			2
DK	SSI	Uffe Braae			2
DK	SSI	Luise Müller			2
DK	SSI	Laura Espenhain			2
DK	SSI	Peter H. S. Andersen			2
DK	SSI	Elsebeth Tvenstrup Jensen			2
DK	SSI	Louise Køhler Olsen			2
DK	SSI	Henrik Bang			2
ES	INIA	Ana de la Torre	Leader of WP4, Institute contact		4

ES	INIA	Irene Iglesias			4
ES	INIA	Antonio Rodríguez			4
ES	UCM	Julio Álvarez Sánchez	Institute contact person		4
ES	UCM	Lucía de Juan Ferré			4
ES	UCM	Kendy Tzu-Yun Teng			4
FR	ANSES	Géraldine Cazeau			?
FR	ANSES	Viviane Henaux			5
FR	ANSES	Adeline Huneau	Leader of WP3, Institute contact		3
FR	ANSES	Renaud Lailler			3
FR	ANSES	Carole Sala			?
FR	ANSES	Briac Virey			3
FR	InVS/SpF	Nathalie Jourdan-da Silva	Institute contact		2
IT	ISS	Rosangela Tozzoli			2
IT	ISS	Gaia Scavia	Institute contact		2
IT	IZS	Luigi Iannetti	Institute contact		2
NL	RIVM	Elisa Beninca			4
NL	RIVM	Eric Evers	Institute contact		5
NL	RIVM	Ingrid Friesema			2
NL	RIVM	Arno Swart			4
NO	NIPH	Gry Grøneng			2,3
NO	NIPH	Zuzana Nordeng			1,2
NO	NIPH	Solveig Jore	Institute contact		1,2
NO	NIPH	Gunnar Rø			3
NO	NIPH	Peter Dougherty			2,3
NO	NIPH	David Swanson			3
NO	NIPH	Clemence Koren			3
NO	NVI	Petter Hopp			3
NO	NVI	Malin Jonsson	Institute contact		3
NO	NVI	Kyrre Kausrud			3
NO	NVI	Madelaine Norström			3
SE	PHAS	Marie Jansson Mörk			2
SE	PHAS	Moa Rehn	Institute contact		2
SE	SVA	Wonhee Cha			3

SE	SVA	Fernanda Dórea			0,3
SE	SVA	Jenny Frössling	Project coordinator, Leader of WP0, Institute contact		0,1,5
SE	SVA	Wiktor Gustafsson			3
SE	SVA	Hyeyoung Kim			4
SE	SVA	Anna Nordenfelt			0
SE	SVA	Thomas Rosendal			5
SE	SVA	Stefan Widgren			4,5
SE	SVA	Estelle Ågren			1,4
UK	APHA	Mark Arnold	Institute contact		5
UK	PHE	Natalie Adams	Institute contact		2
UK	PHE	Lesley Larkin			2

5.1.4.3.3.2 Summary of the work carried out in the Project

Background
Zoonotic foodborne diseases constitute a serious **one health problem** throughout the world and are expected to stay high on the infectious diseases risk agenda, fuelled by factors such as globalised trade with food and live animals, modern intensified food production systems, international travel, and the continuous adaptation of known and arising pathogens and hazards, including increased antibiotic resistance. Foodborne diseases, being very prevalent and giving rise to sequelae, pose a serious problem to public health and have significant economic implications in terms of cost of care, loss of productivity and in the agricultural and food industry sectors.

In order to limit the damage and cost caused by zoonotic diseases, it is better to prevent than to cure. **Disease surveillance is a crucial prerequisite** for prevention; it allows us to be able to analyse and understand trends and patterns of disease occurrence and to choose and evaluate intervention strategies. However, surveillance systems are not always working well and they also come with a price tag. Also digital data sources continues to develop, also within the food chain, and should be utilised if possible for surveillance purposes. It is therefore **important that we continuously develop the ways we do surveillance and that we have methods to measure and compare the performance of different surveillance activities**. This has been the overall **aim** of the **NOVA** project suite.

Methods
NOVA has included project participants from 19 public health and veterinary institutes in 10 countries over a project period of 3½ years. As one of the larger OHEJP projects, it has addressed improvement of disease surveillance through a large number of separate projects, organised into five main themes, as reflected in its overall work package structure. Below, three results from each theme, which we find particularly interesting, are highlighted. Results are summarised in more depths in Section 2 of the Final Report and presented through published and in prep papers and the formal project deliverable reports.

Results
Theme **A**. **Basic aspects** (and issues in connection with) performing **One Health surveillance** (WP1):

- Surveillance across the foodchain is as yet far from used enough; it holds (and is perceived by

actors as holding) much potential.

- Focus group studies highlighted a complex pattern of barriers and opportunities for OH surveillance. In particular relating to data governance.
- A glossary of terms was developed (together with other OHEJP projects) to help overcome barriers between veterinary, food and human surveillance activities.

Theme **B**. Use of **electronic traces of purchase of food** for surveillance and outbreak investigations (WP2):

- Electronic records of the foods we all buy in shops are a powerful tool in the hands of the disease detectives that investigate foodborne outbreaks. A network to promote use hereof was built.
- Methods of analyses of big datasets on supermarket purchases can automatically find vehicles and calculate odds ratio's therefore – tapping into supermarket purchase information should be used regularly in the future for foodborne outbreak investigations.
- Foodborne outbreaks in care institutions – hospitals and elderly homes – occur, are preventable and sometimes traceable via electronic invoices food sales.

Theme **C**. **One Health** developments of **syndromic surveillance** methods (WP3):

- Individual monitoring of *Campylobacter* detections in major broiler slaughterhouses proved effective for enhancing early detection of *Campylobacter* outbreaks in humans
- Parallel monitoring of indicators on cattle health, *Salmonella* detection in food, and human gastro-intestinal syndromes revealed simultaneous temporal abnormalities. Simultaneous alarms may be used to trigger and orientate field epidemiological investigations.
- Predictions based on models combining veterinary, meteorological and medical data in Norway were used to develop a dashboard to provide stakeholders with predictions of gastrointestinal outbreaks one week ahead of other systems.

Theme **D**. Use and development of **spatial risk mapping** (WP4):

- Development of spatial disease distribution models (for *Salmonella*) have identified provinces in Spain at risk, while taking into account the effect of potential risk factors.
- Risk-based surveillance of *Salmonella*, based on a spatial stochastic disease spread model, was economically favourable -- it relied on only one third of the samples used by traditional surveillance, although the capacity for case detection was also reduced.
- Models and scripts that have been developed for salmonellosis can be applied for other zoonotic diseases and many geographical regions in Europe, depending on data availability.

Theme **E**. Modelling the **cost efficiency** of surveillance programs (WP5):

- By combining models for disease transmission (SIR) simulating emerging food safety issues with models simulating sampling schedules and models of laboratory procedures, we determined the time such systems will need to detect an emerging infection – thereby providing a tool to optimise emerging risk surveillance systems.
- It is possible (by modelling) to make a holistic assessment of how new laboratory technologies can be used to improve the performance of surveillance systems before the technology is actually implemented in a population.

- We've shown that the performance of surveillance programs of foodborne diseases in any part of the food production can (and should) be measured as the effect on the human risk using an QMRA approach, and the cost of surveillance (both monitoring and control) per prevented human case.

Discussion

Overall, NOVA has contributed to the development of several novel methods that can be used to make surveillance and response more efficient and cost-effective. In parallel to strengthening of modelling and data frameworks, we have explored and started the process to incorporate new data sources in the everyday surveillance that our institutes are involved in. We conclude that modelling the full chain from primary production to the consumer, including environmental aspects, is possible but remains a challenge. Our collaboration confirm that a multidisciplinary approach is needed to combine and understand information from the different surveillance components.

5.1.4.3.3.3 Work carried out in the JRP, scientific results and integrative outcomes

WP0. Coordination and project management

WP0-T1- Project management

Monthly meetings with WP leaders were held throughout the project, starting already November 2017. For coordination purposes, the coordinator and/or other members from the coordination group attended the official kick-off of meeting of the full OHEJP consortium, and various information meetings within the OHEJP over the years, such as the Project Leaders' Forum. Especially at the start of the OHEJP collaboration meetings with representatives from the ORION and COHESIVE projects were necessary to sort out potential overlap and create synergies between the projects. We have also attended a OHEJP Programme Managers' Committee meeting and a few OHEJP stakeholders' meetings.

Apart from these meetings, project management has included reporting, finding platforms for sharing of information and documents and to summarise information about the project e.g. for the websites of the OHEJP and our institutes. Management has also involved work with the ethics self-assessment and the data management plan (DMP). The DMP work required attendance of educational sessions organised by the OHEJP management, and additional meetings to plan and complete this work process.

A request to extend the project was sent to OHEJP management in May. Due to slow recruiting processes in several partner institutes, we decided already in 2019 that we would ask for an extension. The COVID-19 pandemic also influenced the work in several partner institutes/countries. The request was endorsed and accepted by the PMT and the members of the SSB in June 2020.

WP0-T2- Organise annual assemblies

The first annual assembly was held in Rome, February 28th to March 1st. The meeting was considered a kick-off meeting where we focused on getting to know each other and pick up the planning of tasks and studies included in the project proposal.

A second annual assembly was held in Brussels, March 7-8, 2019. The purpose of the meeting was to meet, plan and discuss ongoing and coming tasks. To focus this work, three specific topics were addressed in the discussions: 1. International aspects and opportunities for international collaboration, 2. Scientific publications, 3. Novelty of methods and approaches used. To share research results across WPs, a scientific webinar was also organised for all project participants in November.

Organisation of a third annual assembly was started at the end of 2019 and was planned to be held in Madrid, 23-24 April 2020. The meeting was cancelled in March due to the COVID-19 pandemic and instead, a shorter online meeting was held on the 23rd of April, with short scientific presentations from the WPs and general information.

In spring 2021 we had a final assembly that again had to be held online. This time we had a two-day meeting, 18-19 May, combining scientific presentations with general information and concluding remarks from the WP leaders.

WP2-T3- Economic reporting and financial management

SVA participated in the start-up financial OHEJP meeting at ANSES 2018 but did not coordinate economic reporting for other partners, as the financial reporting for the joint research project within the OHEJP are performed by each partner institute involved. However, in response to a request by the OHEJP coordination in 2020, the project partners were asked to report any costs that had been wasted due to the COVID-19 pandemic. Only two partner institutes reported such costs. Later, the OHEJP coordination also requested that the budget for the third year of the project were updated by each partner and specific budget files were filled in and sent to OHEJP centrally in June. The conclusion from this update was that partners plan to not underspend but to use potentially remaining resources during the extension period in the fourth year.

WP1. Food chain surveillance mapping

WP1-T1- Definition of a joint food borne zoonosis surveillance terminology

Given the work on a Med-Vet glossary of the participants of ORION WP2 in which members of NOVA WP1 also participated in the first year, in order to complete the deliverable of this WP, a collaboration was established with the ORION team, as well as COHESIVE.

WP1-T1 has collected 274 terms from partners leading all WPs. The NOVA Glossary was delivered to the ORION project leader, BfR, in the second half of June 2019 in order to incorporate the NOVA Glossary into the common OH Glossary. In the period between July and October 2019, there were several exchanges with BfR in order to adjust the NOVA Glossary into the common version. The final result can be found here: https://aginfra.d4science.org/web/orionknowledgehub/

The OHEJP Glossary has 3 main functionalities. First, it is a collection of One Health related terms and definitions in the sectors public health, animal health and food safety. Second, it highlights similarities and differences of terms and definitions between the sectors. Finally, it provides an infrastructure to references, search and filter terms, and definitions. As an outcome of this cooperation (of all three projects), a joint article was published in May 2021.

WP1-T2- Mapping of surveillance: data, regulatory framework, key stakeholders, opportunities and barriers

The objective of this task was to identify barriers and opportunities for an integrated One Health surveillance of food-borne diseases in selected EU countries. The task was split into two qualitative research studies: one targeting responsible experts and officials (coordination/administration level) of surveillance activities across the food chain in four different countries, and one targeting veterinary practitioners (field level) in one country. The results from these studies can be used to support revision of existing systems, or development of new systems for food-borne disease surveillance from a One Health (OH) perspective.

Coordination level study

In order to identify barriers and opportunities in food-borne disease surveillance from a One Health perspective, information was collected through interviews with professionals with selected profiles on coordination/administration level and from four countries (Belgium, France, Sweden, and Norway). The study methods and preparations included: 1. Mapping of an existing food chain (used as a visual tool for cueing), 2. Development of an interview guide document, 3. Demographic questionnaire, 4. Excel files for collection of data, 5. Training on performing interviews and qualitative research, 6. Interview try-outs.

The profiles of the professionals interviewed in each of the four countries were the following:

I. A human medicine epidemiologist (e.g. the head of such a unit or an experienced professional with knowledge of different diseases and managing them)

II. A person from administration in charge of planning the annual programmed food chain surveillance (expected to be place at the Min. for Agriculture)

III. A veterinary medicine epidemiologist (same as for human epidemiologist)

IV. A transversal coordinator of a national agency, positioned on the link between risk analysis and risk management

V. Persons from laboratories, ideally the coordinators of the human and veterinary National Reference laboratories (RNL).

The participants selected to be interviewed were identified and contacted by the WP1 members representing each country thanks to their professional network. The 20 interviews were performed by three researchers who received the same training on performing interviews. More detailed information is provided in the respective deliverable report, while a manuscript is being drafted.

According to the analysis, which was performed following the thematic analysis methodology, the barriers and opportunities identified by the interviewees were grouped in the following themes: 1. Data governance, 2. Set-up and operations of the surveillance system, 3. Coordination, 4. Communication, 5. Regulations (political legislations and procedures), 6. Industry's challenges, 7. Funding, 8. Training and education.

For all countries, the barriers and opportunities pointed out as being most important belonged to the themes covering Data governance and Set-up and operations of the surveillance system.

Therefore, the results show that there is room for improvement in food-borne disease surveillance of microbiological pathogens towards a One-Health approach, especially regarding the themes identified as most. An analysis of the collected information is also being performed per identified theme, taking into account the particularities of each country.

Field level study

Early detection of zoonotic and emerging infections is critical for successful handling of outbreaks and control of foodborne diseases. Veterinary practitioners have a key role in the detection of such diseases in livestock. Our objective was to explore how the clinical surveillance and reporting zoonotic and regulated diseases in livestock are perceived and performed by veterinarians in Sweden.

In this study, we conducted in-depth face-to-face interviews with 13 field veterinarians selected based on socio-demographic characteristics. The veterinarians were working in large animal practice. A semi-structured interview guide with open-ended questions and prompts was used to collect qualitative data. The interviews started with general questions to capture the veterinarians' profiles. Thereafter, interviewees were asked to describe their actions in cases of suspected emerging and zoonotic infections under regulation. The discussions followed topics that included their awareness of major disease threats, their perceived role in diseases surveillance, and views on its feasibility. The interviews were transcribed in verbatim followed by thematic analysis of the transcripts using a phenomenological approach.

Themes that commonly appeared were veterinarians, for various reasons, disregarding clinical signs possibly associated with the emerging and zoonotic infections; difficulties to take samples under field circumstances and to send these samples for diagnostic tests; and aspects related to a communication network involving those taking part of the surveillance system.

These results can serve as a baseline for the design of questionnaire-based surveys, also targeting

veterinarians in other countries, as a complementary quantitative approach to investigate clinical surveillance. The results provide suggestions for improvements in the reporting chain. The final goal is to increase the likelihood that a veterinary practitioner promptly contacts animal health authorities when they encounter animals showing signs indicative of diseases under national or EU legislation.

WP2. Analysis of food purchase data

Those who like to read detective novels or watch police investigation films, know that the investigators often decide to 'follow the money'. The clever police team follows the electronic trail that money transfer leaves when they investigate and solve complicated criminal cases. When trying to crack the sometimes very complicated epidemiological problems of foodborne diseases and foodborne outbreak investigations, one might wonder if not the same logic may be applied – just for food and not money. In this work package, the overall aim was to explore the possibilities of working with electronic records of food purchase and food movements for obtaining a better understanding of the transmission of foodborne diseases.

Work package 2 (WP2) of NOVA aimed to elucidate whether the surveillance potential of food consumption, as reflected by consumer purchasing patterns, may serve as a digital tool to aid our understanding of both outbreaks and general risk factors of foodborne zoonoses. Understanding food consumption patterns is an important part of understanding foodborne diseases, and modern society opens new possibilities to do this using existing data flows. With recent advents of electronic storage of purchase receipts, several countries and food business operators have obtained large but currently unused datasets, which can contribute important information. WP2 was divided into five separate tasks, I) Usage & barriers, II) Outbreak investigations, III) Big data analysis, IV) Hospital outbreaks and V) Trace-back and risk mapping. These five subtasks are described individually below.

WP2-T1- Data availability of purchase and consumer data for outbreak investigations (Formerly: Data availability and barriers)

Some EU countries have several years of experience with use of supermarket purchase data or other consumer data for the investigation of foodborne outbreaks, whereas others have never used it. The objective of WP2.1 was to map actors involved in obtaining and using purchase and consumer data, get an overview of the data already used, the context they may have been used in and the experience with these data for outbreak investigations. A second objective was to try to map possible barriers and obstacles when obtaining and/or using these purchase data and other consumer data. This was done through two questionnaire surveys inquiring about purchase data. These comprised data from supermarkets, wholesale, the Internet, fast food chains, and organizations who have access to these data (e.g. market surveys and scanning data companies/apps). Other consumer data comprised catering companies for health care institutions, schools, etc., and in house food preparation in health care institutions, schools, etc. All (public health) partners of EJP-NOVA received an email with the links to questionnaires. Secondly, we approached the institute representatives included in One Health EJP and the members of the ECDC Food- & Waterborne Disease (FWD) network via the Epidemic Intelligence Information System for FWD (EPIS-FWD) to complete one or both questionnaires or to give names of (possible) contacts who could.

Eleven countries completed one or both questionnaires on use of purchase and consumer data in (outbreak) investigations and other research. Seven countries reported to have actually used these data in one or more studies. The two main aims for using purchase and other consumer data are source hypothesis-generation and food trace-back investigation. It is seen as an alternative tool to solve outbreaks with advantages including: that it mitigates issues around the inability of people to remember everything they have eaten one or more weeks ago, especially stealth food products and also that it may provide good data which can lead to strong evidence pointing to a product. Purchase data of supermarket chains could become available for use in almost all countries, often with national

coverage. Other purchase and consumer data are less often already available and are more often local or regional. To gather purchase data, member/loyalty, credit and cash cards are crucial, as data could not be extracted for any of the data sources when the purchases are payed cash. The most experience appear to be in the area of the supermarkets, mainly chains, but also consortia and individual supermarkets. Overall, actors needed to obtain and use these data were national public health institute – often together with regional public health institute(s) – and the national food safety authority. Depending on where the purchase or consumer data is obtained from, also the company/institution, chain, consortium or interest group is involved.

Three groups of disadvantages were experienced when using the data, namely obstacles on the level of the supermarket/company/institution, the cases, and the handling of the data. Within all responding countries, eight of 11 countries and six of nine countries saw practical and/or technical obstacles for obtaining and using purchase and other consumer data, respectively, with the main threat being a too high workload. Of all obstacles, privacy legislation was conceived as the most important barrier as it was the only barrier mentioned by more than half of the countries.

WP2-T2- Food purchase data for outbreak investigations

Foodborne outbreaks occur with a high frequency in Europe and constitute a high, although partly preventable, burden to health and economy. Unfortunately, the majority of outbreaks are not fully resolved, in part because outbreak investigations are very time consuming and often rely on patient interviews that can be difficult to perform in sufficient scale and in a timely manner. There is therefore much to gain, if information could be easily gathered in an electronic format on which foods, the patients that are part of outbreaks, have bought in supermarkets or shops prior to becoming ill. Then it would theoretically be possible to find the foods, which the patients have in common and perhaps even to do a statistical comparison of purchase frequency with a non-patient consumer population. The aim of WP2.2 was to describe this line of investigation as an outbreak methodology, describe its prior use, and map the obstacles that lies in the way of a wider use and to produce recommendations for how it could be applied in EU member states. This was thus to a large degree a review and networking exercise.

As a first step a structured review of the already published literature was done with the purpose of obtaining an overview of the degree to which the method has been used and how it has found application so far. This review was published as Møller et al (2017) in Eurosurveillance. For the period 2006–17, scientific articles were found describing 20 outbreak investigations. They were presented and the benefits and shortcomings of the method was discussed in the paper. The term ‘consumer purchase data’ (CPD) was introduced to refer to the method.

As a next step, we invited epidemiologists with experience with CPD to take part in a network. We reached out via the European Public health institutes and the EPIET alumni organisation (EAN) and succeeded in creating a small working group from eight European countries plus Canada. In online consultations, this group has tried to answer the central questions of “When is this method working?” and: “When is it not useful and why?” with the aim of suggesting ways to facilitate its use. It was generally found that perceived obstacles related mostly to lack of guidance in how to use CPD rather than legal issues. A manuscript, which based on these consolations contain a description of a framework for use of CPD as an outbreak investigation method has been submitted. We hope that the work we have so far initiated will lead to a resurgence of use of CPD for outbreak investigations and that this network will keep growing.

WP2-T3- Working with large datasets and novel ways of analysed consumer purchase data (Formerly: Big data analysis of risk factors for sporadic disease)

The previous task dealt with concrete individual outbreak situations. It is conceivable, however, that

access to large datasets could lead to conduction of semi-automated outbreak investigations being possible and that CPD could also be used for general analytical purposes. This was the focus of the work conducted in WP2.3. Two paths were followed:

In one path, we aimed to set-up a case-control study of risk factors for sporadic disease using only CPD. Salmonella was chosen as the model organism and the project concerned Denmark only. The aim has been to build a digital solution into which Danish residents by invitation (cases of salmonella and comparable persons without known infection selected from the population register) can sign up. This solution includes a way of safely logging in to a secure website where the user is presented with study objectives and information about legal rights and with the possibility of further opening up for investigator access into past purchase records. This is possible if the user is already subscribing to a third party solution (an app) that stores the results of credit card purchases – something about 20% of the Danish population do. This technical solution (which was built with additional support from funding outside of NOVA) is now working and is legally compliant. Construction has involved overcoming a large number of practical, technical and legal challenges. Also, the case-control study is ethically and data-technically approved, a draft of a protocol study has been prepared for submission, and invitational letters have been drafted. However, due to the exceptional covid-19 situation, the final legal approval of the data collection platform, that were to be used to collect consumer data, was not approved until early June 2021. Thus, running the case control study has been postponed until end of 2021/beginning of 2022 after the end of NOVA. This is in other words a sustainable activity!

A second independent path has involved trying to obtain large real consumer dataset from Scandinavian supermarket chains. Initially work was done using a very large dataset from a major Danish supermarket chain, however, this work had to stop halfway into the project because of GDPR issues. Following a year of negotiations by the FHI, a second large anonymized consumer purchase dataset was offered to our use by a group of Norwegian supermarket chains. It contains individual weekly purchase histories available for analysis (920,834 unique customers from six Norwegian municipalities in 2019, totalling 222 mill purchases of 30,929 different products). The results of the analysis was presented at the OH_EJP annual meeting 2021 with the title “Simulation and identification of foodborne outbreaks in real consumer purchase data“, and a manuscript is in preparation. The study aimed to use consumer purchase data to simulate outbreaks, identify outbreak vehicles using regression, and examine how performance of selected analytic strategies varied across outbreak parameters.

The simulations generated outbreaks with different attack rates, proportion of background cases (where CPD was not available), and food expiry dates. Further analysis was conducted to evaluate the effect of Item purchase frequency. The proportion of background cases (i.e., those with no available consumer data), was the most important parameter for the number of cases needed to point towards a possible source of the outbreak. Sensitivity analysis was also conducted to address different attack rates (0.01-0.1), expiry length (0-300 days) and incubation time (0-10 days). Not surprisingly, the higher the item detail level (primary product group eg. ‘diary’, secondary product group eg. ‘milk’, and GTIN level: product ID, and brand specific product), the less cases were needed before it was possible to point towards an outbreak source. Thus a logistic regression method can identify outbreak vehicles in a real-world CPD, and is, until further methods are explored, a good consensus candidate. Also, analysis of outbreaks using product groups with a granularity that is comparable to most trawling questionnaires, reduces performance compared to outbreaks analysed on item level. The study also indicated that CPD analysis requires participation from all major grocery chains for optimal performance.

WP2-T4- Foodborne outbreaks within healthcare institutions and use of electronic data of patient meals for their control (Formerly: Food distribution data for hospital outbreaks)

The studies described above targeted illness in the community, however foodborne outbreaks may

also take place in hospital and other healthcare settings. For these, individual CPD may not be relevant, but data flows of food purchase by the institutions would be the target of investigation. To explore the possibilities within this area was the focus of WP2.4. It contained two connected but separate paths:

This first aimed to describe the epidemiology of healthcare-associated foodborne outbreaks (HA-FBOs) by conducting a literature review and analysing surveillance data on HA-FBOs. This work has been accepted for publication (Eurosurveillance, 2020, Boone et al). It found 85 HA-FBOs that could be included in an analysis in which the top three associated pathogens were *Salmonella spp*., norovirus and *Listeria monocytogenes*. High mortality was reported in listeriosis HA-FBOs. The most frequently reported food categories for HA-FBOs were mixed foods, such as ready-to-eat sandwiches, vegetables and fruits, and meat and meat products. Associated risk factors included consumption of contaminated ready-to-eat food including risky foods, unprocessed contaminated foods, inadequate heat treatment, insufficient hygiene of kitchen and equipment and pathogen carriers among food handlers. Based on this analysis, it was concluded that the food supply chain for HCFs must be strictly controlled, HCFs should adhere to food safety measures and hold regular food safety trainings for HCF staff. To increase the early detection of HA-FBOs, surveillance of healthcare associated infections should be integrated with surveillance of foodborne diseases which necessitates interdisciplinary collaboration and exchange of information between hospital hygienists, food safety and public health authorities.

The second past aimed to assess whether electronic data of patient meals served in healthcare facilities (HCF) are available and if these data can be used to prevent HA-FBOs and support outbreak investigations.

The study included electronic data records of meals served to patients in hospitals and nursing homes. To investigate the availability, accessibility and usefulness of patient food menu data for outbreak investigations of HA-FBOs, a survey was conducted jointly among 22 HCFs in Italy and 13 HCFs in Germany. HCFs could directly link food menu data to individual patients in 17/19 hospitals in Italy and 3/7 hospitals in Germany, while in nursing homes this linkage was possible in only 1/3, and 1/7 nursing homes Italy and in Germany, respectively. A large variability was reported in the format and the storage duration of patient food data of HCFs. Searchable databases to store patient menu data were more frequently reported among Italian hospitals (15/19) than in German hospitals (3/7), whereas such databases were not reported by nursing homes. Both in Italian and German HCFs, food considered as risky for vulnerable persons were offered on the menu. The survey indicated that the availability and usability of patient food data to support HA-FBO investigations were insufficient in German and Italian HCFs, especially in nursing homes. Therefore, standards should be developed to directly link hospital menu data with individual patients, and to harmonise storage duration of patient food data. The survey suggested a need to increase the awareness to avoid high-risk foods on the menu in HCFs.

An online workshop with the healthcare professionals from the Italian HCFs who participated in the survey was organised by the ISS on the 19th of March 2021. In this workshop, HA-FBOs were described, results of the survey in Italy and in Germany were presented, and traceability to support HA-FBO investigations discussed. In addition, focus group discussions were organised with the participants in order to identify strong and weak points for the identification and control of HA-FBOs. Participants highlighted the importance of electronic food traceability data for patient meals instead of paper-based data, and the need for training and information on food safety both for healthcare workers and patients/caregivers. In addition, a contribution was made to a publication on a listeriosis outbreak investigation involving HCFs in Germany. In this outbreak, prospective calculation of disease association was not feasible because of the scarcity of hospital menu data. The likely food vehicle for this outbreak was identified because of the identification of HCFs and the following food supply analysis of the involved HCFs. To fine-tune recommendations on the fitness of patient meal data for HA-FBO investigations, it would be useful to set up additional discussions with members of the OHEJP network.

WP2-T5- Trace back and food risk mapping

Besides data records of food purchases, trace data on a larger geographical scale may also be used to help locate sources of foodborne outbreaks. This was the focus on WP2.5 which centred on the further development of a previously developed risk tool by the BfR: "FoodChain-Lab". The FoodChain-Lab web application (FCL Web) is an intuitive and user-friendly app that can support public health institutes during foodborne outbreak investigations. It analyses suspicious food items by back- and forward-tracing in the supply chain. The aim of this work was to provide a faster recognition of products potentially involved in foodborne outbreaks. To this end, we implemented the model developed by Kausrud et al. (NVI) that calculates the likelihood of products to be the source of a foodborne-related outbreak based on sales data. This calculation allows investigators to save precious time and resources during outbreaks, since it helps to prioritize food items that should be sampled for laboratory analysis.

Since the calculation of the original model isn't an easy task especially for users lacking IT-skills, we wanted to provide a user-friendly and intuitive solution to facilitate the access to this method. Therefore, we developed a single page user interface using state of the art technology. We created a step by step guide on how to run the model, how to adjust model parameters for improving accuracy, and how the input data should be structured. The user can grade the results of the calculation by reviewing additional information on the data and results displayed on a map. Using EUROSTAT's Local Administrative Unit (LAU) data set, we improved the original model by making it available for the whole European Union, since the original model was designed for Norway.

Besides usability, we also invested quite some effort to make the application as sustainable and secure as possible. Therefore, we followed a modular based approach. This means that elements of the web application are reusable, exchangeable and upgradeable. We re-implemented the original model into a module that can be easily integrated into other solutions. At the same time, it increases the calculation performance of the model and provides reproducible results. Another effect of this modular based approach is that we can calculate the model entirely on the user's machine within the web browser. This increases security, since sensitive data used in the model does not leave the local computer. Hence, the risk of data leakage or hacks of data bases is reduced.

The implementation of the advanced likelihood model into FCL Web increases the availability of this model and provides investigators easy, fast, secure and reliable usage to improve outbreak investigation workflows. It can be accessed at this web address: https://fcl-portal.bfr.berlin. In conclusion, integration of a likelihood model for food risk mapping into the state-of-the-art tracing tool FoodChain-Lab allows for open access use in the scientific community.

WP3. Syndromic surveillance

WP3-T1- Identify the opportunities for SyS of FBD

The objective of this task was to identify potential data sources in Member States with partners contributing to WP3, in order to develop specific surveillance components for FBD based on "secondary data" in tasks 3.2 and 3.3. "Secondary data" are already available information, sometimes collected for other purposes than health surveillance that may be of interest for health surveillance.

WP3-T1-ST1- Food chain mapping

The methodology for the inventory of data sources was defined in the NOVA kick-off meeting in March 2018. We drew a comprehensive map of the food chain, from primary production to human consumption (JRP6-WP3-T1-ST1). A general flow chart of the food chain and its environment was drawn, and information flows associated to the material flows were identified.

WP3-T1-ST2- Data source screening: availability, quality and suitability for SyS

In parallel with the food-chain mapping, we worked on a detailed inventory of data sources existing along this food producing continuum. A table was designed to collect data potentially useful and available for data-driven surveillance of FBD. The table was filled in based on expertise of NOVA WP3 members. Forty-nine data sources were identified as relevant for *Salmonella* surveillance along the food chain in the four countries: 28 were dedicated to animal health, 13 to public health, one in feed, one in the environment sector, and six covered at least two sectors. A wide diversity of data sources was observed, from operational surveillance systems producing daily alerts to isolated databases with no centralisation of data. Potential gaps in surveillance coverage were identified. The general flow chart provided a convenient framework to identify existing data sources for inter-sectoral foodborne zoonosis surveillance. This facilitated pinpointing possible but yet unused data sources. As the identified data sources are planned to be used in task 3.2. for the development of univariate surveillance modules, quality of the data for that purpose were critically assessed (JRP6-WP3-T1-ST2).

WP3-T2- Univariate syndromic surveillance development for FBD (and AMR)

Tasks 2 and 3 were launched at the end of Year 1. Task 1 showed that the three countries participating in T2 and T3 do not have the same level of progress in the implementation and use of SyS monitoring tools. We therefore put in place different strategies depending on the degree of progress. However, we chose the same case study in all three countries: the monitoring of *Salmonella* and *Campylobacter* from animal and food production to human population. We evaluated univariate syndromic surveillance data of many kinds and origins, from high to very low specificity. For example, we tested datasets of *Campylobacter* isolations on broiler carcasses but also syndromic data (digestive symptoms). Datasets had thus various statistical characteristics like stationarity, trend, seasonality, average number of events, etc. These characteristics influenced the results of detection algorithms, based on their configuration. In this context, we applied a wide range of detection algorithms based on various statistical theories with various sensitivity and specificity in order to highlight diverse statistical anomalies. These algorithms were all effective in different ways, with their performance generally being complementary. In order to enhance sensitivity of detection algorithms and thus to increase relevance of identified events, three main points were raised: the combination of several algorithms, the overlap of multiple datasets and, the use of various temporal and spatial resolutions.

WP3-T3- Evaluation of multivariate syndromic surveillance for FBD

We worked on the methods to integrate signals from different univariate syndromic surveillance systems to improve outbreak detection in humans. In Sweden, an explanatory multivariate SyS was developed, in which the value of evidence (Bayesian likelihood ratio) for an outbreak was assessed for each time point (week), by using a Bayesian statistical model. The model could take multiple time series data (human *Campylobacter* cases, positive *Campylobacter* tests in broilers, and weather data) together and the outcome was available at national level. The Norwegian veterinary and public health institutes worked on adding veterinary data (*Campylobacter* in poultry) and environmental data (rainfall, temperature) to the current syndromic surveillance system for human gastro-intestinal outbreaks. We compared several methods for outbreak prediction. A real-time pilot study was planned for spring/summer 2020 but had to be postponed due to COVID 19 crisis. A dashboard was developed for evaluating the possible usefulness and benefits of having this system running in real-time. ANSES worked on the development of detection algorithms which processed data from i) bovine sector (cattle mortality, *Salmonella* isolations in cattle, reports on cattle diseases), ii) food sector (*Salmonella* isolations in beef and dairy products) and iii) human health (number of human gastro-intestinal outbreaks).

WP4. Spatial risk mapping

WP4-T1- Identification of spatial relationships and patterns in Salmonella prevalence

WP4-T1-ST1- Surveillance in high prevalence regions to detect introduction and changes in prevalence.

The high prevalence region chosen for studies in this task was Spain. Surveillance data (animal, human and food) from national official sources have been recorded and spatially analysed to identify possible links between the main stakeholders to strength the surveillance efforts under the one health perspective.

Recorded surveillance information on human cases could only be linked to the administrative level NUTS 3. A descriptive analysis of the clinical data (hospital burden including hospitalization rates and case-fatality rates, costs, and risk factors) was performed to evaluate changes in the burden of the disease.

Recorded surveillance information on food could not been properly linked to their geographical location as that information was found missing in most cases. From the exploration of the surveillance sources, we concluded that even if food alerts were mainly in meat (particularly pork and poultry with a positive rate around 7%, Salmonella-related food-borne outbreaks were mainly related to eggs.

Recorded surveillance information on pigs (isolation and antimicrobial susceptibility typing of *Salmonella*) have been georeferenced and explored to identify a spatial pattern in the probability of isolation of *Salmonella*. Results suggested a West-East increasing risk of Salmonella infection in swine at the province level in Spain, with certain serotype-specific patterns differing (i.e., higher chance of retrieving certain serotypes in certain regions). Association of risk areas with human cases could not be explored because human data does not contain epidemiological information on the possible source of salmonellosis infection.

WP4-T1-ST2- Surveillance in low prevalence regions to reduce prevalence.

The task aimed to develop a disease spread model in the Swedish context (representing a low prevalence region), evaluate different surveillance options, and find direct risks for human salmonellosis using Danish data. Salmonella control has already begun since the 1960s in Sweden, including all serotypes and all animal species. The program gradually reduced the prevalence of Salmonella in Swedish cattle, with the number of cattle found in control programs since the mid-1990s maintained at a low but steady level. To assess potential surveillance strategies, both the within- and between-herd transmission dynamics of S. Dublin infection need to be considered. Within-herd transmission in dairy herds has been extensively studied but approaches to model the between-herd spread of this infection still lack in the literature. In this study, a two-stage transmission model for S. Dublin infections in dairy farms has been developed in the SimInf framework, which is suitable for evaluating the effectiveness of different surveillance strategies in a hypoendemic context. Alternative surveillance options (both traditional and risk-based) to detect infected herds have been evaluated deterministically using simulated disease data. Traditional surveillance could detect 25% of the infected herds after one year, while risk-based surveillance detected around 15% of the cases. However, risk-based surveillance was economically more convenient, as it utilized one-third of the samples used by traditional surveillance. The task of finding direct risks for human salmonellosis using Danish data was delayed due to the difficulty in data sharing (the inability to share sensitive data between SVA and SSI and the inability to share data due to the Covid-19 between SVA and Swedish public health agency). For that reason, the goal of the task was partially modified to develop a method that can measure risks using the Swedish bulk milk screening data instead of human cases. The developed method allows to describe the spatial distribution of S. Dublin in Sweden and quantify associations between S. Dublin-positive herds and hypothesized risk factors. The spatial scan statistical analysis was adopted to evaluate the spatial clustering of S. Dublin and its temporal changes. To evaluate the effect of potential risk factors on the risk of S. Dublin, Bayesian regression models using the INLA-SPDE approach were developed. Two positive clustered areas were detected, which means that these areas are more likely to have high numbers of seropositive cattle to S. Dublin compared to

other regions. These areas are characterized by slightly bigger herd size, high temperature, low precipitation, and fewer contacts with other herds. It remains unclear which factors may be risk factors for spreading S. Dublin infections between cattle herds. Human case data were not available to assess the direct risks for human salmonellosis, but the methodologies applicable to human cases have been sufficiently tested using animal data. The models and methods developed in this sub-task can be applied to other similar diseases or other regions.

WP4-T2- Risk of introduction of Salmonella in pig farms through animal feed.

The focus of this deliverable was changed from its original objective due to the decision to ban formaldehyde in the EU, which was the main scenario that was going to be addressed originally. Because of this, an alternative study, based on the assessment of the usefulness of surveillance AMR data for monitoring emergence of *Salmonella* strains of animal origin, was performed. Preliminary studies performed on resistance data determined in *Salmonella* strains from poultry demonstrated the usefulness of multivariate approaches to detect the emergence of AMR profiles ('resistotypes') that could be then used as targets of surveillance projects (Alvarez et al., 2019). These preliminary studies, conducted on isolates of poultry origin since a large strain panel was available, were further extended for the analysis of swine *Salmonella* strains collected between 2001 and 2017 and subjected to serotyping and antimicrobial susceptibility testing in the frame of the surveillance program for antimicrobial resistance in *Salmonella*.

The analysis carried out in a total of 1,318 pig strains belonging to 63 different serotypes included data on AMR to up to 12 different antimicrobials. Resistance data was analyzed using generalized estimating equations in order to assess the existence of trends in the proportion of resistance strain for each of the antimicrobial, and Bayesian network analysis and hierarchical clustering analysis in order to identify links between the presentation of specific patterns of resistance. Furthermore, the spatial distribution of strains presenting specific resistance patterns was analyzed through the use of empirical Bayesian smoothing methods to identify resistance-resistotype specific spatial patterns. The analyses revealed specific groups of phenotypic resistances that were typically presented simultaneously beyond the ones already expected (e.g., antimicrobials belonging to the same class such as nalidixic acid and ciprofloxacin). This suggests the existence of AMR markers that are probably associated in the bacterial genome, such as those found in Salmonella Genomic Island 1 (with resistant determinants to beta-lactams, phenicols, aminoglycosides, sulfonamides and tetracyclines). We found that certain serotypes were typically associated with such presentations, and had an uneven spatial distribution over the study period.

In addition, assistance in the development of an IT tool for spatial identification of soil vulnerability areas as regards antibiotic residues and resistances has been done.

WP4-T3- Role of the environment in the occurrence and maintenance of Salmonella infection in extensive farming.

Under this task salmonella data in outdoor pigs and wild boar gathered from official surveillance plans in Spain were explored. Data were analysed and displayed in interactive maps to extend the exploration and interpretation of the Salmonella occurrence (story map).

To explore the role of wildlife, a cartographic map of hotspot areas for Salmonella transmission between wild boar and outdoor pigs was produced based on the extensive pig farm and wild boar density (updated map from Bosch et al, 2015). Spatial autocorrelation was analysed using the Getis and Ord statistical test. The weight of each variable was quantified through a sensitivity analysis (1,000 iterations, Monte Carlo method). The "Density of extensive pig farms" showed a greater influence (0.79) compared to "Density of wild boar" (0.41). Results did not reveal an association between hot spots and salmonella in wild boar, probably due to the scarcity of salmonella occurrence data in

extensive pig farms (<1% of total extensive production in the country).

Machine learning algorithms have been implemented to evaluate whether data of salmonella infected farms could be explained by environmental drivers such as temperature, humidity, precipitation or type of soil and vegetation. By using an existing modelling library ('tidymodels'), we were able to apply several machine learning models in one workflow. In particular, random forests and boosted regression trees were explored. Model outcomes were assessed using cross-validation techniques. The model was first successfully validated on surrogate data, and subsequently applied to intensive and extensive farming data. No clear link was identified between infected farms and environmental variables. In particular wild boar density was not identified as a driver. The likely explanation for the failure to detect risk-factors is that the intensive farming system is not affected by environmental factors, while the data on the extensive farming systems were too scarce. As a second case study, we applied the model to data on Salmonella infection in wild boar. Also here, no clear risk-factors could be identified, likely due to the small number of data points. Although the model has not yielded any clearly identified drivers, the developed framework is a valuable outcome that can be readily applied to other settings where there is an interest to correlate spatial absence/presence data on the occurrence of pathogens with environmental and climatic variables.

WP5. Evaluation of surveillance programs & cost efficiency

WP5-T1- Adapt infectious disease models for assessing the effect of surveillance programs in primary animal production on consumer exposure to foodborne pathogens.

This task has been addressed from several different perspectives including a focus on 1. development of model-based approaches to evaluate surveillance in primary production and 2. Models of disease surveillance in primary production that extend to include human exposure.

In the development work on evaluation of surveillance in primary production, methods were established whereby disease spread and surveillance could be simulated simultaneously to efficiently account for both temporally dynamic disease spread and compare various risk-based targeting methods and sampling intensity.

In the task, we worked on three different models:

1) To investigate the utility of these new methods, a model was established of the spread of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in the Swedish cattle population. The model illustrated that the approach allowed to compare surveillance strategies on the standard scale of sensitivity of detection of the disease in the population, but also time to detection, cumulative hazard of detection and the number of herds infected at the time of detection. The method established a foundation to be able to model surveillance strategies and the subsequent downstream effects of interventions and how they could depress the prevalence of disease or the likelihood of disease extinction. In the example of MAP spread in Swedish cattle, the work also illustrated the concept that 'risk based' targeting of herds for surveillance is not always an effective strategy. The results indicate that for MAP, sampling herds randomly resulted in faster detection compared to targeting herds biased towards those that purchase more animals (high in-degree). This finding may be related to the disease dynamics of MAP which is a very slow spreading disease and it is hypothesized that this form of risk based sampling would be more beneficial for a disease with a faster spread because the nodes with higher network centrality are more likely to become infected whereas with slow spread it is more valuable to utilize a strategy that values sampling of more unique herds (Rosendal et al., 2020). The new modelling methods have been made openly available to the community both through publication and sharing of open source developed tools under the GPL3 licence (https://github.com/trosendal/paratb).

2. The same modelling approach was also applied for modelling the Salmonella surveillance in Danish broiler production, with focus on the parent flocks. When a parent flock becomes infected, the infection can be transmitted vertically to the broiler flocks, before the parent flock is detected and

destroyed, including the eggs at the hatchery. To address this issue, we developed a stochastic dynamic modelling of transmission of Salmonella in parent flocks and combined that with the relation between flock prevalence and test sensitivity for environmental samples in the flock. The model was used to investigate the power of the current and alternative sampling schedules. The results show that in the initial phase of spread of infection it is crucial to collect as much sample material as possible in the flocks to detect infection. The model was developed so that it gave parameter estimates relevant for the decision makers. In this case, the decision needs to be taken is that in a situation when a parent flock is detected as Salmonella positive, which eggs should be destroyed at the hatchery to avoid that Salmonella in transferred from the parent to the broiler flock. The parameter estimated as a response to this was the likelihood of infection of eggs at different ages in relation to the date when the flock was tested positive for Salmonella (Apenteng et al., 2020).

3. To explore the potential impact of a national control programme for Salmonella in pigs, a previously developed model for transmission of *Salmonella* in a breeder-finisher pig herd (Hill et al. 2016) was adapted to include surveillance. The surveillance consisted of quarterly pooled faecal sampling for Salmonella Typhimurium (ST) using a PCR test, and control measures were implemented on farms where the number of positive pools was above a specified threshold. To represent the national population of finisher pigs, the model was run many times, with the prevalence of breeding sows drawn from a probability distribution, this distribution being estimated from a Bayesian model applied to the 2008 EU baseline survey for breeding pigs. The model predicted the impact on the infection prevalence of ST in slaughter pigs for different thresholds of the number of positive pools for control of Salmonella on the farm to be implemented. It showed that the prevalence of ST in slaughter pigs in GB would be significantly reduced by targeting control on farms where there were 5 positive pools (out of 5).

WP5-T2- Assessing the effect of using metagenomics in surveillance of foodborne zoonoses.

Traditionally, the surveillance of foodborne zoonotic antimicrobial resistance is based on phenotypic testing of indicator and zoonotic bacteria isolated in the food production chain. The procedure is that samples are collected at different steps in the production line. The presence of antimicrobial resistance has then been investigated both in zoonotic bacteria such as Salmonella, and in indicator bacteria such as E. coli and Enterobacter using different cultivation methods (e.g. MIC).

During the last years, the technological development in sequencing of genetic material directly in the sample matrix, gives the opportunity to investigate the total genetic material of a mixed community of organisms in a sample matrix, such as faecal or environmental samples - metagenomics. Thereby, we can investigate both cultivatable and uncultivatable microorganisms. Metagenomics is a molecular tool used to analyse the mixed genomic materials extracted from environmental samples, which provides quantitative information about presence of genes, including genes coding for antimicrobial resistance (AMR).

In this task we have focused on the surveillance of AMR in the pig production using metagenomics, and how this can be utilised in a national surveillance program with the aim to detect newly introduced AMR and to detect significant increase or decrease in AMR widespread in the pig population.

The modelling approach in this task in similar to the approach in task 1, in the way that we simulate the spread and occurrence of AMR genes in the population. In addition to this, we integrate how pooling of samples and stochastic variation in the sequencing procedure influenced the surveillance data.

The output of the simulation model is counts per million (CPM) of the gene(s) of interest detected per million gene fragment sequenced in a matrix, and the interpretation is on population level. The limit of detection of a gene is 1 count, but because the number of gene fragments sequenced per sample

(sequencing depth) is varying, the limit of detection is varying between analyses. The output from the model depends on i) the actual occurrence of the gene(s) in the population (farm prevalence, within farm prevalence), the concentration of the gene(s) in the animal (in the simulation we collect faecal samples), numbers of sample collected at each time of sampling and pooled, and sequence depth used for the pooled sample. The model has a stochastic nature, taking into account variation due to sampling and sequencing depth. To model the increase in the occurrence of the gene(s) in the population by time, we used a Susceptible-Infected (SI) model describing the spread between farms.

The input parameters describing the occurrence of gene(s) were based on existing data from research studies and expert.

The number of samples collected, pooling and sequencing depth are variables we can decide in the design of the surveillance. Different scenarios were simulated mimicking realistic scenarios for these variables.

The output data from the model represents surveillance data. The surveillance data was compared to the true status in the population (given as input parameters describing the occurrence of the gene(s) in the population). The comparison was done both retrospectively, using time-series analysis to calculate the time between true increase of occurrence and detected increase of occurrence, and prospectively, forecasting the occurrence based on surveillance data, and compared that with the true future (given as the input parameter in the model).

The overall results from the modelling, shows that an increased occurrence of AMR gene(s) was difficult to detect during the initial stage of increase (either after introduction or after increase in the occurrence of already present AMR-genes). The major reason for lack of detection in the earlier stage was that the collected samples did not contain the gene(s). An increased number of samples that is pooled and a simultaneously increase in the sequencing depth could partly reduce the time gap. When the increase in occurrence took place in a low rate, were it takes 5-10 years before all farms has the gene, to keep the time gap between introduction and detection short, relatively more samples in the pool and increased sequencing depth are needed compared to a gene with a faster increase in occurrence.

The application of forecasting algorithms shows that it is possible to forecast the occurrence of AMR-genes in the population, but the precision is varying depending on several factors such as the dynamics of the spread and variability due to sampling.

WP5-T3- Modelling the effect of surveillance programs in the food production on human health. Risk based sampling in the retail phase

The risk-based sampling approach focuses on the question how to subdivide sampling capacity the best in order to monitor as much disability-adjusted life years (DALYs) as possible. The basic idea is that sampling for those pathogen-product combinations is preferred, for which surveillance is cheapest in terms of costs / DALYs monitored. In this study we focus on Salmonella in pig meat in the retail phase. Costs per sample refer to microbiological analysis. The number of samples is set using the criterion of a constant relative uncertainty of estimated pathogen prevalence. The number of DALYS is estimated using an overall pathogen-food animal value, which is subdivided over products using exposure assessment estimates. Risk based sampling methodology was applied to France, United Kingdom, Denmark, Sweden and The Netherlands and this led to two improvements in the methodology, so that the number of food product categories and the number of inhabitants of a country can be taken into account. Ranking of food product groups in terms of costs / DALYs monitored was performed for the respective countries both separately and in combination.

Cost-effectiveness of sampling in the retail phase

We also explored a method to evaluate the cost-effectiveness of product testing in the pre-retail and

retail phase, by food industry and the government, respectively, related to food product withdrawal. The criterion is here costs / DALYs evaded. We focused again on Salmonella in pig meat. Costs refer to microbiological analysis, and the number of samples taken as reported. The effect includes a positive effect on public health, expressed in DALYs, as batches with a positive test will not reach the consumer, or will be heat-treated first. Cost-effectiveness can be compared with a criterion set by WHO. This comparison indicates that the short-term effect of product testing is not cost-effective, the more since it is suspected that the present calculations give too favourable an estimate. On the other hand, uncertainty of the calculations is large. We did not incorporate the long-term effect of product testing on hygiene performance of food industry, which is difficult to quantify.

Cost-effectiveness of sampling in the farm phase

We explored a method to evaluate the cost-effectiveness of surveillance in the farm phase, focusing on Salmonella in pigs, and applying it to The Netherlands and Great Britain. The criterion is costs / DALYs evaded. Costs refer to the number of samples taken, and the costs of microbiological analysis per sample. To calculate DALYs evaded, it is assumed that pig meat from Salmonella test-positive farms does not reach the consumer. It is calculated as a decrease in Salmonella prevalence at animal level as a result of surveillance, which is carried through the slaughterhouse- and consumer phase to result in a reduction of DALYs.

It is shown that cost-effectiveness is independent of the number of farms sampled, and decreases with the number of samples taken per farm. Cost-effectiveness is more favourable (lower) for GB than for The Netherlands, 4.78E3 and 8.70E3 euros/DALY, respectively, for comparable scenarios. Both the costs and the DALYs evaded are higher for GB, being a larger country, but DALYs evaded with a higher factor than costs. It must be stressed, however, that the calculations have a high uncertainty.

Cost-effectiveness of sampling in the farm phase is much more favourable than in the (pre-)retail phase, being about 8.5E3 euro's/DALY vs 6.08E4 or 2.26E5 euro's/DALY (depending on sampling system)(Evers et al., 2020). The value of 8.5E3 euros/DALY is also lower than 4.6E4 euros/DALY, the standard value set by WHO for cost-effectiveness of an intervention (WHO Europe, 2014).

Additional data collection and conceptual model improvement will increase reliability of the calculation results.

5.1.4.3.3.4 Project self-assessment

Main objectives initially proposed have been accomplished. We have had a collaborative partnership with several new collaborations initiated between veterinary and medicine institutes within countries, as well as between countries. A number of novel tools have been developed, both for research and surveillance (model scripts) and dissemination (story map). Improvement of operational surveillance systems in human health were obtained by adding surveillance components relative to animal health and to environmental conditions.

Covid-19

One of the main problems that occurred during the project period was the covid-19 pandemic. The project was formed to include partners from both animal and public health and from several member states. We were happy to create such a diverse group, but many experts and researchers in NOVA had never worked together before the start of the project. Therefore, it took some time before all participants understood and agreed on the practical implementation of all tasks and subtasks in the project plan. In the second year, work started to speed up, but when the pandemic hit many tasks slowed down and it was a serious disturbance to the project. Well-established everyday working routines were altered, face-to-face meetings were not possible, and many experts, especially in the public health institutes, had to prioritise work tasks directly related to covid-19. The extension of the project into 2021 helped us finish our deliverables but did not fully compensate for the impaired connection in our newly formed network. However, many new links have been created and there are

several ideas about how we can capture potential synergies between work that we did and plan for that future, and how we can continue our collaboration.

Data access and quality

Several WPs encountered difficulties to access data and share data between institutions, also within country, due to confidentiality, budget, slow retrieval processes, or data ownership issues. These difficulties were not a surprise, in fact data access was already a focus in our WPs. These obstacles were handled in different ways, for example by aggregating data or changing to another disease of interest. This was not necessarily a problem, as the focus of NOVA has been the epidemiological methods, not one specific disease. However, aggregation of data leads to a loss of information and in a few tasks (in WP4 and WP5), the difficulty or slow process to share human case data resulted in less multidisciplinary outcomes. To compensate for this we have chosen to build tools and models that are general enough to be used for other diseases and data. One approach has been to include synthetic human case data in a model framework, so that the model is fully developed and ready to use once real data are available. In WP2, progress to access large food consumer data was made, but was later withdrawn.

In WP3 problems of data quality were encountered when setting up systems for using secondary data for surveillance. Specific collaborations had to be built with the data owners to improve the usability of their data for an objective of surveillance.

Other deviations and/or problems encountered:

One challenge that appeared already at the start of the project related to the work on one health terminology (WP1, T1). We soon found out that similar work was planned in other OHEJP projects and in the beginning it was unclear how this overlap should be handled. After discussion we concluded that we wanted to avoid any double work, and that it made sense to contribute to one glossary. The ORION project had a glossary with a broader scope in their plan, and in the end, ORION took full lead in this work. The involvement of NOVA and role in the collaborative work was negotiated and focused on terms related specifically to surveillance.

Deliverable 4.6. was replaced because of changes of legislation in the product that was going to be evaluated (formaldehyde). Alternatively, studies on the use of surveillance AMR data for monitoring emergence of Salmonella strains in swine were conducted.

In WP5 task 1, the transmission model for Salmonella in pigs was originally intended to be a between-herd transmission model. However, **staff departures and delays to recruitment** meant that we had to adapt an existing within herd transmission model. This meant that we had to restrict the evaluation of control measures to finisher pigs, rather than also considering breeding herds.

In WP task 2, **no historical samples were available** that could be re-analysed using new sequencing methods and therefore it was not possible to conduct a comparison based on true emerging health problems in the human population and if it would have been possible to detect it the food production before it became a human health issue. Instead, the task was utilising simulation techniques to estimate how quick an emerging problem can be detected in the primary production.

5.1.4.3.3.5 Progress of the project: milestones and deliverables

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
06	D-JRP6-0.1	Documentation of consortium assembly and steering committee meeting	M3	M3		Public https://zenodo.org/record/5497264#.YTsQwp0zY-U Available on OHEJP	8 (meeting documentation)
06	D-JRP6-0.2	Documentation of consortium assembly and steering committee meeting	M20	M20		Public https://zenodo.org/record/5497532#.YTsQip0zY-U Available on OHEJP	8 (meeting documentation)
06	D-JRP6-0.3	Documentation of consortium assembly and steering committee meeting	M35	M35		Public https://zenodo.org/record/5497307#.YTsRLZ0zY-U Available on OHEJP	8 (meeting documentation)
06	D-JRP6-0.4	Documentation of consortium assembly and steering committee meeting	M42	M45		Public https://zenodo.org/record/5499696#.YTsPCRlxd3g Available on OHEJP	8 (meeting documentation)
06	D-JRP6-1.1	Glossary of terms based on a common food borne zoonosis surveillance terminology	M24	M24		Public https://zenodo.org/record/3734079#.XoL5CIgza70 Available on OHEJP	1, 8 (glossary)

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06	D-JRP6-1.2	Mapping of food chain surveillance across countries	M41	M42		Public https://zenodo.org/record/5045224#.YQ5754gzZPY Available on OHEJP	1, 8 (interviews)
06	D-JRP6-2.1	Description by member state of data sources, agreements and organisations (e.g. retailer chains).	M10	M12		Public https://zenodo.org/record/5497406#.YTsOexlxdPY Available on OHEJP	
06	D-JRP6-2.2	Description by member state of barriers for use: legal, political, economic, practical and technical obstacles.	M24	M24		Public https://zenodo.org/record/5497418#.YTsZSJ0zY-U Available on OHEJP	
06	D-JRP6-2.3	Structured review of the field.	M5	M5		Public https://zenodo.org/record/5497396#.YTsZhp0zY-U Available on OHEJP	
06	D-JRP6-2.5	Description of data infrastructure module, including an electronic informed consent tool (Original title: Data	M12	M12		Public https://zenodo.org/record/5497425#.YTsZCp0zY-U Available on OHEJP	

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		infrastructure built, electronic informed consent tool in function.)					
06	D-JRP6-2.6	Case control study of food risk factors for sporadic Salmonella infections. (Formerly: Case control study of food risk factors for sporadic campylobacter infections)	M42	M45		Public https://zenodo.org/record/5500418#.YTt8-hlxd3g Available on OHEJP Deadline pushed forward as part of extension of the project	
06	D-JRP6-2.4	Simulation analyses to assess the use of consumer purchase data as an analytical tool for outbreak investigations (Formerly: Assessment of the use of the method as an analytical tool, rather than merely	M42	M45		Public https://zenodo.org/record/5500406#.YTt78Rlxd3g Available on OHEJP Due to the COVID-19 outbreak and the impact that has had on the possibilities of the SSI to engage in large population studies, this project may need to be redefined. Also, work has been paused throughout the spring of 2020, because the researchers involved have been moved to work on the COVID-19 response exclusively. We	4, 7, 9

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention: **public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)**, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
		hypothesis-generation)				would like to propose to move the deliverable and the corresponding milestone (they are part of the same project) to the end of the new project period, i.e. Month 42	
06	D-JRP6-2.10	FoodChain-Lab web application: Description and documentation of software, development of tutorials and training material (Formerly: Description and documentation of software, development of tutorials and training material)	M42	M45		Public https://zenodo.org/record/5497431#.YTsVWJ0zY-U Available on OHEJP Deadline pushed forward as part of extension of the project	
06	D-JRP6-2.8	Epidemiological analysis of existing surveillance data regarding ha-FBO and foods involved.	M10	M12		Confidential Available on OHEJP, NOVA private group only	

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06	D-JRP6-2.9	Studies using the German results extended to the partner institutes.	M24	M24		Confidential Available on OHEJP, NOVA private group only	
06	D-JRP6-3.1	Full mapping of the chain process for three main productions in E.U	M8	M8		Public https://zenodo.org/record/3734082#.XoL5b4gza70 Available on OHEJP	1
06	D-JRP6-3.2	Data inventory with assessment of availability, quality and fitness for SS	M10	M10		Public https://zenodo.org/record/3734084#.XoL5q4gza70 Available on OHEJP	1
06	D-JRP-3.3	Description of the SS components implemented and guidelines for their use	M24	M24		Public https://zenodo.org/record/5497514#.YTsXGZ0zY-U Available on OHEJP	1,4
06	D-JRP6-3.4	Recommendations about the quality standardisation of data produced across the food chain for their use in SyS ()	M30	M30		Public https://zenodo.org/record/5497473#.YTsYyp0zY-U Available on OHEJP	1, 4

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
06	D-JRP6-3.5	Contribution of multiple syndromic surveillance components in the FBD surveillance	M42	M42		Public https://zenodo.org/record/5497483#.YTsUx50zY-U Available on OHEJP Due to the COVID-19 crisis, NIPH employs two-part time workers for working on NOVA since December 2020.	4,5
06	D-JRP6-4.1.	Maps for *Salmonella* prevalence geographical patterns in intensive livestock and slaughterhouses completed in high prevalence regions	M12	M12		Public https://zenodo.org/record/5497488#.YTsUpZ0zY-U Available on OHEJP	5,6
06	D-JRP6-4.2.	Identification of periods with higher probability of detection of infection identified in high prevalence regions and temporal evidences for an association with human cases	M24	M24		Public https://zenodo.org/record/5497506#.YTsUdZ0zY-U Available on OHEJP	5,6

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06	D-JRP6-4.3.	Assessment of the spatio-temporal infection dynamics model in *Salmonella* in low prevalence regions.	M12	M12		Public https://zenodo.org/record/5497494#.YTsTa50zY-U Available on OHEJP	5,6
06	D-JRP6-4.4.	Assessment of the spatio-temporal infection dynamics model of Salmonella in low prevalence regions – Evaluation of optimal surveillance strategies	M24 (part1), M36 (part2)	M24 (part1), M36 (part2)		Public https://zenodo.org/record/5499762#.YTsgjhlxeMp Available on OHEJP	5,6
06	D-JRP6-4.5.	Characterization of the spatial network structure of the pig industry in a Mediterranean scenario and *Salmonella* data mapped and analysed.	M24	2019-12-20		Public https://zenodo.org/record/5497518#.YTsTQ50zY-U Available on OHEJP	5,6

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
06	D-JRP6-4.6.	Assessment of the potential effect of the withdrawal of the use of formaldehyde-based feed treatments done	M42	2021-06-05		Public https://zenodo.org/record/5497496#.YTsTAZ0zY-U Focus changed to: Assessment of the usefulness of antimicrobial susceptibility test results for the optimization of surveillance programs for Salmonella of swine origin	5,6
06	D-JRP6-4.7.	*Salmonella* data in extensive farming in Mediterranean scenario mapped and analysed.	M12	2018-12-15		Public https://zenodo.org/record/5497498#.YTsS1J0zY-U Available on OHEJP	5,6
06	D-JRP6-4.8.	Cartographic map of hot spot areas for *Salmonella* transmission between wild boars and low biosecurity systems.	M24	2019-12-20		Public https://zenodo.org/record/5497504#.YTsSlZ0zY-U Available on OHEJP	5,6
06	D-JRP6-4.9.	Potential new environmental surveillance indicators identified.	M42	M42		Public https://zenodo.org/record/5497471#.YTsSZJ0zY-U Available on OHEJP	5,6

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06	D-JRP6-5.1	Report comparing performance of surveillance strategies	M42	M45		Public https://zenodo.org/record/5497957#.YTsSLp0zY-U Available on OHEJP D-JRP6-5.1 - Report comparing performance of surveillance strategies" and "D-JRP6-5.2 - Recommendations for metrics to evaluate surveillance performance, will be joined into one deliverable. Deadline after extension of the project is M42 (36+6).	1, 5, 7, 9
06	D-JRP6-5.2	Recommendations for metrics to evaluate surveillance performance	M42	M45		Public https://zenodo.org/record/5497957#.YTsSLp0zY-U Available on OHEJP D-JRP6-5.1 - Report comparing performance of surveillance strategies" and "D-JRP6-5.2 - Recommendations for metrics to evaluate surveillance performance, will be joined into one deliverable. Deadline after extension of the project is M42 (36+6).	1, 5, 7, 9
06	D-JRP6-5.3	An assessment of the public health effects of very different surveillance strategies to detect emerging foodborne infections in	M42	M45		Due to lag in recruitment this activity started later than first planned	2, 5, 9

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted : Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
		a MS or at European level.					
06	D-JRP6-5.4	Report assessing the quantitative effect on human health of changing surveillance capacity across different sources in an MS	M42	M42		Public https://zenodo.org/record/5497485#.YTsRx50zY-U Available on OHEJP Deadline pushed forward as part of extension of the project.	5, 6
06	D-JRP6-5.5	A practical risk based sampling approach that combines exposure to zoonoses with disease burden and costs for sampling at a European level.	M42	M42		Public https://zenodo.org/record/5497451#.YTsRe50zY-U Available on OHEJP Deadline pushed forward as part of extension of the project.	1, 5, 6

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
06	M-FBZ1.NOVA.1	Consortium assembly and steering committee meeting	M2	Yes		
06	M-FBZ1.NOVA.2	Meeting for information exchange (data, literature, data bases) exchange on exposure assessment, DALY's, consumption data and food handling at home	M6	Yes		
06	M-FBZ1.NOVA.3	Food Chain mapping completed, data sources identification advanced, and ready to start developing the SS components	M7	Yes		
06	M-FBZ1.NOVA.4	Pilot, data availability.	M9	Yes		
06	M-FBZ1.NOVA.5	Case-study choice	M9	Yes		
06	M-FBZ1.NOVA.6	Surveillance strategies to implement into the models agreed	M12	Yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
06	M-FBZ1.NOVA.7	Basic model describing models describing spread of emerging zoonoses in immunological naïve animal population established	M12	Yes		
06	M-FBZ1.NOVA.8	SOP, best practice description.	M16	Yes		
06	M-FBZ1.NOVA.9	Development of method to handle time and GTIN.	M17	Yes		
06	M-FBZ1.NOVA.10	Prospectively calculation of disease association measures and retrospective analysis of food consumption patterns in hospitals.	M24	Yes		
06	M-FBZ1.NOVA.11	complete specification of the infectious disease models	M18	Yes		
06	M-FBZ1.NOVA.12	Submodels to be integrated in the model framework identified and coded in common language	M18	Yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
06	M-FBZ1.NOVA.13	Consortium assembly and steering committee meeting	M19	Yes		
06	M-FBZ1.NOVA.14	SS components developed and tested	M22	Yes		
06	M-FBZ1.NOVA.15	Glossary of surveillance terminology for foodborne surveillance is disseminated.	M24	Yes		
06	M-FBZ1.NOVA.16	Result reporting on the spatio-temporal patterns of infection distribution in intensive pig farms, slaughterhouses and human cases in high prevalence regions and evidences for an association between them.	M24	Yes		
06	M-FBZ1.NOVA.17	Result reporting on the spatio-temporal patterns of infection distribution in intensive pig farms, slaughterhouses and	M24	Yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
		human cases in low prevalence regions and evaluation of optimal surveillance strategies.				
06	M-FBZ1.NOVA.18	Result reporting on hot spot areas for Salmonella transmission between wild boars and low biosecurity systems.	M30	Yes		
06	M-FBZ1.NOVA.19	The model parts to detect emerging pathogens in the food-chain and detecting human cases, respectively implemented in the model.	M24	Yes		
06	M-FBZ1.NOVA.20	Interim report for a practical risk based sampling approach that combines exposure to zoonoses with disease burden and costs for sampling at a European level.	M24	Yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
06	M-FBZ1.NOVA.21	Integration into FoodChain-Lab, release as cloud service.	M27	Yes		
06	M-FBZ1.NOVA.22	Case control study of food risk factors for sporadic salmonella infections.	M40	Yes		Due to the COVID-19 outbreak and the impact that has had on the possibilities of the SSI to engage in large population studies, this project may need to be redefined. Also, work has been paused throughout the spring of 2020, because the researchers involved have been moved to work on the COVID-19 response exclusively. We would like to propose to move the deliverable and the corresponding milestone (they are part of the same project) to the end of the new project period, i.e. Month 40
06	M-FBZ1.NOVA.23	surveillance layer added to models	M30	Yes		
06	M-FBZ1.NOVA.24	A dynamic modelling layer of surveillance is overlaid the submodels	M30	Yes		
06	M-FBZ1.NOVA.25	initial set of results circulated to project team	M36	Yes		
06	M-FBZ1.NOVA.26	Consortium assembly and steering committee meeting	M34	Yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
06	M-FBZ1.NOVA.27	Integration of multiple sources developed and tested	M36	Yes		
06	M-FBZ1.NOVA.28	Result reporting on risk of Salmonella introduction in pig farms by animal feed and the potential effect of the withdrawal of the use of formaldehyde-based feed treatments.	M42	Yes		
06	M-FBZ1.NOVA.29	Result reporting on new environmental surveillance indicators.	M42	Yes		

5.1.4.3.3.6 Follow-up of the recommendations and comments by the Ethics Advisors

Comments already followed up and accepted by the Ethics Advisors.

5.1.4.3.3.7 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Identifying emerging trends in antimicrobial resistance using Salmonella surveillance data in	YES		Yes (processing fees: 3750€)

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
poultry in Spain. TBDE, 67(1):250-262. DOI: 10.1111/tbed.13346 https://zenodo.org/record/3660026#.XvyQnCgzZM0			
Climatic and topographic tolerance limits of wild boar in Eurasia: Implications for their expansion. Geography, Environment, Sustainability, 13(1):107-114. https://doi.org/10.24057/2071-9388-2019-52 https://zenodo.org/record/4244729#.X6LYN4hKjcc	YES		YES (no processing fee)
Spatial trends in Salmonella infection in pigs in Spain. Frontiers in veterinary science. In Press. (A). ISSN: 2297-1769. DOI: 10.3389/fvets.2020.00345 https://zenodo.org/record/4244797#.X6Li_IhKjcc	YES		YES, processing fee 2048 € (2490 USD)
An outbreak of monophasic Salmonella Typhimurium associated with raw pork sausage and other pork products, Denmark 2018–19 https://zenodo.org/record/4249319#.X6UlWmhKjcc	YES		YES (no processing fee for this publication)
Analysis of consumer food purchase data used for outbreak investigations, a review https://zenodo.org/record/3747633#.XpCQ_sgzZM0	YES		YES (no processing fee)
Non-Typhi, non-Paratyphi Salmonella related hospitalisations in Spain: trends, comorbidities, risk factors for worse prognosis and hospital costs. 10.1007/s10096-018-3433-1	YES	YES Embargo length = 24 months Manuscript release date = 20 November 2018	

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
https://zenodo.org/record/4244788#.X6LhGYhKjcc			
Salmonella Surveillance Systems in Swine and Humans in Spain: A Review. 10.3390/vetsci6010020 https://zenodo.org/record/3635293#.XjlyUGhKhPY	YES		YES (no processing fee)
Using stochastic dynamic modelling to estimate the sensitivity of current and alternative surveillance program of Salmonella in conventional broiler production 10.1038/s41598-020-76514-3 https://zenodo.org/record/4271431#.X65ljTiWxPY	YES		YES, processing fee 1570€
Modelling spread and surveillance of Mycobacterium avium subsp. paratuberculosis in the Swedish cattle trade network https://doi.org/10.1016/j.prevetmed.2020.105152 https://zenodo.org/record/4450338	YES		YES, processing fee 2837€ (3450 USD), excluding taxes
A one health glossary to support communication and information exchange between the human health, animal health and food safety sectors. https://doi.org/10.1016/j.onehlt.2021.100263 https://zenodo.org/record/4769914#.YT9EEp0za70	YES		YES (no processing fee)
What motivates and prevents Swedish veterinarians reporting disease suspicions?	YES	Not yet published	Not yet published

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Manuscript in preparation by Oliviera V.H.S., Ågren E.C.C., Frössling J., and Nöremark M. Part of D-JRP6-1.2			

Additional output

The OHEJP glossary is available at the ORION project knowledge hub:

https://aginfra.d4science.org/web/orionknowledgehub/

STORY MAP: https://arcg.is/1zHSub. Summary of main outputs, available online.

SCRIPT REPOSITORY: https://github.com/SVA-SE/NOVA. Contains code to explore disease surveillance options in a low prevalence setting of Salmonella Dublin in cattle.

SCRIPT REPOSITORY: soon available. Contains code to evaluate whether data of salmonella infected farms could be explained by environmental drivers.

Boone I., Wilking H., Eckmanns T., Stark K., Ethelberg S. and Haller S. (2019). Healthcare-associated foodborne outbreaks in OECD countries and a special focus on Germany: current status. 1st Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats (22nd-24th May 2019, Dublin, Ireland). Oral communication. https://zenodo.org/record/5244658

Cha, W., Dórea, F., Grøneng, G.M., Rø, G., Hopp, P., Jonsson, M., Dryselius, R. Development of One Health syndromic surveillance for Campylobacter in Norway and Sweden. In: Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats; May 27-29, 2020; Online meeting.

Gustafsson, W., Andersson, M.G. Designing multivariate syndromic surveillance for animal diseases in Sweden. In: Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats; May 27-29, 2020; Online meeting.

De la Torre, A; Rodriguez, A; Álvarez, J; Teng, K; Swart, A; Kim H; Beninca, E. Novel approaches for design and evaluation of cost-effective surveillance across the food chain: a story map. ESRI conference. 21-22 october 2020. Madrid, Spain. Online meeting.

Iglesias, M. Martínez, J. Álvarez, K. Teng, A. de la Torre. Spatial distribution and temporal trends for salmonella in pork and humans (2010-2015). I Congreso Virtual de la Sociedad Española de Epidemiología (SEE) y da Associação Portuguesa de Epidemiologia (APE), 21-30 Octubre 2020. Online meeting.

Boone I., D'Errico M. L., Iannetti L., Scavia G., Tozzoli R., Ethelberg S., Eckmanns T., Stark K., Wilking H. and Haller S. (2020). Electronic data on food served in healthcare facilities in Italy and Germany. Feasibility evaluation to support investigations of healthcare associated foodborne outbreaks. 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats (Prague, Czech Republic) (27th – 29th May, Online Meeting). Oral communication. https://zenodo.org/record/5244713

Iannetti, L., Boone I., D'Errico M.L., D'Orsi F., Ricchiuti L., Centorotola G., Pomilio F. and Cornacchia A. (2021). Foodborne outbreaks surveillance in hospitals and nursery homes: investigation on catering data. 14th European Public Health Conference (Dublin, Republic of Ireland, 20th-21st November 2021, online meeting). Poster. https://ephconference.eu/programme-at-a-glance-83. Abstract reference S202100519

Boone I., D'Errico M L., Iannetti L., Ethelberg S., Eckmanns T., Rosner B., Stark K., Haller S., Wilking H. (2021). Training for the Public Health Service in Germany. Healthcare-associated food-borne outbreaks. 24–26 March 2021, Online meeting. Oral communication. https://www.bfr.bund.de/cm/343/oegd-2021-programm.pdf

Boone I., D'Errico M L., Scavia G., Tozzoli R., Iannetti L., Haller S., Wilking H. Nova Project: results of the

study in German Hospitals and Nursing homes. Scavia G., D'Errico M.L., Cattaneo C., Anniballi F., Tozzoli R., Boone I., Iannetti L. (2021). Workshop for healthcare practitioners: foodborne outbreak preparedness in healthcare settings. Rome, Online Workshop, 19 March 2021. Oral Presentation

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

We suggest that project outcomes are shared through the NOVA Story Map. Story maps are ideal for dissemination of main achievements to national and international stakeholders and citizens, providing an interactive interface to bring them knowledge through a user-friendly and easy way. It could be published on the OHEJP twitter account.

Most of the modelling tools (scripts) developed in NOVA are based on freeware and available through open script repositories (see Additional output above).

Are there any outcomes of this project that are already discussed or even implemented and in use at any institute of the project consortium, at stakeholders' organisations (ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), or at the level of national authorities?

- Deliverables have been and will be shared with **national governments** and **private stakeholder**, specifically with the data owners who shared the data to feed the analysis. In some cases they have been involved in data and results discussion and appears as co-authors of the scientific publications generated.
    - Within NOVA, Anses explored several data sources regarding Salmonella detection in farm animals, food and humans in France. We developed a combination of several algorithms and tools for result visualization that will be transmitted to data owners in order to improve their surveillance systems (2022). The interest of such algorithms is discussed with the National Platform for Animal Health Surveillance (ESA) and the Health monitoring platform for the Food Supply Chain (SCA).
- **Swedish Board of Agriculture**. Our models and methods have been used as effective tools for the national surveillance plan that is adopted by the Swedish Board of Agriculture based on recommendations from SVA
- **Animal health organizations**; Växa Sverige and Gård & Djurhälsan (Sweden)
- **Dutch ministry of public health, welfare & sports, Dutch wildlife health center and centre for monitoring of vectors**
- **Spanish Agriculture Ministry**
- **Spanish Health Ministry**
- **Junta de Andalucía**, Spain
- **French surveillance networks on animal health (ESA) and on food safety (SCA)**. Results from NOVA on *Salmonella* surveillance has been transferred to the ESA and SCA networks. Further works will follow.
- **Ministry of Environment and Food of Denmark**. NOVA results used in prediction or the occurrence of AMR in Danish pig production when altering the AMU.

- **Danish National Food Authorities**. As a response to the work done in WP5 task 1 about Salmonella in poultry production, the Danish national food authorities have in cooperation with the industry revised the legislation about how to handle Salmonella in both the parental and the production part of the production. Three executive orders has been revised based on the output from the research:
    - Control of Salmonella in slaughter poultry
    - Control of Salmonella in hatching egg production
    - Control of Salmonella in the production of eggs for consumption
- **Meat industry and Food Safety Authority, the Netherlands**. The use of the Cost-effectiveness measured as [DALY/Euro] for evaluating the performance of surveillance of foodborne diseases (and other diseases) in the food chain (more precisely the retail phase) was discussed with meat industry and food safety authority in the Netherlands.
- Story map link was shared with **EFSA** after the second annual meeting. We have also had contact with EFSA's Advisory Forum Task Force on Data Collection and Data Modelling

5.1.4.3.3.8 One Health impact

Overall summary of One Health impact

The project contains cultivation of new methods as well as more efficient utilisation of existing data and methods. It offers ideas for direct surveillance tools, but also for tools directed towards evaluation of surveillance systems. The tools and methods are primarily developed with a focus on the currently most important/frequent zoonotic diseases but may also be adapted to control other hazards or emerging agents.

The surveillance of consumer purchase patterns is currently non-existent in Europe. Results from WP2 shows that this genuinely new surveillance approach is potentially valuable and cost-effective. The methods/tools for surveillance of food exposures and trace-back developed, includes tools that presently do not exist anywhere in the world (WP2).

This project's development and combination of syndromic surveillance systems, machine-learning methods, and use of new data sources, is a first bridge across the Med-Vet gap in the syndromic surveillance context (WP3).

Understanding the geographical transmission of diseases is a cornerstone in epidemiology, yet surprisingly rarely used in surveillance practise – the focus on spatial mapping and analysis will provide better possibilities for actual utilisation in the zoonotic disease community (WP4). It will be possible to apply developed models or analytical methods cross-over to other diseases or objects (different animal species or human).

Mathematical modelling is another cornerstone of modern disease transmission understanding that we are now using to find ways to actually measure surveillance performance, and thereby be able to compare the cost-efficiency of different surveillance strategies, considering potential disease spread across the food chain (WP5).

Finally, our mapping of available data sources and identification of surveillance key actors across the food chain, including the underlying reasons for sub-optimal surveillance, should help clarify surveillance structures and challenges across the EU. Moreover, the development of a One Health glossary is useful in promoting communication between different sectors (WP1).

Specific impact points

- The identification and reporting of barriers and opportunities in food-borne disease surveillance from a One Health point of view is the result of a research performed for the first time using a scientific methodology (WP1). This information is already requested from national authorities (e.g. Belgium, Sweden, France), while other projects have contacted WP1 members for collaboration and consultation on similar or following tasks (e.g. Matrix).

- Within NOVA, Anses has explored several data sources regarding **Salmonella detection in farm animals, food and humans in France**. We developed a combination of several algorithms and tools for result visualization that will be **transmitted to data owners** (National Authorities, National Laboratories and private stakeholders) in order to **improve their surveillance systems**. Specific detection algorithms that can process temporal signals from correlated time series simultaneously are under development. The interest of such algorithms will be discussed with the National Platform for Animal Health Surveillance (ESA) and the Health monitoring platform for the Food Supply Chain (SCA) in France.

- Methods developed in NOVA WP3 are also used in a **national Campylobacter project in Sweden**. One of the aims for this project is to assess the temporal correlation between Campylobacter surveillance data in broilers and human campylobacteriosis incidence.

- In Norway, we made a **One Health surveillance based on the use of gastrointestinal consultations in human (NorSySS)** and Campylobacter surveillance data from broiler flocks. We combined the data sources with weather data to improve detection and prediction of outbreaks. The NorSySS data was already in daily use for syndromic surveillance for people in Norway and any improvements found can be implemented in the day-to-day surveillance activities. The website and the models will continue to live beyond the OHEJP NOVA project and also be extended further in the OHEJP MATRIX project.

- Analyses carried out in task 4.1 will help to assess the reliability of the current surveillance system to capture the underlying variability in terms of serotypes and antimicrobial resistance for Salmonella strains recovered from swine. This can help to assess the power of the sample to describe the spatio-temporal epidemiology of Salmonella infection in a high prevalence area (Spain) in swine, and to identify factors associated with the emergence of specific serotypes/strains in specific areas/farms/production systems in the country but not others.

- Defra (UK) have plans to consider a national control programme (NCP) for Salmonella in pigs. The models built as part of WP5 for the simulation of surveillance strategies in pigs will be useful for evaluating options for such an NCP. The model and its results have been presented to **Defra**. Part of the modelling for Salmonella in pigs required Bayesian estimates of pig slaughter prevalence, derived from abattoir surveys – these estimates were useful for another EU project (**COMPARE**), for the **source attribution** task.

- The Danish Veterinary and Food Administration and the Danish poultry industry has based on the results from the model developed in WP5-task1, **decided about strategies for destroying eggs at the hatcheries after detection of Salmonella** in parent flock, including compensation to the producer. The Danish poultry industry is pioneering industry for controlling Salmonella

in the poultry sector.

- The work conducted in WP5 T2 is a part of the **ongoing revision of surveillance of AMR in the Danish animal and food production (DANMAP)**. In the future, this surveillance will be based on **metagenomics** rather than cultivation, that is used today. Thereby the approach and opportunities for collection of samples will be changed. The integration of the surveillance in the animal and food production will increase the opportunity to get the food production more integrated with other sectors of the One Health concept.

- In Sweden, output from NOVA has been used to support the Swedish Board of Agriculture and the animal health organisations in their work **to adapt surveillance activities and Swedish legislation to the new Animal Health Law** within the EU. The models have been used to support decisions about the target confidence level of future surveillance and to compare surveillance alternatives (e.g. different sample size, sample type, frequency of testing).

- As a response to the work done in WP5 task 1 about Salmonella in poultry production, the Danish national food authorities have in cooperation with the industry **revised the legislation about the monitoring and management of Salmonella** in both the parental and the production part of the poultry production.

- Calculations showed that sampling in the veterinary (farm) phase is much more cost-effective (DALY/Euro) than in the retail phase, given that the sampling result has consequences in terms of animal/product withdrawal.

5.1.4.3.3.9 Data Management Plan

The DMP has been uploaded OHEJP project group site. Contact details are all included in the DMP, and data FAIRness aspects (fulfilments or shortcomings) listed for all appropriate data sources.

At the start of the project, there was no common OHEJP template or system available. Experience from setting up DMPs was also limited within the consortium. After some training, and guidance provided by the responsible person within the OHEJP management, we chose to use an overarching plan/template with detailed Excel-files for each WP and task to keep track of how data is handled. Potential updates have then been handled by each WP.

5.1.4.3.3.10 List of dissemination and communication activities

Name of the activity:	SVA research day		
Date:	November 16-17, 2020		
Place:	SVA, Uppsala, Sweden		
Specify the Dissemination and Communication activities linked to the One Health EJP project			
	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	No
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	Yes
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	Yes	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other	Poster
Communication Campaign (e.g. Radio, TV)	No		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)	80	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	ANSES scientific meeting
Date:	September 2021 (on line sessions)
Place:	Maisons-Alfort, France

Is syndromic surveillance suitable for surveillance of food-born zoonosis?			
	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	No
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	Yes
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other	No
Communication Campaign (e.g. Radio, TV)	No		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)	200	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	The One Health EJP Annual Scientific Meeting
Date:	9-11 June 2021
Place:	Copenhagen · Denmark

MAPPING THE FOOD CHAIN TO IDENTIFY NEW OPPORTUNITIES FOR FOODBORNE ZOONOSIS SURVEILLANCE			
	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	No
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	Yes
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other	Poster
Communication Campaign (e.g. Radio, TV)	No		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)	300	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	The One Health EJP Annual Scientific Meeting
Date:	9-11 June 2021
Place:	Copenhagen · Denmark

Is syndromic surveillance suitable for surveillance of food-born zoonosis?			
	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	No
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	Yes
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other	No
Communication Campaign (e.g. Radio, TV)	No		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)	300	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	*De la Torre, A. Role of the wildlife-livestock interface in zoonosis spreading.* *Risk Assessment Research Assembly (RARA)* *https://www.efsa.europa.eu/sites/default/files/event/180207/180207-Posters-Presentations.pdf*
Date:	*7th February 2018*
Place:	*Utrecht, Netherlands*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other (presentations book)***	*Yes*
Communication Campaign (e.g. Radio, TV)	*No*		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***50***	***Media***	
Industry		***Investors***	*25*
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers	***25***		

Name of the activity:	*Martínez, M; Álvarez, J; Garrido, M; de la Torre, A. Monitoring systems of salmonella in Spain to assess a "one health" approach towards a potential risk to humans from ingestion of contaminated pork meat. International Meeting on Emerging Diseases and Surveillance. http://imed.isid.org/downloads/PosterAbstracts2018.pdf*
Date:	*November 9-12, 2018*
Place:	*Vienna, Austria*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other (abstract book)***	*Yes*
Communication Campaign (e.g. Radio, TV)	*No*	***Other (QDR code)***	*Yes*

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***1000***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	Second Annual Assembly
Date:	7-8 March 2019
Place:	Eurostation Brussels (Sciensano)

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	yes	Participation to a Conference	No
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (presentations book)	Yes
Communication Campaign (e.g. Radio, TV)	No		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	39	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Project presentation at OHEJP PMC meeting
Date:	9 May 2019
Place:	ANSES, Paris

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	No
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	yes
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	Nos
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	?	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	OHEJP: How is Sciensano involved?
Date:	5 June 2019
Place:	Sciensano Uccle, Brussels

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	Nos
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	35	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	11		

Name of the activity:	XXXVII Annual scientific meeting from SEE XVIII Congress SESPAS - XIV Congress APE
Date:	September 2019
Place:	Oviedo, Spain

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	Yes*
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	500	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

* Iglesias, I; Martínez, M; de la Torre, A. 2019. Soluciones a la falta de datos en fauna silvestre dentro del concepto one health aplicando el análisis espacial. Gaceta Sanitaria, 33:210. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=2ahUKEwiW66jj8JvmAhUSkRQKHcHNDHwQFjAAegQIBRAC&url=http%3A%2F%2Fwww.gacetasanitaria.org%2Findex.php%3Fp%3Drevista%26tipo%3Dpdf-simple%26pii%3DX0213911119000670&usg=AOvVaw1Hup8osFJVc40rDWJAQ9oQ

* Martínez, M; Álvarez, J; Garrido, M; de la Torre, A. 2019. "One health" concept applied to surveillance systems of Salmonella in swine and humans. Gaceta Sanitaria, 33:79. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=2ahUKEwiW66jj8JvmAhUSkRQKHcHNDHwQFjAAegQIBRAC&url=http%3A%2F%2Fwww.gacetasanitaria.org%2Findex.php%3Fp%3Drevista%26tipo%3Dpdf-simple%26pii%3DX0213911119000670&usg=AOvVaw1Hup8osFJVc40rDWJAQ9oQ

Name of the activity:	GeoVet 2019
Date:	October 8-10, 2019
Place:	Davis, California, USA.

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	Yes*
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	1000	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

* Teng K, Martinez Aviles M, Ugarte M, Barcena C, De La Torre A, Lopez G and Alvarez J (2019). O, Salmonella, Where Art Thou? Modelling Salmonella infection in swine farms in Spain using Hamiltonian Monte Carlo methods. Front. Vet. Sci. Conference Abstract: GeoVet 2019. Novel spatio-temporal approaches in the era of Big Data. doi: 10.3389/conf.fvets.2019.05.00007

Name of the activity:	SETAC Europe 29thAnnual Meeting		
Date:	26-30 May 2019.		
Place:	Helsinki, Finland		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	No
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

* Carballo, M; Esperón, E; de la Torre, A. 2019. Use of mapping tools for defyning scenarios for the environmental risk assessment of antibiotic residues, antimicrobial resistances and resistance genes. SETAC Europe 29thAnnual Meeting. 26-30 May 2019. Helsinki, Finland

Name of the activity:	Project presentation at SVA Research Day
Date:	Nov 12, 2019
Place:	Uppsala, Sweden

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	Yes
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	No
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	60	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Norwegian Public Health Conference
Date:	2019, Oct 15
Place:	Oslo, Norway

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	Yes	Pitch Event	No No
Training	No	Trade Fair	
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	No
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	100	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	SVA research day
Date:	November 16-17, 2020
Place:	SVA, Uppsala, Sweden

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	No
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	Yes
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	Yes	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other	Poster
Communication Campaign (e.g. Radio, TV)	No		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	80	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	ESRI conference		
Date:	21-22 october 2020		
Place:	On line meeting		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)	100	Media	20
Industry	100	Investors	
Civil Society		Customers	300
General Public		Other	
Policy Makers	50		

Name of the activity:	I Congreso Virtual de la Sociedad Española de Epidemiología (SEE) y da Associação Portuguesa de Epidemiologia (APE)
Date:	21-30 Octubre 2020
Place:	On line meeting

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	350	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	ESRI conference
Date:	October 2020
Place:	https://arcg.is/1zHSub

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website	Yes	Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	100	Media	
Industry	50	Investors	
Civil Society		Customers	300
General Public	50	Other	
Policy Makers	50		

Name of the activity:		Rodríguez, A; Iglesias, I; de la Torre, A. Food surveillance data on campylobacter and salmonella in Spain (2016-2018): spatial visualization and descriptive analysis Annual scientific meeting of the one health European joint programme on food-borne zoonoses, antimicrobial resistance and emerging threats
Date:		9-11 June 2021
Place:		Copenhagen, Denmark.

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

		Yes / No		Yes / No
Organisation of a Conference	*No*		Participation to a Conference	*Yes*
Organisation of a Workshop	*No*		Participation to a Workshop	*No*
Press release	*No*		Participation to an Event other than a Conference or a Workshop	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*		Video/Film	*No*
Exhibition	*No*		Brokerage Event	*No*
Flyer	*No*		Pitch Event	*No*
Training	*No*		Trade Fair	*No*
Social Media	*No*		Participation in activities organized jointly with other H2020 projects	*No*
Website	*No*		Other (abstract book)	*Yes*
Communication Campaign (e.g. Radio, TV)	*No*		Other (QDR code)	

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

		Number		Number
Scientific Community (Higher Education, Research)	200		Media	
Industry			Investors	
Civil Society			Customers	
General Public			Other	
Policy Makers				

Name of the activity:		Rodríguez, A; de la Torre, A. Aimed surveillance of veterinary antibiotics in agricultural and pastural land based on an IT tool. ASM Satellite Workshop.
Date:		7-8 June 2021
Place:		Virtual meeting

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories				
		Yes / No		Yes / No
Organisation of a Conference	*No*		Participation to a Conference	*Yes*
Organisation of a Workshop	*No*		Participation to a Workshop	*No*
Press release	*No*		Participation to an Event other than a Conference or a Workshop	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*		Video/Film	*No*
Exhibition	*No*		Brokerage Event	*No*
Flyer	*No*		Pitch Event	*No*
Training	*No*		Trade Fair	*No*
Social Media	*No*		Participation in activities organized jointly with other H2020 projects	*No*
Website	*No*		Other (abstract book)	*Yes*
Communication Campaign (e.g. Radio, TV)	*No*		Other (QDR code)	

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories				
		Number		Number
Scientific Community (Higher Education, Research)	50		Media	
Industry			Investors	
Civil Society			Customers	
General Public			Other	
Policy Makers	50			

Name of the activity:	Sykdomspulsen One Health – A real time surveillance system in an infrastructure coping with half a million analysis a day C Koren, D Swanson, G M Groneng, G Ro, P Hopp,M Jonsson, RA White Norwegian Institute of Public Health, Norwegian Veterinary Institute
Date:	10-06-2021
Place:	Copenhagen, Denmark

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	No
Organisation of a Workshop	Yes	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	Nos
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	50	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Development of One Health syndromic surveillance for Campylobacter in Norway and Sweden. Cha, W., Dórea, F., Grøneng, G.M., Rø, G., Hopp, P., Jonsson, M., Dryselius, R. 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats
Date:	May 27-29, 2020
Place:	Online meeting.

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	Nos
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	200	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	Designing multivariate syndromic surveillance for animal diseases in Sweden Gustafsson, W., Andersson, M.G. 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats
Date:	May 27-29, 2020
Place:	Online meeting.

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference	No	Participation to a Conference	Yes
Organisation of a Workshop	No	Participation to a Workshop	No
Press release	No	Participation to an Event other than a Conference or a Workshop	No
Non-scientific and non-peer-reviewed publication (popularised publication)	No	Video/Film	No
Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	No	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other (abstract book)	Nos
Communication Campaign (e.g. Radio, TV)	No	Other (QDR code)	No

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	200	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

5.1.4.3.4 JRP07-FBZ2-LISTADAPT – Final Report

5.1.4.3.4.1 Consortium composition

Project Coordinator and applicants

Coordinator : Dr Sophie Roussel, French Agency for Food, Environmental and Occupational Health & Safety, Anses, Maisons-Alfort Laboratory for Food Safety, Unit SEL (*Salmonella and Listeria*) F-94701 Maisons-Alfort, France

Appliquants :

- Dr René Hendriksen (DTU), DK
- Dr Susanne Thisted Lambertz , National Food Agency (NFA), SE
- Dr Ariane Pietzka (AGES), AT
- Dr Franceso Pomilio (IZSAM), IT
- Dr Cesare Camma (IZSAM), IT
- Dr Taran Skjerdal (NVI), NO
- Dr Renáta Karpiskova, (VRI), CZ
- Dr Pascal Piveteau (INRAE), FR
- Dr Michel Hébraud (INRAE), FR
- Dr Christophe Soumet (ANSES), FR
- Dr Arnaud Bridier (ANSES), FR
- Dr Yannick Blanchard (ANSES), FR
- Benjamin Félix (ANSES), FR

Dr Sophie Roussel is a senior scientist in charge of the research team of the Laboratory of Food Safety of ANSES Maisons-Alfort (France). Sophie Roussel has focused for ten years her research on the genetic structure of populations of *Lm* strains, through complementary molecular typing approaches: PFGE, MLST and WGS. **Her expertise will be instrumental for WPs 1, 4 and 5.**

Benjamin FELIX is a project leader in the Laboratory of Food Safety of Maisons-Alfort (France). He has been working for ANSES for eight years in the L*isteria* Team in the scope of the European Union Reference Laboratory. His research deals with *Lm* genetic diversity in food, food processing, animal and environment, in particular by using WGS. **His expertise will be instrumental for WP1, WP2 (T2.1) and WP4 (T4.1 and T4.2).**

The Listeria team is the French National Reference Laboratory (NRL) and the European Reference Laboratory (EURL) for *Listeria monocytogenes* in the food chain. The team has access to a wide variety of strains from foods, animals as well as those from human illness across the European Union.

Through existing research collaborations and partnerships, Sophie Roussel and Benjamin Felix have a unique *Lm* food and food processing plant strain collection, characterized both at phenotypic and genotypic levels. This collection is representative of the genetic diversity observed in France in the food

sector (Maury et al., 2016). Of this collection, two hundred complete genomes are available. Moreover, Sophie Roussel and Benjamin Felix are also able to provide sequences of strains considered as persistent in the food processing plants. **All these sequences will be used for WPs.** Moreover, the Laboratory of Food Safety of Maisons-Alfort harbors well-equipped microbiology labs, in which all the **bacteriology and molecular biology techniques necessary** for the **WP 1** (T1.1) of this project are operational.

Dr Yannick Blanchard is the head of the **national ANSES platform for NGS** and transcriptomic analyses. The platform has on site a NGS sequencer (Proton – Life technologies), the computing capacity (Dell server) and bioinformatics skills for the treatment of the WGS data. The platform is member of a scientific community (Biogenouest) which provides access to additional sequencing equipment's (Hiseq, Miseq). The platform is fully equipped for transcriptomic studies with hybridization station (Tecan) and autoloader scanner for microarray (Innopsys) and software's for data extraction. WGS training sessions are regularly organized by Yannick's team. **All this expertise in WGS will be useful for the WP 2, WP4 and WP5.**

Dr Christophe Soumet is the head of the Unit called antimicrobial, biocide, resistance and residue of ANSES. He has been working in the field of **microbial ecology of food industry** for the last 15 years. Since 2002, his main interests and activities include **characterization of antimicrobial resistance and biocides resistance of different bacterial species by phenotypic and molecular biology** techniques to study changes in microbiota from food environment after biocidal treatment. **Given as his expertise, Christophe is the leader of the WP3 and is involved in WP4 (T4.1 and T4.2).**

Dr Arnaud Bridier is a microbiologist. He focused a part of his research on the understanding of mechanisms governing biofilms development and resistance to antimicrobials and biocides at INRAE Micalis. Currently, he works with Christophe Soumet on the impact of cleaning/disinfection treatments on the emergence of resistance of *Lm* and cross-resistance to antibiotics using an integrative approach associating meta-omics, molecular biology and imaging. **The expertise of Arnaud will be used in WP3 and WP4 (T T4.1 and T4.2).**

Dr. Ariane Pietzka is the head of the **National Reference Laboratory and National Public health Laboratory** for *Lm* at the Institute for Medical Microbiology and Hygiene in the Austrian Agency for Health and Food Safety (AGES, Austria). Ariane has developed expertise on new techniques and typing methods including WGS-based methods. Her laboratory is using WGS for the routine surveillance of clinical and food strains. Her lab has a MiSeq and the data is analyzed mainly *via* SeqSphere. **This expertise in WGS will be useful for WP2, WP4 and WP5. Sequences of clinical and food strains will be used in "Listadapt".**

Her laboratory is hosting the "Special Listeria Culture Collection" (SLCC), which was assembled by Prof. H.P. Seeliger. The collection contains about 6000 strains, isolated between 1921 and 1987. The isolates are derived from various sources, including humans, animals, food or the environment. **A part of this collection will be used in WP1.**

Prof. DVM. Renáta Karpíšková is the head of NRL and NPHL for *Listeria monocytogenes*, in Czech Republic. She has an experience in the field of food and waterborne borne diseases, outbreak detection, molecular epidemiology, methods of foodborne pathogen detection and **typing**, **antimicrobial resistance** and **food safety**. **Her expertise will be used in WP1, WP4 and WP5.** Her laboratory has access to a large collection of animal and environmental strains. These strains will be used in WP1.

Dr. Rene Hendriksen has been employed at the DTU Food and has a background as laboratory technologist. At the DTU Food, Research Group of Genomic Epidemiology, he is currently employed as senior scientist. His research focus in **global epidemiology, surveillance, antimicrobial resistance, and population structure** of mainly foodborne and waterborne pathogens. He chairs the **working group for proficiency testing of the GMI initiativ**e. **Given his expertise in WGS, René is the leader of WP4**

and is closely involved in WP2 and WP5.

Dr Michel Hebraud is a Research Director recruited within INRAE in 1989. He works in the Research Unit of Microbiology (UR454) at the INRA Centre of Auvergne-Rhône-Alpes, site of Theix. He is the scientific responsible of the **proteomic component of the "platform of metabolism exploration (PFEM)"**. His research activities have focused on the adaptation of *Listeria monocytogenes* to food processing plants. In this context, his work concerned the molecular determinants involved in biofilm formation, the physiology of bacteria in biofilms, their resistance to cleaning disinfection treatments and the mechanisms by which some of them face to technological stresses. **His expertise will be instrumental for all the WP3 and WP4 (T4.1 and T4.2).**

Dr Pascal Piveteau has built a strong expertise on the ecology of zoonotic agents. He is the group leader of the team working on the **ecology** of *Listeria monocytogenes* in the farm environment in INRAE, Dijon. He has been working on the investigation of reservoirs of several bacterial pathogens in outdoor environments and has specialized in the microbial ecology of soil. His research focuses on both **intrinsic and extrinsic factors that drive the fate of pathogenic bacteria** in this habitat. His expertise will be instrumental for the entire proposal, in particular **for WP1, WP3 and WP4 (T4.1 and T4.2).**

Dr. Francesco Pomilio is Responsible of the Hygiene in Food Technology and Animal Feeds Unit at IZSAM and he works in the **Italian National Reference Laboratory for** *Listeria monocytogenes*. His main areas of expertise are related to human and animal food safety,) validation of- microbiological analytical methods, challenge test and shelf life studies in food for human consumption, development of the Italian database of isolates and improvements of the SEAP (Italian data collection system on Foodborne pathogens), and prevalence and persistence of *L. monocytogenes* in producing factories. **Franceso' expertise will be instrumental for WP1, WP4 and WP5.**

Dr. Cesare Cammà (MSc) is the head of the Genomic unit at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM). His research activities have focused on the development of molecular methods for diagnosis of infectious animal diseases, including zoonosis and in the molecular characterization of viral and bacterial strains by means of several DNA sequence-based subtyping techniques including whole genome sequencing. Specifically, 3 molecular biologists, 1 biotechnologist and 3 bio-informaticians are employed in the Genomic unit and the laboratory is equipped with an Ion Torrent Personal Genome Machine (Life Technologies), a NextSeq 500 desktop sequencing system (Illumina), a MinION device (Nanopore). In addition, two multi-capillary DNA sequencers, the AB Library Builder™ System and two liquid handling workstations are also available. Since January 2015 and up to July 2016, the Genomic unit processed 2440 viral and bacterial strains. IZSAM has an advanced IT infrastructure, including firewalls, automated back-up services and storage units for storage and bioinformatic analysis of NGS data, including metagenomics data. Servers are managed using the virtualization technology and Internet access is provided by the high speed GARR network. The laboratory has participated to two Proficiency Testing Trials for whole genome sequencing and data analysis organized by Global Microbial Identifier (GMI). **His expertise will be instrumental for WP2, WP4 and WP5.**

Dr Taran Skjerdal has more than twenty years' experience of research from research institutes and companies within the areas food quality, food safety, risk assessment and multidiciplinary decision support. The last 10 years she has worked at the Norwegian Veterinary Institute with food safety and bacteriology with focus on growth, survival, pathogenicity and adaptation of *Listeria* and *Staphylococcus* during food processing. The work includes research activities and support to food authorities. **Her expertise will be useful for WP1, WP3 and WP4. Taran is the leader of the WP1.**

NVI has a large and growing collection of *Listeria* strains, in particular of isolates from meat and meat processing facilities and isolates from animals. **These isolates will be used in WP1**

Dr Live L. Nesse, DVM, PhD started working in research 35 years ago. She works at the Norwegian Veterinary Institute for 15 years. She has focused on the importance of bacteria's biofilm forming

abilities for persistence in various environments, gene transfer in biofilms and anti-biofilm strategies. She has been the manager of numerous research projects with both national and international partners, and is presently the manager of a large Norwegian project studying persistence of Listeria in fish processing facilities with emphasis on the possible role of biofilm. **Her expertise will be useful for WP3.**

Dr Karin Lagesen has a PhD in bioinformatics, and has been employed at the Norwegian Veterinary Institute since 2015. She also holds a 20% position at the Dept. of Informatics at the University of Oslo (since 2010), where her focus is on education in bioinformatics. Her work has been focused around genomics and metagenomics, with an emphasis on bacterial comparative genomics. **Her expertise will be instrumental for WP4 and WP2.**

Dr Susanne Thisted Lambertz is a senior microbiologist at the Swedish National Food Agency (NFA) and at the head of the NRL for *Listeria monocytogenes*. During 2016, she performed a **nationwide *Listeria monocytogenes* (*Lm*) survey analyzing risk products.** In parallel environmental samples are taken at the **producing plants**, and in addition isolates from listeriosis patients are collected. **In collaboration with the Public Health Agency** of Sweden, all recovered isolates are being typed. Recently, she investigated two large listeriosis outbreaks. The associated isolates have been typed. Continuously her team types *Lm* isolates originating from the **food companies** own control. **Her expertise will be useful for WP1.** Her close collaboration with the Public Health Agency, Swedish Veterinary Institute (SVA), enable us to have access to sequences of clinical strains. *Suzanne died one year after the start of the project and was replaced by Dr. Monica Ricao.*

5.1.4.3.4.2 Summary of the work carried out in the Project

The LISTADAPT project aims to decipher the molecular mechanisms of adaptation seen in *Listeria monocytogenes* (*Lm*) to its various ecological niches by comparing both phenotypic and genotypic data from a large, balanced set of strains from human clinical cases, animals, food and environments in several European countries. With 21 partner institutes from public health, veterinary health, food and environment laboratories, the project compiled a dataset of 1575 *Lm* strains and their genomes. These strains were collected from 20 European countries, which ensured that the dataset was representative of the large number of clonal complexes (i) occurring worldwide (ii) covering many diverse habitats (iii) balanced between ecological niches and geographical regions. The aim of this dataset is to contribute to the understanding of *Lm* and improve surveillance, therefore it was important to make all of this information available to the scientific community (it has been submitted to the European Nucleotide Archive (ENA)).

Of the 1575 strains collected, a subset of 200 was selected from 33 clonal complexes known to be the most prevalent in Europe, with a balance between three ecological niches: environment, animal and food. Phenotypic tests were performed on these strains to investigate the strain's ability to survive in soil and subsequently the strains were categorised into three groups depending on survival. No source and no clonal complex were specifically associated to soil fitness. These 200 strains showed the same growth kinetics at pH 7 while there was more variation at pH 5.4. The deviating strains belonged to different CC groups and niches.. Antimicrobial susceptibility testing also played a significant role in the LISTADAPT project with 11 antibiotics and 4 biocides being tested against the panel of 200 strains. Overall, results revealed that strains isolated from food had overall higher minimum inhibitory concentrations (MICs) for the following biocides: quaternary ammonia compounds and peracetic acid compared to strains isolated from animal or the environment. Conversely, no significant differences were observed for MIC of antibiotics from strains from different niches and from different clonal complexes. Interestingly, repeated exposure to quaternary ammonia compounds recurrently led to a decrease of susceptibility toward ciprofloxacin, a fluoroquinolone antibiotic, largely used in human and veterinary medicine and considered as a critically important antimicrobial. Additionally, these lower levels of susceptibility to ciprofloxacin remained stable in most strains even after subculture without biocide selection pressure, suggesting an adaptation involving modifications at the genetic level.

Genomic analysis suggested that the accessory genome was associated with biocide tolerance, often linked to prophages and mobile genetic elements, thus demonstrating the adaptability of *Lm* and the need to further characterise and understand these important organisms.

The 200 strains characterized in details both at phenotypic and genotypic level will help to assess the true importance of these strains as sources of foodborne infections for public health. Genetic mechanisms for the survival and adaptation of *Lm* (i) in food processing environment (ii) in wild and farming animals (iii) in natural and farming environments are investigated here. The genes could be used as targets for developing rapid monitoring tests. With a view to controlling risks in agricultural and agri-food systems, this project will make it possible to assess the relevance of monitoring plans, for instance in agricultural soils.

5.1.4.3.4.3 Work carried out in the JRP, scientific results and integrative outcomes

WP0: Coordination (M1-M30)

WP1: Constitution of a strains collection representative of the different reservoirs of Listeria monocytogenes (M1-M12)

WP0: Coordination (M1-M30)

The LISTADAPT leader, Laurent Guillier, left the project during the summer of year 2 (Y2) for another position in ANSES. The project lead has been transferred to Sophie Roussel, the successor of Laurent Guillier. Sophie Roussel is a senior scientist that has maintained close relationships with all the LISTADAPT partners. Indeed, she was a scientist at Anses from 2007 to 2017 working as the head of the molecular typing team of different food-borne bacteria, including *Listeria monocytogenes*. She was closely involved in European projects funded by EFSA and by the European Union (Horizon 2020 PHC7- "COMPARE"). In 2016, with her team, she wrote and set up LISTADAPT. Sophie Roussel worked then during two years (2017-2019) as Research Director at INRAE and was coordinator of the International Centre of Microbial Resources (CIRM) set up by INRAE. In this frame, she was involved in different European programs (H2020 -"CIRCLES" project and H2020 EJP One Health "CARE").

As LISTADAPT leader, Sophie Roussel coordinated and organised two face-to-face meetings (M25): (i) the first – one day -17th January 2020- meeting ANSES-INRAE specifically dedicated to WP3-WP4 (ii) the second - half a day -24th January 2020- with all the partners. Sophie Roussel was invited to present the project (i) during the annual European workshop EURL / NRL Listeria in January 2020 (see JRP7-WP5-T4: Dissemination) (ii) during meetings with different scientific partners such as the LIBio, a laboratory of Lorraine University (24th November 2029) and the main professional federations of the French food industry /ADEPALE) (26th November 2020). In addition, Sophie Roussel was invited by the ANSES management team to present the project to the scientific organisation committee of the Anses (26th May 2020 -60 participants).

WP1: Constitution of a strains collection representative of the different reservoirs of Listeria monocytogenes (M1-M12)

JRP7-WP1-T1: Strain collection (M1-M12)

The LISTADAPT strain collection, unique in Europe, is composed of two compartments, one of which (First compartment C1) very original, as it regroups 847 strains isolated in the environment (soil, river, farm environment) along with strains from wild and farm animals (both healthy and animal presenting clinical symptoms)..The second compartment (C2) is composed of 728 strains from five ready-to-eat (RTE) food categories from LISTADAPT partners (Table 1). All the 1575 strains are now centralized, long-term maintained and stored within the ANSES bacterial collection. The future use of some of these strains is protected by MTAs (Material Transfer Agreement). At ANSES, typing data (MLST data and cgMLST data) and associated epidemiological information of all the strains are centralized in a molecular database under the software BioNumerics version 7.6.3. A standardized nomenclature has been established with well-defined categories for all epidata (country, origin, matrix and animal

condition) in order to improve the homogeneity of strains IDs.

Table 1: Repartition by compartments and sub-compartments of strains from the whole LISTADAPT collection (1,575)

Animal and environment (C1)				**Food products and food production environment (C2)**					
Farm animals	Wild animals	Soil and farm environment	Total	Meat	Fish	Dairy	Vegetables	Composite dish	Total
459	194	194	847	246	165	119	95	103	728

JRP7-WP1-T2: Campaigns to collect additional animal and environmental strains (M1-M10)
JRP7-WP1-T2-ST1: External collaborations (M1-M2)

To increase the size and representativeness of the collection, the LISTADAPT consortium performed an extensive review of all recent collections of published and unpublished *Lm* strains and then contacted researchers in charge of these collections. Finally, 14 external partners, food and veterinary laboratories and research institutes, all dealing with *Lm* hazards in Europe, collaborated with the LISTADAPT consortium (Tables 2 and 3). The initial collection included more strains from animals with listeriosis-associated clinical symptoms than without symptoms. In order to reduce the number of strains originating from animals with listeriosis while maintaining maximum diversity of the dataset, we adopted an original method to select the strains based on metadata (e.g., type of sample, geographic location, isolation time, molecular typing data such as PFGE profiles, animal species and geographic sampling location). This method relies on Gower's coefficient (GC), which is a dissimilarity measure: the "distance" between two units is the sum of all the variable-specific distances (associated with metadata categories). Because this collaborative work, we increased the representativeness and the diversity of animal and environmental *Lm* strains (strains from more countries at the European Union (EU) level and more partners at a national level). We constructed a large dataset comprising 344 animal and environmental *Lm* strains from 6 European countries and published collections (Table 2), as well as 373 animal and environmental *Lm* strains from 15 European countries and non-published collections (Table 3).

Table 2: List of 344 animal and environmental *Listeria monocytogenes* strains from published microbial collections.

Partner	**Country (country code)**	**Category**	**Origin of isolation**	**No. of strains**	**Isolation year**	**References**
Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine of Helsinki (n=132)	Finland (FI)	Wild animals	Wild birds, hare, reindeer [NS]	49	1998, 2001	10,14
		Farm animals	Cow, cow milk in bulk tank and pigs [NS]	62	1981–2011	14,43-46
			Cow (aborted fetus) [CS]	4	1984–1987	14,44,45

		Soil and farm environment	Silage[1] and soil	17	1987–2004	
Faculty of Veterinary Medicine, University of Munich (n=31)	Germany (DE)	Wild animals	Deer and wild boar [NS]	31	2011–2012	6
Norwegian Veterinary Institute (n=29)	Norway (NO)	Wild animals	Slugs	29	2012	11
Department of Applied Microbiology and Human Nutrition ZUT (n=55)	Poland (PL)	Soil and farm environment	Soil from agricultural area	46	2010–2012	26
			Soil from park on city outskirts	9	2015–2016	
Department of Animal Health, NEIKER (n=71)	Spain (ES)	Farm animals	Cow, sheep and poultry feces [NS]	71	2004–2005, 2014–2016	4,47,48
Veterinary Faculty, University of Ljubljana (n=26)	Slovenia (SI)	Farm animals	Cow and sheep [CS]	13	2011–2015	25
		Soil and farm environment	Farm environment, water, pond	2	2008, 2014	
		Wild animals	Fox brain [NS]	11	2014	

CS, Clinical Symptoms. The reported clinical symptoms included rhombencephalitis, abortion, septicemia and mastitis/subclinical mastitis. The type of clinical samples included cerebellum/brain tissue, aborted fetus, fetal membrane, liver, internal organs, feces and milk. NS, No listeriosis-associated Symptoms[1] Strains isolated from silage were considered as originating from the farm environment since silage mainly includes fermented forage crops collected directly from fields.

Table 3: List of 373 animal and environment *Listeria monocytogenes* strains from non-published collections

Partner	Country	Category	Origin of isolation	No. of strains	Isolation year
Not communicated by the authors (n=96)	Belgium (BE)	Farm animals	Cow [NS]	96	2017–2018
Veterinary Research Institute (n=14)	Czech Republic (CZ)	Farm animals	Cow, pig [NS]	6	2013–2014
		Soil and farm environment	Mud, algae from pond, soil from farm, decaying vegetation	8	2010, 2014
State veterinary institute (n=7)	Czech Republic (CZ)	Wild animals	Gerbil, mouflon [NS]	3	Unknown
		Farm animals	Cow, sheep [NS]	4	Unknown
Veterinary and Food Laboratory (n=25)	Estonia (EE)	Farm animals	Cow, sheep, goat [CS]	24	2014–2018
		Wild animals	Deer [CS]	1	2018
Faculty of Veterinary Medicine/ Department of Food Hygiene and	Finland (FI)	Farm animals	Cow, pork, goat milk, sheep [NS]	7	1987, 1995, 1998
		Wild animals	Hare, birds feces [NS]	4	1986, 1987

Environmental Health, Helsinki (n=24)		Soil and farm environment	Silage[1] and farm environment	13	2003, 2014–2015
Laboratory for Food Safety ANSES (n=25)	France (FR)	Farm animals	Cow, poultry [NS], horse [CS]	8	2003, 2014, 2015, 2018
		Wild animals	Hare [NS]	3	1986, 1996, 2015
		Soil and farm environment	Manure, soil	14	2004, 2006, 2009, 2012
Research Unit OPAALE INRAE (n=6)	France (FR)	Soil and farm environment	Soil, compost, pasture	6	2011, 2012, 2013, 2018
Institute of Food Safety and Food Hygiene, Faculty of Veterinary Medicine, Freie Universität Berlin (n=15)	Germany (DE)	Farm animals	Pig and sheep at slaughterhouse retention area or immediately after slaughter [NS]	15	2009, 2018–2019
Institute of Food Safety, Animal Health and Environment BIOR (n=24)	Latvia (LV)	Farm animals	Cow, goat, sheep, pig [CS]	24	2013–2018
Animal Pathology Laboratory INIAV (n=13)	Portugal (PT)	Farm animals	Cow, goat, sheep, zoo animals [CS]	12	2017–2019
		Soil and farm environment	Corn silage	1	2019
Veterinary Faculty, University of Ljubljana (n=53)	Slovenia (SI)	Farm animals	Cow, sheep, goat [CS]	27	2011–2015, 2018–2019
		Wild animals	Fox [NS]	2	2015
		Soil and farm environment	Cattle farm environment	1	2013
	Croatia (HR)	Farm animals	Cow, sheep, goat [CS]	23	2010, 2016–2017
Department of Biology, Swedish Food Agency (n=16)	Sweden (SE)	Wild animals	Deer, rook, moose, wild boar [NS]	13	Unknown
		Farm animals	Poultry [NS], goat, sheep [CS]	3	Unknown
State Veterinary and Food Institute Dolny Kubin (n=22)	Slovakia (SK)	Farm animals	Sheep, goat [CS]	20	2016–2018
		Soil and farm environment	Feed	2	2015, 2017
Laboratory Feed and Food and Product Safety VWA (n=33)	The Netherlands (NL)	Farm animals	Cow, sheep, goat, poultry [NS]	33	2016–2018

CS, Clinical Symptoms. The reported clinical symptoms included rhombencephalitis, abortion, septicemia and mastitis/subclinical mastitis. The type of clinical samples included cerebellum/brain tissue, aborted fetus, fetal membrane, liver, internal organs, feces and milk ; NS, No listeriosis-associated Symptoms ; [1] Strains isolated from silage were considered as originating from the farm environment since silage mainly includes fermented forage crops collected directly from fields.

JRP7-WP1-T2-ST2: Sampling campaigns (M1-M10)

Soil, farm, and wild animal samples were collected in eight European countries (Table 4) in 2018 and in 2019. For the collection of soil samples, the LISTADAPT project members raised awareness and organised crowd-sampling campaigns. All the soil samples were collected from agricultural or wild areas according to a common procedure provided to the samplers based on the existing recommendations reported in the literature. The integration of feedback from samplers enabled a continuous improvement of the sampling protocol. The sampling campaigns were conducted in 17 areas in seven EU member states, Norway and Switzerland, namely AT, CH, CZ, FR, IT, NO, SE, SI and SK, resulting in the isolation of 75 *Lm* strains. Out of the 1752 available sampling records, the overall prevalence was 3%. We confirm in the present study the low prevalence of *Lm* in soil reported in the literature (below 1% and up to 6% depending on soil type).

Regarding the subcompartments of farm and wild animal, 55 *Lm* strains were isolated from sampling campaigns. Three campaigns targeting shelled gastropods sampled in IT, SK and CH resulted in the isolation of six strains. Sampling campaigns were also carried out for wild deer and reindeer feces in

Southern Norway, and from cattle, roe deer, wild boar, wolf, bear and fox feces in the Abruzzo and Molise regions of Italy. Of the 2577 samples collected from vertebrates during the campaign conducted in IT and NO 40 isolates were detected, with an overall prevalence of 1.5%.

Table 4: List of 130 animal and environment strains from sampling campaigns

Partner	Country (country code)	Category	Origin of isolation	No. of strains	Isolation year
Austrian Agency for Health and Food Safety AGES (n=1)	Austria (AT)	Soil and farm environment	Meadow	1	2018
Veterinary Research Institute (n=21)	Czech Republic (CZ)	Natural and farm environment	Soil, river bank, pond, decaying vegetation, manure	18	2016–2018
	Slovakia (SK)	Natural and farm environment	Soil, river bank	3	2016
Research Unit OPAALE INRAE (n=30)	France (FR)	Soil and farm environment	Soil, compost, pasture	30	2018–2019
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G.Caporale (n=59)	Italy (IT)	Farm animals	Cow, goat, sheep [CS]	7	2014–2018
		Soil and farm environment	Soil and river water	16	2016–2018
		Wild animals	Fox, wolf, porcupine, badger, bear, snail, crayfish, roe deer, wild boar [NS]	36	2014, 2016–2018
Norwegian Veterinary Institute (n=10)	Norway (NO)	Wild animals	Deer and reindeer [NS]	10	2017–2018
Veterinary Faculty, University of Ljubljana (n=3)	Slovenia (SI)	Soil and farm environment	Water pond and soil	3	2018
State Veterinary and Food Institute Dolny Kubin (n=1)	Slovakia (SK)	Wild animal	Snail	1	2019
Department of Microbiology, National Food Agency (n=1)	Sweden (SE)	Soil and farm environment	Pasture	1	2018
Agroscope (n=4)	Switzerland (CH)	Soil and farm environment	Pasture soil	3	2019
		Wild animal	Snail	1	2019

CS, Clinical Symptoms. The reported clinical symptoms included rhombencephalitis, abortion, septicemia and mastitis/subclinical mastitis. The type of clinical samples included cerebellum/brain tissue, aborted fetus, fetal membrane, liver, internal organs, feces and milk.NS, No listeriosis-associated Symptoms

JRP7-WP1-T3: Strategy for sequencing (M1-M12)

All the strains collected during the Tasks 1 and 2 have been sequenced in WP2.

WP2: Whole genome sequencing of Listeria monocytogenes strains (M2-M16)

***JRP7-WP2-T1**: Purification of Lm DNA from 2000 Lm strains (M2-M14)*

***JRP7-WP2-T1-ST1**: First batch Purification of DNA from Lm strains available (M2-M4)*

The first batch of 140 strains has been used to purify DNA at month 10. Four additional batches of strains have been used at months 11-12, for a total number of 546 strains

***JRP7-WP2-T1-ST2**: Second batch Purification of DNA from additional Lm strains (M13-M14)*

The second batch of strains has been used to purify DNA at month 24.

***JRP7-WP2-T1-ST3**: Purification of DNA from routine surveillance systems at IZSAM, DTU, AGES (M1-M12)*

DNA from strains gathered during routine surveillance were also made available by ANSES, IZSAM, DTU and AGES.

***JRP7-WP2-T2**: Whole Genome Sequencing (WGS) (M3-M14)*

***JRP7-WP2-T2-ST1**: First batch WGS for available Lm strains (M3-M6)*

The first batch of 140 strains was sequenced in November 2018.

JRP7-WP2-T2-ST2: Second batch WGS for additional *Lm* stains (M13-M14)

The second (and last) batch of strains was sequenced in February 2020.

***JRP7-WP2-T2-ST3**: Ad hoc WGS (M3-M14)*

Completed. Four LISTADAPT partners (AGES, IZSAM, ANSES and DTU) mainly performed the sequencing. All the genomes were centralized in a database at ANSES. (SeeWP2-T3).

***JRP7-WP2-T3**: Genome Assembling and Annotation (M5-M16)*

The next generation sequencing (NGS) paired-reads (2 × 150 bp) were generated during the project with Illumina platforms. The genomes were all *de novo* assembled and annotated with a harmonized in-house workflow named ARTwork (Assembly of reads and typing workflow) used in the ANSES Laboratory for Food Safety. In addition to *de novo* assembly, the ARTwork pipeline also performs genome annotation using Prokka. This whole genome sequencing (WGS) workflow has been described in detail in previous publications including the integrated bioinformatics tools and their corresponding versions, enabling repeatability and comparability of the results (Table 5). Assembled genome files will be publicly available in FASTA format through Figshare. Different WGS metrics and quality criteria were employed in the ARTwork pipeline to ensure high-quality WGS data. Reads with an estimated depth of coverage <30× (as estimated by BBmap[40]) as well as contigs and scaffolds with a length of < 200 bp were excluded (n=22). Draft genomes with a total length outside the range of 2.7–3.3 Mb and with a total number of scaffolds > 200 (n=46) were also excluded. In addition, inter- and intra-species contamination of reads was determined using the recently developed ConFindr software (v0.5.1). Since recently demonstrated, inter-and-intra species contamination of 10 single nucleotide variants (SNVs) assessed by ConFindr in the conserved core genes does not significantly impact cluster analysis. We decided to exclude all

genomes presenting SNVs lower than this cut-off (n=12) as well as various read- or assembly-related errors (n=34). The employed WGS metrics and quality criteria of the complete LISTADAPT genome dataset were reported (available at https://figshare.com/s/6582ab54ce4fabfa1fc8). After quality control of NGS and WGS data, the final LISTADAPT dataset included 1575 genomes. All metadata and WGS data collected herein were centralized and processed with standardized criteria for common nomenclature and NGS/WGS quality control before sharing between project partners. Reads normalized to 100× coverage, draft assemblies and annotated genomes were also centralized at the MongoDB database located at ANSES (Maisons-Alfort Laboratory for Food Safety). Raw (non-normalized) reads for all the *Lm* strains sequenced in the LISTADAPT collection (n=1508) were submitted to the NCBI Sequence Read Archive (SRA) for sharing with the LISTADAPT project's partners. Raw (non-normalized) reads for 67 *Lm* food strains obtained from previous publications were submitted to the NCBI Sequence Read Archive (SRA) database and were linked to their existing accession numbers (available at https://figshare.com/s/6582ab54ce4fabfa1fc8).

Table 5: Bioinformatics tools implemented in the ARTwork pipeline and their versions

Application	Software	Version
Read mapping	BBMap	38.22-0
Read normalization	BBNorm	38.22-0
Quality assessment of reads	FastQC	0.11.8
Trimming of low-quality reads	Trimmomatic	0.38
De novo assembly	SPAdes	3.13.0
MLST prediction	MLST	2.16.1
Retrieval of the closest reference	Mash	2.0
Reference-based scaffolding	MeDuSa	1.3
Gap closing	GapCloser	2.04
Trimming of contigs <200 bp	Biopython	
Quality assessment of the assembly	QUAST	5.0.2
Genome annotation	Prokka	1.13.3

While carrying out a final check on the results obtained during LISTADAPT, we became aware of a correspondence problem between certain strains and their genomes. We are currently fully mobilizing at the level of the ANSES team to resolve as quickly as possible this concern of the integrity of our genome database. Some publications are pending until the problem be solved.

WP3 Phenotypic characterisation of Listeria monocytogenes strains (M1-M29)

JRP7-WP3-T1: Strategy for selection of strains for phenotyping (M1-M12)

A panel of 200 *Lm* strains among the 1575 collected and sequenced for the project has been selected on the ground of their reservoirs, sub-reservoirs, sampling area and clonal complex (Figure 1). The three LISTADAPT partners (INRAE, NVI and ANSES) involved in phenotypic characterization (WP3)

received the first set of 100 strains (those isolated from food) in May 2018 then the second (those isolated from environment) in April 2019. The selection was done according to the MLST-CC data, using statistical tests. The most prevalent CCs were selected.

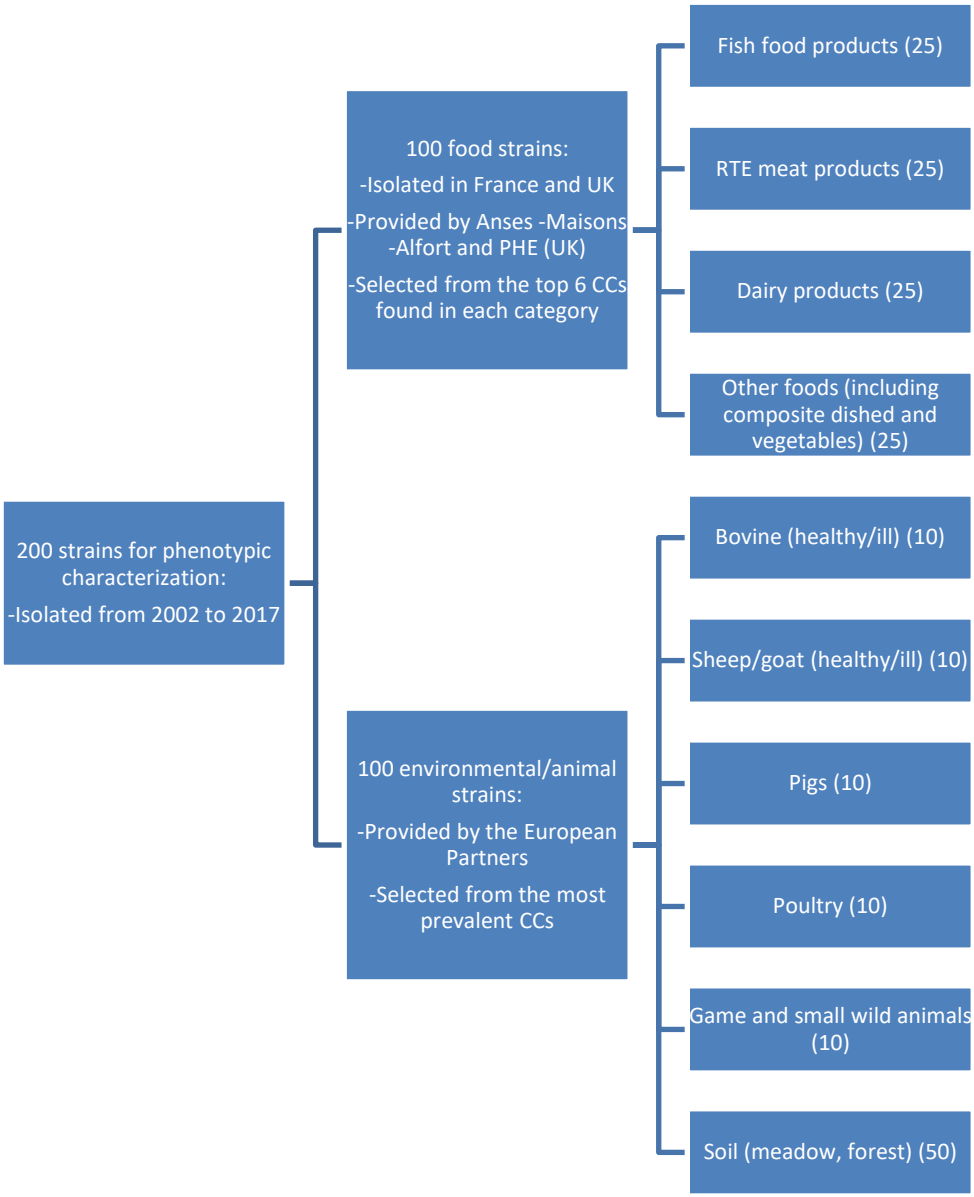


Figure 1: Description of the 200 strains selected

JRP7-WP3-T2: The effects of biocides on Lm strains adaptation (M3-M29)
JRP7-WP3-T2-ST1: Antibiotics and biocides resistance profiles of Lm strains (M3-M22)

The characterization of MICs (minimal inhibitory concentrations) values for 11 antibiotics and 8 biocides was achieved in August 2019. The results were summarized in a paper (cf point 7).

JRP7-WP3-T2-ST2: Adaptation to biocides and cross-resistance development to antibiotics of relevant Lm strains (M12-M22)

The impact of exposure to biocides on the antibiotic susceptibilities of *Lm* was also investigated.. We found that *Lm* strains isolated from food exhibited overall a lower susceptibility (higher MIC) for ammonium quaternary compounds (QACs) and peracetic acid (PAC) than strains isolated from animals and natural environments. We also showed that repeated exposure to QACs recurrently led to a decrease in susceptibility toward ciprofloxacin (CIP), a fluoroquinolone antibiotic, largely used in

human medicine.

JRP7-WP3-T2-ST3: The effect of biocides on Lm strains in biofilm (M12-M29)

The purpose of the study was to investigate phenotypical differences between the strains in terms of MBC-B (Minimum bactericidal concentration when the strain was in biofilm). Thirty-four isolates from food and twenty-four isolates from animals were cultivated in microtiter plates to form biofilm and then tested for survival after exposure to seven different concentrations of three disinfectants; Didectyl Dimethyl Ammonium Chloride (DDAC), Sodium hypochlorite (HS) and Hydrogen peroxide (Hper). We found the variation to be larger among the strains from wild animals than among the strains strains. The strains from the wild animals also seem to be more tolerant to disinfectants and in several cases the strains from wild animals tolerated twice the concentration of disinfectants compared to isolates from food. This pattern was true for all three disinfectants. The result was unexpected and did not support the hypothesis that exposure to biocides on *Lm* strains in biofilm is a significant selective pressure that reduces the diversity of strain.
Possible reasons for the results could be that the wild strains may have produced biofilm which is different from the one produced biofilm, that wild strains may be more stable as they are selected for survival by less excess of nutrients, or block variations in the design of experiments.

JRP7-WP3-T3: Bacterial adhesion and biofilm formation of Lm strains (M3-M29)

The ability of strains to adhere and to form biofilm was evaluated by using two complementary approaches: the BioFilm Ring Test™ (BRT) and crystal violet (CV) methods. These methods allow characterizing early and late/mature states of biofilm development, respectively. The BRT device (Chavant et al., *J. Microbiol. Meth*., 2007) makes it possible to evaluate the capacity of a bacterial strain to adhere to an abiotic support and start forming cellular aggregates. For each strain, the BRT were carried out after 6, 24 and 48 h of culture in BHI medium, in microplate wells incubated at 20°C, with three replicates for each time. The CV method is directly related to the biomass formed by bacterial cells in biofilm. In this case, the biomass of the biofilms formed by the different strains growing in BHI medium at 20°C in microplate wells was evaluated after 24 and 48 h of incubation. Six replicates were carried out for each of the two times. The results obtained were centralized in a database.

JRP7-WP3-T4: Survival and persistence of Lm strains in different ecological niches (M3-M29)
JRP7-WP3-T4-ST1: Survival of Lm in food products and gastro-intestinal environment (M3-M29)
Growth in the presence of preservatives

The primary purpose of the study was to investigate whether the genetic variation had any influence on the responses. The second was to see whether the strains isolated from food responded differently than the strains from nature and animals. An effect was expected as some of the strains from food probably had been isolated from foods with preservatives.

All strains grew well in BHIB at 12 °C. The growth was also significant at 4 °C, but much slower, as expected.

At pH 7, no difference was observed between the 200 strains. The additives had no impact on the growth. The latter was reasonable, as the fraction of undissociated acids at this pH was close to zero at neutral pH. At pH 4.5, the strains responded differently, and a clear effect of the additives was observed. Some strains showed a longer lagphase, some a slower increase in optical density, and some did not reach as high maximum optical density. The inhibiting effect of the additives followed this order: high concentration of acetate, low concentration of acetate, high concentration of lactate and finally low concentration of lactate. This order was the same as the concentration of undissociated acids in the four cases.

A co-variation between CC groups and the phenotypic response of the isolates was however not seen (Figure 2). The more than 200 isolates studied represented 26 CC groups. Isolates with different

sensitivity to 2000 ppm of acetate were present in 16 of the CC groups, some of them often found in food. The reasons for the different responses is therefore connected to other characteristics in the genome, which will be further explored in GWAS analyses. The impact of the results is that challenge studies of foods and development and validation of predictive models should take strain variation into account when targeted to food with low pH.

The impact of CC group, isolation step in the farm-to-fork chain and possible genetic markers for high or low tolerance of preservatives will be systemised and analysed with classical and bioinformatics tools.

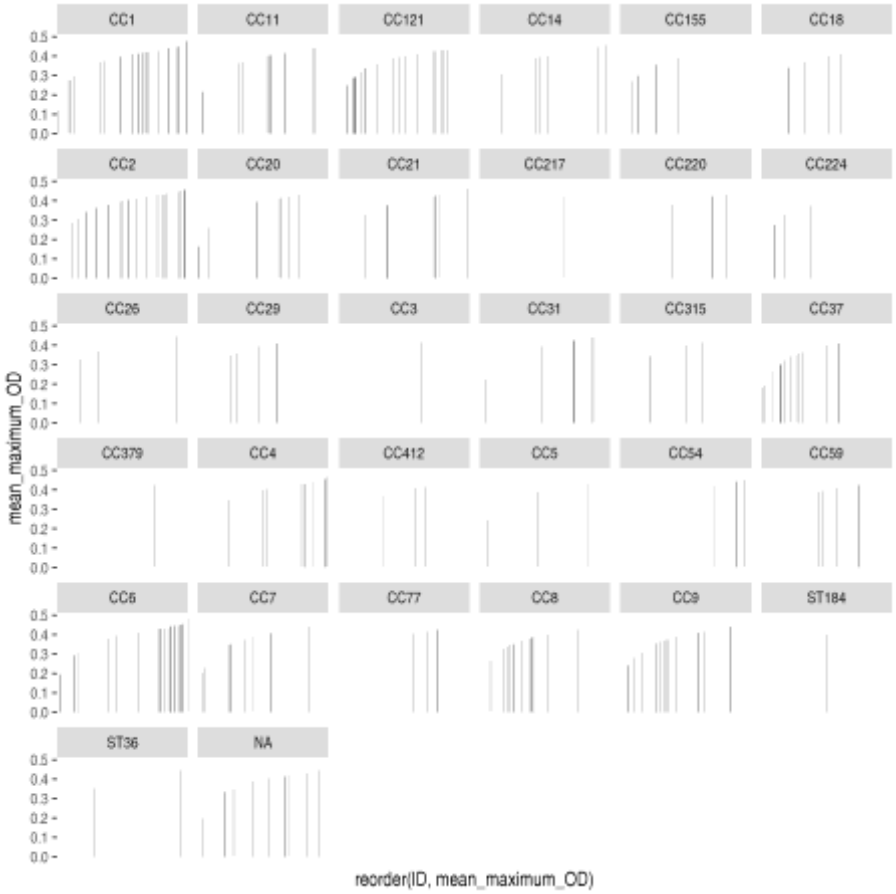


Figure 2: Maximum optical density of Lm strains after growth at 12°C in Brain heart infusion broth (BHIB) at pH 5.4 supplemented with 2000 ppm of sodium acetate. The results are presented per CC group, and each column represent one strain.

The results were presented in an oral presentation at the EJP conference in May 2020 : Skjerdal T, Fagereng T, Osland AM, Lagesen K, Fiskerbeck E, Nesse L, Sévellec Y, Felix B, Roussel S. 2020. Phenotypical responses to stress of in *Listeria monocytogenes* strains of different Clonal Complexes isolated along the nature-to-farm-to-fork chain. 27-29 May. Second annual meeting EJP (virtual meeting).

Survival during in-vitro digestion

In order to cause foodborne illness, the *Listeria* has to pass the digestion tract and invade the organs. The survival during digestion is therefore essential for the pathogenicity potential of the strain, as a strain that do not pass this hurdle will not cause illness even if it has high virulence genes. The purpose was to study the survival of a fraction of the strain library in an in-vitro digestion model. As the purpose

of ListAdapt was to detect differences between strains, we chose to study more strains and fewer replicates, in order to search for links between responses and DNA sequences in later bioinformatics studies.

The concentration of alive *L. monocytogenes* decreased with app 1 to 5 log_{10} units cfu/g during the gastric phase. The strains showed some difference also during the intestinal phase, but smaller than in the gastric phase. The model is complex in the sense that enzyme activities and several concentrations have to matched, which in turn makes it challenging to standardise between experiments. There are indication of a higher tolerance to stomach acid among isolates from some CC-groups, but the data need to be analysed further assessed both with bioinformactic and phenotypical methods before conclusion.

JRP7-WP3-T4-ST2: Survival of Lm in soil microcosm (M3-M16)

The ability of *Lm* to survive in soil was strain dependent. Survival ranged from zero to 22% (Figure 3).

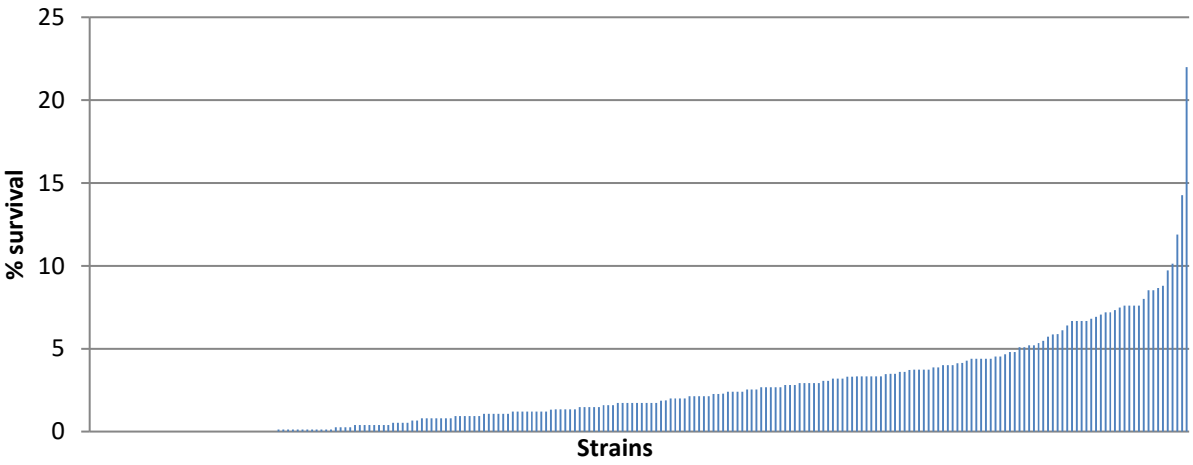


Figure 3. Soil survival phenotype of 230 isolates of *Listeria monocytogenes.*

Ascending Hierarchical Clustering clearly identified 3 groups of phenotypes (Figure 4), possibly indicating that some isolates (Phenotype 3) may be better competitors in complex habitats such as soil.

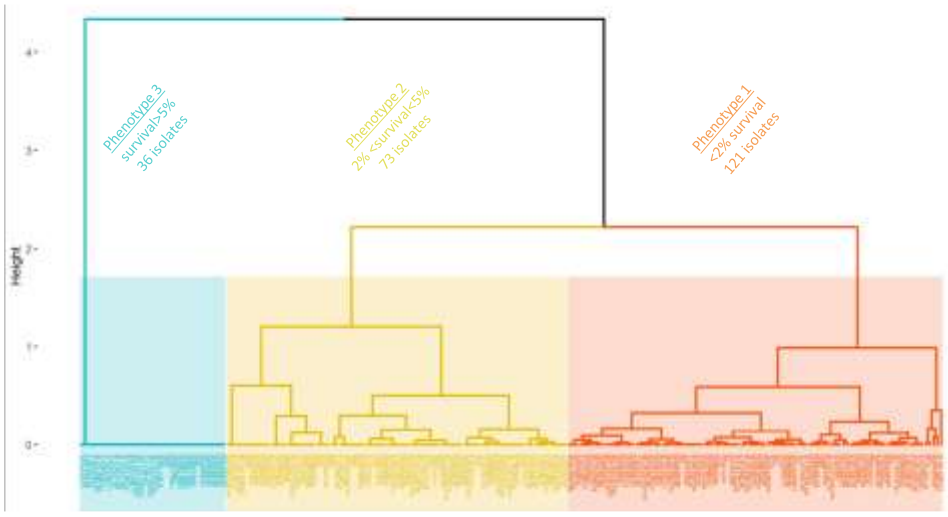


Figure 4 Ascending Hierarchical Clustering of soil survival data.

WP4: Identification of genetic traits in Listeria monocytogenes underlying adaptation to the ecological niches (M1-M30)

JRP7-WP4-T1: Analyze the distribution / prevalence of clonal complexes among the reservoirs (M1-M14)

This characterization helped to determine the two sets of strains for phenotypic studies.The 1575 strains clustered into 109 MLST STs, which belonged to 52 CCs and 23 singleton STs. For 38 strains, the allele profile was unknown (a presumable novel ST) or incomplete (When six out of seven MLST alleles were present, a CC was assigned when possible). We analysed in depth the strains genetic diversity between the three compartments and we showed the obtained results during (i) a 2-days-meeting at ANSES in April (2019) including all the partners and some external partners and (ii) the EJP general meeting in Dublin (2019) (cf Point 7).

JRP7-WP4-T2: Literature search of genes or genetic mechanisms responsible for virulence, adaptation and survival (M9-M12)

The list of genes involved in adaptation and survival was produced from research data obtained during the H2020 COMPARE project (2014-2019), coordinated by DTU (DK).

JRP7-WP4-T3: Biostatistics analysis of annotated genomes (M6-M29)
JRP7-WP4-T3-ST1: Identification of statistically relevant methods and development of analysis (M6-M16)

During the kick-off meeting of Y1 (March 2018), a list of relevant tools for identifying markers of adaptation to niches (environment, food industry) was established. The LISTADAPT partners has identified two alternative methods (DBGWAS and machine learning method from DTU) (Jaillard et al., 2018). Within the full lists, at least three methods are tested (Machine learning, GWAS based on presence/absence matrix and TreeWAS for SNP) (Brynildsdrud et al., 2016; Collins et al., 2018). For the research of genes identified in JRP7-WP4-T2, the LISTADAPT partners have chosen to use ABRICATE method (https://github.com/tseemann/abricate).

- Scoary:https://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-1108-8
- TreeWAS:https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1005958
- DBGWAS:https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1007758

JRP7-WP4-T3-ST2: Processing of all isolates (M22-M27)

Completed

JRP7-WP4-T4: Comparative analysis of phenotypic data / genotypic data (M24-M29)

The results obtained in tasks WP3-T4-ST1; T3 and T2-ST3 are compiled with all the genomic data for a mixomics approach. All the data are centralized in a database.

Antibiotics and biocides resistance profiles of *Lm* strains

Genome Wide Association Study revealed that accessory mobile elements including transposon Tn6188 and plasmid pLMST6 were associated with benzalkonium-chloride (BC), a quaternary ammonium compound (QAC) widely used in food processing. The results (genomic and phenotypic data) were summarized in a publication (cf point 7).

Adaptation to biocides and cross-resistance development to antibiotics of relevant *Lm* strains

Genomic investigations on the strain adapted to biocides revealed important insertions and deletion in the Internalin sequence. Those variations impacted mainly Internalin of unknown function. Several biocide-adapted-strains presented also deletion of transposons or plasmids. An analysis on SNPs

revealed that resistance to QACs are likely to be caused by the inactivation of a multidrug resistance efflux pump regulator by the insertion of a stop codon, hence explaining the increased resistance to biocide and to fluoroquinolones. A scientific publication combining the phenotypic and genotypic data is in preparation for the review "Frontiers in Microbiology" (cf Point 7).

Survival in soil

We have investigated the correlation between the phenotypes obtained for survival in soil and the characteristics of the genomes from the 200 isolates. Genome Wide Association Studies (GWAS) analysis did not evidence any link between the origin, the lineage or CC and the fitness in soil. This data suggest that the ability to survive in the soil is linked to multiple small effect genetic factors such as variations of transcriptional regulators, stress resistance proteins and cell wall proteins. However, GWAS applied on smaller and more genetically homogeneous subset (strains from the same CC) successfully identified phage-related- genes associated with soil survival rate. In particular, in the CC6, phenotype 1 was associated with the presence of a lysogenic phage corresponding to LP-030-3 and with variations in the transcriptional regulator BglG. A scientific publication ANSES/INRAE combining the phenotypic and genotypic data is in preparation for the review "Frontiers in Microbiology" (cf Point 7).

Bacterial adhesion and biofilm formation of *Lm* strains

A statistical study of all these results is planned in June in order to highlight possible correlations between the origin or clonal complex and the ability to rapidly form a biofilm, and/or a significant biofilm. A publication combining the phenotypic and genotypic data is planned (meeting ANSES/INRAE 12th January 2021).

Survival of *Lm* in food products and gastro-intestinal environment

Genomic analysis are in progress at NVI. A publication combining the phenotypic and genotypic data is planned.

WP5 : Trainings and dissemination (M1-M30)

JRP7-WP5-T1: Implementation of a workshop (M1-M2)

Statistical and bio-informatic tools useful for the project were discussed during the Kick-off meeting (March 2018).

JRP7-WP5-T2: Trainings (M3-M6)

The LISTADAPT coordinator organized a training session, in April 2019, in parallel with the Y2-meeting. It aimed to train 20 participants to R package methods.

JRP7-WP5-T3: Proficiency Testing Trials (M19-M22)

We decided that there will be no Proficiency testing trial WGS as planned. However, as part of the activities carried out by ANSES as EURL / NRL *Listeria*, a PT trial WGS was organized in 2018, then in 2019, during which four LISTADAPT partners (AGES, IZSAM, NVI, ANSES) participated.

JRP7-WP5-T4: Dissémination (M1-M36)

Congresses and Publications –see Point 7

5.1.4.3.4.1 Project self-assessment

As planned, LISTADAPT stimulated Reference laboratories to use WGS for the surveillance of *Lm* in their countries, as recommended by EFSA and ECDC. LISTADAPT allows continuing stimulation or implementation of WGS as the method of surveillance of *Lm* in the European countries, as recommended by the EFSA Biohaz panel in 2019 (https://doi.org/10.2903/j.efsa.2019.5898).

We had originally planned for WP1 to end in December 2018 (M12). However, it was very challenging to collect *Lm* strains isolated from natural environment. That is why we needed to (i) look for more partners (ii) collaborate with them and (ii) perform additional sampling campaigns. This is the reason why WP1 has been extended until December 2019 (M24). This has led to a substantial delay in the project in particular (i) in obtaining all the genomes assembled and annotated (completed in M28 instead of M16) (ii) in selection of the second batch of strains for WP3 (completed in M16) and (iii) in genomic analysis for WP4.

Last January 2021, we were performing validation tests on an in-house tool, “Genolisteria” which is a high-throughput real-time PCR allowing accurate identification of the most prevalent clonal complexes reported from human cases and food in Europe. Two of the strains analysed with Genolisteria (frozen corn outbreak) were previously sequenced, in the framework of our LRUE Listeria activities, and were included in the LISTADAPT dataset. We observed that the clonal complexes predicted by *in silico* analysis of these two strains did not match the results obtained by Genolisteria. For the moment, and despite the efforts of our team and those of the ANSES sequencing platform, we do not know the origin of the problem. These discrepancies led us to verify all the genomes obtained in Listadapt-WP2 according to the "historical" data available. A total of 211 strains were sequenced again and for 125 strains, the genomes obtained were not the same and these new genomes corresponded either to historical data or to data obtained by Genolisteria.

5.1.4.3.4.2 Progress of the project: milestones and deliverables

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted : Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
07	D-JRP7-0.1	Consortium agreement	1				
07	D-JRP7-0.2	Internal reporting templates	3	3		https://zenodo.org/record/3733303#.X_2-W-hKjcc	
07	D-JRP7-0.3	Plan for dissemination and exploitation of results.	14	16		The dissemination plan was validated during the face-to-face meeting of April 2019. https://zenodo.org/record/3733315#.X_2-d-hKjcc	
07	D-JRP7-1.1	Description of the panel of strains already sequenced	1	1		Sequenced strains were mainly isolated from food industry/ready-to-eat food. These strains were described with metadata https://zenodo.org/record/3733281#.X_2_f-hKjcc	3
07	D-JRP7-1.2	Description of the first panel of strains available to sequence	3	3		https://zenodo.org/record/3686994#.X_2_JuhKjcc	3
07	D-JRP7-1.3	Description of the second panel of strains to sequence.	12	24		The last panel of strains to sequence was sent in December (11 December 2019) Deliverable uploaded on web site on 18 December 2019.	3

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
						https://zenodo.org/record/3733232#.X_2-tOhKjcc	
07	D-JRP7-1.4	Report on strain collection and strategy for selection of strains for sequencing.	14	14		The strategy was presented during the OHEJP conference in May 2019 (Dublin). Deliverable uploaded on website on 16 December 2019 https://zenodo.org/record/3734093#.X_2-ZuhKjcc	3, 4
07	D-JRP7-2.1	Annotation of *Lm* genomes already sequenced (genomes available before the start of the project).	26	31		Confidential until publication in Scientific data is accepted. https://zenodo.org/record/3747482#.XpBuGsgzZM0	3
07	D-JRP7-2.2	Annotation of the *Lm* assembled genomes from 1st batch sequencing.	26	26		Confidential until publication in Scientific data is accepted. https://zenodo.org/record/3747493#.XpBun8gzZM0	3
07	D-JRP7-2.3	Annotation of the *Lm* assembled genomes from 2nd batch sequencing.	26	26		https://zenodo.org/record/3747502#.XpBwJMgzZM0	3
07	D-JRP7-2.4	Annotation of the *Lm* assembled genomes from *ad hoc* WGS	26	31		https://zenodo.org/record/3747504#.XpB04sgzZM0	2, 3
07	D-JRP7-3.1	Resistance profiles to biocide and antibiotics for the 200 *Lm* strains.	12	20		-Deliverable uploaded on web site on 17 December 2019.	3

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted : Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
						Poster presented during the OHEJP conference in May 2019 (Dublin) and during the IAFP's European Symposium on Food safety in April 2019 (Nantes). Confidential until publication in Pathogens is accepted https://zenodo.org/record/3686934#.X_2_NehKjcc	
07	D-JRP7-3.2	Assessment of the ability to adapt to biocides and develop cross-resistance to antibiotics for some illustrative *Lm* strains.	25	26		Confidential until publication in Frontiers in Microbiology is accepted. https://zenodo.org/record/3828737#.X_2-p-hKjcc	9
07	D-JRP7-3.3	Data on the effect of biocides on *Lm* strains in biofilm.	29	26		Confidential until publication in Scientific data is accepted. https://zenodo.org/record/3734064#.X_2-BOhKjcc	9
07	D-JRP7-3.4	Biofilms phenotypes for the 200 *Lm* strains.	33	37		Task completed in December 2020 (meeting Anses/INRAE 12th January 2020). Confidential until publication is accepted. Public. Deliverable uploaded on web site on 22 January 2021. https://zenodo.org/record/4461726#.YA6j_ehKhM0	9
07	D-JRP7-3.5	Collection of data on survival of *Lm* as planktonic	27	27		Confidential until publication in Scientific data is accepted.	9

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
		cells in various ecological niches.				Public https://zenodo.org/record/3686947#.XoIDllgza70	
07	D-JRP7-3.6	Collection of data on survival of *Lm* in soil microcosms.	22	20		-Deliverable uploaded on web site on 17 December 2019. -Poster presented at ISOPOL XX in September 2019 (Toronto). https://zenodo.org/record/3686947#.X_2_TOhKjcc	3
07	D-JRP7-4.1	Bibliographic study on catalogues of genes	13	13		https://zenodo.org/record/3733308#.X_2-luhKjcc	3
07	D-JRP7-4.2	Report on prevalence and distribution of clonal complexes among the reservoirs.	26	26		Public https://zenodo.org/record/3734095#.X_2-KOhKjcc	9
07	D-JRP7-4.3	Software chosen for bioinformatics analysis.	16	16		-Validated during the LISTADAPT meeting (April 2019). -Deliverable uploaded on website on 16 December 2019 https://zenodo.org/record/3733311#.X_2_vehKjcc	2
07	D-JRP7-5.1	"LISTADAPT" workshop program.	2			No workshop was organised. Exchanges and discussions were held during the kick off meeting on methodologies and bioinformatics and statistical tools used in LISTADAPT; https://zenodo.org/record/3733319#.X_2-OOhKjcc	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted : Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories * *(1 to 8) (several categories may be applicable)*
07	D-JRP7-5.2	Minutes of the training sessions.	10			There has been no minutes on the training sessions. https://zenodo.org/record/3733322#.X_2-hehKjcc	
07	D-JRP7-5.3	Publications and communications	36			Public. Cf point 7	8, 1,3,4

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
07	M-JRP7-1	Kick off meeting	2	Yes		6th March 2018, located at Anses, Maisons-Alfort, France
07	M-JRP7-2	Selection of the 200 *Lm* strains based on genomic analyses in WP2.	3	Yes		100 strains have been selected based on their genomic characteristics and context of isolation. These strains correspond to samples collected along the food production chain. They were sent to WP3 partners in April 2018; For the remaining 100 strains (from environment and from animals), the selection was based on the prevalence of CCs and chosen to best represent the diversity at the European level. The strains were sent to WP3 partners in May 2019.
07	M-JRP7-3	Workshop done	3	Yes		Discussions on statistical and bioinformatics methods were held during the Kick off meeting.
07	M-JRP7-4	DNA prepared for 1st batch WGS	4	Yes		The first DNA were prepared in September 2018
07	M-JRP7-5	Strategy for selection of strains for sequencing in place	5	Yes		An original algorithm was developed for selecting strain based on meta-data describing the context of isolation of the strains
07	M-JRP7-6	WGS raw data produced	6	Yes		The sequencing was reported of many months
07	M-JRP7-7	Face-to-face meeting -2018	8	Yes		Kick off meeting / 6th March 2018, located at Anses, Maisons-Alfort, France

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
07	M-JRP7-8	First batch Lm genomes assembly completed	8	Yes		Assembly and annotation with a harmonized in-house workflow named ARTwork. All the genomes are centralized in a database.
07	M-JRP7-9	Identification of Lm strains sequenced to be annotated	10	Yes		Assembly and annotation with a harmonized in-house workflow named ARTwork. All the genomes are centralized in a database.
07	M-JRP7-10	First batch Lm genomes annotation completed	10	Yes		Assembly and annotation with a harmonized in-house workflow named ARTwork. All the genomes are centralized in a database.
07	M-JRP7-11	WGS Training session done	10	Yes		Done
07	M-JRP7-12	All the strain collected and centralized at ANSES	12	Yes		All the 1575 strains are centralized, long-term maintained and stored within the ANSES bacterial collection. The future use of some of these strains is protected by MTAs. Typing data and associated epidemiological information of all the strains are centralized in a molecular database under the software BioNumerics. A standardized nomenclature has been established. (country, origin, matrix and animal condition…).
07	M-JRP7-13	DNA prepared for 2nd batch WGS	12	Yes		Done
07	M-JRP7-14	Selection of some representative *Lm* strains for the study of adaptation to biocides.	12	Yes		Thirty-four isolates have been tested on three disinfectants (seven different concentrations). The disinfectants used were Didectyl Dimethyl Ammonium

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
						Chloride (DDAC), Sodium hypochlorite (HS) and Hydrogen peroxide (Hper).
07	M-JRP7-15	WGS raw data produced.	25	Yes		Done
07	M-JRP7-16	Second batch *Lm* genomes assembly completed.	26	Yes		Assembly and annotation with a harmonized in-house workflow named ARTwork. All the genomes are centralized in a database.
07	M-JRP7-17	The database includes MLST data (CC and ST) of all the strains	14	Yes		
07	M-JRP7-18	Second batch of *Lm* genomes annotation completed.	26	Yes		Assembly and annotation with a harmonized in-house workflow named ARTwork. All the genomes are centralized in a database.
07	M-JRP7-19	*Ad hoc* batch of *Lm* genomes assembly completed	25	Yes		Assembly and annotation with a harmonized in-house workflow named ARTwork. All the genomes are centralized in a database.
07	M-JRP7-20	Bioinformatic analysis done for all the strains	28	Yes		
07	M-JRP7-22	Ad hoc batch Lm genomes annotation completed	20	Yes		Assembly and annotation with a harmonized in-house workflow named ARTwork. All the genomes are centralized in a database.
07	M-JRP7-23	Face-to face meeting -2020	25	Yes		
07	M-JRP7-24	WGS Proficiency Testing trial (PTtrial) done	26	No		Given the delay in the project, we decided that there will be no Proficiency testing trial WGS as planned. However, as part of the activities carried out by ANSES as EURL / NRL Listeria, a PT trial WGS was organized in

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
						2018, then in 2019, during which four LISTADAPT NRL partners (AGES, IZSAM, NVI, ANSES) participated.

5.1.4.3.4.3 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors already accepted the comments. Therefore, this part of the report can be closed.

5.1.4.3.4.4 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
First report on the occurrence of Listeria monocytogenes ST121 strain in a dolphin brain. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7601084/ https://zenodo.org/record/4244051#.X6J4fziWxM1	YES	Gold	1250
Comparative analysis of genetic determinants encoding cadmium, arsenic and benzalkonium chloride resistance in Listeria monocytogenes of human, food and environmental origin Front. Microbiol., 14 January 2021 https://doi.org/10.3389/fmicb.2020.599882 https://zenodo.org/record/4456720#.YLDmBzhDtM0	YES	Gold	1000

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Exposure to quaternary ammonium compounds selects resistance to ciprofloxacin in *Listeria monocytogenes*. *Pathogens*. 10, (2) 220. https://doi.org/ 10.3390/pathogens10020220 https://doi.org/10.5281/zenodo.5471681	YES	Gold	1100
Genetic Characterization of a Listeria monocytogenes Serotype IVb Variant 1 Strain Isolated from Vegetal Matrix in Italy. Microbiol Resour Announc. 2020 Aug 13;9(33):e00782-20. DOI: 10.1128/MRA.00782-20 https://doi.org/10.5281/zenodo.5472980	YES	Gold	500
A European-wide dataset to uncover adaptives traits of *Listeria monocytogenes* to diverse ecological niches. Accepted in the review Scientific Data in December 2020	YES	Gold	1000
Genomics elements located in the accessory repertoire drive the adaptation to biocides in Listeria monocytogenes strains from different ecological niches. Accepted in the review Food Microbiology in December 2020. https://doi.org/10.1016/j.fm.2021.103757	YES	Gold	850
Investigation of genome characteristics underlying fitness of *Listeria monocytogenes* in soil. In preparation for Frontiers in Microbiology.	YES	Gold	1200

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Assessment of the ability to adapt to biocides and develop cross-resistance to antibiotics for *Listeria monocytogenes* strains. In preparation for Frontiers in Microbiology.	YES	Gold	1200
Genomic diversity of *Listeria monocytogenes* strains isolated in Slovakia during 2010-2020. Will be submitted in June 2021 –Frontiers in Microbiology	YES	gold	1200
Draft genomes of two Listeria monocytogenes strains isolated from invasive snails Arion vulgaris in Austria. MRA00375-21R1. https://doi.org/10.5281/zenodo.5472856	YES	gold	1100

Oral communications:

2020

- Skjerdal T, Fagereng T, Osland AM, Lagesen K, Fiskerbeck E, Nesse L, Sévellec Y, Felix B, Roussel S. 2020. Phenotypical responses to stress of in *Listeria monocytogenes* strains of different Clonal Complexes isolated along the nature-to-farm-to-fork chain. 27-29 May. Second annual meeting EJP (virtual meeting)

- Sévellec Y, Ascencio Schuttz E, Félix B, Guillier L, Roussel S, Piveteau P. Comparative Analysis of the genomic diversity of *Listeria monocytogenes* in soil and water and food processing through pan Genome Wide Association Study. 27-29 May. Second annual meeting EJP (virtual meeting)

- Palma F, Guérin A, Radomski N, Bridier A, Sévellec Y, Félix B, Soumet c, Guillier L , Roussel S. Deciphering the Biocide-Resistance of *Listeria monocytogenes* Strains from Europe through Genome-Wide Associations at the pangenomic scale. 27-29 May. Second annual meeting EJP (virtual meeting)

- Guérin A, Palma F Le Grandois P, Bridier A, Soumet C, Sevellec Y, Roussel S. Exposure to quaternary ammonium compounds show resistance to ciprofloxacin for *Listeria monocytogenes* from diverse ecological niches. 27-29 May. Second annual meeting EJP (virtual meeting)

- Sept 2020: Food Micro Next Generation Challenges in Food Microbiology –Athènes. Reported in September 2021/Three abstracts submitted (Y. Sévellec, S. Roussel)

- Sévellec, Y. An insight in Listeria genomes. Terramo. annual workshop of the ItNRL Lm. 16th September 2020

2019

- Felix B, Feurer C, Maillet A, Guillier L, Boscher E, Kerouanton A, Denis M and Roussel S (2019). Population genetic structure of *Listeria monocytogenes* strains isolated from the pig and pork production chain in France. 26-27 August 2019, Safepork 2019, Berlin.

- Félix B, Feurer C, Maillet A, Desmonts M H, Hickey B, Jankuloski D, Karpíšková R, Skjerdal T, Denis M, Gareis M, Zdovc I, Pietzka A and Guillier L (2019). Typing and persistence of Listeria monocytogenes strains in food processing environments, prophages identified as major persistence markers. ISOPOL XX 2019, 24 – 27 September 2019, Toronto.

- Guillier L (2019). Assessment of the Genomic Diversity of a Large Collection of *Listeria monocytogenes* Strains Isolated in EU Natural Environments. OHEJP Annual scientific meeting, Dublin, 22-24 May 2019

- Guillier L (2019). Proposal of an Original Method for Selecting Strains to Include in Source Tracking or Source Attribution Based on their Metadata. OHEJP Annual scientific meeting, Dublin, 22-24 May 2019

- Sévellec, Y. 2019. Listadapt- Study of the Genetic diversity of *Listeria monocytogenes* from environment to human; Terramo. annual workshop of the ItNRL Lm. 4th July 2019

2018

- Felix, B., 2018. Population genetic structure of *Listeria monocytogenes* strains isolated from the pig and pork production chain in France, presented at Food Micro September 2018, Berlin (Germany).

- S. Antoci, V. A. Acciari, V. Di Marzio, I. Del Matto, G. Centorotola, M. Torresi, C. Marfoglia, G.

- Iannitto, A. Ruolo, G. A. Santarelli, G. Migliorati, F. Pomilio. Preliminary results on prevalence and persistence of *Listeria monocytogenes* in different dairy and meat processing plants in Central Italy, presented at "International meeting on emerging diseases and surveillance" November 2018, Vienna (Austria).

Posters:

2021

- Taran Skjerdal#, Tone Fagereng, Eve Fiskebeck, Karin Lagesen, Sophie Rousse. Impact of genetic variation of 200 Listeria monocytogenes strains on the growth in presence of preservatives. OH-EJP annual scientific meeting Copenhagen, 9-11 June 2021.

- Toresi M, Rinaldi A, Marzio D et al. Genomic features of two main Clonal Complex groups of Listeria monocytogenes strains isolated from European wild animal. 6th World One Health Congress; 30 October - 3 November 2020.

2019

- Torresi M., Rinaldi A., Cammà C., Di Pasquale A., Skjerdal T., Lagesen K., Felix B., Sevellec Y., Guillier L., Leroux A., Ricao M., Boysen M., Lindström M., Castro H., Korkeala H., Gareis M., Frank E., Bulawova H., Brychta M., Amar C., Grant K., Pate M., Zdovc I., Pomilio F (2019). Diversity of *Listeria monocytogenes* associated with wild animals: focusing on CCs with a wide capacity of adaptation. International Symposium on Problems of Listeria and Listeriosis ISOPOL XX. 24-27 September 2019 Toronto

- Oevermann A, Hurtado A, Papić B, Karpíšková R, Piveteau P, Wullings B, Bulawova H, Castro H, Lindström M, Korkeala H, Šteingolde Ž, Bērziņš A, Avsejenko J, Kramarenko T, Cabanova L, Szymczak B, Torresi M, Leroux A, Sevellec Y, Guillier L and Félix B (2019). European-wide study reveals high prevalence of hypervirulent Listeria monocytogenes clones in farmed ruminants and their environment. ISOPOL XX -24 – 27 September 2019 Toronto

- Skjerdal T, Sevellec Y, Guillier L, , Zdovc I, Pate M, Torresi M, Riacao M, Boysen M, Lindstrøm M, Castro H, Gareis M, Bulawova H, Amar C, Grant K, Leroux A, Pomillo F, Camma C, Di Pasquale A, Lagesen K, Osland Mohr A, Rinaldi A, Karpiskova R, Pietzka A, Ruppitsch W, Szymczak B, Ascencio-Schultz E, Piveteau P and Felix B (2019). Occurrence and diversity of *Listeria monocytogenes* strains in environment and wild life in Europe. ISOPOL XX -24 – 27 September 2019 Toronto

- Ascencio-Schultz E, Gal L, Garmyn D, Szymczak B, Karpiskova R, Pietzka A, Ruppitsch W, Boysen

M, Pomilio F, Torresi M, Camma C, Di Pasquale A, Pate M, Skjerdal T, Sevellec, Y., Felix B, Guillier L and Piveteau P (2019). Investigation of genome characteristics underlying fitness of *Listeria monocytogenes* in soil. ISOPOL XX -24 – 27 September 2019 Toronto

- Sévellec Y, Torresi M, Orsini M, Centorotola G, Bilei S, Senese M, Terracciano G, Felix B, Guillier L and Pomilio F (2019). Investigation of a dolphin infection by *Listeria monocytogenes* CC121. ISOPOL XX -24 – 27 September 2019 Toronto

- Vranckx K, Sevellec Y, Deneweth J and Felix B. Phages in Listeria: Who are they, what do they do? ISOPOL XX -24 – 27 September 2019 Toronto

- Guérin A, Le Grandois P, Bridier A, Soumet C (2019). Evolution of antibiotics and biocides resistance of *Listeria monocytogenes* from diverse ecological niches following in vitro exposure to biocides disinfectants. ISOPOL XX -24 – 27 September 2019 Toronto

- Guérin A, Le Grandois P, Bridier A, Soumet C (2019). Evolution of antibiotics and biocides resistance of *Listeria monocytogenes* from diverse ecological niches following in vitro exposure to biocides disinfectants. IAFP's European Symposium on Food Safety, Nantes, 24-26 April 2019

- Guérin A, Le Grandois P, Bridier A, Soumet C (2019). Evolution of antibiotics and biocides resistance of *Listeria monocytogenes* from diverse ecological niches following in vitro exposure to biocides disinfectants. OHEJP Annual Scientific meeting, Dublin, 22-24 May 2019

2018

- Felix B, Feurer C, Maillet A, Guillier L, Boscher E, Kerouanton A, Denis M, Roussel S. 2018. Population genetic structure of *Listeria monocytogenes* strains isolated from the pig and pork production chain in France. IAFP EU Stockholm 25-27 April 2018

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

D-JRP7-1.4 Report on strain collection and strategy for selection of strains for sequencing.

Scientific Paper : "A European-wide dataset to uncover adaptive traits of *Listeria monocytogenes* to diverse ecological niches." Accepted in the review Scientific Data

LISTADAPT made it possible to strengthen the collaboration between the consortium and a team of DTU (Danish Technical University, DK). This team (Research Group for Genomic Epidemiology, expert in machine learning (Njage P et al. 2019. Risk Analysis 39:1397-1413) asked the coordinator to provide them the 1575 genomes and metadata in order to develop a tool predicting the resistance of *Lm* strains to biocides used in food industry.

D-JRP7-3.1, 3.2, 3. 3, 3. 4, 3.5, 3.6-Phenotypic data of 200 strains isolated from food, animals and environment.

This output included all the phenotypic data obtained (resistance to antibiotics, effects of biocides on *Lm* strains adaptation; bacterial adhesion and biofilm formation of *Lm* strains, survival and persistence

of *Lm* strains in different ecological niches). This data is very valuable, as the 200 strains panel has been selected to be balanced between reservoirs, sub-reservoirs, sampling area and CCs with high and low degree of genetic relatedness

All the phenotypic data obtained is centralized in a database. This data will be compiled with all the genomic data for a mixomics approach.

The results obtained on the phenotypic data will be valuable for EURL activities such as selection of strains for challenge testing of low pH foods, and for risk assessments of foods with preservatives. Further, these results can be used to assess the validity of predictive models that have been developed with few strains for strains with other genetic characteristics.

ADEPALE, a professional federation of the French food industry, is very interested in the results. Following the presentation of the project by the coordinator in November 2020, they asked to give them a presentation in May 2021 on all the combined phenotypic/genotypic data obtained. They want to understand the mechanisms of adaptation of the strains in the plant chain from the soil to the finished plant products and wish to set up a research collaborative project with ANSES on this subject.

Are there any outcomes of this project that are already discussed or even implemented and in use at any institute of the project consortium, at stakeholders' organisations (ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), or at the level of national authorities?

A large part of the 1575 genomes is used in an important research project (2020-2024) between INRAE and Anses (funded the French Research Agency-ANR). This new project aims to develop tools for the detection and quantification of persistent intracellular *Lm*, in order to progress in the knowledge of the biology of this phenomenon, on the mechanisms leading to asymptomatic carriage in host, and to propose diagnostic, therapeutic and preventive solutions for at-risk human populations (such as pregnant women and the elderly) and farm animals. This project also aims at studying the variability of this intracellular persistence phenotype according to the genetic and ecological diversity of *Lm* strains.

The collection of 1575 genomes has been explored using a machine-learning approach recently implemented at DTU-Food (Njage et al., 2019). Complementary to GWAS analyses, this approach allows in silico prediction of phenotypical properties (survival in sol, adhesion, biofilm formation…) for the entire collection, including isolates that will not have been characterized at the phenotypic level. Several machine-learning methods (gradient-boosting, random forest, support vector machine) will be tested within the SEL Unit, in close collaboration with the DTU-Food.

Some of these strains and genomes are used in the One Health EJP Project "CARE" (2020-2023) (Cross-sectoral framework for quality Assurance Resources for countries in the European Union).

5.1.4.3.4.5 One Health impact

The detailed characterization of these strains at phenotypic and genotypic level will help to assess the true importance of these strains as sources of foodborne infections for public health. Mechanisms for the survival and adaptation of Lm (i) in food processing environment (ii) in wild and farming animals (iii) in natural and farming environments will be identified. The genes identified will be used as targets for developing rapid monitoring tests. With a view to controlling risks in agricultural and agri-food systems, this project will make it possible to assess the relevance of monitoring plans, for instance in agricultural soils.

The results obtained in WP3-T4-ST1 will be valuable for EURL activities such as selection of strains for challenge testing of low pH foods, and for risk assessments of foods with preservatives. Further, these results can be used to assess the validity of predictive models that have been developed with few

strains for strains with other genetic characteristics.

With LISTADAPT project, we are looking for molecular markers of interest such as for instance mobile genetic elements harbouring antimicrobial resistance factors as well as provide insight into the population structure and evolutionary history of *Lm* for epidemiologic investigation. This information could be used for the development of **new diagnostic tests** to screen food, processing environment and animal reservoirs for contamination by *Lm* strains. These new tests represent **key tools** to improve the *Lm* surveillance system and to assist the food industry decision-making around food processing for improving food safety. For instance, a professional federation of the French food industry is interested in results obtained in W3/WP4. Following the presentation of the project in November 2020, they asked the coordinator to give them a presentation in 2022 on the results obtained in WP4. They want to understand the mechanisms of adaptation of the strains in the plant chain from the soil to the finished plant products and wish to set up a research collaborative project with Sophie's team on this subject.

The LISTADAPT project makes it possible bridging the gap between "Med" and "Vet". Listdadapt strengthened the collaboration between Anses and the Institute of Microbiology and Parasitology of the Veterinary Faculty of the University of Ljubljana. A project entitled "Detection of Listeria monocytogenes clonal complexes in animal and food samples using real-time PCR" was submitted in April 2021 in response to the call PHC Proteus (Programme Proteus 2022 | Campus France). Moreover a joint paper Anses/ Institute of Food Safety, Animal Health and Environment BIOR, Latvia; entitled "Characterization and genetic diversity of Listeria monocytogenes isolated of cattle abortions in Latvia, 2013-2018" has been submitted in July 2021 at the Journal Veterinary sciences.

This is the first time a project has focused on such a large and diverse collection of *Lm* strains isolated from farming/wild animals and farming/wild environment in different European countries. Most of these strains and genomes are very useful for the One Health EJP Project "CARE" (2020-2023) (Cross-sectoral framework for quality Assurance Resources for countries in the European Union).

5.1.4.3.4.6 Data Management Plan

All the produced genomes will be available to the scientific community (umbrella *Bioproject* in European Nucleotide Archive (ENA)) as soon as we have finished checking all the genomes.

The paper, in revision in the review Scientific Data, lists all the strains, metadata and genomes produced during LISTADAPT. All the produced genomes will be available to the scientific community (umbrella *Bioproject* in European Nucleotide Archive (ENA)) as soon as the paper is definitely accepted.

5.1.4.3.4.7 List of dissemination and communication activities

Name of the activity:	Poster / IAFP's European Symposium on Food Safety		
Date:	24–26 April 2019		
Place:	La Cité des Congrès de Nantes, France		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	

Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)	300	Media	X
Industry	15	Investors	X
Civil Society	X	Customers	X
General Public	X	Other	X
Policy Makers	10		X

Name of the activity:	1 Poster and 1 oral communication / OHEJP Annual Scientific meeting
Date:	22-24 May 2019
Place:	Teagasc Conference Centre, Ashtown, Dublin

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	300	Media	
Industry	5	Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	5		

Name of the activity:	Oral presentation at OHEJP Annual Scientific meeting: Proposal of an Original Method for Selecting Strains to Include in Source Tracking or Source Attribution Based on their Metadata
Date:	22-24 May 2019
Place:	Teagasc Conference Centre, Ashtown, Dublin

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	300	Media	
Industry	5	Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	5		

Name of the activity:	Oral presentation OHEJP Annual Scientific meeting: First Assessment of the Genomic Diversity of a Large Collection of *Listeria monocytogenes* Strains Isolated in EU Natural Environments
Date:	22-24 May 2019
Place:	Teagasc Conference Centre, Ashtown, Dublin

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	300	Media	
Industry	5	Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers	5		

Name of the activity:	1 Oral presentation and 6 posters International Symposium on Problems of *Listeria* and Listeriosis (ISOPOL)
Date:	September 24 - 27, 2019
Place:	The Peter Gilgan Centre for Research and Learning, Toronto

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	Yes
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		Number
Scientific Community (Higher Education, Research)	500	Media	3
Industry	20	Investors	0
Civil Society	0	Customers	0
General Public	0	Other	0
Policy Makers	20		X

Name of the activity:	*IAFP's European Symposium on Food Safety*		
Date:	*April 25 – April 27 2018*		
Place:	*Brewery Conference Centre, Stockholm, Sweden*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***
General Public	***X***	***Other***	***X***
Policy Makers	***X***		***X***

Name of the activity:	*FoodMicro conference*		
Date:	*September 2018*		
Place:	*Berlin*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***
General Public	***X***	***Other***	***X***
Policy Makers	***X***		***X***

Name of the activity:	*International meeting on emerging diseases and surveillance".*
Date:	*9th-12th November.*
Place:	*Vienna*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***
General Public	***X***	***Other***	***X***
Policy Makers	***X***		***X***

5.1.4.3.5 JRP10-FBZ3-MOMIR – Final Report

5.1.4.3.5.1 Consortium composition

Participant No *	Participant organisation name	Country
Dr. Velge Philippe (COORDINATOR) Dr. Laroche Béatrice	INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE (INRA)	France
Dr. Martine Denis	AGENCE NATIONALE DE SECURITE SANITAIRE DE L'ALIMENTATION, DE L'ENVIRONNEMENT ET DU TRAVAIL (ANSES)	France
Pr Hristo Daskalov	National Centre of Food Safety, NDRVMI, BFSA	Bulgaria
Dr. Rychlik Ivan	VETERINARY RESEARCH INSTITUTE (VRI)	Czech Republic
Pr Rodríguez Bertos, Antonio	VISAVET HEALTH SURVEILLANCE CENTRE-UNIVERSIDAD COMPLUTENSE DE MADRID (VISAVET-UCM)	Spain
Pr La Ragione Roberto	UNIVERSITY OF SURREY, School of Veterinary Medicine	United Kingdom
Dr Barbara Chirullo	Istituto Superiore di Sanità (ISS)	Italy
Pr Alborali Giovanni	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER)	Italy
Dr Hagenaars Thomas J.	Dienst Landbouwkundig Onderzoek (CVI/ DLO) including Wageningen Bioveterinary Research (formerly CVI)	The Nederlands
Dr Naseer, Mohammed Umaer	Norvegian Institute of Public Health (NIPH)	Norway
Pr de Jong, Mart	Netherlands Centre for One Health (NCOH)	The Nederlands

Short CV of each participating key scientist (at least one per participating organization)

This part summarizes the major activities and competencies of the person over the last 5 years. Include information that indicates the coordinator's skill to manage international projects.

Partner 18 INRAE (Coordination):

Partner 18-Tours: Dr. **Philippe Velge** (male, 56 years old) has been graduated from the Lille University in life science and Health. He is Director of Research at INRA. He was Deputy Director of the Federative Research Institute: IFR136 "Agents transmissibles et Infectiologie" between 2008 and 2012, and was co-responsible for the "pole bactériologie" of the ISP department (40 permanent scientists).

Since 2000, he is the head of the group "Signalisation, Portage et Virulence Bacterienne" within the Infectiology and Public Health department (ISP). Currently, this research group consists of 7 permanent Scientists and 3 permanent technicians. This research group has analyzed the virulence mechanisms of *Salmonella* and the asymptomatic carrier state induced in chicken.

He is an expert in analysis the interactions of bacteria with the host. He has worked mainly on the virulence mechanisms of *Listeria monocytogenes* and *Salmonella* both *in vitro* and *in vivo*. He also analyzes the *Salmonella* carrier state in poultry. His fields of interest are Microbiology, Cellular Microbiology and Infectiology.

He participated in four European projects: Heritability (1998-2001), Novacsal (1998-2002), Salarray

2000-2004), Supasalvac (2003-2008), two Network of excellence (Eadgene, 2005-2009; Eadgene-S (2009 – 2012)) and an Integrated Project SABRE (2006-2010). He coordinated the ANR project Genanimal "Resisal" (2006-2009) and 3 other National projects. He has participated to three Transnational Emida or Anihwa projects: Heathy gut (2010-2014), coordinated by P. Barrow (U. of Nottingham, UK), DIFAGH (2012-2015) coordinated by A. Smith (Oxford U., UK), AWAP (2016-2018) coordinated by M. Dawkins (Oxford U., UK), and one European FP7 project Prohealth coordinated by I. Kyriazakis (Newcastle University, UK).

Dr P. Velge has more than 90 articles published in books or in international journals (h-index of 24 in Scopus).

Partner 18-Tours: Dr. **Mustapha BERRI** (gender: M) received his PhD degree in Biochemistry in 1995 from the University of Blaise-Pascal of Clermont-Ferrand in France. He completed also a three years postdoctoral training at the University of Madison-Wisconsin in the USA in 1998, and joined the French National Institute of Agronomic Research as a biochemist to work at Lymphocytes and Mucosal Immunity laboratory previously directed by Dr. Henri Salmon. His scientific research interests involved the development and trafficking of immune cells within mucosal surfaces of pig as a model. He used cellular and molecular immunology approaches to characterize the basic mechanisms underlying the induction and dissemination of mucosal immune response between the respiratory, the gastrointestinal tracts and the mammary gland. His team have improved the scientific knowledge to elicit and enhance maternal lactogenic protection in sows to develop earlier active mucosal immunity in the neonate piglet in order to increase the animal's resistance at the weaning, and to decrease the use of antibiotics. His research is also focusing to study the cellular and molecular host/pathogen interactions in the pig model, and to test candidate vaccines or new potential therapeutic molecules such as probiotics, prebiotics or feed additives. He coordinated research programs funded by the French minister of agriculture and participated in several scientific projects including ANR-funded Sus-Flora and Piglet Biota. He is the author of 50 scientific publications and a co-inventor of 4 exclusive operating licences granted to private companies.

Partner 18-Jouy: Dr **Béatrice Laroche** (female, 57 years old) graduated from the Ecole Normale Supérieure and Paris 6 University in Applied Mathematics. After several years spent in R&D in industry and in university, she was appointed research director in the Applied Mathematics and Computer Science department at INRA in 2011 in the MaIAGE research unit of Jouy-en-Josas. She is the author of more than 40 articles in peer reviewed proceedings and journals. She is or has been involved in various collaborations based on epidemic as well as digestive physiology, system biology and gut microbiota modelling, in the framework of projects funded by INRA (gut metagenomic data integration through modelling), by the French National Research Agency (ANR AlimIntest 2007-2009 digestive physiology and microbiota modelling, CADENCE 2016-2019 epidemic modelling at landscape scale, WP leader in DALLISH, 2016-2019, modelling & system biology) and by the EU (COST Infogest, 2011-2015). She is currently a member of the coordination team of a national INRA program, "Holobionts and Microbial Flux within Agri-food Systems", focused on microbial communities in interaction with their host in agricultural systems.

Partner 1 ANSES: **Martine DENIS** (female, 52 years old, HDR), is Doctor in Biological Sciences from the university of Rennes, France. She is Deputy Head of the research unit "Hygiene and Quality of poultry and pork products" director of research project in pig production and responsible of the NRL of *Campylobacter,* at ANSES Laboratory from Ploufragan. The unit (30 peoples including 9 Scientifics) is National Reference laboratory for *Campylobacter*, *Salmonella*, avian salmonellosis and avian botulism, and develops its research activities for the Control of zoonotic bacterial agents through a multidisciplinary approach in the poultry and pig productions. The projects focus on *Campylobacter*, *Salmonella*, *Yersinia*, *Listeria* and *Clostridium botulinum*. Her research activities are divided into three

thematic areas : 1) Prevalence, risk factors and molecular epidemiology for obtaining data on prevalence, quantification, risk factors and genetic diversity along all the food chain, and tracking bacteria from reservoir, animal, to human (development of techniques for detection / numeration / typing (classical or molecular methods), 2) Host-pathogen relationship for a- obtaining knowledges on colonization of animals by bacteria, on the spread of bacteria between animals in link or not with the digestive microbiota, on the relationship of the pathogen with different matrices, b- characterizing the field strains and human strains by studying virulence mechanisms / phenotypic characteristics of the strains (development of animal models / methods for virulence and phenotypic characters), 3) Control measures at farm by reducing excretion of bacteria by animals under the action of molecules, feed or vaccination, at abattoir by reducing the spread of bacteria in slaughterhouse under the action of technological parameters (development of animal models / technological tools). She is the author of more than 50 scientific publications.

Annaëlle KEROUANTON (female, 44 years old) is project leader in the the research unit “Hygiene and Quality of poultry and pork products”. She received her PhD degree in life and health science in 1999. She is researcher at the French Agency for Food, Environmental and Occupational Health & Safety since 2000. She has been currently involved in national research or European projects on foodborne pathogens (*Salmonella*, *Campylobacter*, *Listeria*). In the research unit “Hygiene and Quality of poultry and pork products”, she is in charge of research projects on foodborne zoonoses related to pigs. Current research interests include characterization of pig response to *Salmonella* infection.

Partner 6: NDRVMI, Prof. Dr **Hristo Yordanov DASKALOV**, PhD, DVM (Male, 61 years old); Head of NRL Salmonella, Campylobacter, Staphylococci and Antimicrobial Resistance; National Centre of Food Safety, NDRVMI, BFSA. Former Deputy President of AGRICULTURAL ACADEMY, BULGARIA, May 2015 – Present (1 year 10 months) Sofia, Bulgaria; Professor at National Center of Food Safety, NDRVI, BFSA, March 2013 – Present (8 years). Head of National Center of Food Safety of Bulgarian Food Safety Agency, Sofia, BULGARIA , December 2011 – Present.
Scientific projects – Heriot-Watt University, Edinburgh, UK, 1996-1998, Colorado State University, CO, Fort Collins, 2003; Bourlag Program, USDA, Colorado State University, CO, Fort Collins, 2006, EFFORT project, 7th frame scientific program EU, 2013-2018, One Health EJP, Horizon 2020, EU, 2018 – 2022.
Publications - https://scholar.google.com/citations?user=lx1_eWcAAAAJ&hl=en;
https://www.researchgate.net/profile/Hristo_Daskalov2

Partner 8: VRI, **Ivan Rychlik** (gender: M) graduated in 1989 at the Faculty of Science, Masaryk's University, Brno, Czech Republic. Since 1989 till presence he has been employed at the Veterinary Research Institute, Brno, Czech Republic where he has been a leader of *Salmonella* group since 2004. Currently this group consists of 15 people. He is an author or coauthor of more than 100 publications listed in Web of Science database with H-index 21. Current research interests include characterization of chicken response to *Salmonella* infection, design of live attenuated *Salmonella* vaccines for oral administration to chickens, analysis of composition and function of chicken gut microbiota, use of defined microbiota members for prevention of chicken colonization with *Salmonella* and *Campylobacter* and chicken immune response to the inoculation with microbiota of different composition.

Partner 16: VISAVET-UCM ; **Lucas Dominguez** (gender: M) is a Doctor in Veterinary Medicine and Head of the VISAVET Health Surveillance Centre. He is full professor at Veterinary Medicine Degree at Complutense University of Madrid being involved as well in Master classes teaching. Throughout his career he has given over 400 lectures and participated in 20 books. At present, he has an index h of 37, 296 published articles having SCI cited over 5000 times. Most of his work has been included in high impact journals in the first quartile of the category of Microbiology and Veterinary Science. He has directed 22 doctoral theses, 7 of them with European Mention, and 6 PhD Extraordinary Award. He

has been principal investigator of 47 research projects and has participated in other 30 projects. He has particular expertise in Foodborne Zoonoses and Antimicrobial Resistance, including *Salmonella, Campylobacter*, *E. coli*, *Yersinia* and *Staphylococcus aureus*. Dr. Lucas Domínguez is in charge of several lines of research on the characterization of important foodborne pathogens for Public Health and the mechanisms involved in the emergence and spread of antimicrobial resistance by using molecular tecniques as well as sequencing.

María Ugarte-Ruiz (gender: F) is Doctor in Veterinary Medicine (2015) and Bachelor of Veterinary Medicine (2003-2008), University Complutense Madrid (UCM). Since 2009, she has been working at VISAVET Health Surveillance Centre and participated as Assistant teacher at Veterinary Medicine Degree at Complutense University of Madrid. She realized stays in the "London School of Hygiene & Tropical Medicine" and the "U.S. Enviromental Protection Agency, Ohio". She is responsible of the Foodborne Zoonoses and Resistance Antimicrobial Unit, which is focused on the study of Foodborne Zoonoses and Antimicrobial Resistance, including *Salmonella, Campylobacter*, *E. coli*, *Yersinia* and *Staphylococcus aureus.* Her research resulting to date in eleven scientists articles, five of them as first author, as well as nine participations in international conferences. She is also co-authored the chapter of a book published by Springer.

Antonio Rodríguez Bertos (gender: M) is professor of the Veterinary Faculty since 1991, obtaining his PhD degree in Veterinary Pathology in 1994. He has published over 40 articles in the JCR and participated in over 20 research projects funded by private and public companies. He is currently Lecture Professor in the Department of Internal Medicine and Animal Surgery (Veterinary Faculty – Complutense University of Madrid). He has a wide knowledge designing experimental procedures to evaluate pathological alterations induced for different bacterial infections. His work as a veterinary technical expert in detection techniques in Spanish National Accreditation Body (ENAC) and EQA (Entity Management System Certification - ISO 9000 and ISO 14000 Environmental Verification) support its capacity as auditor evaluator. Recently, Dr. Rodriguez-Bertos has started a new research line in the spectrophotometry field in veterinary by using MALDI TOF Imaging, which could be applied as an useful tool in Healthy Gut studies.

Susana Gómez-Barrero (gender: F) is Doctor in Biochemistry and Molecular Biology. Her career has been centered in Human Genetics, diagnosis and research in hereditary diseases. She has won a National research award on Darier-White disease, and has directed a doctoral Thesis of Medicine and Surgery. Since 2007, she has been working at VISAVET Health Surveillance Centre, involved in several lines of research based on molecular characterization of several zoonosis, mainly *Salmonella, Campylobacter*, *E. coli* and *Staphylococcus aureus*. She has contributed to the writing and performance of several research projects, she is co-author of several published articles and participated in a number of Congresses.

Partner 22: University of Surrey; **Prof Roberto Marcello La Ragione** (gender: M) is a specialist in veterinary microbiology and pathology with special interest in food-borne pathogens and antimicrobial resistance. After a PhD thesis at the government Veterinary Laboratories Agency (VLA) (1996-99), a post-doctoral position at Royal Holloway (University of London) (1999-2001), he was appointed as a Senior Scientist in the Department of bacterial diseases, VLA, Weybridge (2001-05). He was Head of pathogenesis and control at the AHVLA (2005-15) and after being Senior Lecturer in veterinary microbiology (2009-10), he was appointed Professor of Veterinary Microbiology and Pathology at the University of Surrey in 2010. He gained the FRCPath in 2010 and in 2012 was appointed the Associate Dean for Veterinary Strategy in the new School of Veterinary Medicine at the University of Surrey. He is the past president of the Med-Vet-Net Association and the Head of Pathology and Infectious Diseases and Deputy head of the School of Veterinary Medicine at the University of Surrey. He is the author of over 160 publications (h-index of 45).

Partner 27: ISS ; **Barbara Chirullo** (gender: F, PhD) is working at the Istituto Superiore di Sanità since 2010. as researcher at the unit Prophylaxis and Control of Bacterial Zoonoses, Department of

Veterinary Public Health and Food Safety, Istituto Superiore di Sanità; Director.

As scientist she is currently involved in the study of the interface between pathogens and immune system as an approach to understand the pathogenicity of infection diseases and to develop new or more efficient vaccines to control infections. From 2019, she is part of the group of expert for the Italian Technical and scientific Committee for Nutrition and Animal Health, sections Veterinary Medicine and Pharmacovigilance, on veterinary medicines, for scientific and technical evaluation on immunological products and, from 2020, of the Group of Experts no. 15V Veterinary vaccines and sera of European Farmacopea.

Paolo Pasquali (gender: M, DVM, PhD) is working at the Istituto Superiore di Sanità since 2000. Director (2008-today), unit Prophylaxis and Control of Bacterial Zoonoses, Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità; Director (2013-today), FAO Reference Centre for Veterinary Public Health, Istituto Superiore di Sanità.

As scientist he is currently involved in the study of the interface between pathogens and immune system as an approach to understand the pathogenicity of infection diseases and to develop new or more efficient vaccines to control infections. He authored or co-authored more than 70 research papers in peer reviewed journals and he is serving as editor in chief of the journal Research in Veterinary Science.

In the last few years he took part as coordinator or key person at several projects funded by national or international organizations

Partner 29: IZLER ; **Alborali Giovanni Loris** (gender: M, DVM) is working in IZSLER since 1999 where he is the head of the department of diagnostic. He is spending the most part of time with porcine and bovine diagnostic, infection disease and health management. The mainly lines of research in which he has participated are the following: Antibiotic- resistance; monitoring program about principal bacterial diseases, with particular attention to the role of respiratory diseases (APP and *H. parasuis*) and enteric diseases (*Salmonella* spp. and *Brachyspira* spp.); Porcine Reproductive and Respiratory Syndrome Virus (PRRSV); Aujeszky Disease Virus (ADV); disease associated with Porcine Circovirus 2 (PCV2) , Swine Influenza Virus.

Partner 30: Wageningen Bioveterinary Research/Wageningen Research; **Thomas J. Hagenaars** (gender: M, PhD) (CVI, Wageningen UR, NL) is a mathematical epidemiologists working as a senior researcher and project leader in veterinary epidemiology group at the Central Veterinary Institute, part of Wageningen UR. He has more than 20 years of experience in research quantifying livestock disease spread using mathematical transmission models, and employing transmission models to assess the effectivity and/or risks of both preventive and reactive intervention strategies. In this research he has modelled the spread of wide variety of infectious agents in livestock and humans, including bacteria, viruses and agents causing transmissible spongiform encephalopathies. Most of this work was carried out to inform veterinary policy making, e.g. of the Dutch Ministry of Economic Affairs. Thomas combines attention to sound modelling with the ability to apply the modelling in a fashion relevant to the practical policy-making context. Much attention in his research is devoted to estimating model parameter values either from appropriate field data or from suitably designed experiments. Thomas is an Associate Editor of “BMC Veterinary Research”.

Partner 32: NIPH; **Mohammed Umaer Naseer** (gender: M, PhD) completed a Philosophiae Doctor (PhD) degree in medical microbiology from the University of Tromsø in Norway (2009) building upon a Masters in Technology (Molecular Biotechnology) from the same institute in 2005. Expert on the molecular epidemiology of antimicrobial resistance. His postdoctoral research focused on the identification of virulence factors, mutation potentials and transfer mechanisms of resistance with emphasis on plasmid biology using whole genome sequencing tools. Since 2013 employed at at the Norwegian Institute of Public Health (NIPH), now at the Department of Zoonotic, Food- and Waterborne Infections. In 2016 completed the European Centre for Disease Prevention and Controls

(ECDC) two-year competency based training programme in public health microbiology (EUPHEM).

Partner 41: NCOH ; Prof **Mart C.M. de Jong** (male, 1957) is currently the chair of quantitative veterinary epidemiology at Wageningen University. His research interest is the quantification of transmission of infectious agents in animal populations and the effect of interventions on this transmission. His publications (2021: number of publications 210, h-index=43, citations: 5,984) are on stochastic/statistical models to quantify transmission from transmission experiments and observational data. Furthermore, most publications are about the application of these methods to data on a large number of infectious agents important for animal husbandry or because they are zoonotic. For example the effects of vaccination on transmission of important OIE listed infections. Currently, the main line of research is on the role of the environmental phase of infectious agents on the transmission. He is an expert member of the official advisory board on animal disease control in the Netherlands.

5.1.4.3.5.2 Summary of the work carried out in the Project

The **MoMIR project** aimed to develop new approaches to predict, identify and prevent the appearance of animal and human super-shedders based on immune response and gut microbiota composition. The vast majority of the planned objectives were achieved despite the many problems encountered during the project, and thanks to the time extension granted to us.

This project led to

1- Reproduce the heterogeneity of *Salmonella* infection, characterized by the super- and low-shedder phenotypes in all the species studied (Human, pig, chicken mouse), beside the use of different infection models**.**
2- Original and interesting results, which allow better understanding of the heterogeneity of excretion and to predict and differentiate between these different shedding phenotypes. Moreover, the project allowed the development of original epidemic mathematical models and methods for data analysis.

Among the most important advances, we can highlight:

- **The key role of animal-animal recontaminations** in the spread of *Salmonella* infection at the flock level, which are mainly related to the presence of Super-Shedders, functioning as a reservoir for the pathogen. Our results, have shown that variation in the presence/absence of bacterial virulence cannot explain the super and low shedding phenotypes. However, in contrast, host factors and gut microbiota composition play a crucial role. For example, the gut microbiota composition before infection determines in chicks the super and low shedder phenotypes. Moreover, although the risk of human-to-human transmission of *Salmonella* from asymptomatic carriers is considered low, our study indicates a 10-fold higher rates (24%) of nontyphoidal *Salmonella* shedding than previously suggested.

- **The development of original mathematical models** of epidemics and methods of analyzing intra-animal transmission data. A mathematical model of indirect transmission of bacteria between broilers was also developed. The model can be used for designing and quantitatively assessing candidate bio-security based intervention strategies against indirect transmission of *Campylobacter* and *Salmonella.*

- **The identification of several control strategies** based on the importance of the ventilation in farms and the identification of the animals at risk. For that purpose, the MoMIR-PPC project identified several predictive biomarkers, based on gut microbiota composition and immune parameters. The identified biomarkers, which still need to be further confirmed, can either predict the levels of *Salmonella* shedding (in pigs or in chickens) if animals are infected, or can differentiate between the *Salmonella* shedding levels. We also identified the major factors associated with the long term shedding of *Salmonella* in humans.

- **The identification of several preventive measures based on the use of pre- and probiotics.** To prevent salmonellosis, we isolated, characterized and tested the efficacy of probiotics and prebiotics for use in pigs and poultry. The studies were undertaken in experimental infections and in field conditions. For example, we were tested the interventions in 72640 chicks in Bulgaria and 130 000 chicks in Czech Republic. These probiotics and prebiotics can be used as an alternative to antibiotics and may help reduce AMR. Samples were also taken from a number of challenge and intervention studies for metagenomic analysis. Finally, a draft inventory of relevant intervention measures against *Salmonella* in poultry has been developed and the cost effectiveness (utility) of intervention strategies using probiotics against Campylobacter has been calculated.
- These results have been or will be disseminated in Joint scientific articles and in congresses.

5.1.4.3.5.3 Work carried out in the JRP, scientific results and integrative outcomes

WP0. Management

WP0-T1- Draft and agree Consortium Agreement

As consortium agreement was signed between the EJP coordinator ANSES and the EJP beneficiaries, we considered at the first MoMIR-PPC meeting that it was unnecessary to sign a particular consortium agreement at the MoMIR-PPC level (**Deliverable.0.2**).

WP0-T2- Produce project-planning, control documentation and Data Management Plan

The project planning has been modified several times due to the left of SAIM from the EJP consortium, and of the Vet-DTU, which has been closed by its Government. The work, which should be performed by these partners, has been taken into account by a new partner (NNDRVMI). We also modified the project planning of the Norwegian Institute of Public Health because Astrid Louise Wester left her institute. The final project planning was decided in June (month 6). The data management plan was updated several times during the project. A final version has been produced in Excel format (**Deliverable.0.1 ; D.4.2**).

WP3-T3- Control and manage activity progresses, the timely delivery of project tasks and outputs

Numerous exchanges have been performed to coordinate the work performed by the members. This has been done during the intermediate and final meetings and during video-conferences. Informal meeting were organized between few partners to exchange information, to define who will test what (especially concerning the pro and prebiotics, which were tested in farms) and to build joint articles. We discussed also how to store and exchange the bacterial strains developed in the project. Many experiments were postponed due to the COVID-19 crisis, which had an impact on the work of other Partners. This was managed through the extension of the project (**Deliverable.0.2**).

WP0-T4- Control and manage the project closure and outputs

The final meeting took place in video-conference on 10-11 May 2021. During this meeting, all partners presented their results, but they also provided the necessary information for the final report. All partners read, completed and validated the final report (**Deliverable.0.3**).

WP1. Risk prediction for Super-shedder animals and human asymptomatic carriers through the use of gut microbiota and immune status analyses (M1-M12)

The goal of this WP was to identify immunological and microbiota markers that are associated to super/low shedding before and/or after infection, which allow us to predict and identify the animals which will be Super shedders. For this WP, we used experimental infection models and also naturally infected animals and humans in “field condition”. Taking into account the great adaptability of

pathogens for their hosts, the virulence and immunogenicity of *Salmonella* strains originated from Super- and low-shedders were compared.

WP1-T1- Predictive immunological markers associated to the high and low shedders in chickens and pigs

The goal of the task was to identify immune markers able to identify the super-Shedders and other able to predict the most susceptible animals and/or the animals that will become super-shedders, if infected. The activities focused on the most prevalent serotypes: *S.* Typhimurium for pig, and *S.* Enteritidis for chicken.

Four Partners were involved in this task (ANSES, ISS, IZLER and INRAE), which were done with pigs (ANSES, ISS, IZLER and INRAE), chickens (INRAE) and mice (ISS). A Post doc who, undertook her PhD in the ISS lab, was recruited by INRAE-Tours to manage part of the work devoted to SAIM.

The results obtained by **ISS** and **IZLER** showed in mice and pigs that IFN gamma gene expression (and protein level) correlates to *Salmonella* burden. Moreover, a higher INF-gamma production increases the ability of animals to control infection. In contrast, a specific pro-inflammatory response and stimulation of inflammation pathways is predictive of super-shedder phenotype (**Deliverable.1.1**).

ANSES performed a trial with a total of 45 piglets divided into five groups: one group with five piglets as control and four groups each with 10 piglets (n=40) as inoculated pigs. At 7 weeks of age, piglets were infected with 10^9 CFU/piglets of a monophasic variant of *Salmonella* Typhimurium strain. Pigs were followed during 3 weeks after inoculation before being necropsied. The level of *Salmonella* shedding allowed us to identify three significantly different classes ($p<0,01$) corresponding to high (n=13), intermediate (n=16) and low shedders(n=11) pigs. Regarding the blood cell number, 12 parameters showed a significant difference (p-value<0.05) between inoculated pigs and controls, at one or several days after infection, most of them were white cells. The difference was also confirmed to be also significant between super- and low-shedders for 5 parameters, Lymphocytes, basophile, Granulocytes, and Haematocrite). Quantification of TNF-α, IL-1β, IL-6 and IFN-γ, showed that a significant difference ($p<0,01$) was observed on the median production level between super- and low-shedders for IL1-β and IL6 at 1DPI (**Deliverable.1.3 ; D.1.7)**. As in part observed in chicks, Low shedders pigs seroconverted earlier than intermediate and high shedders pigs (**Deliverable.2.11**). The results obtained by **ANSES** and **INRAE** suggested that the expression of one immune related gene could be a predictive biomarkers (**Deliverable.1.6**). The expression of several host genes could be biomarkers able to characterize the low- and super-shedder phenotypes. Moreover, a strong pro-inflammatory immune response is correlated to the super-shedder phenotype. This is counter-intuitive but could be explained by the articles describing that *Salmonella* requires intestinal inflammation to sustain its replication in the intestinal tract. Indeed, intestinal inflammation leads to availability of nutrients that are otherwise not accessible in the uninflamed gut and to a better competition with the resident microbiota by creating microaerophilic conditions.

The results obtained from the chick studies performed by **INRAE** showed that the number of blood immune cells circulating in the blood could not be used as a predictive marker for the appearance of the low and super-shedder phenotypes (**Deliverable.1.6**). Moreover, the analysis has shown no differences, after infection, when low and super-shedders are compared with the control group. Similarly, to identify predictive immunological markers associated to high and low shedders, we analysed the differences of the humoral immune response of low and high-shedders and compared their response to non-infected chicks by measuring the production of total (non-specific) IgA, IgY, IgM before and after infection. Generally, the analysis has revealed that the total antibody responses follow a classical pattern with a rise in levels of total IgM preceding the rise in IgY. The total immunoglobulin response was low before infection and increase after infection. Moreover, no significant differences were observed between naïve/low shedders and high shedders. This result shows that level of

immunoglobulin cannot be a predictive marker for low shedders and high shedders. Interestingly, a statistically significant increase in IgY level has been observed at 7 days post infection in high-shedders when compared with low-shedders and control groups. Finally, the evaluation of anti-*Salmonella* LPS IgA levels has revealed a lack of response in all the samples analyzed except for two of them, suggesting that anti-*Salmonella* IgA response is not a good candidate to investigate differences between low-shedders and high-shedders in contrast to total IgM response(**Deliverable.1.6 ; D1.7; 2.11**). This work had been performed in collaboration with the Bernd Kaspers's lab (U. Munich), **during a short stay, funded by EJP**.

In the same way, the immuno-histological work performed by **VISAVET-UCM** on caecal samples did not show any major histopathological changes between low and super-shedders. In contrast, the inoculation of four commensal bacteria the day of hatch, and which protect chicks from *Salmonella* infection (see WP2), modified both the number of immune cells circulating in the blood during *Salmonella* infection and the host immune response to the pathogen (**Deliverable.2.6**).

WP1-T2- Predictive microbiota markers associated to the high and low shedders in chickens and pigs

The goal of the task was to analyse the gut microbiota composition before and after *Salmonella* infection to identify predictive microbiota markers associated to the super and low shedders in chickens and pigs. Samples from representative pigs and chickens originated from activities of task 1.1 were analyzed based on Illumina sequencing of V3/V4 variable region of 16S rRNA genes. The same experiments were used to analyse both the immune response and the gut microbiota composition.
Four Partners (ANSES, ISS, IZLER and INRAE) were involved in this task for the animal experiments performed with pigs and chicks. UoS sequenced all samples from pigs while VRI sequenced all samples from chicks. Sequencing data were analyzed by UoS, VRI and INRAE.

The work performed by **INRAE** and **VRI** with chicks demonstrated the role of gut microbiota in the susceptibility to *S.* Enteritidis infection and in the appearance of the low and super-shedder phenotypes. We especially demonstrated that (1) axenic and antibiotic-treated chicks are more prone to become super-shedders; (2) super or low-shedder phenotypes can be acquired through microbiota transfer; (3) specific gut microbiota taxonomic features determine whether the chicks develop a low- and super-shedder phenotype after *Salmonella* infection in isolator **Figure 1** (**Deliverable.1.8**). It has been especially described that presence of *Enterococcus* could be a predictive markers of low *Salmonella* susceptibility (**Deliverable.1.9; 1.10**). This study demonstrates the key role plays by gut microbiota composition in the heterogeneity of infection and the possibility to increase resistance to *Salmonella* colonization with pro and prebiotics (see WP2). An article describing this work has been accepted for publication in Microbial Biotechnol.

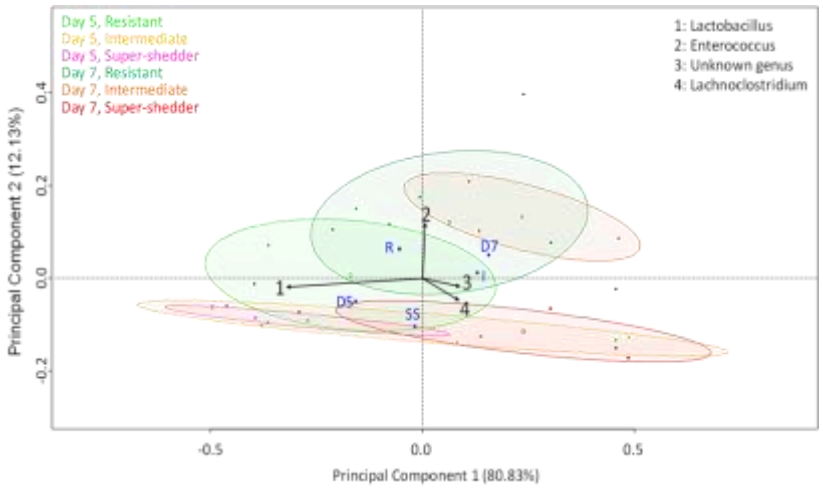


Figure 1: PCA analysis of the genus frequencies observed before infection (i.e. at 5 and 7 days of age), in order to highlight predictive markers among the three shedding level categories (Low (resistant), intermediate and high (super) shedder phenotypes). Chicks were orally infected at 7 days of age and fecal samples were recovered before and after Salmonella infection. Gut microbiota composition was analyzed by 16S rDNA sequencing.

For pigs, **ANSES**, **UoS** and **INRAE** showed that numerous taxa are specific to the low and super-shedder phenotypes (**Deliverable.1.9; 1.10**). A Co-inertia analysis between the immune genes dCt and microbiota features abundances, conducted at **INRAE** did not show a correlation between the gut microbiota composition and immune gene expression (**Deliverable.1.15**). A joint article is in preparation.

Similarly, **ISS** and **IZLER** performed experiments in pigs and sent the samples to **UoS** for microbial community analysis. The samples have been analysed in conjunction with metadata related to the animal's overall health and shedding status, to test the hypotheses regarding the association of the gut microbiome with *Salmonella* shedding status. No clear correlation was observed (**Deliverable.1.9 ; 1.10**). However, It should be noted that both studies were carried out using pigs with different genetic backgrounds and *Salmonella* strains, therefore this created an opportunity to test the association between the microbiome and *Salmonella* shedding in two different scenarios.

WP1-T3- Risk factors associated with prolonged convalescent *Salmonella* shedding in humans FHI driven this Task.
The goal of the task was to investigate the risks factors associated with chronic *Salmonella* infection in human population. This Task was managed by NIPH. A cohort study was carried out on all nonthyphoidal- (NT) salmonellosis cases registered in the Norwegian Surveillance System for Communicable Diseases (MSIS). All potential participants were asked to send a stool sample and to respond to an online/paper questionnaire to collect information. Stool samples were processed at **NIPH** for *Salmonella* growth. Participants with long-term shedding (stool samples positive for *Salmonella* growth by direct culture approximately 5 weeks post infection) were compared to participants with short-term shedding of *Salmonella* (stool samples negative for *Salmonella* growth by direct culture approximately 5 weeks post infection) in order to identify associated factors with long-term shedding. (**Deliverable.1.11**).

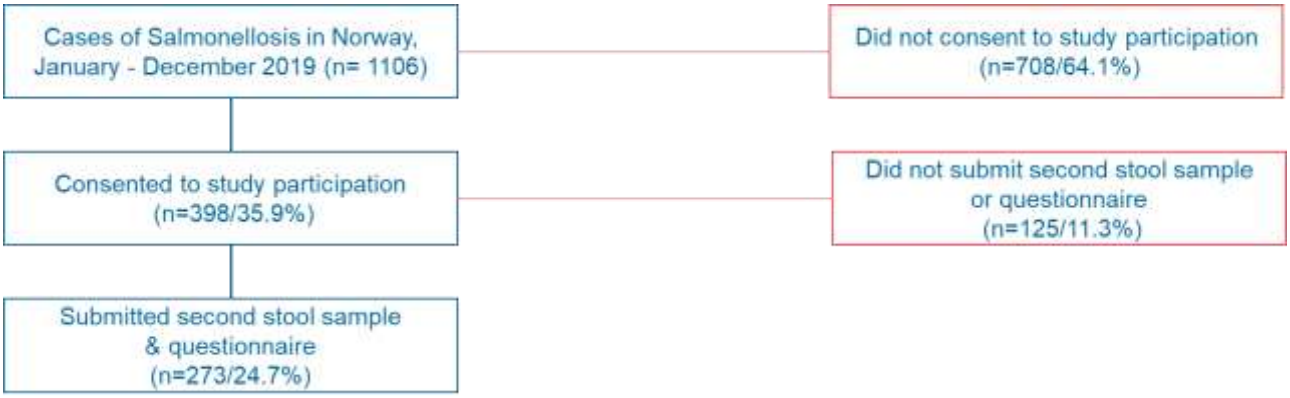


Figure 2: Process describing collection of data

Furthermore, all initial *Salmonella* isolates submitted to NRL were serotyped, screened for antibiotic susceptibility and whole genome sequenced (Illumina) (**Deliverable.1.12**). The analysis of host risk factors associated with long-term shedding shows that the percentage of long-term shedders is higher

(25%) than previously reported (1-2.2 %) (Gal-Mor O. *Clin Microbiol Rev*. 2018;32(1):e00088-18). Moreover, NIPH identified the main risk factors to become long term shedders (**Deliverable.1.12**):
- *Y*ounger people are *more at risk* (OR 3.5, 95%CI 1.05- 11.56)
- A lactose free diet (OR 5.19, 95%CI 1,21 - 22,31)
- Taking regular medication (OR 2.51, 95%CI 1,42 - 4,41)
- Taking food supplements, vitamins and health foods (OR 2.45, 95% CI 1,09 - 5,49)

No specific pathogen characteristic was identified: no association was found between serotype, sequence or Complex type and long-term shedding. The association with Fosfomycin resistance is tentative due to the small sample size. In conclusion, these results suggested that long term shedding seems more dependent of host factors than pathogen characteristics (**Deliverable.1.5**).
Recent studies indicate that transmission of *Salmonella* is controlled by indigenous intestinal microbiota. We had planned to select pairs of cases (Long-term shedders and Short-term shedders) that shared pathogen or host specific characteristics, with the aim to analyze the host microbiota. However, due to the shortening of the sample collection period, the sample size was considerably reduced and we were unable to perform analytical statistical analysis. The findings are based on univariable and descriptive analysis, and as result, the scientific merit of completing metagenomics is compromised. As our assessment was that metagenomic analyses of fecal samples would not add weight to our descriptive findings, we have therefore concluded that more data is needed to substantiate the associations that have been uncovered in our study before metagenomics will add scientific value.

WP1-T4- Virulence of *Salmonella* strains originated from high and low shedders

The goal of the task was to determine whether the super and low-shedder phenotypes could be related to a modification of the virulence level of the *Salmonella* strains during infection.

In vitro virulence of *S*. Typhimurium and *S*. Enteritidis strains collected from super-shedders and low-shedders pigs (**ANSES**) and chickens (**INRAE**) were evaluated by **INRAE** in pig and chicken epithelial and macrophage cell lines.
Salmonella strains from two super-shedders and two low-shedders animals were used in each experiment in comparison with the strain*,* which was inoculated. **INRAE** evaluated *Salmonella* adhesion, invasion and intracellular multiplication to an intestinal pig epithelial cell line (IPEC-1) and a pig macrophage cell line (3D4) as well as with a chicken epithelial cell line (LMH). No differences were observed between *Salmonella* strains*,* whatever the serotype, for any of the parameters evaluated (**Deliverable.1.5**). In addition, the gene expression of pro-inflammatory and anti-inflammatory genes was evaluated. No differences were observed in the gene expression of CXCL8, IL8 or TGFβ for any of the conditions evaluated. In conclusion, our data do not show any alteration in the *in vitro* virulence of *Salmonella* strains from super-shedder and low-shedder origin (**Deliverable.1.14**). These results strongly suggested that the super-shedder and low-shedder phenotypes are not related to a modification of the virulence level of the bacterial strains. These results are in line with those obtained in human (**Deliverable.1.15**).

WP2. Prevention of the appearance of Super-shedder animals and asymptomatic carriage in humans and animals by modifying feed and/or microbiota (M1-M12)

The goal of this WP was focused on the development of preventive and control measures. We tested especially the protective activity of pre-biotics, pro-biotics or nutraceutical formula and assess their effects on immune response, gut microbiota composition, gut physiology and animal performance.

WP2-T1- Development and use of probiotics in chickens and pigs

In this Task, we identified and characterized probiotics and tested their protective effect in pigs and chickens.

Four Partners were involved in this task (**VRI**, **UoS**, **NDRVMI** and **INRAE**), which were done with pigs and chickens in field and experimental conditions.

VRI has developed an extensive library of commensal bacteria from chicken gut, which currently consists of more than 450 isolates with known genomic sequences (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP101913&o=acc_s%3Aa). These strains were used for oral inoculation of chicks on the day of hatching followed by verification of their presence 7 days later. The conclusion is that in this type of experimental design, bacterial species expressing outer membrane, i.e. Bacteroidetes, Proteobacteria and Veillonellaceae, can efficiently and persistently colonise chicken caeca. Rather unexpectedly, they never succeeded with the colonisation of newly hatched chicks with Gram positive bacteria from phylum Firmicutes and families Lachnospiraceae, Ruminococcaceae or Lactobacillaceae, despite the fact that these species are common microbiota members. Recently **VRI** changed the protocol for culture of gut anaerobes and this seems to provide novel opportunities for culture of yet unculturable species. This modification consisted of culture under microaerophillic conditions and using Karmali agar. Several experiments have been performed to understand 1- why the inoculation of Lachnospiraceae, Ruminococcaceae or Lactobacillaceae which are otherwise common in gut microbiota on chickens 1 to 2 weeks old, were unable to colonise chicks after oral inoculation and 2- the origin of these bacteria in the caeca of chickens.

Other experiments were performed to determine whether the colonisation of chicken intestine with some strains modified on the long term the faecal and/or caecal microbiota composition. For this purpose defined mixtures consisting of bacterial species which can colonise chicken caecum after a single dose on day of hatch were gradually tested in real commercial farms. Altogether, over 130 000 chickens were treated with different mixtures consisting mainly of different *Bacteroides, Prevotella* and *Megamonas* species. Due to the financial scopes, these experiments were co-funded also by other projects of **VRI**. Central issue when upscaling from laboratory to field level was the administration of chicken gut anaerobes to flocks consisting of more than 10, 000 chickens. This was done via drinking water, resuspension of liquid cultures to pre-starter feed, jellyfying liquid probiotic cultures before their spread over the pre-starter feed or even feed fermentation by the probiotic strains. The control of gut colonisation showed that tested probiotic strains persist in the caeca of treated broilers till day 35 of life. Moreover, since some of the flocks were formed by reproductive birds, bacterial species were detected in these flocks till week 20 of their life. Thus, the persistence of selected and tested species seemed to be quite long if not permanent (**Deliverable.2.3; D.2.6; D2.5**).

UoS isolated a large panel of potential probiotic candidates from pig faeces. Following identification by 16s and basic *in vitro* characterisation a subpanel was selected for further study. A panel of 30 lactobacilli were phenotypically and genotypically characterised. These studies included growth curves, survival in acid/bile, whole genome sequencing and tissue culture studies (pig cell line) to determine safety and inhibitory ability of whole cells and CFS against *Salmonella* adhesion/invasion (ongoing). To date the studies have indicated that a number of strains are suitable probiotics that meet the EFSA requirements. Four probiotic strains (2 isolated from chicken faeces in a related project) and 2 isolated from pig) were sent to Bulgaria (**NDRVMI**) for efficacy evaluation in chickens and pigs, respectively (**Deliverable.2.3; D.2.6**). If required, strain will also be shared with the EU AVANT H2020 project."

NDRVMI has analysed the effect in field conditions of several probiotics and prebiotics obtained from the **UoS**. They used 4 groups of day-old broilers (18,160 in each group). In groups with probiotics 1 and 2, the birds were raised without antibiotics, as well as any other side effects. Growth rates and food consumption were also excellent and mortality was a little lower than in the control group (where antibiotics were still used). In the third experimental group, where probiotics 1 and 2 were used and a prebiotic was added to stimulate probiotic microorganisms, there were some issues in the middle of the experiment with a sharp increase in mortality observed, this was due to the presence of a

pathogenic strain of *E. coli* and for 5 days they were treated with an antibiotic after which the mixture of probiotic 1 and 2 and prebiotic were delivered until the time of slaughter. This group was not a linear hybrid Ross like the other groups, but a linear hybrid Cobb, which appeared to be much more susceptible to disease. The experiment was repeated only with this compromised third group and the control, as the linear hybrid was Ross. Probiotics were used on farms with a long history of *Salmonella* infection and periodic treatment with antibiotics allows the birds to be free of *salmonella* at the time of slaughter. It is noteworthy that *Salmonella* were not detected only in group with probiotic 1, which leads us to think that probiotic 1 has a more pronounced anti-*Salmonella* effect in the gastrointestinal tract. However, the combination of the two probiotics did not result in the elimination of *S.* Infantis throughout the growing period (**Deliverable.2.5; D.2.7**). Moreover, in the group with both probiotics and prebiotic a reduced incidence of intestinal disorders and better food conversion ratios was observed compared to the control group (**Deliverable.2.5; D.2.6 ; D.2.7**).

The experiments with pigs started on September 2020, when 3 experimental groups of weaned pigs were formed, 25 in a group and transferred to individual boxes without a connection between boxes. An additional group of pigs of the same age, reared conventionally (in the same way as those receiving the intervention) on the farm, were used as controls. The pigs in all four experimental groups received the same feed etc., with the only difference being the respective probiotic or a mixture of the two probiotics tested with the addition of prebiotic fed through the drinking water. Particular attention was paid to the safety of supplying a sufficient amount of water containing at least one billion living cells in a milliliter of broth culture dissolved in drinking water. It should be noted that treatment of pigs with *Lactobacillus reuteri* SAP 3319 or a combination of both probiotics and a prebiotic resulted in a reduced incidence of intestinal disorders and the absence of *Salmonella* in the faeces (**Deliverable.2.5 ; D.2.6 ; D.2.7**).

After completion of the experiment with the broilers and the pigs, all samples were sent to the **UoS** for 16s microbial community analysis, which is ongoing (**Deliverable.2.8 ; D.2.9**).

In WP1 **INRAE** identified *Enterococcus faecium* as a predictive biomarker for the most resistant phenotype. To test the protective activity of this commensal bacteria against *Salmonella* colonization, a candidate *E. faecium* strain was isolated, purified and orally inoculated to a group of 30 chicks reared in a large isolator (CFU=1x109 per chick) at 1 and 6 days of age (i.e. before Salmonella infection) (**Deliverable.2.3**). However, no significant difference was observed between the *Salmonella* shedding levels of the *E. faecium*-inoculated and control groups (at any time point). To go further we tested the impact of four commensal bacteria containing *E. Faecium* but also *E. coli*, *Lactobacillus* and *Clostridium* strains. When administrated the day of hatching, this cocktail induced a partial protection against *Salmonella* infection at 7 days of age (**Deliverable.2.5**). The data obtained shows the protective effect of the four commensal bacteria on *Salmonella* excretion and their major impact, on the long term, on gut microbiota composition and immune response. This study paved the way for developing a protective mix of probiotics. An article describing this work has been accepted for publication in Microbial Biotechnol. The impact of the four commensal bacteria on the gut microbiota composition and the immune response was done by **INRAE** with a 16S metabarcoding approach in collaboration with **VRI** and with the Biomark approach. One publication is in preparation (**Deliverable.2.5; D.2.6 ; D.2.10**).

<u>WP2-T2- Use of pre-biotics and nutraceutical already defined by the consortium partners in chicken and pig</u>

The goal of this Task is to measure the effect of several pre-biotics and nutraceuticals on intestinal barrier, gut microbiota composition and protection against pathogens in farms and in experimental conditions.

Mainly three Partners (**VRI, VISAVET-UCM and NDRVMI)** were involved in this task, which was undertaken with pigs and chickens in field and experimental conditions.
To identify bacterial species, which are dependent on particular supplements or growth substrates, **VRI** tested specific supplementation of nutrient broths used for growth of commensal strains. At the beginning of the experiment, caecal contents of adult hen are decimally diluted and inoculated to nutrient broth. Nutrient broths are then anaerobically incubated and after 3 days, microbiota composition is determined by 16S rRNA sequencing. WCHA agar and BHI medium supplemented with lactate, glucose, starch, cellulose, mucin, bile salts, panthenol, biotin, vitamin B12 or whole vitamin B complex were tested. In addition, in the last experiment **VRI** inoculated BHI supplemented with sodium acetate, propionate, lactate, succinate, pyruvate, fumarate, ascorbate, glucose, maltose, saccharose, trehalose, fucose, rhamnose, pectin and inulin. These experiments allowed us to i) determine metabolic potential of individual gut microbiota members, ii) define conditions under which it had been possible to enrich target gut anaerobes and obtain them in pure cultures (see WP2-T1) and iii) map characteristics of different supplements which can be used as prebiotics (**Deliverable.2.6**).

VISAVET-UCM tested the protective effect of fermented defatted “alperujo” against *Salmonella* infection in chickens. The objective of these experiments was to analyze the modification of the microbiota and intestinal mucosa changes before and after the challenging, comparing treated and control groups.

The first experiments performed with laying hens demonstrated the positive effect of fermented defatted “alperujo”. Indeed, a significant increase of duodenal villi height and crypt depth (duodenum and cecum) was observed in the treated group at early life stages. A higher abundance of Actinobacteria, Firmicutes and Proteobacteria was also assessed in the treated group at early life stages as well as decreased number of broken eggs in the treated group (**Deliverable.2.6**).

A second experiment performed with broilers leads to similar conclusion. Significant increase of duodenal villi height was observed in the treated group from 14 to 35 days-old, as well as significant body weight increase at 35 days-old. Fermented defatted “alperujo”consumption also favours microbiota modulation, that may control the spread of pathogenic bacteria by means of competitive exclusion.

Several experiments have demonstrated, in vitro, the effect of “alperujo” on *Salmonella*. To test its effect *in vivo*, chicks were divided in two groups: one that was fed with conventional feed and a second one that was fed with “alperujo” since their arrival. After a week of adaptation, animals were challenged with a *Salmonella* strain resistant to colistin. Besides, a subgroup of animals was challenged after 21 days consuming the “alperujo”. The analysis of the data shows a significant reduction of *Salmonella* in the cecum, with both traditional culture and qPCR approaches, at 7 and 14 dpi. However, no significant differences were observed at 21, 28, and 35 dpi (28, 35, and 42 days old, respectively) in the cecal *Salmonella* load among groups by culture ($p > 0.05$) or by qPCR ($p > 0.05$) (see Figure) (**Deliverable.2.7**).

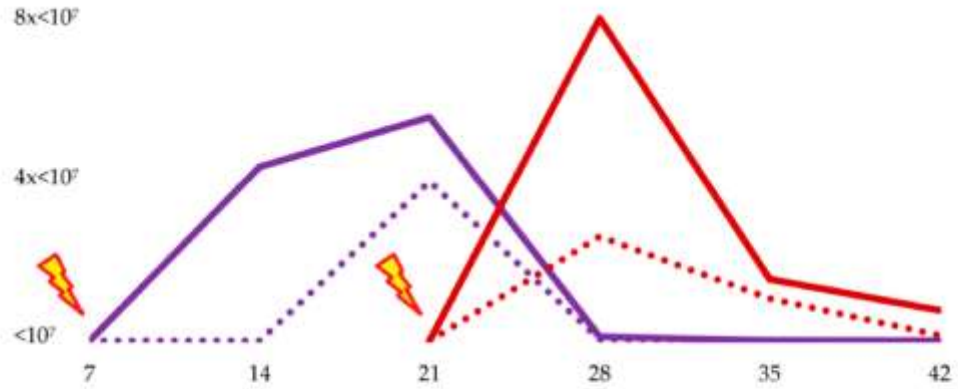


Figure 1. Results of the *Salmonella* spp. count in selective agar in broilers challenged with *Salmonella* Typhimurium at day 7 (purple lines) and day 21 (red lines) of life. Continuous lines represent control groups, whereas discontinuous lines represent treated groups, fed with fermented defatted 'alperujo'. The *Salmonella* spp. count by culture (CFU/g) is shown on the vertical axis, and samplings (7, 14, 21, 35, or 42) on the horizontal axis.

The intestinal histopathology measures revealed only few differences in the duodenum between the control and the treated group. In the ceaca, there was a reduction in the intensity of lamina propria lymphocytic infiltration and GALT hyperplasia in the treated group compared to the control group. In 7-day-old challenged chickens, duodenum villi height was significantly improved in treated chickens on days 7, 14, 28, 35, and 42 ($p < 0.05$). Similarly, the crypts in the duodenum were deeper in in all treated samplings ($p < 0.05$). Regarding ceca morphology, crypts were seen to be deeper in 7-, 21- and 42-day-old treated chickens ($p < 0.05$) but not at 28 days of life. Broilers in the treated group challenged at 21 days of age showed a significant improvement in the duodenum villi height at 28, 35, and 42 days of life ($p < 0.05$). The depth of the crypts in the duodenum was significantly improved by the treatment on days 28 and 42 ($p < 0.05$). The ceecal crypt was deeper in treated chickens on days 35 and 42 of life ($p < 0.05$).

Treatment with fermented defatted "alperujo" has no significant impact on caecal microbiota (**Deliverable.2.8**). In chickens challenged at 7 or at 21 days of age, there were no statistically-significant differences among the groups established.

WP3. Modelling the transmission of zoonotic agents to improve intervention strategies on livestock farms (M1-M12)

The third WP was focused on the development of multiscale computational models (from within-host to between- host scale) of heterogeneous pathogen transmission by integrating data from WP1 and 2 and already available datasets by consortium partners in order to design and assess intervention strategies. The models was designed to allow assessment of the efficiency and/or cost-effectiveness of different intervention strategies.

WP3-T1- Transmission modelling at within-host and between-host scales

The goal of the task was to develop simplified, but relevant models of the host including key features of the gut microbiota dynamics and key features of the immune system response.

Three partners (NCOH-WU, INRA-Jouy, WUR-WBVR) were involved in this Task with fruitful discussion with the other partners.

WP3-T1-ST1- Within-host scale: modelling individual responses and shedding

INRAE-Jouy designed a generic mathematical model of the dynamic interplay between the gut microbiota, the pathogen and the host's immune response at the within and between-host scale. A paper has been published (**Deliverable.3.1**).

To improve the model with a better representation of the microbiota and pathogen dynamic, **INRAE-Jouy** completed the realization of a C++ software and the corresponding Matlab and R plugins allowing to analyse time series of microbial concentrations. This software implements a non parametric, robust inference approach for fitting Generalized Lotka-Volterra model on time series data. It will be made publicly available in a near future on INRAE gitlab repository (https://forgemia.inra.fr/), a publication is currently being written.

The model was used to analyze a first set of time series of gut microbiota composition in chicken (with **INRAE-Tours** and **VRI**) in order to infer interactions between the pathogen (here *Salmonella*) and the resident microbiota. However, the results were not satisfactory because the time series were very short. In collaboration with **UoS** and **INRAE-Tours** we recently started analysing longer microbiota time series from WP1 (ANSES pig experiment) with this software, together with other data analysis approaches, to better understand and model the interplay between of the immune response and the microbiota. To conclude, this task allowed the development of original epidemic models and methods for data analysis. However, the opportunity to work on the pig data came very late in the course of the project, so we will be able to provide an output on data analysis only close or after the end of the project.

WP3-T1-ST2- Between-host scale: modelling transmission, linked to within-host results
Mathematical modelling analyses were carried out of the outcomes of tailor-made experiments studying the indirect transmission of Campylobacter between broilers. In previous research also consisting of a combination of experiments and mathematical modelling, a mathematical model of indirect transmission of bacteria between broilers was developed. This model assumes that bacteria are transferred from inoculated animals (source animals) to spatially separated susceptible animals (recipient animals) through random displacement of infectious material in the environment in combination with a loss of viability of the bacteria in time. Technically this model uses diffusion equations to describe the random displacement of material in the environment between the source and recipient animals. New experiments carried out during the MoMIR project served to validate and refine this mathematical model by testing predictions in an experimental setting, and to do so consisted of four different spatial setups that were each studied in two repeat animal rooms. . The refined model can be used for designing and quantitatively assessing candidate bio-security based intervention strategies against indirect transmission of *Campylobacter* and *Salmonella* (**Deliverable.3.2**).
This model not only allows to test biosecurity/hygiene measures, but also offers desired biological insight. In particular it explains the distance-dependence of the indirect transmission rate observed in the experiments, as well as an observed distance-dependent time delay of transmission. The model does so using only three parameters: 1) a 'loss-of-viability' rate describing how fast the pathogen is becoming inactivated in the environment; 2) a diffusion coefficient describing how the spatial distribution of infectious material in the environment changes in time; 3) a transmission parameter summarizing host-dependent processes (shedding and dose response). As a consequence, the model enables to quantify processes occurring in the environment separately from host-dependent processes.

WP3-T2- Interventions strategies: Identification and evaluation tools
The goal of the task was to include the different other intervention strategies with those described in WP3-T1. Three partners (WUR-WLR, WUR-WEcR and INRAE) were involved in this Task.

WP3-T2-ST1- Systematic inventory of relevant intervention measures

A systematic inventory of relevant intervention measures against *Salmonella* in broilers and laying hens has been developed.

WP3-T2-ST2- Inclusion of potential interventions into the modelling

At within-host scale, **INRAE-Jouy** obtained first encouraging results on the use of microbiota and inflammatory response data in **ANSES** pig experiment to discriminate pigs according to their shedding pattern, in collaboration with **UoS** and **INRAE-Tours**. A publication is currently started. The resulting knowledge, as well as knowledge from probiotic strategies in WP2 will be used in the future to include probiotic based strategies in the model developed in WP3-T1-ST1.

WP3-T2-ST3- Development of economic analysis tools

The economic analysis tools allow to evaluate the cost effectiveness (utility) of intervention strategies using probiotics to reduce *Campylobacter* prevalence in broilers. The work performed by WUR WEcR has been done with results of other MoMIR work packages and model developed in CamCon1) (EU 7th framework programme). The tools determine the cost per averted disease burden (in €/Disability-Adjusted Life Year, DALY), by taking into account the attribution of DALY's to broiler sector, and correcting for import/export. The cost-utility is obtained by calculating the cost per averted disease burden as a function of the on-farm efficacy. The model has been parameterized for four different European countries (Denmark, Netherlands, Poland, Spain) spanning a range of differently structured poultry sectors: taking into account differences in technical and economic farm performance, and scale of poultry production. A scientific paper has been published (**Deliverable.3.3 ; D.3.5 ; D.3.6**). The main results obtained with the model suggested:

- Assuming an efficacy between 10% and 20%, costs per avoided DALY were lowest in Poland and Spain (4,000–30,000 Euro per avoided DALY) and highest in the Netherlands and Denmark (70,000–340,000 Euro per avoided DALY)
- In Poland and Spain, using probiotics is a moderately expensive intervention if efficacy is more than 10%, otherwise it is relatively expensive
- In the Netherlands and Denmark, using probiotics is a relatively expensive intervention irrespective of efficacy
- However, if probiotics were assumed to enhance broiler performance, the intervention would become relatively cost-effective even at low efficacy levels of 1 to 10%.

WP4. Communication and Dissemination for Impact (M1-M12)

WP4-T1- Dissemination of data within the project and management of data

Participation to the OHEJP-ASM conferences in Dublin (2019) and to the virtual conferences (2020 and 2021) with several posters and conferences.

As described in the report, Partners have exchanged for analysis numerous samples and thus the corresponding data from animal experiments. A synthetic Excel file summarized the animal experiments as well as the samples recovered (**Deliverable.4.3**). UoS and VRI have sequenced the samples from pigs and chickens, respectively. UoS, INRAE-Tours and VRI Have analyzed the data and thus the gut microbiota composition of the different samples. VISAVET-UCM has performed histological analyses, INRAE-Tours has performed virulence assays with strains recovered by other Partners. INRAE-Tours and ISS have performed immune responses analyses. UoS, IZLER and ISS have sequenced and analyzed the data and thus the gut microbiota composition of the different samples. IZLER and NDRVMI have performed field experiments with strains of UoS. Data obtained by other Partners during experimental infections have been sent to INRAE-Jouy and to U. Wageningen. Consequently, an impressive collaboration has made it possible to analyze many parameters of the in vivo experiments performed with pigs and chickens.

WP4-T2- Dissemination of data outside the project and management of data

Participation to several congresses outside the EJP. Several articles have been accepted for publication in open access journals (**Deliverable.4.4**). These articles often involved two or more partners. Several joint articles are in preparation. Due to the Covid19 crisis the high strategic meeting has been cancelled and the budget devoted to this activity has been reimburse to EJP (INRAE) and (NCOH)). After the international lockdown, we will disseminate our results in international congresses and to stakeholders (**Deliverable.4.5**).

Project self-assessment

The vast majority of the planned objectives are achieved despite the many problems encountered during the project, and thanks to the time extension granted to us.

An **exceptional extension of 12 months** of the project at no extra expense has been indeed obtained by the EJP Project Management Team. This extension was motivated by:

- The withdrawal of SAIM from the EJP consortium after the approval of our project. Part of the work has been performed by INRAE with the help of a Post doctorant.

- Astrid-Louise Webster has left the NIPH, just before the beginning of the project, so that we have adapted the project with Anke-Stüken. These modifications have delayed the ethics committee approvals, resulting of one year delay in the human experiments, but the majority of the aims have been achieved.

- The fact that after the beginning of the project, Vet-DTU has been closed by its Government. To compensate for this loss, we looked for new partners in the EJP consortium and in June 2018 (6 months after the beginning of the project), H. Daskalov (NDRVMI) has joined our consortium. Consequently, part of the work related to WP2 has been delayed by one year.

- Finally, several partners had had difficulties to obtain the ethics committee approval for animal experiments, which has also delayed some experiments.

Moreover, a supplementary **6 months extension**, in addition to our previously 1 year extension has been obtained because some partners have been highly impacted by the COVID-19 crisis. Indeed, several experiments had been cancelled or postponed (especially those with humans and in field conditions) due to the lockdown. Finally, the cancellation of all the meetings has led us to cancel the high strategic meeting with stakeholders (the money was returned). **This project led to:**

- Reproduce the different shedding patterns in all the animal species and with all the models developed.

- -Original and interesting results, which allow better understanding of the heterogeneity of excretion and to predict and differentiate between these different shedding phenotypes. These results have been or will be published in international scientific journals and at OHEJP ASM congresses (1st, 2nd, 3rd ed.). The project allowed the development of original epidemic mathematical models and methods for data analysis.

- Important and fruitful exchanges, where one partner analyzed the samples from another partner and which led to joint articles. Moreover, the exchanges between experimental scientists and mathematicians offers a great opportunity to test our methods on very complete datasets.

Several difficulties have been detected regarding the variability of the gut microbiota composition of chicks from one experiment to another, which raises the question of how can we standardise or normalise the gut microbiota of farm animals? Another observation has been that analysis of immune response in blood of chicks seems less relevant than the mucosal immune response (this is not true for pigs).

Several interesting results have been obtained from a One Health perspective, but as different protocols and methods were used in pigs, chickens and humans, it has been difficult to generalize the results obtained in humans to farm animals and vice versa.

5.1.4.3.5.4 Progress of the project: milestones and deliverables

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
10	D.0.1	Project initiation, Kick off meeting, Project-planning and management documentation	2	2		https://zenodo.org/record/5079519#.YOWoQzNxdPY	
10	D.0.2	Approved and signed Consortium Agreement	6	1		This has been done at the EJP level	
10	D.0.3	Minutes of project meetings (Kick off, midterm and final meeting)	2, 13, 24	2, 24, 41		https://zenodo.org/record/3685528#.XlOVuWhKiUk	report
10	D.0.4	Financial and scientific reports for the EJP Coordinator (Intermediated report)	12, 24	12, 24, 36, 42		https://zenodo.org/record/3685530#.XlOV_WhKiUk	report
10	D-1.1	Panel of immunological markers to assess in pig and chicken	3	6		https://zenodo.org/record/5082034#.YObIwzNxdPY	3
10	D-1.2	Development of the immune-signature chips	12			This deliverable has been cancelled due to the left of SAIM at the beginning of the project (see new version of June 2018).	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
10	D-1.3	Identification of risk factors for shedding of *Salmonella* in pigs and poultry farms	12	41		https://zenodo.org/record/5081949#.YObAjjNxdPY Another article is in preparation	5
10	D-1.4	Identification and purification of commensal bacteria present before *Salmonella* colonization in low shedders (first round)	12	36		https://doi.org/10.1111/1751-7915.13621 https://zenodo.org/record/4005830#.X0kCUMgzbcc	3
10	D-1.4	Identification and purification of commensal bacteria present before *Salmonella* colonization in low shedders (second round)	20	41		https://doi.org/10.1111/1751-7915.13621 https://zenodo.org/record/4005830#.X0kCUMgzbcc	3
10	In AWP2021 : D-1.04	*In vitro* virulence levels of different *Salmonella* strains recovered from high and low shedders in animals and humans (first round)	36	41		this deliverable has been merged with D-JRP10-1.5 Confidential	8 report
10	D-1.5	*In vitro* virulence levels of different *Salmonella* strains recovered from	36	41		https://zenodo.org/record/5082103#.YObSKTNxdPY CO: until the publication. Only a short version has been uploaded on Zenodo	8 report

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
		high and low shedders in animals and humans (second round)				Joint publication is in preparation with ANSES, UoS and INRAE (in AWP2021 : D-1.05)	
10	D-1.6	Definition of predictive immunological markers associated to the high and low shedders in chickens and in pigs.	20, 34	36		this deliverable has been merged with D-JRP10-1.7	
10	D-1.7	Definition of immunological markers associated to the high and low shedders in chickens and in pigs.	20, 34	36		https://zenodo.org/record/5079723#.YOWv1zNxdPY CO: until the publication. Only a short version has been uploaded on Zenodo Joint publication is in preparation with ANSES, UoS and INRAE	1 ; 7 ; 8 report
10	D-1.8	Characterization, evolution and comparison of the microbiome of pig and poultry with different shedding status of *Salmonella*	18	NO		Delayed due to Covid19, No clear comparison can be done due to the differences in age between chicks and pigs. No Deliverable	
10	D-1.9	Definition of predictive microbiota markers associated to the high and low shedders in chickens and in pigs.	40	36		(in AWP2021 : D-1.09) https://zenodo.org/record/5082178#.YObcrzNxdPY One publication accepted on chicks https://doi.org/10.1111/1751-7915.13621 https://zenodo.org/record/4005830#.X0kCUMgzbcc, others are in preparation	1 ; 7 ; 8 report

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
10	D-1.10	Definition of microbiota markers associated to the high and low shedders in chickens and in pigs.	40	36		This Deliverable has been merged with D1.9 One publication accepted: https://doi.org/10.1111/1751-7915.13621 https://zenodo.org/record/4005830#.X0kCUMgzbcc , others are in preparation	1 ; 7 ; 8 report
10	D-JRP10-1.11	Recovery of all human samples	42	42		Delayed due to Covid19, This Deliverable has been merged with D1.12	3
10	D-JRP10-1.12	Antimicrobial susceptibility testing, serotyping and whole genome sequencing of all *Salmonella* isolates from participants submitted to the Norwegian Reference laboratory	34	42		Delayed due to Covid19, https://zenodo.org/record/5078733#.YOWeazNxdPY A manuscript is in preparation	
10	D-1.12	Identification risk factors for patients with long term shedding	20	42		Due to change of project leaders at the NIPH,, this Deliverable has been modified and merged with the D-JRP10-1.12 (see version of June 2018).	
10	D-1.13	Description of the differences in immunological, gut-microbiota markers but	22		cancelled	Due to change of project leaders at the NIPH, this Deliverable has been cancelled	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
		also in resistome before and after travel to high-risk areas					
10	D-1.14	Identification, from *in vitro* studies, of immune parameters related to high and low shedders	42	36		This Deliverable has been merged with D1.7 No difference has been detected in chicks. One joint publication is in preparation	8 report
10	D-1.15	Understanding whether high and low shedder phenotypes are mainly related to *Salmonella* strain variation, immune status, or microbiota composition.	24	42		https://zenodo.org/record/5079547#.YOWqlTNxdPY One joint publication is in preparation	report
10	D.1.16	A predictor, based on the designed set of mimotopes, using machine learning techniques to discriminate between the diagnostic groups.	24		cancelled	This deliverable has been cancelled due to the left of SAIM at the beginning of the project (see new version of June 2018). The immunoglobulin response cannot be used as a predictive factor	
10	D-2.1	*In vitro* effect of already characterized probiotics on *Salmonella* growth and cell invasion	36	42		Delayed due to Covid19, (in AWP2021 : D2.01) CO: until the publication. Only a short version has been uploaded on Zenodo	8 report

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
						https://zenodo.org/record/5085459#.YOhVtzNxdPY	
10	D-JRP10-2.11	Evolution of the immune-signature in pig, chicken and/ or human according to the context (infection, treatment…)	34	36		This Deliverable has been merged with D2.10 The immunoglobulin response cannot be used as a predictive factor	report
10	D.2.2	Description of the microbiome and resistome in farms	12, 36	46		The experiment has been delayed due to the Covid19. The experiments have been completed. The gut microbiota were sequenced, Data analysis is ongoing This Deliverable has been merged with D-2.8	
10	D-2.3	Characterization of protective commensal bacteria able to inhibit *Salmonella* colonization (two rounds)	40	40		https://zenodo.org/record/5082203#.YObhrDNxdPY One article accepted for chicken, https://doi.org/10.1111/1751-7915.13621 https://zenodo.org/record/4005830#.X0kCUMgzbcc One joint publication on pigs is in preparation (in AWP2021 : D2.04)	7,8
10	D-2.4	Determine the influence of defined and undefined probiotics on the microbiome signature, the immune response, gut physiology and welfare of pig and/ or chicken	42	42		This deliverable has been merged with D-JRP10-2.5 (in AWP2021 : D2.05)	7

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
10	D-2.5	Impact of defined and undefined probiotics on *Salmonella* colonization in pig and chicken	42	42		https://zenodo.org/record/5082213#.YObjrTNxdPY (in AWP2021 : D2.06) One article accepted https://doi.org/10.1111/1751-7915.13621 https://zenodo.org/record/4005830#.X0kCUMgzbcc One article in preparation.	7
10	D.2.6	Impact of pre-biotics or feed on the immune parameters, the microbiome and resistome, gut physiology and welfare of pig and chicken.	42	42		This Deliverable has been merged with D2.08 (D2.7) (in AWP2021 : D2.07) One article accepted. https://zenodo.org/record/4114070 https://doi.org/10.1016/j.psj.2020.07.015 https://doi.org/10.3390/antibiotics8040215 https://zenodo.org/record/3648214#.XvyTJygzZM0	7
10	D.2.7	Impact of pre-biotics or feed on *Salmonella* colonization in pig, chicken and human. Effect on the super-shedders and low-shedders.	42	36		https://zenodo.org/record/5079336#.YOWkYDNxdPY (in AWP2021 : D2.08) One article accepted. https://zenodo.org/record/4114070	7
10	D.2.8	Dynamics of the microbiome and resistome in the different groups and conditions	24	46		The experiments have been delayed due to the Covid19. They are now completed. The gut microbiota were sequenced, Data analysis is ongoing This Deliverable has been merged with D-2.2	5

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
10	D.2.9	Validation of immunological and microbiota markers, identified in WP1 and associated to high/low shedding, in the control groups of WP2	24		No done	Delayed due to Covid19, The great variability in the gut microbiota composition hampered us to conclude.	
10	D.2.10	Evolution of the immune-signature in pig, chicken and/ or human according to the context (infection, treatment…)	20	42		https://zenodo.org/record/5079202#.YOWilzNxdPY Delayed due to Covid19, One article in preparation Not done for human	5
10	D.2.11	Differences in the resistome before and after travel to high-risk areas and after pre-biotic administration	24		cancelled	Due to change of project leaders at the NIPH, this Deliverable has been cancelled (see version of June 2018)	
10	D-3.1	Models at within-host scale taking into account the interactions between infection and microbiota and mechanisms of interventions	22	42		https://doi.org/10.1051/proc/202067015 https://zenodo.org/record/4244169#.X6KF4TiWxM0	5
10	D-3.2	Models at between-host scale, linking to the within-host modelling	22	42		https://doi.org/10.1051/proc/202067015 https://zenodo.org/record/4244169#.X6KF4TiWxM0	5, 7

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
		and taking into account the mechanisms of interventions					
10	D-3.3	Intervention measures inventory	40		46	(in AWP2021 : D3.03) https://zenodo.org/record/5500249#.YTtd-YgzY2w	5, 7
10	D-3.4	Economic analysis tools combined with D-JRP10-3.06	30			(in AWP2021 :D3.04) https://doi.org/10.1016/j.psj.2020.05.003 https://zenodo.org/record/4244748#.X6LaaIhKjcc	
10	D-3.5	Definition of intervention measures to target super-shedders	42			(in AWP2021 :D3.05) https://doi.org/10.1016/j.psj.2020.07.015	5, 7
10	D-3.6	Evaluation scheme for cost effectiveness of the intervention strategies combined with D-JRP10-3.04	30			(in AWP2021 :D3.06) https://doi.org/10.1016/j.psj.2020.05.003 https://zenodo.org/record/4244748#.X6LaaIhKjcc	5, 7
10	D-4.1	Development and production of MoMIR-PPC website	4	12		We have used the EJP website	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention:* ***public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)****, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
10	D-4.2	Data management policy and strategies	4	42		The final DMP version has been sent	report
10	D-4.3	Creation of a database of each animal group included in the study (age, conformation, diet, clinical status, previous antibiotic treatments, infectious status, etc.)	2	36		https://zenodo.org/record/5082319#.YObuYDNxdPY Only a short version has been uploaded on Zenodo	3
10	D-4.4	Publication, and presentation at conferences	24	42		Information included in this report, see paragraph 5: Publication and additional output… Several publications will be finished after the end of the project	5
10	D-4.5	Dissemination to lay-public communities, to policy-makers and regulators, farmers and companies	24	42		Information included in this report, see paragraph 8. Several disseminations will be finished after the end of the project	5

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
10	M-FBZ3-MoMIR-PPC.1	Update of the members of the steering committee and of the leader and deputy leader for the WPs and tasks	2	yes		
10	M-FBZ3-MoMIR-PPC.2	Organization of the consortium kick off meeting	1	yes		
10	M-FBZ3-MoMIR-PPC.3	Protocols and ethical committee requests for the different experiments	1	yes		
10	M-FBZ3-MoMIR-PPC.4	Recovery of samples from the first round of experimentally infected animals	8	Yes		
10	M-FBZ3-MoMIR-PPC.5	Identification and selection of farms	4	yes		
10	M-FBZ3-MoMIR-PPC.6	Recovery of samples from selected farms ; Identification of super-shedders and low-shedders in poultry and pig farms	8	yes		
10	M-FBZ3-MoMIR-PPC.7	Comparison of immune response of high and low shedders in chickens and pigs	11	Yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
10	M-FBZ3-MoMIR-PPC.8	Histological characterization of different intestinal lesions between high and low shedder in chickens and pigs. Immunohistochemical studies on intestinal mucosa.	12	Yes		
10	M-FBZ3-MoMIR-PPC.9	Comparison of microbiota composition of high and low shedders in chickens and pigs.	11	Yes		
10	M-FBZ3-MoMIR-PPC.10	Comparison of the predictive markers obtained in pigs with those obtained in chickens.	12	Yes		
10	M-J FBZ3-MoMIR-PPC.11 (or 10?)	*In vitro* infection of cell lines and organoids with the *Salmonella* strains recovered from high and low shedders in animals and humans (from the first experiments)	36	Yes		
10	M-J FBZ3-MoMIR-PPC.12	Comparison of the transcriptomic immune response induced *in vitro* between different strains to identify immunological markers	12	Yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
10	M-J FBZ3-MoMIR-PPC.13	Four sets of NGS derived 10^5 mimotope sequences – positively and negatively enriched in IgM and IgA	8	NO		Due to the left of SAIM, this milestone has been cancelled. The role of immunoglobulin levels was investigated, but these levels cannot be used neither as a predictive marker nor as a marker to differentiate between shedding phenotypes
10	M-J FBZ3-MoMIR-PPC.14	A selected set of 100 mimotopes for the immune-signature chips.	12	NO		Due to the left of SAIM, this milestone has been cancelled (see version of June 2018). The role of immunoglobulin levels was investigated, but these levels cannot be used neither as a predictive marker nor as a marker to differentiate between shedding phenotypes
10	M-J FBZ3-MoMIR-PPC.15	Inclusion and sampling of volunteers	12	yes		
10	M-J FBZ3-MoMIR-PPC.16	Selective cultivation of commensal bacteria correlated to low shedding in pig and/ or chicken	10	yes		
10	M-J FBZ3-MoMIR-PPC.17	Define the panel of probiotics for use in pigs and chickens	2	yes		
10	M-J FBZ3-MoMIR-PPC.18	Define the panel of pre-biotics and feed for use in pigs, chickens and humans	2	yes		
10	M-J FBZ3-MoMIR-PPC.19	Recovery of samples from experimentally infected	8	Yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
		animals and from farms, pretreated with pre-biotics or neutraceuticals				
10	M-J FBZ3-MoMIR-PPC.20	First version of within-host models completed	12	yes		
10	M-J FBZ3-MoMIR-PPC.21	First version of between-host models completed	12	Yes		
10	M-J FBZ3-MoMIR-PPC.22	First inventory of intervention measures completed	12	Yes		
10	M-J FBZ3-MoMIR-PPC.23 (or 21?)	First version of economic analysis tools completed	12	Yes		
10	M-FBZ3-MoMIR-PPC.23 (or 22)	Organization of consortium meetings (intermediate and closure)	40	Yes		
10	M-FBZ3-MoMIR-PPC.24 (or 12 ?)	Comparison of immune response of high and low shedders in chickens and pigs	36	Yes		
10	M-FBZ3-MoMIR-PPC.25	MALDI TOF Imaging processing and workflow on the paraffin wax slides of intestine wall in high and low shedder from chickens and pigs	18	In part		Only the histological analyses were done on all samples

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
10	M-FBZ3-MoMIR-PPC.26	Comparison of microbiota composition of high and low shedders in chickens and pigs.	18	yes		
10	M-FBZ3-MoMIR-PPC.27	Comparison of the predictive markers obtained in pigs with those obtained in chickens.	19	yes		Poor correlations have been observed due to the different ages of pigs and chickens
10	M-FBZ3-MoMIR-PPC.28	*In vitro* infection of several cell lines and organoids with the different *Salmonella* strains recovered from high and low shedders in animals and humans (second round)	18	Yes		
10	M-J FBZ3-MoMIR-PPC.29	Comparison of the transcriptomic immune response induced *in vitro* between different strains to identify immunological markers	20	yes		
10	M-J FBZ3-MoMIR-PPC.30	Comparison of the immune signature of high and low shedders in chicken and pig.	18	Yes		
10	M-J FBZ3-MoMIR-PPC.31	Inclusion and sampling of volunteers	15	yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
10	M-J FBZ3-MoMIR-PPC.32	First selective cultivation of *Salmonella* from human rectal swabs to be use in WP1	13	NO		The experiment with human was postponed due to the Covid19. The *Salmonella* strains were not included in the analysis
10	M-J FBZ3-MoMIR-PPC.33	Selective cultivation of *Salmonella* and antibiotic-resistant bacteria from 1000 human rectal swabs	20	yes		
10	M-J FBZ3-MoMIR-PPC.33	Recovery of samples from experimentally infected animals and from farms, pretreated with probiotics	16	yes		
10	M-J FBZ3-MoMIR-PPC.34	Updated version of within-host models completed	21	yes		
10	M-J FBZ3-MoMIR-PPC.35	Updated version of between-host models completed	21	yes		
10	M-J FBZ3-MoMIR-PPC.22 (or 32?)	Final inventory of intervention measures completed	39	yes		
10	M-J FBZ3-MoMIR-PPC.36	Updated version of economic analysis tools completed	22	Yes		
10	M-J FBZ3-MoMIR-PPC.37	Potential interventions included in between-host modelling	22	yes		

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
10	M-J FBZ3-MoMIR-PPC.38	Organization of the high strategy meeting	24	NO		Due to the lockdown, this meeting has been cancelled and the money returned

5.1.4.3.5.5 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors already accepted your comments. Therefore, this part of the report can be closed.

5.1.4.3.5.6 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
A Multi-Scale Epidemic Model of Salmonella infection with Heterogeneous Shedding. A first draft was accessible on HAL repository. https://doi.org/10.1051/proc/202067015 https://zenodo.org/record/4244169#.X6KF4TiWxM0	YES	GREEN 0 MONTHS	
Reduction of Salmonella Typhimurium Cecal Colonisation and Improvement of Intestinal Health in Broilers Supplemented with Fermented Defatted 'Alperujo', an Olive Oil By-Product. 10.3390/ani10101931 https://zenodo.org/record/4114070	YES		GOLD – 1,334.20 €
Cost-effectiveness analysis of using probiotics to control Campylobacter in broilers (submitted). https://doi.org/10.1016/j.psj.2020.05.003 https://zenodo.org/record/4244748#.X6LaaIhKjcc https://doi.org/10.1016/j.psj.2020.07.015	YES		GOLD – 2000 €

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Dietary Supplementation with Fermented Defatted 'Alperujo' Induces Modifications of the Intestinal Mucosa and Cecal Microbiota of Broiler Chickens. https://doi.org/10.1016/j.psj.2020.07.015 https://zenodo.org/record/4017817#.X6LRjYhKjcc	YES		GOLD – 1 720 €
Effects on Intestinal Mucosal Morphology, Productive Parameters and Microbiota Composition after Supplementation with Fermented Defatted Alperujo (FDA) in Laying Hens. https://doi.org/10.3390/antibiotics8040215 https://zenodo.org/record/3648214#.XvyTJygzZM0	No		GOLD – 435.89 €
Gut microbiota composition before infection determines the *Salmonella* super- and low-shedder phenotypes in chicken. https://doi.org/10.1111/1751-7915.13621 https://zenodo.org/record/4005830#.X0kCUMgzbcc	YES		GOLD – 2 100 €

2018

Oral presentation

A spatially structured model to investigate key drivers of the gut microbiota biogeography" B. Laroche, S. Labarthe et al. Oral presentation MB2 worshop in Besançon, 2018, "Modelling the gut microbiota"

2019

Poster

F. Kempf, P. Menanteau, I. Rychlik, E. Guitton, J. Trotereau, I. Virlogeux-Payant, P. Velge (2019). *Faecal gut microbiota composition of chicks can predict resistance to* Salmonella *Enteritidis colonization*. Poster communication at Gordon Research Conference "Molecular Mechanisms, Evolution and Treatment of *Salmonella*"

Poster in OHEJP ASM 2019. Rebollada-Merino, A.; Ugarte-Ruiz, M.; Mayoral-Alegre, F.J.; Maasoumi-Nouha, N.; Tomé-Sánchez, I.; Rivero, E.; Porras-González, N.; García, M.; Domínguez, L.; Rodríguez-Bertos, A. Changes on Caecal Mucosa Morphology and Microbiota in Laying Hens Supplemented with a Nutraceutical Product Obtained from Olive Oil Production

2020

Poster

Participation to the ASM virtual EJP OneHealth meeting (UoS, INRAE)
Posters/

Metagenomic Analysis of The Pig Gut Microbiota and association with *Salmonella* status. Poster. Guido Cordoni, Daniel L Horton, Helen Louise Brown, Roberto La Ragione
https://www.researchgate.net/publication/342123925_Metagenomic_Analysis_of_The_Pig_Gut_Microbiota_and_association_with_Salmonella_status/stats

Metagenomic Analysis of The Pig Gut Microbiota and Association With *Salmonella* Status. Presentation. RCE 2020 event at UoS. Guido Cordoni, Daniel L Horton, Helen Louise Brown, Roberto La Ragione.
https://www.researchgate.net/publication/346943667_Metagenomic_Analysis_of_The_Pig_Gut_Microbiota_and_Association_With_Salmonella_Status

Velge P. Faecal gut microbiota composition determines susceptibility to *Salmonella* Enteritidis primo colonization. ." One Health EJP Annual Scientific meeting, Virtual, Pragues, 27th-29th May 2020.

Poster in OHEJP ASM 2020. Rebollada-Merino, A.; Ugarte-Ruiz, M.; Miguela-Villoldo, P.; Fernández-Manzano, A.; García, N.; Gómez, S.; Bárcena, C.; de Juan, L.; Domínguez, L.; Rodríguez-Bertos, A. Fermented Defatted Alperujo (FDA) Reduces Histopathological Lesions and Excretion in Laying Hens Naturally Infected with *Brachyspira spp*.

Oral presentation

Porcs artificiellement contaminés par *Salmonella*: liens entre niveau d'excrétion, microbiote et immunité. A. Kerouanton. 2020, Meeting ANSES Ploufragan : Information and exchanges meeting pig farming sector.

2021

Poster

F. Kempf, R. Drumo, Anne Marie Chaussé, T. Kubasova, I. Caballero, S. Roche, P. Menanteau, E. Guitton, I. Rychlik, P. Velge-Immune response, gut microbial composition, *Salmonella* super-and low-shedder phenotypes can be modulated by the inoculation of four commensal bacteria in chicken. One Health

EJP Annual Scientific meeting, Virtual, Copenhagen, 9th-11th June 2021. **Poster**

16S RRNA MICROBIAL COMMUNITY ANALYSIS AND RELATIONSHIP WITH SALMONELLA SUPER SHEDDER STATUS IN PIGS Guido Cordoni One Health EJP Annual Scientific Meeting 2021 (Poster)

Albena Dimitrova, Gergana Mateva, Gergana Krumova-Valcheva, Eva Gyurova, Mihail Milanov, Helen Brown, Guido Cordoni, Daniel Horton, Roberto La Ragione, Hristo Daskalov. INFLUENCE OF ORAL ADMINISTRATION OF LACTOBACILLUS REUTERI PROBIOTIC STRAINS AND GOS PREBIOTIC ON THE PRESENCE OF SALMONELLA SPP. IN FATENNING PIGS. One Health EJP Annual Scientific meeting, Virtual, Copenhagen, 9th-11th June 2021. **Poster**
Gergana Mateva, Krasen Penchev, Gergana Krumova-Valcheva, Albena Dimitrova, Eva Gyurova, Mihail Milanov, Helen Brown, Guido Cordoni, Jade Passey, Daniel Horton, Roberto La Ragione, Hristo Daskalov. INFLUENCE OF ORAL ADMINISTRATION OF LACTOBACILLUS REUTERI PROBIOTIC STRAINS AND GOS PREBIOTIC ON THE PRESENCE OF SALMONELLA SPP. IN BROILERS. One Health EJP Annual Scientific meeting, Virtual, Copenhagen, 9th-11th June 2021. **Poster**

Poster in OHEJP ASM 2021. Rebollada-Merino, A.; Ugarte-Ruiz, M.; Miguela-Villoldo, P.; Domínguez, L.; Rodríguez-Bertos, A. Histomorphological Changes in the Bursa of Fabrizio Associated with *Salmonella* Typhimurium Infection in Animals Feed with a Nutraceutical Derived from the Olive Oil Production

Oral presentation

Oral presentation

Oral communication in Programa de Doctorado en Veterinaria. Rebollada-Merino, A. Efecto sobre la mucosa intestinal y la microbiota cecal del alperujo fermentado desengrasado en pollos de engorde experimentalmente infectados con *Salmonella* Typhimurium (2020)

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

Key role of animal-animal recontaminations in the spread of Salmonella infection at the flock level. The high level of Salmonella transmission between chicks is related to the presence of Super-Shedders, functioning as a reservoir for the pathogen. Salmonella can be transmitted by air through contaminated dust particles. It is crucial to decrease these transfers (Importance of ventilation on farms) and to identify the animals at risk.

Identification of predictive biomarkers, based on gut microbiota composition and immune parameters, which can either predict the levels of *Salmonella* shedding (in pigs or in chickens), or which can differentiate the Salmonella shedding levels (in pigs or in chickens).

In humans, Long-term Salmonella shedder phenotype may be associated to host factors such as young age, disease manifestation, taking food supplements and regular medication. Moreover, although the risk of person-to-person transmission of Salmonella from asymptomatic carriers is considered low, our study indicates a 10-fold higher rate (24%) of non-typhoidal Salmonella shedding than previously suggested. Our results support current recommended control measures to prevent the occurrence of secondary cases especially in children. Presentation at ECDC Nordic Mini Module.

Are there any outcomes of this project that are already discussed or even implemented and in use at any institute of the project consortium, at stakeholders' organisations (ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), or at the level of national authorities?

Participation to PoC projects COOPERATE funded by stakeholders in Paris-Saclay University (starting 2021). Interest of stakeholders for OTU clustering and for detection method. Design of probiotic consortia consortia based on endogeneous microbiota bacteria

Development of probiotics for use in pigs and poultry, which can be used as an alternative to antibiotics and which may reduce AMR.

With approval of national authorities **VRI** tested the developed probiotics in more than a half million of broilers in 2020. Farmers are interested in further collaboration as the product may be used instead of antibiotic treatment during first days of life. The probiotic product is being prepared for registration in the Czech Republic though we are still improving its commercial formula – lyophilisation of strict anaerobes without losing viability is quite challenging.

Collaboration with commercial companies on the use of prebiotics (UoS, UCM-VISAVET). UoS will communicate results to stakeholders FSA, Defra and VMD.

5.1.4.3.5.7 One Health impact

Asymptomatic carrier animals and humans are a serious food safety and health issue resulting in a significant loss to agri- food industry as well as a substantial burden on the healthcare system. The MoMIR-PPC project has led to major advances 1- in the understanding of heterogeneity of *Salmonella* infection, which has been confirmed in all models studied: human, pig and chicken ; 2- in the control measures, which could be used, and in the identifications of the super-shedder animals; 3- in the development of prevention measures able to either predict the most susceptible animals (or flocks) or to prevent infection by the use of pre and probiotics.

- **Understanding heterogeneity of *Salmonella* infection.** This project has shown the key role of animal-animal recontaminations in the spread of *Salmonella* infection at the flock level. The high level of Salmonella transmission is related to the presence of Super-Shedders, functioning as a reservoir for the pathogen. The data have shown that the gut microbiota composition before infection determines in chicks the super and low shedder phenotypes. **It is thus possible to prevent infection** with pre and probiotics or nutraceuticals. Moreover, although the risk of human-to-human transmission of *Salmonella* from asymptomatic carriers is considered low, our study indicates a 10-fold higher rates (24%) of non-typhoidal *Salmonella* shedding than previously suggested. Our results support current recommended control measures to prevent the occurrence of secondary cases especially in children. **The results obtained will have direct applications for the control measures**.

- **Modelisation of infection**. The project allowed the development of original epidemic models and methods for data analysis based on a generic mathematical model of the dynamic interplay between the gut microbiota, the pathogen and the host's immune response at the within and between-host scale. **This model will help to decipher the impact of modifications based on gut microbiota and immune response.**

- **Modelisation of transmission**. A mathematical model of indirect transmission of bacteria between broilers was developed. The results showed no transmission at longer distances (above 130 cm), which is consistent with the existence of a threshold distance. **The model can be used for designing and quantitatively assessing candidate bio-security based intervention strategies against indirect transmission of *Campylobacter* and *Salmonella*.**

- **Control strategies**. In general, the control of Salmonella is based upon the implementation of preventive actions throughout the whole production chain. More specifically**,** measures should be addressed to (i) the prevention of introduction of *Salmonella* into the herd/ flock, (ii) the prevention of within-herd transmission, and (iii) the increase of the resistance to the infection. Our results have shown at least in chicks, that Salmonella can be transmitted by air through

contaminated dust particles. It is thus crucial to decrease these transfers with the important ventilation on farms. It is also important to identify the animals at risk. For that purpose, **the MoMIR-PPC project identified several predictive biomarkers**, based on gut microbiota composition and immune parameters. These biomarkers, which need to be confirmed, can either predict the levels of *Salmonella* shedding (in pigs or in chickens) if animals are infected, or can differentiate the *Salmonella* shedding levels.

- **The cost effectiveness (utility) of intervention strategies** using probiotics to reduce Campylobacter prevalence in broilers was evaluated. Interesting results showed that using probiotics is a moderately expensive intervention in Poland and Spain, if efficacy is more than 10%, otherwise it is relatively expensive. In contrast, in the Netherlands and Denmark, using probiotics is a relatively expensive intervention irrespective of efficacy. However, if probiotics were assumed to enhance broiler performance, the intervention would become relatively cost-effective even at low efficacy levels of 1 to 10%.

- **Prevention measures**. To prevent salmonellosis, we purified, characterized and tested the efficacy of probiotics and prebiotics for use in pigs and poultry, in experimental infection but also in field conditions. For example they were tested on 72640 chicks in Bulgaria and 130 000 chicks in Czech Republic. **These probiotics and prebiotics can be used as an alternative to antibiotics and may reduce AMR.**

The benefits of our project for the livestock industries, authorities, veterinarians and medical doctors should be rapid as we have already identified new control measures for risk managers for the prevention and control of salmonellosis. It will also have a short- and long-term impact on the industry, especially with regard to pro and prebiotics. The existence of a market for well characterized and defined probiotics is attested by the success with undefined avian caecal cultures in competitive exclusion, which are now banned by the EU. The results obtained will be disseminated to stakeholders through specialized congresses like the “Animal microbiome congresses” and the “International Conference on Poultry Intestinal Health”.

5.1.4.3.5.8 Data Management Plan

The MoMIR-PPC project gathers numerous approaches (from animal experiments to mathematical models), numerous disciples and numerous methodologies. It has been difficult to harmonize all the data, but the FAIR principles have been applied depending of the discipline and the rules of Institutes. The DMP has been described in the final version of the Excel file.

Concerning the data: All are **findable** and included either in the lab notebook of the partners or deposited in public databases. For the data in lab notebook, they are not freely available until the end of the project or the publication but authorized parties are able to identify and use it through the naming convention (i.e., project, pathogen, host and year). The vast majority of the data are in Excel files, not coded and thus easily **accessible and interoperable**, but difficult to use without explanation from the partner who produced them. In contrast, the personal data of the participants of the human part of our project are anonymized. Two security systems have been used to ensure limited access to these personal data. After publication, these data are **Reusable**, particularly those deposited in public databases. If the data are not published yet, they are in institutional repositories only accessible to institutional persons. The relation between the data and the person in charge is described in the Excel file. All publications are (or will be) in open access journals.

5.1.4.3.5.9 List of dissemination and communication activities

Name of the activity:	CIAG Prévenir et guérir les maladies infectieuses dans le concept One Health. « L'approche microbiote : Stratégies pour prédire et prévenir les infections à *Salmonella* chez le poulet »
Date:	*21 June 2018*
Place:	*Tours, France*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	*yes*
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***
General Public	***X***	***Other***	***X***
Policy Makers	***X***		

Name of the activity:	Animal Microbiome congress

	« Faecal gut microbiota composition of chicks can predict the super-shedder phenotype of *Salmonella* Enteritidis"
Date:	*20-21 June 2018*
Place:	*Paris, France*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*YES*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***
General Public	***X***	***Other***	***X***
Policy Makers	***X***		

Name of the activity:	*diseminación*. IV VETINDOC PhDay, *Facultad de Veterinaria*. - Poster communication. *(Identificación y caracterización de bacterias resistentes a la colistina. Evaluación de su persistencia y posible)*
Date:	*27/06/2018*
Place:	*Universidad Complutense, Spain, Madrid.*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	*YES*
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other : Poster***	*Yes*
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***
General Public	***X***	***Other***	***X***
Policy Makers	***X***		

Name of the activity:	XXII Congreso de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica, Sociedad Española de Enfermedades Infecciosas – Oral communication. (Nuevos determinantes de la resistencia a la colistina en *Escherichia coli* de origen animal)
Date:	*24/05/2018*
Place:	*y Microbiología Clínica, Spain, Bilbao*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*YES*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other : oral communication***	*YES*
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***
General Public	***X***	***Other***	***X***

Policy Makers	***X***		

Name of the activity:	European Congress of Clinical Microbiology and Infectious Diseases – Poster communication. (Whole genome sequencing analysis of *Salmonella enterica* serotype Choleraesuis isolates in Spain provides insight into possible transmission chains)
Date:	*22/04/2018*
Place:	*European Society of Clinical Microbiology and Infectious Diseases, Spain, Madrid*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*YES*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other : POSTER***	*YES*
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***

General Public	***X***	***Other***	***X***
Policy Makers	***X***		

Name of the activity:	Congrès Mbio : Les microbiotes et la santé humaine, animale et environnementale : Prévention et traitements du futur - poster presentation (Les niveaux d'excrétion de Salmonella sont liés à la composition du microbiota intestinal chez le Poulet.)
Date:	*19 - 20 June 2018*
Place:	*Biocitech, Cité des entreprises de santé et de biotechnologies, Romainville*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*YES*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other : Poster***	*YES*
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	*X*
Industry	***X***	***Investors***	*X*

Civil Society	***X***	***Customers***	*X*
General Public	***X***	***Other***	*X*
Policy Makers	***X***		

Name of the activity:	Velge, P., Kempf, F., Menanteau, P., Beaumont, C., Leterrier, C., Virlogeux-Payant, I. (2018). L'approche microbiote : stratégies pour prédire et prévenir les infections à *Salmonella* chez le poulet. In: Prévenir et guérir les maladies infectieuses dans le concept One Health (p. 37-47). *Innovations Agronomiques, 66*.
Date:	*2018*
Place:	*Innovations Agronomiques, 66.*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)	*YES*	***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*

Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***
General Public	***X***	***Other***	***X***
Policy Makers	***X***		

Name of the activity:	International symposium *Salmonella* and salmonellosis - oral communication (*Salmonella* shedding levels are related to the chicken gut microbiota composition)
Date:	*24-26 September 2018*
Place:	*Saint-Malo, France*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference	*yes*	***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***X***	***Media***	***X***
Industry	***X***	***Investors***	***X***
Civil Society	***X***	***Customers***	***X***
General Public	***X***	***Other***	***X***
Policy Makers	***X***		

Name of the activity:	Association of Veterinary Students - RVC - AMR and biofilms		
Date:	*January 2019*		
Place:	*Please update*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)		***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	***Microbiology Society 2019 Annual conference. Flash poster presentation titled "Creation and characterisation of probiotic libraries for use in pigs"***		
Date:	*March 2019*		
Place:	*Please update*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)		***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	***One health EJP annual scientific meeting: oral presentation titled "Creation and characterisation of probiotic libraries for use to control zoonotic pathogens in pigs"***
Date:	*21-24 May 2019*
Place:	*Teagasc conference centre Dublin*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***300***	***Media***	
Industry	***5***	***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers	***5***		

Name of the activity:	*1st Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Poster communication.*
Date:	*22-24 May*
Place:	*Dublin, Ireland.*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***300***	***Media***	
Industry	***5***	***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers	***5***		

Name of the activity:	Microbiote et résistance du poulet aux salmonelles. 4ème journée thématique "Je suis un écosystème : le microbiote dans tous ses états" dans le cadre du Réseau Thématique de Recherche soutenu par la Région Centre Val de Loire
Date:	*28 June 2019*
Place:	*Château de Beaulieu, Joué les Tours*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***30***	***Media***	
Industry	***5***	***Investors***	
Civil Society		***Customers***	
General Public	***10***	***Other***	
Policy Makers			

Name of the activity:	*Faecal gut microbiota composition of chicks can predict resistance to Salmonella Enteritidis colonization. Presented at NEM Research Network,)*
Date:	*2019-05-21 - 2019-05-22*
Place:	*Nantes, FRA*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***60***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*Salmonella resistant vs super-shedder broilers: how can we improve birds' resistance. Presented at Phileo symposium "Animal health with less ATB and more food safety Latest technologies"*
Date:	*2019-04-02 - 2019-04-02*
Place:	*Rome, ITA*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***10***	***Media***	
Industry	***60***	***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*Faecal gut microbiota composition of chicks can predict resistance to Salmonella Enteritidis colonization. Poster communication at Gordon Research Conference "Molecular Mechanisms, Evolution and Treatment of Salmonella"*
Date:	*2019-06-02 - 2019-06-07*
Place:	*Easton, MA, US*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***200***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*Faecal gut microbiota composition of chicks can predict resistance to* Salmonella *Enteritidis colonization. Oral presentation at the 6th international conference on poultry intestinal Health*
Date:	*2019-04-03 - 2019-04-05*
Place:	*Roma*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***400***	***Media***	
Industry	***360***	***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*1st Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Poster communication. Rebollada-Merino A., Ugarte-Ruiz M., Mayoral-Alegre F.J.,Maasoumi-Nouha N., Tomé-Sánchez I., Rivero E., Porras-González N., García M., Domínguez L. and Rodríguez-Bertos A. Changes on Caecal Mucosa Morphology and Microbiota in Laying Hens Supplemented with a Nutraceutical Product Obtained from Olive Oil Production*
Date:	*22-24 May*
Place:	*Dublin, Ireland.*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***300***	***Media***	
Industry	***5***	***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers	***5***		

Name of the activity:	*Poster presentation* *13th International Symposium On Epidemiology and Control Of Foodborne Pathogens In Pork (Safepork)* *Kerouanton A., Souchaud F., Houdayer C., Houard E., Nagard B., Guionnet J-M., Fougeroux A., Paboeuf F. and Denis M. Pigs infected experimentally with the same dose of monophasic variant of Salmonella Typhimurium exhibit different shedding levels.*
Date:	*26-29 septembre **2019***
Place:	*Berlin, Germany*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)		***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	***One health EJP annual scientific meeting: oral presentation titled "Faecal gut microbiota composition determines susceptibility to Salmonella Enteritidis primo-colonization."***
Date:	*27th-29th May 2020*
Place:	*Virtual conference in Pragues*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	*yes*
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)		***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

5.1.4.3.6 JRP12-R2-AMRSH5-FARMED

5.1.4.3.6.1 Summary of the work carried out in the Project

Unfortunately the COVID-19 situation in Europe has further delayed the FARMED project, as many institutes have had restricted access to their laboratories for non-essential research work. The majority of the FARMED project is based on wet laboratory work; therefore, the lockdowns or limited laboratories access has impacted the project. In spite of this we have had some progress to the FARMED objectives.

FARMED aims to assess the feasibility of long-read sequencing to rapidly characterise the metagenome and resistome of samples, on-site, in order to provide investigators with the correct information to apply the most appropriate control measures.

Bioinformatics analysis of long read sequencing of a simple (pond water) or complex (animal faeces) matrix spiked with a defined microbial community (DMC) (supplied by BfR), DNA extracted using the preferred method in each institute, highlighted the differences of microbiomes achieved by each institute (WP1). Further work is currently underway to understand why these differences have occurred even though the 'same' samples were used. The consortium has selected 3 lab-based DNA extraction kits, which will be assessed by a minimum of three institutes, in order to determine the usability as well as consistency of these methods. To aid this assessment, the same commercially available defined community will be used by all institutes. Institutes are currently applying one of these lab-based methods on sample matrices most relevant to their institutes. Further work is still required to optimise as well as automate the DNA sequence analysis methods, such that the analysis can be performed on 'basic' computers by non-bioinformaticians (WP2).

Two instruments capable of on-site DNA extractions are being trialled for their suitability to produce DNA of sufficient quantity and quality for DNA sequencing. Preliminary analysis using these instruments has yielded DNA for single isolates suitable for long read sequencing, but current optimisation is underway to achieve the appropriate quality of metagenomic DNA (WP3).

One peer-reviewed publication and a poster have been presented highlighting the use of long read metagenome sequencing to characterise the microbiome as well as identify the presence of genetically modified microorganisms *Bacillus subtilis*, containing AMR genes on the chromosome and on a plasmid, in a feed additive.

5.1.4.3.6.2 Progress of the project: description of activities

WP1 - Assess feasibility of Long-read metagenome sequencing on exemplar matrices. Investigate the use of Hi-C metagenomics.

JRP12-WP1-T1-Assess feasibility /perform long-read metagenomics MinION from 'defined' microbial community.

A thorough evaluation of the short (Illumina) and long (ONT) read sequencing of a defined mock community (DMC) consisting of 6 species (four Gram-negative isolates and two Gram-positive isolates) with known complete genome sequences and different AMR profiles, spiked into water buffalo faeces or pond water at different concentrations, was performed. The sequencing data from six partners were loaded up to the Owncloud repository, where they have been analysed using the KMA pipeline (details of the analysis methods are given in WP2), to allow inter-partner comparison of richness (observed species) and diversity. Analysis showed that long-read sequencing using ONT technology was able to identify some of the bacterial species contained in the DMC, but not all, which appeared to be dependent on the DNA extraction method used. Work is underway to determine the underlying cause of false positives in the blank samples.

Regardless of the DNA sequencing method, sequencing comparisons showed that microbial profiles clustered by laboratory (i.e. by project partner) and was likely due to the different DNA extraction

method employed by each institute. The AMR profiles appeared to be less affected by institute or DNA extraction method, using the Illumina sequencing compared to the ONT sequencing.

Based on this, the workflow amongst the consortium will be further streamlined, concentrating on using three commercially available DNA extraction kits. This will be done using real-life 'simple' and 'complex' matrices (see T2 and T3). To achieve a consistent output between different users and allow comparison of methods between partners in the consortium, a commercially available mock community (ZymoBIOMICS Gut Microbiome Standard) will be used.

Some additional qPCR analyses are being performed to evaluate the extraction effectiveness, compared to the sequencing reads output. Additionally, the impact of the sequencing coverage (i.e. 3 samples per MinION flow cell) is being investigated (by Sciensano).

JRP12-WP1-T2- Assess feasibility /perform long-read metagenomics MinION from 'simple' sample matrices.

This task is behind due to the delay in finalisation of the DMC samples (task WP1.1) (caused by the COVID situation), to determine which DNA extraction and bioinformatics analysis methods should be explored further, with institutes starting to analyse their own 'real' simple samples, which includes drinking water, river water and milk. The commercial mock community will be used to spike some samples to allow comparison amongst institutes.

JRP12-WP1-T3- Assess feasibility/perform long-read metagenomics MinION from 'complex' sample matrices.

This task is behind due to the delay in finalisation of the DMC samples (task WP1.1) (caused by the COVID situation), to determine which DNA extraction and bioinformatics analysis methods should be explored further, with each institute starting to analyse their own 'real' complex samples, which includes faeces, food/feed, boot socks and wastewater. Once again the commercial mock community will be used to spike some samples to allow comparison amongst institutes.

BfR is planning to spike both complex sample matrices with and without pre-existing bacterial species. Specifically, BfR will spike faecal material containing existing multidrug resistant *Salmonella* and *E. coli* from calves, cattle or pigs ("real life") as well as faecal material free from *Salmonella*, ESBL and/or carbapenemase producing Enterobacteriaceae as well as isolates harbouring *mcr-1* to *mcr-9* genes. Furthermore, other complex matrices such as food will be tested. Here, insect-based foods and sesame-based tahini will be spiked. Both food types are also "real life" matrices, as previous analyses have shown a contamination with *Bacillus* and *Salmonella*, respectively. For all these different matrices, subsequent sequencing and comparisons with project partners will determine to what extent spiked-in bacterial species, "real life" bacterial species and AMR genes can be identified.

Sciensano performed the analysis of the complex feed additive matrix using shotgun metagenomics. Feed additives (e.g. vitamins) are often produced through the use of genetically modified microorganisms (GMMs) to replace chemical synthesis methods, as this is more practical and requires fewer resources. The presence of a genetically modified microorganism (GMM) or its DNA, often harbouring AMR genes, in microbial fermentation products is prohibited by European regulations. If no isolate of the GMM can be obtained, a metagenomics approach is the only way to demonstrate the presence of a GMM in a microbial fermentation product, with characterization based on detection of AMR genes and vectors, species and unnatural associations in the GMM genome. We based the evaluation of the feasibility of this new approach on the investigation of a previously analysed sample containing a GMM *Bacillus subtilis* (rapid alert to the competent authorities (EU): RASFF 2014.1249) overproducing vitamin B2 (riboflavin), isolated and fully characterized at that time. The method was applied to a sample positive for some qPCR markers but for which no isolate could be obtained. The short and long read sequencing technologies were compared for their performances, including the newly released Flongle, as a smaller and cost-effective alternative. The analysis was finalized and the results published in Buytaers et al. 2021 (Food Chemistry: Molecular Sciences, Volume 2, 30 July 2021,

100023). Altogether, our method allowed to predict the presence of a GMM in the sample, based on the simultaneous detection of AMR genes or vectors in species previously described as common GMM producers, and the encounter of unnatural associations in the genome. Generally, this open approach can in the future be applied to other GMM used to produce fermentation products like food enzymes.

JRP12-WP1-T4- Perform Hi-C metagenomics

This task is delayed due to COVID restrictions affecting access to laboratories.

At BfR, initial development of the Hi-C workflow has shown that the ProxiMeta Hi-C Library Prep Kit (PhaseGenomics) requires an already sequenced library as well as ample starting material for library preparation. To this extent, BfR will prepare a Hi-C library for the already sequenced DMC samples. To establish the method (D-JRP12-WP1.2 and M-JRP12-06), this Hi-C library will then be compared to existing short-read and long read sequencing data to determine whether it is possible to associate AMR genes to bacterial species as well as the genes' locations on the chromosome or plasmid. Once the feasibility of Hi-C has been evaluated, further testing and optimisation of workflow will be undertaken by task partners, using 'complex' matrices spiked with a mock community. All participating project partners will use the same Hi-C library preparation workflow and sequencing in order to obtain comparable sequencing results. A first discussion on Hi-C sequencing was held on June 7th. For the coordination of participating project partners, the BfR will organise additional (virtual) meetings in order to discuss practical details for laboratory workflows and subsequent data analysis.

WP2 - Bioinformatics tools to analyse the sequencing data and defining the characteristics within the sample.

JRP12-WP2-T1- Development/adaptation of a pipeline that can predict species within sample/matrix

To ensure consistency of results, DTU performed preliminary KMA analysis using the entire DMC dataset of raw short (Illumina) and long (Nanopore) read sequencing data generated by the consortium in WP1.1. The community analysis showed that the species richness of taxa varied between the FARMED consortium, while the diversity of identified bacterial taxa appeared to be consistent between samples. Microbial community analyses showed that bacterial communities that were sequenced by the same institute were more similar to each other, even if they were from different DMC faecal samples, compared to the same sample that were sequenced at another institute. This was observed using both sequencing platform outputs, Illumina and Oxford Nanopore, suggesting the difference were largely influenced by the different DNA extraction and library preparation practices used at each institute. The spiked bacteria were detected from the Nanopore sequencing at a range of different number of reads between the different institutes, only two bacterial taxa were not detected by three institutes when the bacterial communities were identified by Oxford Nanopore sequencing. In the case of the blank samples (FB/WB) we would expect to identify some of the spiked bacteria such as *E. coli* in the samples as these are part of the normal microbiome.

It is important to understand the impact of chosen methodology, such as the different extraction methods, sequencing library preparations, sequence base-calling, as well as the data analysis method, will have on the results obtained. APHA has compared and evaluated the choice of sequencing library preparation kit (slower ligation library kit vs faster rapid library kit) as well as the basecalling (fast vs high accuracy) approach, both can be time-consuming stages thereby impacting timely delivery of results. Using the same DNA extraction method on the same DMC samples from WP1.1, we compared the two options for library preparation and base calling, which will be finalised later this year.

Future work will compare and identify appropriate reference databases, define the sequence quality thresholds, normalisation steps required, as well as how to present the results of the microbiome analysis.

JRP12-WP2-T2- Development/adaptation of a Resfinder-'like' pipeline for identification of AMR for long-read sequences

Following on from WP2.1, the AMR profiles of the DMC dataset were compared. Unlike the bacterial species, the resistome profiles of the same samples were more similar and this was regardless of the institute. Additional work is needed to define the threshold required.

JRP12-WP2-T3- Benchmarking of tools with spiked samples in WP2

The entire DMC dataset, which includes the raw sequencing data files as well as the KMA output files, was compiled for evaluations and comparison, and analysis conducted by DTU (cfr WP1-T1).

JRP12-WP2-T4- Test protocols on site

This task is delayed until an analysis workflow is established.

WP3 - Implementation of on-site protocols for long-read metagenomic DNA sequencing

JRP12-WP3-T1- Literature search & Harmonisation of on-site DNA isolation

The literature report on DNA extraction was finalised and uploaded Zenodo: https://doi.org/10.5281/zenodo.4724557.

JRP12-WP3-T2- Investigation of on-site equipment for DNA extraction and sequencing (original title: Investigate the use of Voltrax)

The Voltrax (from ONT) was expected to be able to perform the metagenome DNA extraction as well as the library preparation, which could directly be loaded onto the MinION for sequencing. However, currently the Voltrax is only able to prepare the DNA sequencing library, which is still valuable for on-site metagenome sequencing. The Voltrax is being tested by WBVR and compared to other sequencing library preparation methods in terms of suitability for on-site use and yield per library per time unit. Other partners (e.g. Sciensano) will investigate the use of Voltrax for library preparation in the near future (linked to WP3-T4). Additionally, the FARMED consortium has identified potential alternatives which are marketed to produce sequence quality DNA from a metagenomics sample which are being investigated. WBVR has tested the USEB DNA extraction method developed by Wageningen University and Research. While the method would be suitable for on-site diagnostics and is currently used for amplification-based detection methods, the yield of the method was 10 to 100 times below the yield of the Qiagen Puregene kit to which it was compared. Efforts to concentrate the material using DNA binding magnetic beads were unsuccessful and the method is therefore considered unsuitable for on-site usage for Nanopore sequencing.

JRP12-WP3-T3- Assess DNA isolation methods for suitability, test on matrices (faeces, blood, dust, (environmental), milk)

At APHA, initial trials of the PDQeX system, using the manufacturer's protocol, to extract DNA from pig faecal samples (collected at APHA) spiked with *E. coli* and MRSA, required further optimisation to increase DNA yields. In partnership with the manufacturer, a new prototype of the extraction tubes, changing sample input amounts and extraction conditions, appear to have increased DNA yield, although still not optimal (~10 ng/µl). Future work aims to optimise this method to achieve large enough quantity of sequence quality DNA.

Similarly as national lockdowns are further eased, other FARMED institutes will begin to assess on-site DNA extraction methods.

JRP12-WP3-T4- Test protocol on site

This task has not started as we still do not yet have a suitable on-site DNA extraction and sequencing workflow.

WP4 - Project management, coordination, and training workshop.

JRP12-WP4-T1- Annual physical project meetings

The FARMED consortium has not yet been able to gather together in person since the start of the project, as soon after, the COVID-19 pandemic started. No plans have been made this year to meet in person, as for many countries only essential travel is currently allowed. We will re-assess the travel

situation later in 2021.

JRP12-WP4-T2- Teleconferences will be organised every 3 months, between partners

The FARMED consortium has held teleconferences to discuss project progress and share experiences and results.

- In February 2021, DTU presented preliminary analysis of the WP1 DMC experiments conducted by members of the consortium. This highlighted the application of long read metagenomics to characterize the metagenome and enabled the consortium to decide on DNA extraction methods to pursue in FARMED.
- In April 2021, WBVR presented the WP3 deliverable reviewing the current scientific literature and overview of commercially available methods for on-site DNA isolation. APHA presented analysis comparing the effects of different sequencing library preparations as well as the sequence base calling approach.
- In June 2021, BfR presented the advantages of the HiC sequencing approach and its applicability to the FARMED objectives to associate AMR plasmids to a host with the microbiome.

JRP12-WP4-T3- Training dissemination of developed protocols

Not applicable.

JRP12-WP4-T4- Annual reports

The Y3 2020 annual report was submitted to OH-EJP in February 2021.

5.1.4.3.6.3 Progress of the research project: deliverables and milestones

Deliverables

Impact of COVID-19 on FARMED consortium partners

The COVID-19 pandemic continues to affect many of the FARMED consortium.

- Some countries still remain (18 months since start of FARMED) in lockdown or restricted access to the laboratories to only undertake essential activities, including some scientist involved in COVID activities. For most, all research activities, including the OHEJP projects were considered as non-essential and could not be continued, i.e. no activity in the R&D labs was allowed. This particularly had a big impact and the majority of FARMED involves laboratory based method optimisation.
- The national lockdowns delayed recruitment of staff or students to deliver FARMED objectives, with many new staff / students not in post until summer 2020. For some staff they had to contribute to the COVID-19 related activities.
- Research activities were able to start in summer 2020, however, homework remained the general rule, and social distancing needed to be assured, meaning only a limited number of people can work in the R&D labs. In winter 2020/21, COVID-19 cases were increasing and national lockdowns were re-introduced.
- In recent months there has been limited availability, in many countries, of various consumables (such as pipette tips) and scientific kits (such as WGS kits), due to the these items being diverted to COVID-19 testing activities.

As such all the milestones and deliverable have been delayed, and will require additional time to complete.

JRP/JIP code	**Project deliverable number** *(Original number, if different from the actual one)*	**Deliverable name** *(Original name, if different from the actual one)*	**Delivery date from AWP 2021 (month)**	**Date delivered on Project Group (month)**	**If deliverable not submitted on time: Forecast delivery date (month)**	**Comments** *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	**Proposed category*** *(1 to 8) (several categories may be applicable)*
12	D-JRP12-1.1	Report on an initial assessment of long-read sequencing for	47		M52	Adapted to M47 in M9 report 2020, so net yet due.	8 (Method application)

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		metagenome (community and AMR) analysis (defined community, spiked and real simple and complex matrices) and comparison to current short-read standard.					
12	D-JRP12-1.2	Report on the feasibility of using Hi-C sequencing for metagenomics to define the context of AMR genes.	46		M51	Adapted to M46 in M9 report 2020, so net yet due.	8 (Method application)
12	D-JRP12-2.1	Optimised long-read sequence bioinformatics tool for the analysis of microbial communities made publicly available.	45		M58	Adapted to M45 in M9 report 2020, so net yet due.	2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats
12	D-JRP12-2.2	Optimised long-read sequence bioinformatics	M58		M58	Adapted to M51 in M9 report 2020, so net yet due.	2. Harmonised protocols and

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		tool for analysis of the resistome made publicly available.					applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats
12	D-JRP12-3.1	Review on current scientific literature and overview of commercially available methods for on-site DNA isolation.	M36	M40		https://doi.org/10.5281/zenodo.4724557	8 (Summary of available methods, not publication)
12	D-JRP12-3.2	Protocol for use of Voltrax on-site for extraction and library preparation from clinical, veterinary and environmental samples, suitable for long-read metagenome sequencing.	M41		M53	Adapted to M43 in M9 report 2020.	2. Harmonised protocols and applied best practice
12	D-JRP12-3.3	Protocol for on-site extraction of high-quality DNA from clinical,	M54		M60		2. Harmonised protocols and applied best

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		veterinary, and environmental samples, suitable for long-read metagenome sequencing.					practice
12	D-JRP12-3.4	Detailed method for on-site long-read sequencing of sample matrix.	M54		M60		2. Harmonised protocols and applied best practice
12	D-JRP12-3.5	Report on initial findings of on-site diagnostic tests and IT solutions for human clinical, veterinary and environmental samples for early warning of emerging resistant pathogens.	M54		M60		3. Databases of reference materials and data, incl. metadata
12	D-JRP12-4.1	Annual communication to stakeholders and OH EJP coordinators	M48, M45		M48, M60		8 (Sharing and communication for application of methods)

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
12	M-JRP16-01	Review on current scientific literature and overview of commercially available methods for on-site DNA isolation presented to FARMED consortium.	M36	No	M40	https://doi.org/10.5281/zenodo.4724557
12	M-JRP16-02	Assessment of long-read sequencing for resolving a 'defined' microbial community and their AMR genes, using current DNA extraction methods; and comparison to current short-read sequencing standard.	M46		M48	Adapted to M46 in M9 report 2020
12	M-JRP16-03	Assessment of long-read sequencing on spiked metagenome samples, using current DNA extraction methods, and comparison to current short-read sequencing standard for resolving the microbial community and AMR content/context.	M46		M48	Adapted to M46 in M9 report 2020
12	M-JRP16-04	Protocols for on-site high quality DNA extraction using Voltrax and library preparation ready for	M43		M50	Adapted to M43 in M9 report 2020

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
		testing on-site				
12	M-JRP16-05	Assessment of long-read sequencing for 'simple' metagenome samples, using current DNA extraction methods, and comparison to current short-read sequencing standard for resolving the microbial community and AMR content/context.	M45		M50	Adapted to M45 in M9 report 2020.
12	M-JRP16-06	Assessment of the feasibility of Hi-C sequencing for metagenomics to define the context of AMR genes.	M46		M51	Adapted to M46 in M9 report 2020.
12	M-JRP16-07	Protocols for extraction of high-quality DNA (using different methods than Voltrax) from clinical, veterinary and environmental samples (simple and complex), suitable for long-read metagenome sequencing distributed amongst FARMED consortium for testing.	M44		M51	

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
12	M-JRP16-08	Assessment of long-read sequencing for 'complex' metagenome samples, using current DNA extraction methods, and comparison to current short-read sequencing standard for resolving the microbial community and AMR content/context.	M47		M52	Adapted to M47 in M9 report 2020.
12	M-JRP16-09	Development/adaptation of a pipeline that can predict species within sample/matrix	M45		M51	
12	M-JRP16-10	Finalised protocol for on-site long-read sequencing of sample matrix distributed amongst FARMED consortium for testing.	M45		M53	
12	M-JRP16-11	Development/adaptation of a Resfinder-'like' pipeline for the identification of AMR from long-read sequences	M51		M55	
12	M-JRP16-12	Hands-on laboratory and bioinformatics workshop organised at DTU and SSI	M54		M59	
12	M-JRP16-13	Benchmarking of bioinformatics tools developed in WP2 with metagenomic sequences gained	M54		M57	

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
		from T1.1, T1.2 and T1.3				
12	M-JRP16-14	Test the bioinformatics tools that were benchmarked and developed in WP2 on a laptop that is on-site (outside of laboratory conditions)	M54		M60	
12	M-JRP16-15	DNA extraction, library preparation and long-read sequencing tested on-site (outside of laboratory conditions)	M54		M58	
12	M-JRP16-16	Voltrax DNA extraction, Voltrax library preparation and long-read sequencing tested on-site (outside of laboratory conditions)	M54		M58	
12	M-JRP16-17	Complete workflow of DNA extraction (with or without Voltrax), library preparation (with or without Voltrax), long-read sequencing and bioinformatics analysis tested on-site for early warning of emerging resistant pathogens.	M54		M60	

5.1.4.3.6.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors already accepted your comments. Therefore, this part of the report has been closed.

5.1.4.3.6.5 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
D-JRP12-WP3.1 Review on current scientific literature and overview of commercially available methods for on-site DNA isolation. https://doi.org/10.5281/zenodo.4724557	Yes	no	Yes, (no cost)
A shotgun metagenomics approach to detect and characterize unauthorized genetically modified microorganisms in microbial fermentation products https://doi.org/10.1016/j.fochms.2021.100023 https://zenodo.org/record/5347021#.YS-kSY4zaM8 Food Chemistry: Molecular Sciences	Yes	No	Yes, (0 euros)

Additional output

Poster of project "FARMED: Long-read metagenomic sequencing workflow for the identification of pathogens/AMR on-site" presented at the One Health EJP Meeting, hosted in Copenhagen and online between 9-11th June 2021.

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

N/A.

5.1.4.3.6.6 Data Management Plan

The FARMED DMP has started to be updated, further changes will be made before data is generated.

The current dataset is not yet ready for sharing publically. The consortium will eventually make this data publically available.

The dataset of the publication Buytaers *et. al.* 2021 is publicly available in NCBI SRA under project PRJNA686880.

5.1.4.3.6.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

Several members of the FARMED consortium are involved in other OHEJP projects. In particular the Full-Force JRP is relevant as the project is utilising long-read sequencing to characterise mobile genetic elements which are associated with driving AMR in commensal and pathogenic Enterobacteriaceae.

Valeria Michelacci (ISS), working at the European Union Reference Laboratory (EURL) for *E. coli* at ISS, is involved in the activities of the steering committee for the development of a joint EFSA-ECDC database for molecular typing data collection, which started working in 2011 on PFGE and MLVA data but is now moving to WGS data collection. The three pathogens object of the surveillance at present are *Listeria monocytogenes*, *Salmonella* and Shiga toxin-producing *E. coli*, that's why these three EURLs are involved. Moreover, since 2017 EURL *E. coli* on a mandate of the European Commission is coordinating the activities of an Inter EURLs working group on Next Generation Sequencing, involving the following EURLs: *E. coli, Listeria, Staphylococcus* (CPS), *Salmonella*, *Campylobacter*, Parasites, Antimicrobial resistance, and foodborne viruses. The aim is to promote the use of NGS across the EURLs' networks of National Reference Laboratories, build NGS capacity within the EU and ensure liaison between the work of the EURLs and the work of EFSA and ECDC on the NGS mandate sent by the Commission. The working group is meeting twice a year (online since 2020). Deliverables produced by now consist in guidelines for the different steps of NGS data production and analysis, information on reference NGS datasets, trainings modules and proficiency test schemes available for NGS. It has never dealt with metagenomics, yet, but it is among the interests of the Working Group for the future. The presence of Valeria Michelacci, among partners of FARMED, in this Working Group will ensure proper exchange of information about the outcomes of the projects and metagenomics-related documents eventually produced in the working group. EFSA and ECDC are observers of the activities of the group.

DTU is a EURL laboratory as well as being certified WHO laboratory.

Sciensano, as NRL-GMO and also part of the European ENGL (European Network of GMO Laboratories), has contacts with the competent authorities at national (FAVV/AFSCA) and EU level, regarding the notification of the presence of GMM in microbial fermentation products. Sciensano is also member of the ENGL working group on “Sequencing strategies for the traceability of GMOs - methods and related quality aspects” and of the ENGL working group on “Detection of genetically modified microorganisms in food and feed", where the use of metagenomics for the identification and characterization of GMM in food/feed is being discussed.

5.1.4.3.7 JRP13-R2-AMRSH5-WORLDCOM

5.1.4.3.7.1 Summary of the work carried out in the Project

The One Health WorldCOM project aims to develop on-site diagnostic tools, linked with mobile referencing technology, for detection of AMR zoonotic pathogens (*Escherichia coli*, *Salmonella*, *Campylobacter*) in agricultural and environmental settings. The impact for One Health EJP and stakeholders will be rapid on-site detection of AMR pathogens in animal and human populations. Technologies under development will facilitate investigation of potential emerging resistances at earlier stages than currently feasible. Data generated will be used to develop machine-learning algorithms to predict AMR in various environments.

At NUIG, the development and optimisation of an internally controlled multiplex LEC-LAMP assay for the differential detection of clinically relevant Extended-Spectrum Beta-Lactamase (ESBL) genes associated with animals and humans has been completed. ESBLs are a group of diverse, complex, plasmid-mediated enzymes produced by organisms such as *E. coli* and *K. pneumoniae* that pose a therapeutic challenge in the treatment of hospitalised and community-based infections. The assay detects and differentiates the cefotaximase (CTX-M) group 1 variants $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$, which potentially could be used to identify AMR in human ($bla_{\text{CTX-M-15}}$) and veterinary ($bla_{\text{CTX-M-1}}$) settings. The development and optimisation of an internally controlled multiplex LEC-LAMP assay for the differential detection of both CTX-M group 1 variants, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$, has been completed. Successful analytical specificity and sensitivity of this assay has been demonstrated using a range of *E. coli* CTX-M environmental isolates collected by NUIG. Additionally, NUIG have completed the development and evaluation of a rapid sample prep protocol for on-site DNA extraction from animal faecal samples. This protocol, in combination with the LEC-LAMP assay, was successfully demonstrated using culture-confirmed CTX-M-1 positive porcine faecal samples provided by FLI. Demonstration of the on-site application of the rapid extraction protocol with the LEC-LAMP assay will be carried out in combination with portable instrumentation.

At the UoS, the AMR LAMP assays have been substantially progressed. Five AMR LAMPs assays have been developed and validated targeting important AMR resistance genes (MCR-1, KPC, OXA-48, OXA-23 and VIM) that are associated with zoonotic bacterial infections. The assays are able to detect target genes within 10 min, with 100% sensitivity and specificity. There has also been significant progress with the water sample preparation methods for LAMP detection of pathogens and AMR genes. The method is rapid (30 min), and sensitive with a detection limit of 10 cfu/mL (spiked bacterial culture). Once coupled with LAMP, the method demonstrated consistent detection of all the target AMR genes from tap water spiked with different Gram-negative bacterial cultures in less than 4 min, and within 35 min of the sample preparation.

Additionally, two machine learning models have been developed to provide automated detection in LAMP diagnostic tests: resulting in high test accuracy for the fluorescence-based data, and promising, preliminary results for the colorimetric data. A literature review is being conducted to identify existing dimensionality reduction techniques and supervised machine learning algorithms as applied to AMR prediction from WGS data, as well as knowledge gaps.

At UT, preparation to test AMR LAMP assays developed at NUIG has started. Genes of CTX-M-1 and 15 are targeted for testing on WGS sequenced *E. coli* isolates (> 300 isolates), gene annotation of assembled WGS indicates presence of CTX-M genes in about 40 to 50 isolates.

5.1.4.3.7.2 Progress of the project: description of activities

WP: 1 Generation of up to date sequence information for selected pathogens and antimicrobial resistance genes

JRP13-WP1-T1- Analysis of publically available sequences for antimicrobial resistance genes associated with Salmonella, Campylobacter and E. coli.

This task has been completed, see 12M report. JRP13-WP1-T2- Targeted and whole genome de novo sequencing of phenotypically characterised isolates from various settings.

At the UoS, no whole genome sequencing has been performed due to the COVID-19 lock down and difficulty in obtaining samples. So, this task is currently postponed. However, publicly available genome databases have been utilised.

NUI Galway previously provided genomic nucleotide sequence information from locally isolated *E. coli* CTX-M environmental samples to a WorldCOM AMR gene sequence database for use in assay development. This nucleotide sequence information included 2 x CTX-M-1, 1 x CTX-M-9, 4 x CTX-M-4, 24 x CTX-M-15, 5 x CTX-M-27 and 1 x CTX-M-65 *E. coli* isolates. At FLI, three cephalosporin-resistant *E. coli* strains isolated from alpacas in Central Germany were short-read whole genome sequenced. Two isolates contained the $bla_{\text{CTX-M-1}}$ allele, the third the $bla_{\text{CTX-M-27}}$ allele. Additionally, 17 cephalosporin-resistant *E. coli* strains isolated from pigs raised in Central Germany were short-read whole genome sequenced. We identified eight isolates with the $bla_{\text{CTX-M-1}}$ allele, seven strains with the $bla_{\text{CTX-M-15}}$ allele and two with the $bla_{\text{CTX-M-27}}$ allele.

At the UT, *de novo* whole genome sequencing (WGS) continued, mostly *E. coli* were isolated and placed in the sequencing pipeline, however due to overload at the local sequencing core facility due to COVID-19 related work next batch of 192 isolates were sequenced. Manuscript about *de novo* WGS of *Campylobacter* spp. Has been submitted to Food Control (Elsevier).

At INSA, *E. coli* and *K. pneumoniae* isolates resistant to 3rd/4th generation-cephalosporins and/or to carbapenemes and/or to colistin from human and animal origin were sequenced using a MiSeq Illumina platform. Overall, three PT WorldCOM AMR gene sequence databases were generated and are available for use. This nucleotide sequence information comprised isolates from clinical settings, including hospital surfaces. Among the 187 isolates (38 *E. coli* and 149 *K. pneumoniae*), 99 showed the presence of the $bla_{\text{KPC-3}}$ carbapenemase; $bla_{\text{NDM-type}}$, $bla_{\text{OXA-48-type}}$, and $bla_{\text{VIM-type}}$ were also identified. Mostly of the isolates were extended-spectrum-β-lactamase (ESBL) co-producers: $bla_{\text{CTX-M-15}}$, n=77; $bla_{\text{OXA-9}}$, n=69. To further complement WP1-T1, INSA is sequencing 15 *Acinetobacter* genomes using a MiSeq Illumina platform.

At UCM, a total of 410 *E. coli* isolates from diverse sources (110 from human, 154 from swine and 146 from broilers) were sequencing using a MiSeq Illumina platform. Regarding the presence of CTX-M genes among the 410 *E. coli* isolates, four isolates contained the $bla_{\text{CTX-M-15}}$ allele, two the $bla_{\text{CTX-M-14}}$ allele and one the $bla_{\text{CTX-M-27}}$ allele. In addition, 9 isolates showed the presence of the colistin resistance gene *mcr-1*. Environmental water samples bearing 16S rRNA 16S methyltrasnferases have been sequenced using Illumina and Nanopore technology and the results were published in Communications Biology (IF 6,268).

Meanwhile, UCM and UT are leading creating and sharing a database draft, with the support of UoS, among the WorldCOM partners to develop a resource of all isolates, their resistance, sequences and share all relevant information. The database draft is currently being further developed and all important and frequently reported ESBL and mobile colistin-mediated resistance genes are included.

The task is still ongoing.

JRP13-WP1-T3- Development of machine learning algorithms for the prediction of anti-microbial resistance. (University of Surrey, VISAVET Health Surveillance Centre UCM)

At the UoS, the machine learning algorithms have two aims: 1) developing algorithms for automating LAMP detection, reducing personnel biases and the need for expertise to analyse the results, and 2) development of algorithms for the prediction of AMR from whole genome sequencing.

For the first aim, algorithms have been developed in collaboration with WP2-T1, specifically for the automated detection of antimicrobial resistance genes based on fluorescence and colorimetric LAMP assays data. For the fluorescence-based detection, amplification and annealing data series were used as the input for a multi-modal neural network model trained using logistic regression. This model was trained and tested using a total of 56 samples, and leave-one-out cross validation estimated a test accuracy of ~ 98 %. With respect to colorimetric-based detection, pre- and post-reaction images of tested samples (i.e., MCR-1 and KPC) were used to train a K-means clustering algorithm to automatically identify colour changes. The images for the samples were pre-processed using colour-based thresholding and contour edge detection to extract representative features for the algorithm. Accordingly, using MCR-1 and KPC LAMP results images as the training and test data, respectively, we confirmed this model is capable of correctly identifying positive samples. This algorithm is still at an early stage of development and requires more data to confirm its sensitivity.

Concerning the second aim commitment of this work package task, predicting antimicrobial resistance using whole genome sequencing (WGS), progress has been delayed due to the other commitments for this work package, the delayed employment date and COVID-19 related restrictions. So far, a data preparation technique for performing dimensionality reduction on WGS data has been identified from the recent literature, based on forming 'k-mer-based representations' and 'compacted de Bruijn graphs', to reduce the number of input features from ~85 million to ~1 million per sample, in some cases. We plan to pursue this strategy further and identify a suitable supervised learning method for predicting AMR especially EBSL in zoonotic pathogens. Also, no whole genome sequencing has been performed, but this task is going to be developed first using publicly available sequences and those from our developed database from WP1-T2 as a training for the algorithm.

This task is still ongoing.

WP2: Assay Development

JRP13-WP2-T1- Development and performance evaluation of singleplex isothermal amplification assays for selected AMR gene targets

At the UoS, five loop-mediated isothermal amplification (LAMP) assays had been developed targeting 1) *mcr-1*, 2) $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-48-like}}$ genes (OXA-48, OXA-232, OXA-181 and OXA-54 encoding genes), 3) $bla_{\text{OXA-23}}$, 4) bla_{KPC} and 5) bla_{VIM}. All five assays had been optimised and validated against a wide range of Gram-positive and Gram-negative bacterial strains to confirm sensitivity and specificity. All five assays showed 100% sensitivity and specificity, and were detected in less than 10 min. The assays were developed and detected both fluorescently and colorimetric. The LAMP detection limit had also been assessed using mcr-1 LAMP. The detection limit of mcr-1 LAMP was assessed using a 10-fold and 2-fold serial dilutions of genomic DNA of *E. coli* NCTC 13846 and shown to be 0.0625 pg of DNA, which was successfully amplified in duplicates in less than 8 min. LAMP primers were also designed targeting *mcr-8*, *mcr-9* and the most frequent alleles of bla_{IMP} (imipenem metallo-β-lactamase), and will be validated once *mcr-8*, *mcr-9* and bla_{IMP}-encoding resistant strains are obtained.

NUI Galway previously completed the design of singleplex LEC-LAMP assays for the detection of CTX-M group 1 variants, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$, using existing genomic sequence data from *E. coli* isolates collected and processed by NUI Galway. Sequence alignment analysis was used to identify single nucleotide polymorphism (SNP) differences between these variants and SNP differences were used to develop LEC-LAMP assays for differential variant detection. A separate LEC-LAMP assay for the detection of an internal amplification control (IAC) template was also developed. Complete analytical specificity for each assay was demonstrated. Analytical sensitivity for each assay demonstrated low limits-of-detection with 10 genome copies detected in under 20 min. These assays would subsequently be used in WP2 Task 3 to develop a multiplex LEC-LAMP assay.

JRP13-WP2-T2- Evaluation and selection of sample preparation methods for use with laboratory on-site tests

At the UoS, the water sample preparation method has been further developed and validated. The method is advantageous in being rapid (30 min). The detection limit of the method was further assessed using tap water spiked with the mcr-1 positive *E. coli* NCTC 13846 bacterial culture at different concentrations (10^7 – 10 cfu/mL). The assay detected up to 10 cfu/mL, as confirmed by culture, of spiked bacterial cells in less than 6 min of the LAMP assay. The water sample preparation method was further validated using tap water spiked with different bacterial cultures of mcr-1 (*E. coli* NCTC 13846), OXA-48 (*E. coli* NCTC 14321), OXA-23 (*A. baumannii* NCTC 13301), KPC (*K. pneumoniae* NCTC 13809 and *E. coli* NCTC 14321) and VIM (*E. cloacae* NCTC 14326) positive strains separately before being detection with the AMR LAMP assays. All AMR targets were detected in less than 4 min of the AMR LAMP assays and within 35 min of sample preparation.

NUIG has completed the development and evaluation of a rapid sample prep protocol for on-site DNA extraction from animal faecal samples. For validation of this method, porcine faecal samples were supplied by FLI. These samples were collected and confirmed positive for CTX-M-1 by picking cultures from Gassner agar plates containing 4 µg/ml Ceftiofur and PCR-amplifying and Sanger-sequencing the bacterial DNA with *bla*$_{\text{CTX-M-1}}$-family-specific primers. Samples contained varying amounts of coliform strains resistant to Ceftiofur, ranging from 25-100%. 200mg of each sample was processed using the rapid sample preparation protocol, a 15 min Chelex/heat lysis DNA extraction method, followed by LEC-LAMP testing. All samples successfully tested positive for CTX-M-1 in under 20 min.

JRP13-WP2-T3- Development and performance evaluation of multiplex assays for pathogens and resistance genes

NUIG has completed the development and evaluation of an internally controlled multiplex LEC-LAMP assay for the differential detection of CTX-M group 1 variants, *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CTX-M-15}}$. The internally controlled LEC-LAMP assay contains fluorescently labelled primer/probes specific to each target: FAM for CTX-M-1; HEX for CTX-M-15; and Cy5 for the internal amplification control (IAC). The LEC-LAMP assay demonstrated complete analytical specificity and differential detection of both variants when tested with environmental *E. coli* isolates collected from Ireland and animal *E. coli* isolates from Central Germany: CTX-M-1 (n=15); CTX-M-15 (n=37); and other CTX-Ms (n=12). The LEC-LAMP assay also demonstrated low-level detection for each variant at 10 genome copies per reaction in approximately 20 min.

So far, FLI has tested the multiplex LEC-LAMP assay on 227 *E. coli* strains which were isolated from porcine faecal samples and are resistant to Ceftiofur. Of these, 150 were positive for *bla*$_{\text{CTX-M-1}}$ and 75 for *bla*$_{\text{CTX-M-15}}$. One of the remaining 2 negative strains contains a sequence-verified *bla*$_{\text{CTX-M-27}}$ allele, the Ceftiofur-resistance mediating gene from the other strain has not been characterised yet. FLI is actively acquiring samples from pig farms and characterizing them for cephalosporin and colistin resistance for sample processing and assay validation.

To complete this work, the multiplex LEC-LAMP assay will be further evaluated with additional isolates provided by INSA (CTX-M-producing isolates others than CTX-M-1/15: CTX-M-9, CTX-M-14 and CTX-M-27).

WP3: Development of Lateral Flow Diagnostics Detection and Mobile Communication system

JRP13-WP3-T1- Development of lateral flow diagnostics detection

At the UoS, AMR LAMP assays have been developed using both fluorescence and colorimetric signals to allow for the immediate detection of the results. With the current developments and the excellent assays performance we are postponing the decision to develop lateral flow detection as it may be redundant and not necessary. However, we plan to expand the current pathogen and AMR LAMP assay targets to further develop a platform that can detect up to ten AMR genes and identify new pathogens of important zoonotic significance. This will significantly improve our developed LAMP platform and enhance its application for the detection of zoonotic pathogens and associated AMR.

JRP13-WP3-T2- Development of mobile technology for communication on-site results

WP4: Evaluation of on-site tests and other tools developed

JRP13-WP4-T1- Feasibility testing of laboratory and on-site tests for detection of pathogens and resistance genes

NUIG is currently developing a mobile workstation using portable instrumentation for the demonstration of on-site application using the developed rapid sample prep protocol and LEC-LAMP assay. The proof-of-principle of this technology will be evaluated on-site using previously confirmed CTX-M-1 positive porcine faecal samples provide by FLI.

JRP13-WP4-T2- Generation of information for dissemination

NUIG is currently preparing a manuscript for publication detailing the development and evaluation of the internally controlled multiplex LEC-LAMP assay for the differential detection of CTX-M group 1 variants, *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CTX-M-15}}$, from porcine faecal samples.

The UoS is currently preparing a manuscript for publication detailing the assays development and evaluations of the five AMR LAMP targets (mcr-1, KPC, OXA-48, OXA-23 and VIM) for the detection of antimicrobial resistance from pathogens and water samples for One Health diagnostic applications.

WP5: Project Management

JRP13-WP5-T1-Project Management

5.1.4.3.7.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
13	D-JRP13-1.1	Generation of a sequence database comprising publically available and newly generated sequences for *E. coli, Salmonella* and *Campylobacter* spp. and resistance genes CTX-M-15, NDM-5, KPC-2, OXA-48 and MCR-1.	30	M33		Zenodo link: https://doi.org/10.5281/zenodo.4019840. Confidential on relevant WorldCOM OHEJP website section, and already shared with all consortium members during development. Public	3
13	D-JRP13-1.2	Novel machine learning algorithms for the prediction and detection of AMR from genomic sequences.	43		M48	Confidential until full validation of the code or publication (except for WORLDCOM or other One Health EJP members). This deliverable has been delayed for several reasons: the late employment date in relation to COVID-19 restrictions, and the other commitments	3

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
						of this work package arising from collaboration with WP2-T1 – automated detection of LAMP assay data.	
13	D-JRP13-2.1	Multiplex real-time isothermal assays for pathogens and antimicrobial resistance genes which will be suitable for use in laboratory and optimised further in WP3 into a format suitable for on-site use	43	43		Confidential until publication	
13	D-JRP13-4.1	Feasibility testing of on-site tests for pathogens and antimicrobial resistance genes complete			58	Confidential until publication	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
13	D-JRP13-5.1	Kick-off meeting organised	25	25		Brief report on KOM uploaded to ZENODO : Public https://doi.org/10.5281/zenodo.5464662	
13	D-JRP13-5.2	DMP reviewed	30	33		The OHEJP WP4 team reviewed and approved WorldCOM's online DMP in the CDP tool in December 2020.	3
13	D-JRP13-5.3	Organisation of project review meeting	45				
13	D-JRP13-5.4	Final 12 month technical and financial reports prepared for Year 3	36	38		Both technical and financial reports have been uploaded to Zenodo and can be accessed at: Public https://zenodo.org/record/5499692#.YTsQPp1KgdU	Other – project management

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
13	M-JPR15-AMR2.1-01	Generation of sequence information for pathogens and resistance genes of interest.	30	Y		Deliverable 1: Prevalence of ESBL subtypes in bacterial pathogens and a sequence database of selected alleles, DOI: 10.5281/zenodo.4019839
13	M-JPR15-AMR2.1-02	Transfer of relevant sequence information for development and training of novel machine learning algorithms.	36	No	M48	
13	M-JPR15-AMR2.1-11	Project review meeting organised	45			To be held in September 2021

5.1.4.3.7.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors already accepted your comments. Therefore, this part of the report has been closed.

5.1.4.3.7.5 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Population genomics and antimicrobial resistance dynamics of Escherichia coli in wastewater and river environments. Delgado-Blas JF, Ovejero CM, David S, Montero N, Calero-Caceres W, Garcillan-Barcia MP, de la Cruz F, Muniesa M, Aanensen DM, Gonzalez-Zorn B. Commun Biol. 2021 Apr 12;4(1):457. doi: 10.1038/s42003-021-01949-x Zenodo link in progress.	YES		YES (2680 euros)

Additional output

Poster

For WP1-T3 - an abstract entitled 'Automated detection of AMR target genes using loop-mediated isothermal amplification (LAMP)' was submitted by PDRA Brian Gardner at UoS to the OHEJP Annual Scientific Meeting 2021, 9th-11th June 2021 in Copenhagen. This was selected as an e-Poster presentation.

For WP2-T2 and WP2-T3, poster entitled "Rapid detection and discrimination of closely related Enterobacteriaceae CTX-M group 1 variants, *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CTX-M-15}}$, using an internally controlled multiplex loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) assay" was presented by Owen Higgins at the 2021 Microbiology Society conference, the OHEJP 2021 ASM, and is accepted for the ECCMID 2021 conference.

For WP2-T1 and WP2-T2, posters entitled ''Development of LAMP assays for the detection of key AMR targets in animal faeces and water samples'' and ''Rapid LAMP detection of key AMR targets for use as bed-side and/or pen-side diagnostics'' were presented by Marwa Hassan at the 2021 Microbiology Society conference and the OHEJP 2021 ASM, respectively.

Oral presentation

For WP2-T1 and WP2-T2 an abstract entitled "Whole-genome sequencing of phenotypically characterized isolates from various settings" was presented by Vera Manageiro at the OHEJP Annual Scientific Meeting 2021, 9th-11th June 2021 in Copenhagen (e-Poster).

5.1.4.3.7.6 Data Management Plan

The WorldCom DMP is uploaded to the CDP tool and has been updated in June 2021.

All generated datasets from the project are described, the locations the data are stored are listed and email addresses for the data controller are indicated in the tool. Links are provided to the open access datasets. Each dataset has been given a unique descriptive identifier.

All generated datasets from the project are described, the locations the data are stored are listed and email addresses for the data controller are indicated in the tool. Links are provided to the open access datasets. Each dataset has been given a unique descriptive identifier.

5.1.4.3.8 JRP14-R2-AMR2.1-FULL-FORCE

5.1.4.3.8.1 Summary of the work carried out in the Project

The goal of the Full Force project is to supply 17 EU partners with a technological toolbox and hands-on training in Single-Molecule Real-Time (SMRT) sequencing, and to apply this knowledge on six study cases and applications in metagenomics and AMR transmission models. Using this state-of-the-art technology, public health and veterinary labs will have the capacity to perform full-length sequencing, and gain detailed insight in mobile genetic elements (MGEs) which carry antimicrobial resistance and virulence genes within and across species.

In Y4, corresponding to the second year of the project, the main focus went to (i) the application of the Full Force Plasmid Assembly (FFPA) pipeline during an internal proficiency test, (ii) start-up of the five study cases in WP2, and (iii) applications on metagenomics datasets (WP3). However, the strong laboratory focus which is inherent to the project set-up continued to suffer from the pandemic. Many consortium members are still reoriented towards Covid-19 surveillance, causing significant delays in deliverables and milestones of the Full Force project, as elaborated more in detail in the sections below. The physical progress meeting which was planned during ECCMID 2021 in Vienna, was cancelled and postponed to a physical meeting on November 22-23 in Brussels. However, we are still confident that most goals of the Full Force project are still within reach, also thanks to a six-month extension which was applied for in May 2021.

- The main goal of the Full Force project is to enable consortium partners to reach a sufficient technical level in plasmid sequencing. To maximize the output from this project, SSI scientists involved in WP1 developed an easy-to-use software package (Full Force Plasmid Assembler), which will automatically perform hybrid assemblies through the build-in UniCycler program from a combination of short and long sequence reads.
- A proficiency test to assess each institute's capacity for SMRT sequencing is currently (M42) ongoing. Five multi-drug resistance *E. coli* strains are send to 16 participating institutes, and results are expected by the end of Y4.
- Although WP2 (five cases studies implementing long-read sequencing on existing datasets) has suffered some delays, substantial progress has been made. In four of the five study cases, phylogenetic analyses on short-read datasets are ongoing to identify reference strains and plasmids for detailed study by long-read technology.
- WP3 (metagenomics studies using long-read technology) has very well advanced. DTU published a manuscript on the plasmidome of sewage samples using methodology which was optimized during the Full Force.
- In the context of WP4, a protocol for harmonized bacterial conjugation has been established. The robustness of this method will be assessed with selected Full Force partners in the second half of Y4, and intense collaboration between partners of WP2 and WP4 are foreseen in M42-54.
- The design and parameterisation of a transmission spread model of pAMR in the simulation framework SimInf is currently ongoing in collaboration between the consortium partners of WP5. The second task in WP5 to perform exposure assessment of horizontal and vertical transmitted amr has been initiated and a first report completed.

5.1.4.3.8.2 Progress of the project: description of activities

WP0 Project management

JRP14-WP0-T1-meetings and telcalls

The annual consortium meeting, planned during ECCMID 2021 (Vienna) was **postponed** due to Covid-

19 measures. This will be replaced by a two-day annual meeting in Brussels on November 22-23, 2021. Regular teleconferences were held to discuss progress and planning of ongoing WPs.

JRP14-WP0-T2-reporting

In total, ten new deliverables were uploaded to Zenodo during 2021. The 9 month report (Y4) was submitted in due time.

JRP14-WP0-T3- central data repository

FULL_FORCE is using a centralized data repository to upload sequence- and metadata which are generated during WP1 and WP2. Originally, we planned to use the AMR data hub of the European COMPARE Consortium and the European Nucleotide Archive (ENA). However, we were not successful in reaching an agreement with ENA, who is still negotiating single-subcontractor model for hubs created during COMPARE. Therefore, we have decided to use a commercial cloud tool (OwnCloud) for temporal data storage during Full Force.

Currently, this cloud stores over 900 Gb of data which is exchanged among partners.

JRP14-WP0-T4-data management plan

The Full force Data Management Plan was constructed in the CDP software and uploaded to https://apps.lisam.com/app/#Apps/CDP. As important part of the DMP, a framework agreement on Material Transfer was drafted, circulated and signed among all 18 participating institutions. This agreement covers all transfer of data and strains during Full Force.

WP1 SMRT implementation

JRP14-WP1-T1-methodology for MGE sequencing

This task was completed during the first annual year of Full Force.

JRP14-WP1-T2-SMRT sequencing workshop

This task was completed during the first annual year of Full Force.

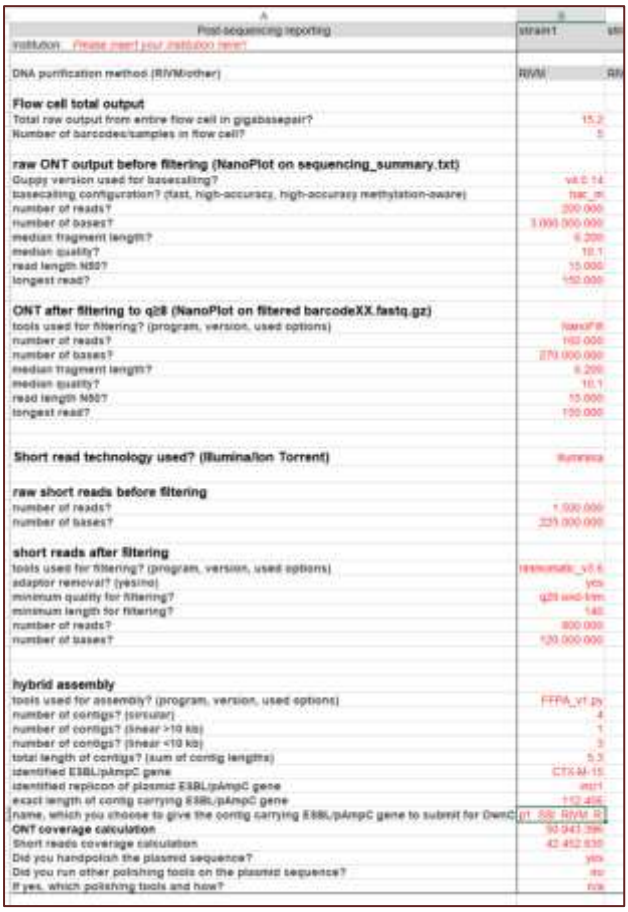
JRP14-WP1-T3-proficiency test for MGE sequencing

Each institution's proficiency in SMRT sequencing will be assessed afterwards using a proficiency test, organised and coordinated by SSI. Given the delay in the workshop, this EQA is being organised in M37-48. Upon discussion during and after the workshop in October 2020, it was decided to include 5 *Escherichia coli* strains from BfR (GER) as reference strains, since they have been sequenced using Illumina, MinION and PacBIO technologies.

In the PT instructions, it was highlighted that the Full Force SMRT protocol by RIVM (current version 2) should be followed to produce the ONT sequencing data, so all data can be aggregated into one common method paper. However, as many partners have already established in-house/homemade sequencing protocol (e.g. for DNA purification or hybrid assembly), the option to benchmark these against the Full Force results was also foreseen.

The strains have been send out mid-November and at time of writing (M42), six institutes have submitted data.

First analyses and sharing of experiences pointed at difficulties with the elaborate RIVM purification protocol, as it gave highly viscous/concentrated DNA samples which clogged the pores despite producing markedly higher N50s. Therefore, data analysis and the resulting publication will be focused on comparison of DNA extraction methodology and influence of minION 9.4 and 10.3 flow cells on full-length plasmid sequencing.



Post-sequencing reporting	strain1
institution (Please insert your institution here!)	
DNA purification method (RIVM/other)	RIVM
Flow cell total output	
Total raw output from entire flow cell in gigabasepair?	15.2
Number of barcodes/samples in flow cell?	5
raw ONT output before filtering (NanoPlot on sequencing_summary.txt)	
Guppy version used for basecalling?	v4.0.14
basecalling configuration? (fast, high-accuracy, high-accuracy methylation-aware)	hac_m
number of reads?	200.000
number of bases?	3.000.000.000
median fragment length?	6.200
median quality?	10.1
read length N50?	15.000
longest read?	150.000
ONT after filtering to q28 (NanoPlot on filtered barcodeXX.fastq.gz)	
tools used for filtering? (program, version, used options)	NanoFilt
number of reads?	160.000
number of bases?	270.000.000
median fragment length?	6.200
median quality?	10.1
read length N50?	15.000
longest read?	150.000
Short read technology used? (Illumina/Ion Torrent)	Illumina
raw short reads before filtering	
number of reads?	1.000.000
number of bases?	225.000.000
short reads after filtering	
tools used for filtering? (program, version, used options)	trimmomatic_v0.6
adaptor removal? (yes/no)	yes
minimum quality for filtering?	q28 and trim
minimum length for filtering?	140
number of reads?	800.000
number of bases?	120.000.000
hybrid assembly	
tools used for assembly? (program, version, used options)	FFPA_v1.py
number of contigs? (circular)	4
number of contigs? (linear >10 kb)	1
number of contigs? (linear <10 kb)	3
total length of contigs? (sum of contig lengths)	5.3
identified ESBL/pAmpC gene	CTX-M-15
identified replicon of plasmid ESBL/pAmpC gene	IncI1
exact length of contig carrying ESBL/pAmpC gene	112.456
name, which you choose to give the contig carrying ESBL/pAmpC gene to submit for DwnC	p1_ESBL RIVM_R
ONT coverage calculation	50.943.396
Short reads coverage calculation	42.452.830
Did you handpolish the plasmid sequence?	yes
Did you run other polishing tools on the plasmid sequence?	no
If yes, which polishing tools and how?	n/a

Figure 1. *Some post-sequencing QC parameters, which are assessed during the EQA.*

WP2 Genome studies

In WP2, the acquired SMRT toolbox will be applied in the (re-)sequencing of AMR strains from various research and surveillance projects in WP2 including EU projects such as EFFORT, COMPARE, ENGAGE and ARDIG, as well as national and EU surveillance activities for which short read sequences are available. In Y4, the focus in WP2 was on **hypothesis generation based on short-read sequence data** for the five defined studies cases (T2.1-2.5). Short-read Illumina data will allow comparison of total plasmid content and phylogenetic relatedness of isolates and based on those results choose a subset of isolates for MinION runs.

JRP14-WP2-T1- MGE evolution in longitudinal sample sets (ARDIG, ABRES)

This task focuses on IncI1 plasmids, and focuses on the research question regarding the European diversity in the complete sequences, and wether these plasmids evolve either host-or country specific. Data on IncI1 containing samples from more than 1,000 longitudinal and annual surveillances was collected (see figure and table below), and MLST type of the host was determined. Phylogenic analysis was performed by Mike Brouwer, showing cross-sectorial and pan-European conservation of certains plasmid lineages. Based on this information, a selection will be made for long-read sequencing (M-JRP19-M12).

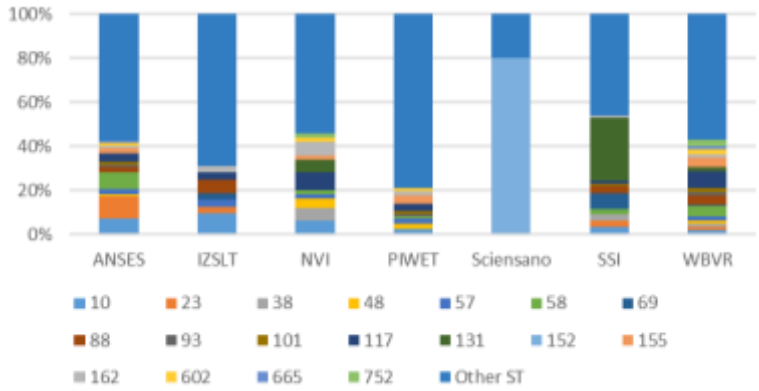


Figure 2. *cgMLST typing of IncI1 positive E. coli and Salmonella strains from EU monitoring, showing vast diversity in host cell types.*

JRP14-WP2-T2- MGE evolution in cross-sectional data sets (EFFORT, ENGAGE & National Surveillance)

Only preliminary actions have been taken due to the long-term absence of task leader Jens (bFR).

JRP14-WP2-T3- Klebsiella pneumoniae: the canary in the coalmine

K. pneumoniae constitute a significant clinical problem and represents a species in which several new AMR genes were first discovered before spreading to other pathogens. *K. pneumoniae* has a wide ecological distribution, diverse DNA composition, AMR gene diversity and high plasmid burden. This is a reason for assuming that the horizontal gene transfer occurs at high frequency in this species. Chromosomal DNA and plasmid DNA will be analysed and information regarding resistance genes and molecular strain typing will be evaluated through SMRT sequencing.

Available strain collections were reported from:

- Human
  - Denmark = 192 isolates
  - Sweden = 215 isolates
  - The Netherlands = 578 isolates
  - Portugal = 144 isolates
- Animal/environment
  - Italy = 15 isolates (cats and dogs)
  - Norway = 2 isolates (dogs)
  - France = 105 isolates (cats and dogs)
  - Germany = 103 isolates (livestock, zoo and environment)
    = 191 isolates (wastewater and slaughterhouse)

PHAS (Alma Brolund) and RIVM performed analyses on this dataset (ST type, and carbapenemase gene content), and found wide variety in sequence types of which some are limited to either human, animal or environment (Figure 2). Likewise, clear association between certain sequence types and carbapenemase genes (e.g., OXA-181 and ST873) was found. More specific phylogenetic analyses are currently (M42) being performed at RIVM (Antoni Hendrickx) to propose a strain selection for long-read sequencing (M-JRP19-M12).

Figure 2. Preliminary analysis of K. pneumoniae strains under study.

JRP14-WP2-T4- AMR in horses: commensals and pathogens

This task objective is to investigate the spread of ESBL-producing Enterobacteriaceae in horses in Europe. The focus will be on blaCTX-M-1 and blaSHV-12 genes. To date, isolates carrying blaCTX-M-1 predominate in horses. This is due to clonal dissemination as well as the spread of closely-related IncHI1 plasmids. The success of this plasmid gene combination might be due to the fact that the IncHI1 plasmid also carries the fos-operon, involved in the metabolism of fructo-oligosaccharides, which could give the plasmid a selective advantage in the gut of the horse. The combination IncHI1+ blaCTX-M-1 appears to be almost exclusively linked to horses. Less is known of the epidemiology of blaSHV-12, which is often associated with IncX3-plasmids.

The inventory of available isolates from horses, both commensals and clinical isolates, among participating partners is completed (n=264). All short-read sequences have been uploaded to owncloud, raw reads are assembled and the plasmidome determined/filtered. The plasmidome is comprised out of 8 clusters.

Based on this information, a selection will be made for long-read sequencing (M-JRP19-M14).

JRP14-WP2-T5- MGEs in Salmonella Infants and Kentucky

Salmonella Infantis is typically most prevalent Salmonella serotype in broilers, and the 4th most prevalent serovar in humans. It is known for increasing drug resistance. In 2007, a first report of megaplasmid pESI (300 kb) was published, while follow-up studies showed that this IncP plasmid contains a *mer* operon, yersiniabactin, toxin-antitoxin systems and AMR genes. Currently it is being

reported in Israel, Italy, Switserland, US, and Hungary[2].

This task examines the European distribution and evolution of this plasmid, and is led by ISS and IZSLT, who previously published on the phylogeny of S. Infantis (Figure 2[3]). During Full Force, data from their publication will be merged with pESI positive *Salmonella Infantis* strains from contributing partners, to study its diversity and evolution.

	ANIMAL		FOOD		OTHER		tot	
	STRAIN	SEQ	STRAIN	SEQ	STRAIN	SEQ	STRAIN	SEQ
SCIENSANO	520	16	2634	15	48	0	3202	31
RIVM*								
SVA	6	0	2	0			8	
PIWET	109	3	137	1	18		265	4
PHAS								
ISS								
IZSLT	29	13	10	5			39	18
INRA								
INSA								
tot	664	32	2783	21	66		3514	53
								25

Figure 2. Phylogeny S. Infantis.

Samples were selected among partners, based on the following criteria:

- Phenotypic markers: SMX-TET-SUL resistance (NAL/CIP)
- Genotypic markers: presence of IncFIB(pN55391), tet(A), sul1 and dfrA14
- In case ESBL profile detected, these isolates should be preferably included.

The number of available isolates are listed in the table on the right, and short reads were uploaded to Owncloud. Based on this information and subsequent phylogenetic analyses (IZSLT), a selection will be made for long-read sequencing (M-JRP19-M13) by IZSLT.

JRP14-WP2-T6- Evaluation of tools for MGE typing

This task has not been started at the time of writing of this report.

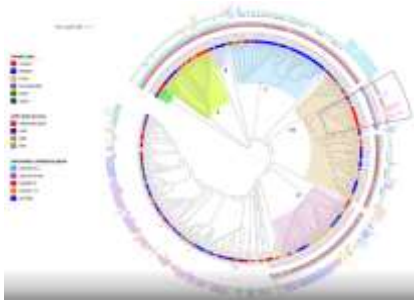
WP3 : Culture-Independent typing and metagenomics

JRP14-WP3-T1-MGE analyses in metagenomic datasets

This task has been completed in the first annual year of Full Force.

JRP14-WP3-T2-culture-independent methods for plasmid identification

The extraction methodology developed during Y3 was applied on waste water samples in Y4. The results were published in MSystems (https://journals.asm.org/doi/10.1128/mSystems.00283-21). This study represents, to the best of our knowledge, the first study to investigate plasmidomes at a global scale using long read sequencing from complex untreated domestic sewage.



WP4 : Functional characterization of AMR mobile genetic elements (MGE)-carrying AMR genes and bacterial host associations

JRP14-WP4-T1-MGE and host selection

As planned, a complete protocol of conjugation assays in liquid cultures has been drafted. This protocol has the objectives (i) to isolate the plasmid of interest in a plasmid-free recipient strain,

[2] Franco A, et al. PLoS One 2015; 10:e0144802. Aviv G, et al. Environ Microbiol 2014; 16:977–994 ; Hindermann D, et al. Front Microbiol 2017; 8:1322; Tate H, et al. Antimicrob Agents Chemother 2017; 61:e00488-17; Carfora V,et al. Front Microbiol 2018; 9:1880

[3] Alba, P. et al. Microbial Genomics 2020: 6.

(ii) to determine if the plasmid is self-transmissible or only mobilizable, and (iii) to determine the conjugation rate (max) based on the Simonsen model and its derivatives (Simonsen et al., Huisman et al.). This protocol is derived from Huisman et al. that provides all tools to estimate conjugation rates and to simulate population dynamics in an R package and Shiny app (https://ibz-shiny.ethz.ch/jhuisman/conjugator/).

An additional protocol for conjugation assays on solid medium is also provided representing a useful alternative protocol for mating assays (see 3.).

In its complete form, the protocol requires 2 conjugation experiments:

- A first one starting from a donor (D) + recipient (R) mating in which D is usually a field strain.
- A second one using transconjugants (T) + R' mating in which R' has a different chromosomal selective marker to the one used in the first experiment.

JRP14-WP4-T2-In vitro characterization

The protocol developed in WP4-T1 will be applied in to (i) a proficiency test, in which conjugation rates of an identical D-R pair will be assessed by five institutes, and (ii) the shufflon regions in IncI1 plasmids studied in task 2.1., i.e. different pilV-Cterm. The latter will be effectuated by constructing plasmid mutants with only one pilV-Cterm, to have all possibilities and compare them also with WT plasmids in mating assays.

JRP14-WP4-T3-Host association studies

This task has not yet been initiated at the time of writing.

WP5 : modelling

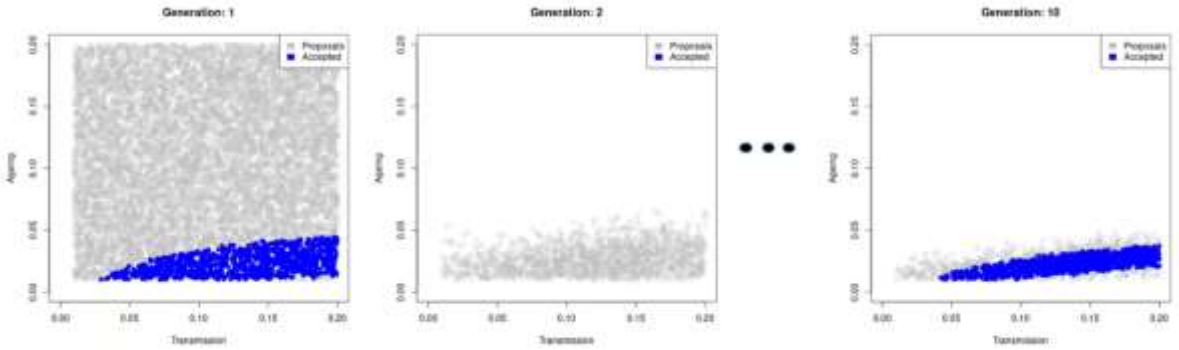
The objectives of WP5 are to address: i) gaps in quantitative knowledge on the spread of pAMR which will be essential to direct future focused research, ii) insight in the uncertainty around the effect of measures reducing pAMR prevalence in the food production chains, and iii) identification of key elements in the production chains to mitigate the risk of human exposure.

Task 5.1 *MODEL DESIGN for AMR TRANSMISSION (M25-M42)*

The parameterisation and design of a transmission spread model of pAMR in the simulation framework SimInf is ongoing. SVA (Stefan Widgren) has coordinated two teleconferences with the task participants. The first teleconference was a startup meeting and the second teleconference was a meeting to discuss horizontal vs. vertical AMR transmission. A necessary but challenging step in stochastic modelling is to determine parameters such that the model generates data that are consistent with observations. Parameterization is preferably conducted within a Bayesian framework and in WP5 we are focusing on using Approximate Bayesian computation (ABC), a recent computational approach for simulation-based inference. Development has been completed to add ABC functionality to the open-source SimInf modelling R package (https://github.com/stewid/SimInf). The figure below illustrates using ABC in SimInf to fit parameters from data of infected chicken broilers published in Dame-Korevar et al. (2017), and a model with susceptible (S) and infected (I) chicken, showing (for example) that the ageing parameter has a tighter posterior distribution compared to the

transmission parameter. Work is ongoing to identify and include other sources of broiler data for parameterisation of more complex models.

Task 5.2 *EXPOSURE ASSESSMENT of HORIZONTAL and VERTICAL TRANSMITTED AMR (M25-M54)*



This task has been initiated. The objective of this task will be on investigating the relative importance of exposure routes from the broiler production chain to humans, as this will indicate where intervention measures to reduce transmission to humans will be most effective, given that an intervention is realistic for the specific exposure route. The terms horizontal and vertical transmission are used in a different way than usual, horizontal transmission meaning AMR transmission from chickens to farmers or slaughterhouse personnel, vertical transmission meaning AMR transmission related to chicken meat consumption. As planned, a report on quantification of horizontal vs. vertical transmission of AMR was completed (D-JRP19-WP5.D2).

5.1.4.3.8.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
14	D-JRP14-0.D1	Start-up meeting report	M27	M27		Confidential due to research updates; 10.5281/zenodo.4275887	8
14	D-JRP14-0.D2	Activity report Y3	M36	M42		Public; 10.5281/zenodo.4896736	8
14	D-JRP14-0.D3	Recorded webinar tutorial ENA AMR data hub	M27	M27		Public; 10.5281/zenodo.4277545	3
14	D-JRP14-0.D4	Annual meeting report with (re-)approval of DMP	M42		M48	Annual meeting postponed to Novermber 2022, Brussels.	
14	D-JRP14-1.D1	Teleconference to assess required protocols and infrastructure	M25	M26		Public; 10.5281/zenodo.3733393	8
14	D-JRP14-1.D2	Teleconference to discuss proposed protocols and infrastructure (follow-up)	M26	M26		Public; 10.5281/zenodo.3759335	8
14	D-JRP14-1.D3	Completion of final protocol for SMRT sequencing	M27	M34		CONFIDENTIAL UNTIL PUBLICATION; 10.5281/zenodo.4277521	2
14	D-JRP14-1.D4	Invitation to workshop delivered to all participating institutions	M28	M27		Public; 10.5281/zenodo.3693741	8
14	D-JRP14-1.D5	Completion of workshop organization plan including selection of course material	M29	M35		CONFIDENTIAL UNTIL PUBLICATION; 10.5281/zenodo.4290698	8

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
14	D-JRP14-1.D6	Selection of proficiency test data	M32	M35		CONFIDENTIAL UNTIL PUBLICATION; 10.5281/zenodo.4290707	8
14	D-JRP14-1.D7	Delivery of analysis results of proficiency test by partners to SSI	M39	M42		CONFIDENTIAL UNTIL PUBLICATION; 10.5281/zenodo.4896743	8
14	D-JRP14-1.D8	Completion of final report of proficiency tests	M40		M48	Proficiency test data will be analysed in Q3 and Q4 of 2021.	
14	D-JRP14-2.D1	Submission of sequence- and metadata of longitudinal samples at ENA hub	M33	M42		CONFIDENTIAL UNTIL PUBLICATION; https://zenodo.org/record/4905398	8
14	D-JRP14-2.D2	Submission of sequence- and metadata of cross-sectional samples at ENA hub	M33		M48	Long-term illness of task leader.	
14	D-JRP14-2.D3	Submission of sequence- and metadata of K. pneumoniae samples at ENA hub	M33	M42		CONFIDENTIAL UNTIL PUBLICATION; https://zenodo.org/record/4896755	8
14	D-JRP14-2.D4	Submission of sequence- and metadata of S. enterica samples at ENA hub	M33	M42		CONFIDENTIAL UNTIL PUBLICATION; https://zenodo.org/record/4896772	8
14	D-JRP14-2.D5	Submission of sequence- and metadata of horse-related samples at ENA hub	M33	M42		CONFIDENTIAL UNTIL PUBLICATION; https://zenodo.org/record/4896789	8
14	D-JRP14-2.D6	List of relevant publicly available and in-house developed tools.	M36		M48	Task will be executed in Q4 of 2021.	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
14	D-JRP14-3.D1	Database construction tailored at MGEs	M36	M36		Public; 10.5281/zenodo.4305711	3
14	D-JRP14-3.D2	Protocol for plasmid DNA extraction from environmental samples	M34	M42		Public; 10.5281/zenodo.4896791	2
14	D-JRP14-3.D3	Publication of the validated method for plasmid DNA extraction on environmental samples.	M40	M42		CONFIDENTIAL UNTIL PUBLICATION; 10.5281/zenodo.4896722	1
14	D-JRP14-4.D1	First collection of type-materials (MGEs and strains) to be shared between involved partners	M30	M42		CONFIDENTIAL UNTIL PUBLICATION; 10.5281/zenodo.44896802	3
14	D-JRP14-4.D2	Refine additional collection of type-materials (MGEs and strains) to be shared between involved partners	M42		M54	Continuously updated, finalization M54	
14	D-JRP14-5.D1	Source code of the implementation of a SimInf model designed for pAMR transmission.	M33	M36		Public; 10.5281/zenodo.4305750	1
14	D-JRP14-5.D2	A report on quantification of horizontal vs. vertical transmission of AMR	M39	M42		Public; 10.5281/zenodo. 4925761	4
14	D-JRP14-5.D3	A parameterised SimInf model designed for pAMR transmission to be studied in	M42		M50	Q4 2021 / Q1 2022.	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		T5.2					

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
14	M-JRP13-M1	Creation of specific data hubs in ENA AMR hub	27	Yes		
14	M-JRP13-M2	A teleconference or physical meeting on horizontal vs. vertical AMR transmission	27	Yes		
14	M-JRP13-M3	Selection of MGEs and host strains to be studied in T4.2	28	Yes		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
14	M-JRP13-M4	A teleconference or physical meeting on input/output relationship between SimInf and sQMRA	29	Yes		
14	M-JRP13-M5	Publication of first version of data management plan	30	Yes		
14	M-JRP13-M6	3-days workshop on SMRT sequencing event	30	Yes		
14	M-JRP13-M7	Shipment of proficiency test data and strains	32	Yes		
14	M-JRP13-M8	Sharing of protocols, recipient- and host-strains, molecular tools	33	Yes		
14	M-JRP13-M9	An implementation of a SimInf model designed for pAMR transmission to be studied in T5.1	33	Yes		
14	M-JRP13-M10	Final selection of longitudinal samples for SMRT sequencing	34	No	M46	Delays in lab work due to pandemic
14	M-JRP13-M11	Final selection of cross-sectional samples for SMRT	34	No	M46	Delays in lab work due to pandemic

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
		sequencing				
14	M-JRP13-M12	Final selection of K. pneumoniae samples for SMRT sequencing	34	No	M46	Delays in lab work due to pandemic
14	M-JRP13-M13	Final selection of S. enterica Infantis and Kentucky samples for SMRT sequencing	34	No	M46	Delays in lab work due to pandemic
14	M-JRP13-M14	Final selection of horse-related samples for SMRT sequencing	34	No	M46	Delays in lab work due to pandemic
14	M-JRP13-M15	Analysis of proficiency test data by all partners	38	No	M46	Delays in lab work due to pandemic
14	M-JRP13-M16	Individual reports of proficiency test sent to partners	40	No	M48	Delays in lab work due to pandemic
14	M-JRP13-M17	Successful adaptation of plasmid purification protocol to field samples	36	Yes		
14	M-JRP13-M18	Selection of additional MGEs and host strains based on	40	Yes		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
		first outcomes of WPs-2 and -3.				

5.1.4.3.8.4 Follow-up of the recommendations and comments by the Ethics Advisors

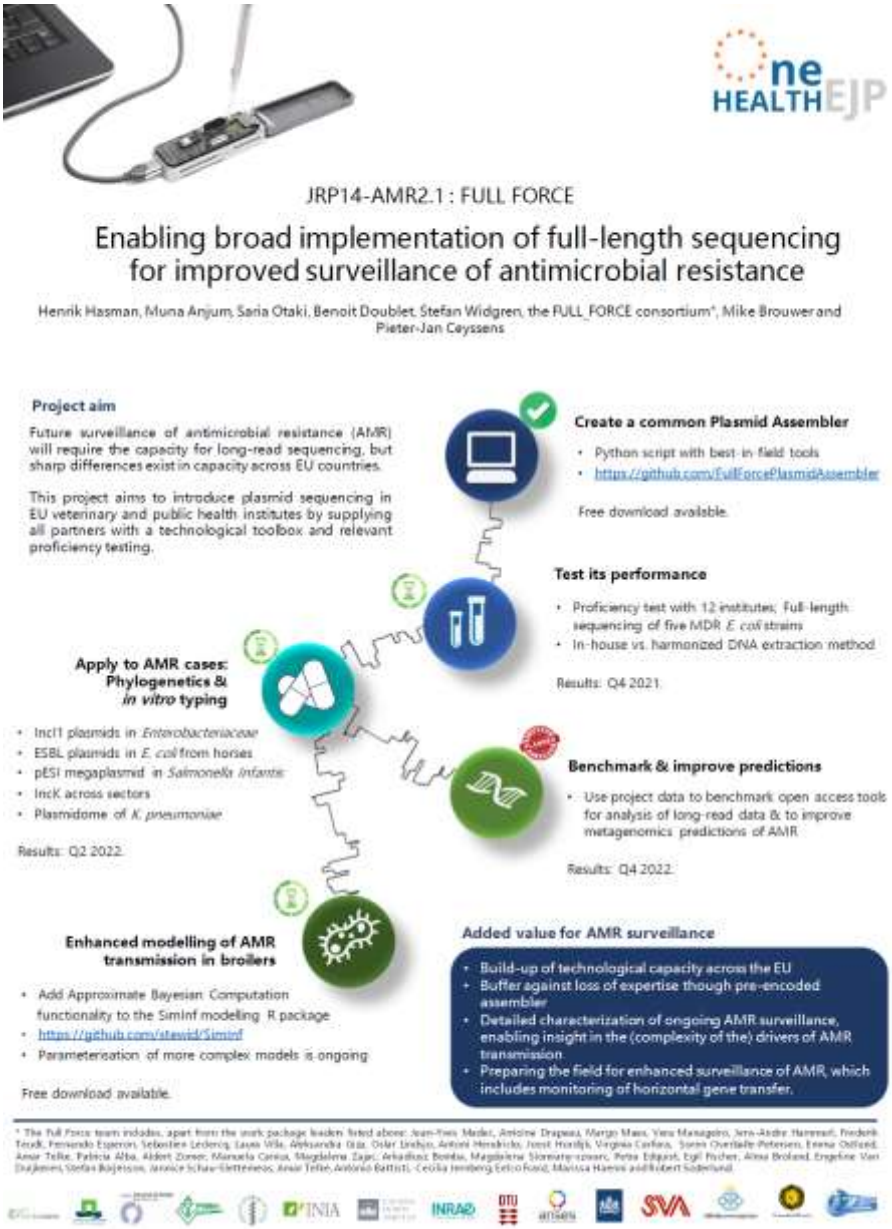
The Ethic Advisors already accepted your comments. Therefore, this part of the report has been closed.

5.1.4.3.8.5 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Diaconu EL, Carfora V, Alba P, Di Matteo P, Stravino F, Buccella C, Dell'Aira E, Onorati R, Sorbara L, Battisti A, Franco A. Novel IncFII plasmid harbouring blaNDM-4 in a carbapenem-resistant Escherichia coli of pig origin, Italy. J Antimicrob Chemother. 2020 Dec 1;75(12):3475-3479. doi: 10.1093/jac/dkaa374. https://zenodo.org/record/4451840	YES		YES (processing charges unknown)
Kirstahler P, Teudt F, Otani S, Aarestrup FM, Pamp SJ. A Peek into the Plasmidome of Global Sewage. mSystems. 2021 May 26:e0028321. doi: 10.1128/mSystems.00283-21. 10.5281/zenodo. 4896722	YES		YES (processing charges unknown)

Additional output

Poster presentation at the ASM meeting (Copenhagen, June 9-11, 2021).



Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

Foreseen for Y5.

5.1.4.3.8.6 Data Management Plan

Full Force's PI followed the training coordinated by the EJP WP4 responsible, Géraldine Boseret on the Data Management Platform based on the CDP software. In September 2020, a complete DMP of Full Force was created and uploaded to https://apps.lisam.com/app/#Apps/CDP. This plan will be updated in M47 during the annual meeting. As important part of the DMP, a framework agreement on Material

Transfer was drafted, circulated and signed among all 18 participating institutions. This agreement covers all transfer of data and strains during Full Force.

Currently, three types of data were generated during Full Force :

- Open source software packages (FFPA, SimInf), which are freely accessible in Github.
- Protocols (plasmid extraction, long-read sequencing), which will become open access upon publication or at the end of the project
- Sequence data (temporarily stored in OwnCloud), which will become open access upon publication or at the end of the project

5.1.4.3.8.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

- The SOLIDNESS network (JPIAMR, 2019-2020) which grouped experts in sequencing, plasmid biology and bioinformatics, and aims to streamline procedures for MGE sequencing. We build on their expertise to organise the PT.
- Cross-sectional and longitudinal bacterial samples of ENGAGE, EFFORT (Horizon 2020, 2013-2018) and ARDIG (OHEJP, JRP2, 2018-2020) projects will be selected for long-read sequencing during WP2.
- The KENTUCKY PhD project (OHEJP, 2020-2022) will use fully sequenced S. Kentucky strains (T2.4) to focus on the cell biology behind MGE transfer.
- Potential collaborations with ECDC and EFSA might be envisioned, for sustainable implementation of long-read sequencing technology in surveillance of AMR in Europe.

5.1.4.3.9 JRP15-R2-AMR2.1-FED-AMR

5.1.4.3.9.1 Summary of the work carried out in the Project

In the second year of the FED-AMR project WP1 has been able to coordinate the scientific and administrative matters between the partners including the scientific meetings and scientific webinars, budget and overall coordination of the project.

In WP2 all samples have been collected; sample cultivation, antimicrobial susceptibility testing (AST) and genomic characterization of the collected bacterial isolates is in progress. The FED-AMR bacterial collection is a diverse set of relevant bacteria, from human, animal and environmental reservoirs that will overall contribute to understand the dissemination of antimicrobial resistance (AMR) within the compartments, reinforcing the One Health approach. In addition, extracellular and total DNA extracted from the different EU samples are being analysed through 16S metagenomics (measurement of bacterial diversity) and through gene enrichment [characterization of AMR genes, including the resistome but also the mobilome, and heavy metal resistance genes (MRGs)]. The first results from WP2 were already presented at the OHEJP ASM2021 and other works were submitted to ECCMID 2021 and accepted for poster presentation.

WP3 has finished JRP15-WP3-T1. Regarding JRP15-WP3-T2, several sampling campaigns were conducted in order to enrich the collection of *Clostridioides difficile* isolates from zoonotic ribotypes. This was done targeting several animal species including previously uncharacterized *C. difficile* compartments, such as llamas and alpacas, but also food, where a study involving sampling of strawberries has been planned, and preliminary experiments to validate the isolation method have been conducted. In addition, environmental isolates obtained from the HOALs (Hydrological Open Air Laboratories) samples were also added to the collection. All these sampling campaigns are concluded, except for the food isolates, and WGS and AMR characterization of *C. difficile* isolates is ongoing. The obtained genomes from important ribotypes from all the partners will be gathered for a genomic study to be undertaken in JRP15-WP3-T3. The first results were presented in the ASM2021 as posters. A scientific manuscript with the results from the Portuguese HOAL is being prepared.

WP4 works with samples collected during the sampling in WP2 and thus, the analysis of antibiotics, elements and herbicides for all regions tested is in progress. The analysis is being performed by each of the three specialized laboratories of the WP (Austria, UK and Poland). Important results were already obtained.

WP5 has finalised all risk assessments for the *in vitro* gut model and set up of the model has begun. There were some delays to WP5 due to personnel needing to prioritise the WP2 sampling time points, which are now finished and currently being processed for sending samples to WP2 and WP4 for further analysis. We prepared the *E. coli* strain and DNA amplicon, encoding rifampicin resistance, to be used in the gut model and confirmed the ability of the *E. coli* strain to acquire antimicrobial resistance from the DNA amplicon, which completed deliverable WP5.1.

WP6 has focused on two main tasks: 1) finalizing the protocol for a systematic review on factors influencing the prevalence of antibiotic resistance in the environment and 2) formulating and validating a mathematical model for the dynamics of microbial communities. Finalizing the protocol has been slightly delayed because of change of co-leading author. It has been a considerable effort, but now we have all comments from multiple co-authors and are essentially ready to start the screening. We also intend to register this protocol with a registry for prospective systematic reviews or submit to a journal to get peer-review feedback. With respect to the task on modelling microbial communities, we have formulated a new model for this and explored other models for comparison purposes to refine our research questions. We have also developed *in-silico* data to validate our approach.

5.1.4.3.9.2 Progress of the project: description of activities

WP1: Project Management and Communication

The main topics of WP1 were coordination due to delays and issues arising from the global pandemic, as well as the coordination of budget shifts and the assessment of needs and requirements of different partners. The Scientific Supervisory Board (SSB) was involved in voting on analysis options (04/11/2020), and on project extension as well as on budgetary issues (20/04/2021).

Despite the negative impact of the COVID-19 pandemic, the partner institutes were able to continue with the longitudinal study, although they confirmed the need of a 6-months extension to compensate the delay in milestones and deliverables and to ensure the project finalization with outstanding outcomes.

JRP15-WP1-T1- Scientific Management (M25-M60)

This task is **ongoing** for the whole project duration (M25 to M60). The experts of the Scientific Supervisory Board (SSB) have participated in decision-making processes affecting the FED-AMR project.

JRP15-WP1-T1-ST1- Coordination of sampling, laboratory experiments and building a database (M25-M33)

This subtask was already **completed** in year 3. Check the 12-months report 2020.

JRP15-WP1-T1-ST2- Webinar forum and Skype meetings for instant scientific interactions (M30-M58)

This subtask is a continuing from Y3. Four webinars were already held in year 3. In the year 4 of the project we continued with the scientific webinars. Up to now, Dr. Maja Rupnik held a webinar on "*Clostridioides difficile* – an environmental perspective". In the upcoming months we are planning to organize one more webinar whose provisional theme will be "Metagenomics and analysis of metagenomics data". The 4th webinar presentation was also recorded as previously indicated with permission of speakers and participants. The recordings were made available to the consortium and are confidential, as they are only meant for the consortium members that could not take part or would like to revisit the webinar. As a general rule, the dissemination outside of the FED-AMR project is not permitted.

The management team at AGES is also organizing monthly online meetings. Minutes of these meetings are registered and shared with the project partners for approval. A definitive version incorporating all suggestions received is distributed and published in the AGES website (https://fed-amr.ages.at). In addition, regular calls occur between the deputy leader of WP1 and the leader of WP1-T1.1 to discuss the evolution of the different WPs and tasks. Such meetings will continue for the duration of the project as necessary in addition to the monthly TCs for progress report.

JRP15-WP1-T1-ST3- Project Meetings (M25-M52)

Due to the COVID pandemic, the next project meeting is postponed to year 5 and is planned to be held in Lisbon. This subtask is **ongoing**.

JRP15-WP1-T2- Administrative Management (M25-M60)

The administrative management (AM) is supported by the infrastructure of the AGES Academy and the secretariat of the AGES Knowledge Transfer Department. The coordination of joint activities in the frame of the FED-AMR project is being coordinated by AGES. Additionally, each partner had appointed an Administrative Representative who is and will be in direct contact with the AGES AM whenever necessary.

As indicated before, considerable effort was put in the assistance with budgetary issues for the entire project and specific partners, which was conducted in close cooperation with the project lead, the scientific manager and the partners in question. Due to the additional load of administrative work

associated with the global pandemic a project management assistant (Krista Rathammer) was hired permanently as support for the AM.

A risk management on a daily basis is also taken care of by the administrative team, in coordination with the leading staff. The overarching risk management strategy initiated in year 3 of the project was put in place by the AM and the Scientific Manager (SM), in consultation with the Scientific Supervisory Board (SSB) to ensure that adverse situations are properly handled along the course of the project, which is being highlighted in the Data Management Plan. This task is **ongoing**.

JRP15-WP1-T3- Data and Protocol Management (M25-M58)

A first version of the Data and Protocol Management Plan was already delivered on M34. In year 4, the DMP has been updated in the new OHEJP data management platform CDP, with details of FED-AMR data throughout the project, with information provided to the leader and deputy leader by task leaders on their datasets. In addition, the task leader and deputy leader have generated a metadata file for all samples collected in WP2. This file will facilitate the introduction of metadata in the CPD platform and data analysis. This task is **ongoing**.

WP2: Field experiments: Determination of the naturally occurring ARG background load and microbial biodiversity in the tested environmental compartments (M25-M54)

WP2 takes place over the first, second and third year of the project (Y3, Y4 and Y5). The end of this WP has been postponed to M54.

JRP15-WP2-T1- Assemble list of sampling compartments and points. Determination of test areas representative for the European regions (North, West, East, South) (M25-M30)

This task was already **completed** in year 3. Check the 12-months report 2020.

JRP15-WP2-T2- Establish common protocol for sampling and data analyses to facilitate comparability of the results between European test areas (North, West, East, South) and local sampling locations (M25-M33)

This task was already **completed** in year 3. Check the 12-months report 2020.

JRP15-WP2-T3- Assess microbial and ARG diversity with NGS in the selected test environments (metagenomics). Compare microbial and ARG diversity between ecosystems and over ecosystem boundaries. Characterization of cultivable environmental bacteria on complete nutrient and minimal media (M27-M48)

Due to the situation caused by the COVID pandemic and the tight budget available for genomic analysis, the FED-AMR consortium decided in Year 3 to introduce changes with respect to this task, as also indicated in task JRP15-WP2-T2 (see 12-months report 2020). Up to now, most samples have been collected and cultivated by the FED-AMR partners. The last sampling was finalized in early June 2021.

As explained in the culture protocol, **isolates** from six bacterial species that may be resistant to one or more critically important antimicrobials (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella spp*, *Staphylococcus aureus* methicillin resistant, *Enterococcus faecium* and *E. faecalis* vancomycin resistant) have been obtained along the sample cultivation process. These bacterial species selected from the WHO Global priority list of antibiotic-resistant bacteria to favour their identification in our human, animal and environmental compartments and to search for equal or similar genetic traits than human pathogenic bacteria. AMR is now being evaluated or even completed in some countries through diverse AST, for a selection of **isolates** gathered in each country. The methodology followed depends on the availability of each test in the participant countries and includes determination of the minimum inhibitory concentration (MICs) by broth microdilution or E-test and/or disc diffusion as preliminary screening. Moreover, some countries have already started to whole genome sequence a selection of their isolates.

In addition, the microbial diversity is being evaluated by detecting the entire 16S region (V1-V9) through 16S metagenomics in the **DNA samples** (extracellular and total DNA), which is more sensitive

than the conventional 16S amplicon sequencing. In parallel, the same samples are being analysed through gene enrichment to detect ARGs (see task WP2-T2 and subtask WP2-T3.2). As explained in Year 3, gene enrichment allows us to dispense with both shotgun metagenomics (WP2-T3.1 task) and qPCR (WP2-T4). It was planned that countries should finish with their DNA shipments to the Austrian company in June of Year 4.

As for the six clinically relevant species isolated from the collected samples, WGS analysis (task WP2.6) is being performed. This task is **ongoing**.

JRP15-WP2-T3-ST1- Shotgun sequencing and bioinformatic analyses of AMR genes and MGEs (M37-M48)

The FED-AMR partners agreed on the evaluation and comparison of ARGs using a novel methodology based on gene capture probes or gene enrichment (see task WP2-T2 and subtask WP2-T3.2). This novel methodology allowed us to analyse the AMR genes and MGEs, and dispense with both conventional shotgun metagenomics (this subtask) and qPCR (task WP2-T4) for the collected samples. So, this subtask does no longer exist in a methodological point of view, but the aim will persist, and **results will come from WP2-T3.ST2.**

JRP15-WP2-T3-ST2- Gene enrichment with gene capture probes (M31-M43)

After confirming in Year 3 the better performance of the gene enrichment methodology over the conventional shotgun metagenomics for detection of the resistome, project partners started sending their DNA samples (both extracellular and total DNAs) to a third party that is performing the gene enrichment and the 16S shotgun metagenomics for all samples in WP2. Last samples will arrive to the facilities of the Austrian company by M43. The target enrichment results for a first batch of samples have been received. These include the ARGs diversity in each of the samples, DNA type (extracellular vs. total) tested, and the raw FASTQ files generated in the target enrichment process for each of the samples, which are being stored both locally and on a cloud server. The former are been currently analysed with statistical software. This task is **ongoing**.

JRP15-WP2-T4- Quantify clinically relevant ARGs in the tested compartments (qPCR; qPCR arrays) (M34-M42)

As explained in the 12M report of Year 3, the detection of ARG through qPCRs was eliminated from the project. The Scientific Supervisory Board (SSB) contributed to this decision-making process. However, the quantification of relevant ARGs in the analysed compartments will be performed, as it will be inferred from the number or reads that cover each detected ARG.

JRP15-WP2-T5- Identify naturally transformable bacterial species in the tested compartments (NGS) (M42-M48)

This task is **ongoing** and relies on the results from previous tasks.

JRP15-WP2-T6- Assess clonal/lineage diversity in selected ARB species (M43-M46)

Phylogenetic trees will be established between bacterial species present in different but interconnected compartments using Illumina 16S targeted amplicon and shotgun sequencing data obtained in **WP2-T3.**

JRP15-WP2-T7- Isolate and assess quantity, diversity and stability of free extracellular ARG encoding DNA in the tested environments. Sequence comparisons (M34-M50)

Following the available DNA extraction protocol, WP2 participants have extracted the exDNA and total DNA from all samples immediately after their collection. Since the sampling campaign finished in Spring 2021, the target enrichment and 16S metagenomics assays started previously in M36 for the first batch of DNA samples.

E. coli, *K. pneumoniae*, *Salmonella spp*, *S. aureus* methicillin resistant, *E. faecium and E. faecalis* vancomycin resistant strains have been retrieved from the collected samples. Antimicrobial susceptibility testing and Whole Genome sequencing is being performed in some institutes in a

selection of strains, where the characterization of the ARGs harboured by those strains is also being investigated.

The start and end of this task was delayed to M34 (year 3) and M50 (year 5), respectively.

WP3- Elucidating the role of Clostridium difficile as an ARG transfer platform over ecosystems boundaries and its linkage between human and non-human (zoonotic) reservoirs (M25-M50)

JRP15-WP3-T1- Epidemiological survey of zoonotic ribotypes across participant countries (M25-M40)

This task was already **completed** in Year 3. Check the 12-months report 2020.

JRP15-WP3-T2- WGS and AMR characterization of human and non-human C. difficile isolates (M34-M48)

At the moment, all the new *C. difficile* strains collected by WP3 participants are being tested to identify their AMR profiles and whole genome sequenced to identify ST and resistance genes. In addition, ribotyping is also being conducted. This task is **ongoing.** The activities are described in more detail per partner below.

At 36-INSA, the new isolates were collected from pets and from food chain animals; in 2021, there were 7 different campaigns of sampling. In total, 271 samples from pets were collected, with a prevalence of *C. difficile* around 9%, so far. Genetic characterization of these isolates showed the presence of diverse toxigenic and non-toxigenic ribotypes (RT001; RT056; RT225; RT010, RT009, RT033, RT106), all of them also found in human samples. Phenotypic AMR revealed the presence of strains resistant to moxifloxacin, to clindamicin and to metronidazole, this last being associated with a previously described MZ-R plasmid. WGS is ongoing.

Regarding food chain animal, several animal species were studied: 56 samples from cattle were collected, with a prevalence of 10.7%. Different zoonotic and toxigenic RTs were found (RT001; RT033; RT078; RT572), again overlapping human strains. In this group, resistance was found to moxifloxacin; pig intensive culture, a total of 141 samples were collected, the culture and study of *C. difficile* strains is still ongoing; the third group consisted of a smaller subgroup of other animals (5 poultry, 14 sheep, 5 goats and 4 gooses), the only positive for *C. difficile* were 2 samples from sheep. WGS of animal isolates, as well as isolates from human cases with zoonotic ribotypes, distributed from 2016-2020 from different Portuguese hospitals, in a total of around 100 isolates, is being carried out, in order to evaluate the extent of genetic overlap between human and non-human *C. difficile* lineages (JRP15-WP3-T3). To support all the WP3 activities involving partner 36-INSA, a DVM (Frederico Alves) was recruited for 6 months (March to August 2021).

At partner institute 9-BfR, the second batch of samples originating from partner 25-NUIG (wastewater collected within WP2) was started to be analysed in June 2021. All of the four samples received are positive for one or more *C. difficile* strains. The characterization of the isolates is ongoing.

Furthermore, strains isolated in Y3 were whole genome sequenced and will be compared with whole genome sequences of other partners in JRP15-WP3-T3.

For Q2 and Q3 2021 a sampling study with strawberries has been planned and preliminary experiments to validate the isolation method have been conducted. For this, strawberries from retail were spiked with *C. difficile* spores of three different test strains at two spiking levels and analysed regarding the recovery rate. Different variants of the sampling procedure were compared. The field study is planned to start in June 2021.

At the institute of partner 10-FLI, samples from WP2 (14-UT and 25-NUIG, human and animal fecal samples, and manure) are analysed for *C. difficile* and isolates are characterized. Strains from the FLI *C. difficile* collection were revived and whole genome sequences were generated for approximately 100 isolates. 48 animal RT 033 strains, all from cattle, were identified and sequenced. To identify unusual, previously uncharacterized *C. difficile* compartments, a collection of 94 fecal samples from

llamas and alpacas was examined: 8 *C. difficile* isolates were obtained and characterized. Phenotypic and genotypic AMR characterization of the *C. difficile* isolates was initiated.

JRP15-WP3-T3- Evaluation of the extent of genetic overlap between human and non-human C. difficile lineages (M41-M52)

At 13-SSI: As part of WP3-T3 where we compare human and veterinary *C. difficile* isolates, we did whole genome sequence of 12 porcine bacteria and 579 human clinical isolates. Porcine bacteria were retrieved from 330 fecal samples (4% positives) collected at farms from different locations in Denmark. The most striking similarities between human and porcine strains were three ST6 and four ST11, three of the ST11 with 2-3 cgMLST allelic differences to closest human isolate. Several different AMR genes were found both complete and partial, which is being explored as part of the resistance gene pool prone for extracellular exchange WP3-T4.

36-INSA and 9-BfR are still sequencing the genome of the isolates. The analysis of WGS data will be performed in the second half of 2021.

At partner 9-BfR: Human RT033 strains were delivered from an external project partner and whole genome sequenced at the BfR. The whole genome sequences will be used for a comparison with RT033 strains from 36-INSA isolated in JRP15-WP3-T4.

At partner 10-FLI: The whole genome sequences will be used for comparison with RT033 strains from 36-INSA and strains collected from 9-BfR.

JRP15-WP3-T4- C. difficile / AMR dissemination between the human, animal and the environment: pig farm as a proof of concept (M34-M50)

This task started on M34 although it was planned to start on M37. Two sampling campaigns have been undertaken, one in the summer and the other in autumn, in the HOALs from Austria and Portugal. For *C. difficile* study, samples were taken from pig barn, pig manure, farmers, wastewater treatment plant, groundwater and superficial water according to the sampling scheme from WP2.3. The partners that could not perform *C. difficile* isolation sent their samples to other partners. Several *C. difficile* strains have already been isolated and currently under study. This task is **ongoing**.

Again, a more detailed summary of activities per partner is described below:

2-AGES: In this task, we aimed to assess the genetic relatedness, genetic and phenotypic resistances of *C. difficile* strains obtained from human, animal and environmental sources to monitor the dissemination of resistant RT 078 /ST 11 *C. difficile* across the human animal wildlife interface. For that, we collected 85 samples from geographically interconnected environmental compartments in an open air laboratory in Austria in 2020. After sample cultivation, the obtained *C. difficile* strains were all Whole Genome Sequenced, ribotyped and tested for antimicrobial susceptibility. In addition, isolates were compared with human and pig isolates gathered between 2016 and 2019.

We recovered seven toxinogenic *C. difficile* RT 078 /ST 11 isolates (manure n= 3, wastewater n= 3, wildlife n=1) from the 85 samples. Five strains were phenotypically resistant to moxifloxacin and one also to clindamycin. All were susceptible to vancomycin, metronidazole and rifampicin and carried *cdeA* (n= 7), tet40 (n=3) and efpA (n=1) as resistance genes (ARGs). A comparison of the genetic and phenotypic features of the RT 078 /ST 11 Austrian FED AMR *C. difficile* strains with the human (n= 20) and pig (n= 20) strains of RT 078 /ST 11 from the external collection was made. Human isolates were toxinogenic (n=20) and resistant to moxifloxacin (n=9), clindamycin (n= 7) and rifampicin (n=1), but no resistances were detected against metronidazole or vancomycin. However, they presented more diversity of ARGs than the environmental isolates.

Core genome MLST based typing revealed that four FED AMR strains clustered with four human isolates from 2018 and 2019. All were resistant to moxifloxacin and/or clindamycin.

cgMLST revealed a cluster of antimicrobial resistant RT 78 /ST 11 *C. difficile* strains from human, animal

and environmental sources, and therefore it suggests again its zoonotic traits despite lacking epidemiological links.

26-INSA: In this task, we have evaluated the diversity and dynamics of *C. difficile* in a farm environment. For that, samples were obtained from an experimental farm and collected from different compartments of animal, human and environmental origin. All samples were broth enriched and cultured on selective medium. Toxins profile was evaluated by multiplex-PCR and genetic diversity by PCR-ribotyping, multilocus variable number repeat analysis (MLVA) and whole genome sequencing; antibiotic susceptibility was performed. A total of 181 samples were included in this study. The overall prevalence of *C. difficile* was 37% (67/181). Positivity rate per compartment was: of 51.7% (31/60) in environment (manure, soil, water and WTP); 32.4% (36/111) in animals (pigs, cattle and sheep), 0% in humans. A total of 267 colonies were studied, among which 169 were positive; 9 different ribotypes were found: RT033 (89.4%), RT126 (2.4%), RT056 (1.8%), RT027 (1.2%), RT720 (1.2%), RT005 (1.2%), RT071 (1.2%), RT643 (1.2%) and RT147 (0.6%).

All RTs were toxigenic (*tcdA+tcdB+*), except RT071; in addition 93.5% of the isolates were also positive for the binary toxin genes (*tcdA+tcdB+cdt+*). Antimicrobial resistance to moxifloxacin was found in 2 samples: 1 RT126 from sheep and 1 RT027 from a forest soil.

The most prevalent ribotype was RT033 (89.4%), found in all compartments linked with the pig barn [pigs, swine cesspool/manure, agricultural soil, control soil and wastewater treatment plant (WTP)]. All the isolates from this RT were positive for all toxin genes (*tcdA+tcdB+cdt+*).

MLVA typing, based on 5 loci, showed that all samples belonged to the same genetic complex (summed tandem-repeat difference STRD≤ 2), except one isolate from the WTP sedimentation tank displaying a STRD=3, but still indicating a high genetic relatedness.

The core-genome SNP based analysis corroborates the global genetic relationship among RT033 strains from different reservoirs (ΔSNPs=3); besides the difference in toxin profile, the RT033 clone presented a high distance from the RT033 reference strain (>990 cgSNPs).

The results obtained so far showed the presence of a predominant clone of the RT033 in all compartments associated with the pig barn, suggesting a transmission cycle originated in these animals. These findings confirm the importance of environmental/animal reservoirs for *C. difficile* dissemination in the community.

WP4- Determination of the selection pressures in the tested compartments of human, animal and environmental ecosystems (M25-M50)

In Y4, and as part of WP2, the collection of environmental samples at the HOALs in Austria and Portugal, the agricultural areas in Czech Republic and Estonia as well as the single compartments in Ireland (wastewater and wildlife) and the UK (pig feces and pig manure) were finalized. At this stage, almost all samples have been dispatched to 34-PIWET (antibiotics), UBA Vienna (herbicides) and 23-UoS (trace element analysis). The analysis of the samples for antibiotics, herbicides and trace elements is still **ongoing**.

JRP15-WP4-T1- Selection of essential antimicrobials to be quantified in the tested compartments (published antibiotic consumption data, farmers' questionnaire, personal experience, expert interviews (veterinarians) (M25-M30)

This task was already **completed** in year 3. Check the 12-months report 2020.

JRP15-WP4-T2- Quantification of four antimicrobial classes (tetracyclines, macrolides, sulphonamides and fluoroquinolones) in aqueous matrices (water) (M31-M50)

This task is **ongoing**.

JRP15-WP4-T3- Quantification of four antimicrobial classes (tetracyclines, macrolides, sulphonamides and fluoroquinolones) in manure (M31-M50)

This task is **ongoing**.
JRP15-WP4-T4- Quantification of four antimicrobial classes (tetracyclines, macrolides, sulphonamides and fluoroquinolones) in faeces (M35-M50)

This task is **ongoing**.
JRP15-WP4-T5- Quantification of four antimicrobial classes (tetracyclines, macrolides, sulphonamides and fluoroquinolones) in soil (M31-M50)

This task is **ongoing**.
JRP15-WP4-T6- Quantification of herbicides in agricultural soil (M31-M50)

This task is **ongoing**
JRP15-WP4-T7- Measurement of the concentration of trace elements in environmental samples gathered across participants countries (M31-M50)

The task leader Monica Felipe-Sotelo is on maternity leave, a new task leader will be announced as soon as a proxy at 23-UoS is found. This task is **ongoing**.

WP5- Identification of environmental conditions modulating transformation frequencies in soil microcosms and an in vitro porcine gut model (poGutMo) (laboratory studies) (M32-M56)

JRP15-WP5-T1 Establish baseline levels of HGT in the model organism (E. coli) arising from transformation in the poGutMo (M32-M49).
JRP15-WP5-T1-ST1 Ability of E. coli to acquire AMR to serve as a donor DNA (M36-M38).

E. coli is generally known to be naturally transformable; however, standard laboratory strains are also known to be naturally competent in rapidly acquiring new genetic material at a relatively high rate. Thus, *E. coli* is commonly selected and used a model bacterial species to study bacterial transformation. *E. coli* K12 is a standard transformation strain that is often used in studying antimicrobial resistance gene transfer. *E. coli* K12-J53 is a derivative strain of K12 that is sodium azide resistant, which is often used as a selection marker. Thus, *E. coli* K12-J53 has been obtained from AGES and is used as a reference strain in the transformation experiments in both the *in vitro* and in the gut fermentation model where sodium azide is used as the first selection marker.

Under antibiotic stress, *E. coli* can undergo spontaneous mutation in the RNA polymerase β subunit (*rpoB*) gene conferring resistance to rifampicin. Using the spontaneous mutant generation method, *E. coli* J53 was used to generate rifampicin resistant mutants so rifampicin can be used as a second selection marker for transformation conjugates. Six rifampicin resistant mutants were generated and named *E. coli* J53-RifR1-6 and a growth curve was performed for both rifampicin resistant strains and the parental J53 strain to confirm the growth rate of mutant strains and ensures the lack of intrinsic fitness burden associated with the mutations. All rifampicin mutant strains grew successfully with minimum or no effect on the growth rate. *E. coli* J53-RifR1 was chosen, cultured and its DNA was extracted to be used as a donor DNA. PCR primers were designed targeting part of the *rpoB* gene (~2250 bp) including all RNA polymerase β subunit clusters where mutation frequently occur. Targeted *rpoB* sequence of both *E. coli* J53 and *E. coli* J53-RifR1 were successfully amplified, purified, and quantified.

Preliminary natural transformation experiments were performed using *E. coli* J53 strain as the recipient strain (rifampicin sensitive) and the *rpoB* DNA amplicon (0.2-0.5 µg) from *E. coli* J53-RifR1 as the donor DNA (rifampicin resistant) in LB broth. Our results showed the successful recovery of *E. coli* J53 that is both sodium azide and rifampicin resistant with controls showing colonies only on sodium azide/MacConkey no.3 agar plates. The transformation experiment was also shown to be dependent on time and DNA concentration as demonstrated in the literature. The transformation frequency was calculated and showed to be about 10^{-6}-10^{-7}. This demonstrates the success of the generation of the rifampicin mutant strain, PCR primer design, amplification of the target sequence and transformation of the *E. coli* J53-RifS with rifampicin resistance containing amplicon, which confirms the suitability of

the strains used to be utilised for transformation experiments in the *in vitro* gut model. In the future, we shall setup the *in vitro* fermentation pig gut model and inoculate it with *E. coli* J53 as the recipient strain (rifampicin sensitive) and the *rpoB* DNA amplicon from *E. coli* J53-RifR1 as the donor DNA (rifampicin resistant) to assess the transformation frequency at different time points and experimental conditions.

This task and the corresponding deliverable D-JRP15-FED-AMR-WP5.1 have been completed.
JRP15-WP5-T1-ST2 Determine the optimal growth parameters for cultivating E. coli strains within the gut model (M40-M45).

We are currently setting up the *in vitro* gut model to include six fermentation vessels, which will allow for more experimental conditions to be tested. The PCR amplification is currently being troubleshooted for upscaling the amplification, in order to use the amplicons as a source of extracellular DNA. The task has started and is still **ongoing**.

Start date delayed to M40. New end month: 45 (Year 4).
JRP15-WP5-T1-ST3 Rates of transformation calculated by taking samples from the gut model and plating on TSC agar plates supplemented with the appropriate antibiotics (M46).

Start date delayed to M46. New end month: 48 (Year 4).
JRP15-WP5-T1-ST4 DNA transfer rates via bacterial conjugation will be calculated using the endpoint method (M45-M48).

Start date delayed to M45. New end month: 48 (Year 4).
JRP15-WP5-T2- Evaluate conditions that drive HGT and the emergence of AMR via transformation (M49-M56)
JRP15-WP5-T2-ST1- Iterative evaluation of candidate drivers (antibiotic, herbicide, cation) of HGT evaluated in the gut model – round 1 (M49-M53)

Start date delayed to M49 (Year 5).
JRP15-WP5-T2-ST2- Iterative evaluation of candidate drivers (antibiotic, herbicide, cation) of HGT evaluated in the gut model – round 2 (M53-M56)

Start date delayed to M53 (Year 5).
JRP15-WP5-T3- Effect of different environmental conditions on the expression of competence genes in E.coli determined using soil microcosms (M44-M52)

This task will start in M44 and will be performed with *A. baylyi* as it is more suitable to show the effects on soil microcosms.

WP6- Probabilistic and mechanistic models of the links between antimicrobial usage in animals, AMR in the environment, and the risks for public health.

JRP15-WP6-T1 Build a probabilistic mathematical model of the emergence of AMR in target bacteria and the relative contribution of transformation and conjugation to ARG acquisition (afterwards: Factors influencing the prevalence of antibiotic resistance in the environment) (M32-M60)

As said in the previous report, due to challenges in obtaining the data required for setting up a machining learning model (in part related to COVID-19 restrictions), we have decided carry out a systematic review of the literature regarding environmental factors of AMR prevalence. The data extracted as part of this systematic review will be used to inform the design of a future machine learning model.
JRP15-WP6-T1-ST1Data Integration, Annotation and Association Analysis (afterwards: Systematic review: environmental factors associated with AMR) (M32-M60)

Start date delayed to M32.

We have received all comments from the co-authors for the protocol for the systematic review and we

are incorporating them. This has resulted in an extensive 9-pages document with many sections (e.g. Introduction, material and methods, appendixes) that can be transferred/adapted to the final manuscript. We have contacted PROSPERO to register the protocol, but since the feedback was that this review is not within the scope of PROSPERO we are exploring other options (e.g. Cochrane) or register the protocol with a journal (e.g. like PLoS One or BMC Open).

In-line with the goal of this WP6.1, a key outcome is to identify the relative importance of HGT mechanisms associated with the spread of antibiotic resistance, i.e. transformation vs. conjugation. The searched databases, specific search strategy, plans for data management and categories of extracted data types are specified in the linked protocol. This deliverable provides this protocol documentation on the UoS GitLab website at the following link: https://gitlab.eps.surrey.ac.uk/bg0013/systematic-review-protocol-amr. This is a private repository, and will remain confidential until publication of the systematic review or registration of the protocol. The protocol can be shared with all members of the FED-AMR or other One-Health EJP members and they can access to the GIT repository if requested. We have also secured a machine-learning based software tool that will aid the leading authors with screening 1000's of potentially relevant articles. We haven't started screening the titles/abstract due to other commitments with WP6-T2. This task is ongoing.

JRP15-WP6-T2 Develop mechanistic models to address key questions regarding the spatio-temporal changes observed in microbiological communities (M34-M60)

We are continuing to review the literature to keep updated with state-of-the-art techniques. We have developed and formulated a mathematical model for microbial communities as well as explored published models and used an existing dataset of time-series bacterial abundances to validate our approach. We have also tested different approaches to infer relevant parameters for published models and we are now focusing on how to adapt these techniques to our model. We now aim to link *in-silico* data with our model to validate our approach. Once this is done, we will start to use the model to address a few conceptual questions. This task is ongoing.

JRP15-WP6-T2-ST1 - Modelling microbial communities I (M34-M60)

Start date was delayed to M34.

In a preliminary analysis performed on the dynamics of microbial community, we showed that these can critically switch from one state to another depending on how antibiotics are administrated.

We aim to use our model with novel data (from UoS or from other FED-AMR partners) rather than the one in the literature. Discussion with our partners on potentially available data is on-going. As discussed above, in-silico data is being generated based on individual-based modelling (i.e., Gillespie algorithm) with parameters matched to experimentally obtained values, including background noise, for increased biological realism. This data shall be used to test our generalised Lotka-Volterra model with a carrying capacity term.

JRP15-WP6-T2-ST2- Modelling microbial communities II (M46-M54)

Start delayed to M46.

5.1.4.3.9.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
15	D-JRP15-FED-AMR - WP1.1	Scientific Supervisory Board (SB) installed. Local administrative representatives nominated (T1, T2)	M25	M25		Public Zenodo: https://doi.org/10.5281/zenodo.5081685	
15	D-JRP15-FED-AMR - WP1.2	Unified sampling and experimental protocols (T1.1.)	M27	M33		Public Zenodo https://doi.org/10.5281/zenodo.5081689	2
15	D-JRP15-FED-AMR - WP1.3	Data and protocol management plan (T3)	M27	M34		Public Zenodo: https://doi.org/10.5281/zenodo.5078099	8
15	D-JRP15-FED-AMR - WP1.4	Webinars (T1.2.)	M30	M31		Zenodo: https://doi.org/10.5281/zenodo.5081710	
15	D-JRP15-FED-AMR - WP1.5	Annual project report	M36	M38		Public Zenodo: https://doi.org/10.5281/zenodo.5078024	8
15	D-JRP15-FED-AMR-WP2.1	List of sampling compartments, points and European test areas and harmonized	M26	M33		Public Zenodo: https://doi.org/10.5281/zenodo.5081756	2

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		protocols in alignment with EFFORT project protocols available in data repository (T2.1, T2.2)					
15	D-JRP15-FED-AMR-WP2.2	Preliminary data collection on ARG prevalence and ARG background load in the compartments analysed so far (T2.4)	M36	M37		Public OHEJP: available Zenodo: https://doi.org/10.5281/zenodo.5078064	3
15	D-JRP15-FED-AMR-WP2.3	Annual report Y3 (WP2)	M39	M38		Public Zenodo: https://doi.org/10.5281/zenodo.5078149	8
15	D-JRP15-FED-AMR-WP2.4	Determination of naturally transformable bacteria in tested environmental compartments (T2.5)	M44		M48		
15	D-JRP15-FED-AMR-WP2.7	Quantity and stability of free extracellular DNA observed in environmental	M42		M50		

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		compartments tested so far (T2.7)					
15	D-JRP15–FED-AMR-WP3.1	Database of zoonotic Clostridium difficile isolates across participant countries (task 3.1)	M36	M36		Public Zenodo: https://zenodo.org/record/5078164#.YTsuIOdCR4E	3
15	D-JRP15-FED-AMR-WP4.1	Standardize protocols for sampling and testing of environmental samples	M26	M30		Public Zenodo: https://doi.org/10.5281/zenodo.5081764	2
15	D-JRP15-FED-AMR-WP5.1	*E. coli* strains demonstrated to be suitable for transformation	M26	M38		Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines. Zenodo: deliverable description https://doi.org/10.5281/zenodo.5121159	10
15	D-JRP15-FED-AMR-WP5.2	Optimal growth parameters for cultivating *E. coli* within the porcine gut model and the time after inoculation at which its	M27		M45	Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		concentration is maximal determined					
15	D-JRP15-FED-AMR-WP5.3	Pilot experiments using PCR amplicons as ARG donors	M28		M46	Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP5.4	Optimal growth parameters for cultivating the clostridial strains within the gut model determined	M30		M47	Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP5.5	Conjugation-mediated HGT between the clostridial donor and recipient strains within the gut model determined	M44		M47	Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP5.6	Clostridial transconjugates characterised by whole-genome sequencing	M43		M48	Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-	Second round of experiments using PCR	M44		M51	Deliverables will be made public, but elements of the data included in the deliverable may be	10

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
	WP5.7	amplicons as ARG donors				embargoed or kept confidential, in line with the OHEJP guidelines.	
15	D-JRP15-FED-AMR-WP5.8	Results from pig gut model regarding HGT by transformation and conjugation in presence of selected antibiotics, herbicides and heavy metals	M44		M52	Deliverables will be made public, but elements of the data included in the deliverable may be embargoed or kept confidential, in line with the OHEJP guidelines.	10
15	D-JRP15-FED-AMR-WP6.1	Main code for the mathematical modelling made available in public repository (e.g. GitHub) with associated documentation (which can be used as "Material and Method" section of the forthcoming publications).	M30	M37		This is now a protocol for systematic review (not a code for mathematical modelling). Confidential until publication or registration of the protocol for the systematic review, except for FED-AMR or other One-Health EJP members. Zenodo: deliverable description https://doi.org/10.5281/zenodo.5139307	3
15	D-JRP15-FED-AMR-WP6.2	Findings presented at one international conference and one	M36		M54	Due to Covid-19 many conferences have been cancelled. Ideally we would like an in person conference but we will keep an eye on	5

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		national conference.				conference opportunities. Findings have been presented internally at University level.	
15	D-JRP15-FED-AMR-WP6.3	Update of codes and documentations in public repository (e.g. GitHub).	M36		M43	Confidential until full validation of the code or publication (except for FED-AMR or other One Health EJP members).	3
15	D-JRP15-FED-AMR-WP6.4	Submission/publication of 1/2 paper(s) on how resilience of microbial communities depends on external environmental drivers and richness and diversity of the community.	M44		M54	Confidential until full validation of the code or publication (except for FED-AMR or other One Health EJP members). This deliverable has been delayed due to difficulties in obtaining the experimental data required for this work, arising from COVID-19 restrictions.	8: Published work
15	D-JRP15-FED-AMR-WP6.5	Main code for the mathematical modelling made available in public repository (e.g. GitHub) with associated documentation (which can be used as "Material and Method"	M44		M54	Confidential until full validation of the code or publication (except for FED-AMR or other One Health EJP members). This deliverable has been delayed due to difficulties in obtaining the experimental data required for this work, arising from COVID-19 restrictions.	3

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		section of the forthcoming publications).					

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
15	M-JRP15-FED-AMR-01	Kick off meeting	M26	Yes		
15	M-JRP15-FED-AMR-02	Database repository active	M27	Yes		
15	M-JRP15-FED-AMR-03	Webinar forums started	M30	Yes		
15	M-JRP15-FED-AMR-04	Interim meeting	M41	No		COVID-19 forced us to postpone the meeting to next year.

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
15	M-JRP15-FED-AMR-07	List of sampling compartments, points and European test areas available. Harmonized protocols for sample collection + transportation, DNA extraction, qPCR, metagenomics, shotgun sequencing, gene capture and bioinformatics and statistical analysis of sequence data available. Alignment with EFFORT project protocols (T2.1, T2.2)	M26	Yes	M33	The list of sampling compartments, points and European test areas have been defined. Harmonized protocols for sample collection and transportation and DNA extraction protocols are already available for all project members. Protocols for WGS, metagenomics, gene capture and bioinformatics were developed. When possible, sampling protocols where aligned with e.g. EFFORT projects.
15	M-JRP15-FED-AMR-08	Start of annual field study (i.e. sampling campaign, sample collection) (T2.3)	M27	Yes	M27	
15	M-JRP15-FED-AMR-09	Start of DNA isolations and cultivation of ARB strains (immediately after reception of the first environmental samples) (T2.3, T2.7)	M27	Yes	M27	
15	M-JRP15-FED-AMR-10	Start of performing qPCRs (T2.4)	M28	Not applicable		
15	M-JRP15-FED-AMR-11	Start collecting data for annual report (WP2)	M34	Yes		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
15	M-JRP15-FED-AMR-12	Starting preparations for gene capture probe approach (T2.3.2)	M35	Yes		
15	M-JRP15-FED-AMR-13	Finishing annual report (WP2)	M36	Yes		Delivered: M37
15	M-JRP15-FED-AMR-14	Starting preparations for shot gun sequencing (T2.3.1)	M37	Not applicable		Shotgun Metagenomics was replaced for target enrichment (see Task 2.3.1)
15	M-JRP15-FED-AMR-15	Stop: field sampling campaign (T2.3), DNA isolations (T2.7)	M40	No	M42	
15	M-JRP15-FED-AMR-16	Starting 16S metagenome analysis of obtained DNA isolates. Batch format (T2.3)	M40	Yes		
15	M-JRP15-FED-AMR-17	Start: Determination of naturally transformable bacteria (T2.5)	M41	No	M42	
15	M-JRP15-FED-AMR-18	Finalizing ARG qPCRs (T2.4)	M42	Not applicable		
15	M-JRP15-FED-AMR-19	Finalizing determination of transformable bacteria (T2.5)	M44	No	M48	Delayed results from sequencing analysis
15	M-JRP15-FED-AMR-20	Start: Assessment of clonal/lineage diversity of ARB (T2.6)	M43			

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
15	M-JRP15-FED-AMR-25	Completed database with zoonotic types	M36	Yes		
15	M-JRP15-FED-AMR-30	Starting the selection of essential antimicrobials to be quantified in the tested compartments	M25	Yes		
15	M-JRP15-FED-AMR-31	Starting the analysis of antimicrobials in aqueous matrices	M27	Yes		
15	M-JRP15-FED-AMR-32	Starting the analysis of antimicrobials in manure	M31	Yes		
15	M-JRP15-FED-AMR-33	Starting the analysis of antimicrobials in faeces	M29	Yes		
15	M-JRP15-FED-AMR-34	Starting the analysis of antimicrobials in soil	M27	Yes		
15	M-JRP15-FED-AMR-35	Starting the quantification of herbicides in agricultural soil	M27	Yes		
15	M-JRP15-FED-AMR-36	Starting the measurement of the concentration of trace elements in environmental samples	M27	Yes		
15	M-JRP15-FED-AMR-37	Bacterial strains supplied to UoS	M25	Yes		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
15	M-JRP15-FED-AMR-38	Porcine gut model set up using faecal samples obtained through WP2, samples stored for trace element analysis (WP4) – experiments can start	M40	Yes	M44	
15	M-JRP15-FED-AMR-39	Samples from gut model experiments stored for trace element analysis (WP4)	M47			
15	M-JRP15-FED-AMR-40	Samples from gut model experiments stored for trace element analysis (WP4)	M52			
15	M-JRP15-FED-AMR-42	DNA sent for whole-genome sequencing	M55			
15	M-JRP15-FED-AMR-43	Antibiotics, herbicides and heavy metals most likely to drive HGT through transformation supplied to UoS	M51			
15	M-JRP15-FED-AMR-44	Samples from gut model experiments stored for trace element analysis (WP4)	M50			
15	M-JRP15-FED-AMR-45	Deliver results from 1st round of gut model experiments to WP6 leader	M49			

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
		for further modelling				
15	M-JRP15-FED-AMR-46	Results from modelling in WP6 communicated to inform parameters for use in 2nd round of gut model experiments	M49			
15	M-JRP15-FED-AMR-47	Deliver results from 2nd round of gut model experiments to WP6 leader for further modelling	M53			
15	M-JRP15-FED-AMR-56	Literature review on fluctuating ecological systems and gut model approach.	M45			

5.1.4.3.9.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors has evaluated the comments you provided last January. All recommendations have been addressed and therefore this part of the report can be closed.

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
(1) Human biological samples The beneficiaries must	The ethical self-assessment was sent out to all partners of the FED-AMR project to	No comment		

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
confirm that appropriate authorizations will be sought to collect the Human samples.	ensure besides visibility and awareness of the high demand and principles of ethical conduct, a basic feedback on the mentioned issues relevant for OHEJP and this project. As no critical paths were identified and no current risks for the further progress of FED-AMR became obvious by this first assessment, the digestion and iterative update of the process are running in regular terms. An amended and clean version of the ethics section will be presented in the regular reporting periods. The first re-evaluation (end date 10th September) revealed no further critical paths and can be found in the annex of this report.			
(2) personal data processing The beneficiaries must confirm that the personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.		**Satisfactory reply**	Closed	Closed
(3) Animals Further details are need on the use of animals which are legal animals and any experimental animals (i.e. pigs). Please clearly state the 3Rs aspects of this work. Please describe how the beneficiaries are complying with access to animal material requirements and animal welfare laws.		**There may be no issues raised but you have not direct answer the question about animal use - please clarify**	FED-AMR complies with the 3Rs (Replace, Reduce and Refine) regarding the use of animals in research. We use mostly molecular and culture approaches. Sampling of wildlife feces occurs without any animal contact since they are collected from the floor. Sampling of pig feces from the rectum was preferred, as stated in the protocols. However, feces were directly collected from the floor or with a plastic bag in the moment of the defecation. Thus, this methodology does	**Satisfactory reply**

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
			not disturb the animal	

5.1.4.3.9.5 Publications and additional outputs

There are no publications yet.

Additional output

Poster

- Abstract describing the FED-AMR project presented in the ASM Annual Meeting, May, **2020** (e-Poster). https://zenodo.org/record/4915927#.YTsr0udCR4E
- Abstract in ECCMID **2021** (e-poster): Antimicrobial resistance and genetic relatedness of environmental bacteria across the animal-human-wildlife interface in Austria (WP2, 2-AGES) https://zenodo.org/record/5499916#.YTs2z-dCR4E
- Abstract presented in Annual Scientific Meeting **2021** (e-poster): Diversity of bacterial communities and genes encoding AMR in different environmental compartments along the food/feed chain (WP2, 36-INSA) https://zenodo.org/record/4916135#.YTsqrudCR4E
- Abstract presented in Annual Scientific Meeting **2021** (e-poster): Dissemination of antimicrobial resistant *Clostridium difficile* RT078/ST11 in Austria across the human-animal-wildlife interface (WP3, 2-AGES) https://zenodo.org/record/4915872#.YTsrmOdCR4E
- Abstract presented in Annual Scientific Meeting **2021** (e-poster): Zoonotic spread of multi-resistant *C. difficile* (WP3, 13-SSI) https://zenodo.org/record/4915901#.YTsqsOdCR4E
- Abstract presented in Annual Scientific Meeting **2021** (e-poster): Diversity and Dynamics of *Clostridioides difficile* in a farm environment PT (WP3, 36-INSA) https://zenodo.org/record/4916135#.YTs1O-dCR4E
- Abstract presented in Annual Scientific Meeting **2021** (e-poster): FED-AMR: Determination of selection pressures for AMR in environmental samples (WP4) https://zenodo.org/record/4916158#.YTs1CudCR4E
- Abstract presented in Annual Scientific Meeting **2021** (e-poster): Ecological modelling of microbial communities subject to perturbation (WP6, 23-UoS) https://zenodo.org/record/4915941#.YTsqs-dCR4E

Other

- Launch of an internal website hosted by AGES that serves as an exchange platform of internal documents. Partners are granted with private access and can downloaded common protocols, minutes from the TCs and other documents.

5.1.4.3.9.6 Data Management Plan

The FED-AMR Data Management Plan was first uploaded in M34 to the online Lisam APP. Since then we have been regularly updating the information. The generation of a metadata template to be used in the Data Management Plan was performed with contribution of all WP2 partners, which will be also very helpful for project data analysis.

FAIR principles are fulfilled in the DMP following the DMP guidelines from OHEJP and according to the few data that the project as at the moment.

5.1.4.3.9.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

- On April 6 2021 partner 2-AGES presented our project to **MediPIET** (Mediterranean and Black Sea Field Epidemiology Training Programme Network), at the Training of Trainers on One Health.
- In the context of WP2, we have established a small collaboration with the **University of Delft** for comparison of our different extracellular DNA extraction methodologies and further analysis with target enrichment will be performed for two water samples that University of Delft.
- Presentation of the FED-AMR project at the One Health Session, Project Review Module 2021, as part of the **ECDC Fellowship Program**.
- **EFSA** is being updated (in)directly on the progress of the FED-AMR project through an stakeholder that belongs to the Advisory Board.

5.1.4.3.10 JRP16-R2-ET2.2-TELE-Vir

5.1.4.3.10.1 Summary of the work carried out in the Project

TELEVIR is a 2.5 year (now 3.0 with an extension of six months) Joint Research Project of the One Health EJP that focusses on developing a fast point-of-evidence (poi) toolbox for identification and characterization of emerging virus threats for human and/or domestic and wildlife animals.

In the TELEVIR project we are combining a suitable field-deployable point-of-care approach, and a direct upload of genomic, phenotypic and epidemiological data into a user-friendly bioinformatics toolkit for fast identification and characterization of new emerging virus threats. We are developing and adapting existing point-of care methods and tools and expand these to a harmonized poi protocol for field analysis. The poi protocol will only require a minimum of laboratory equipment and will be designed to be compatible with MinION sequencing technology. Moreover, we are combining and integrating in the poi toolbox phenotypic and epidemiological data to aid risk assessment and management. The poi toolbox will be made available to other interested national and international parties for example shared with established networks.

The worldwide SARS-CoV-2 pandemic has had a great impact on the TELEVIR project. There has been national lockdowns, laboratories have been closed or allocated for SARS-CoV-2 diagnostics. There has been a worldwide shortage of basic laboratory reagents and equipment, which has influenced the ability to perform basic laboratory experiments. However, the SARS-CoV-2 pandemic has also a positive influence on the project, as we demonstrated that we can inactivate the SARS-CoV-2, besides other RNA viruses being non-infectious and can develop alternative methods for NA extraction (WP3), which is in line with the development of a field-based protocol for MinION sequencing using a minimum of laboratory equipment (the poi tool box).

Our first annual meeting from the TELEVIR project was held online at the end of January 2021, due to the SARS-CoV-2 pandemic. We agreed on that, we will keep our strategy and will focus on RNA viruses as the newly emerged SARS-CoV-2, but also on DNA viruses to apply our metagenomics approach. WP2 supports this approach by providing proof of concept for bioinformatic platforms using coronaviruses, and influenza viruses as main models. The pandemic has both given new opportunities in this area with overwhelming volumes of data becoming available as well as challenges caused by data quality, and logistical constraints and restrictions. In this context, the TELEVIR platform is being built to enhance the genome-based viral surveillance, by facilitating both sequencing data analysis and output navigation and interpretation.

Challenged by the SARS-CoV-2 pandemic, the TELEVIR consortium has shown impressive resourcefulness and adaptability. Deliverables and reports of the first year were reached and submitted. The TELEVIR consortium meets every month to exchange, to discuss problems and to find solutions to guarantee the best possible progress and success of the project. Results are disseminated as scientific publications and presentation at workshops, webinars and conferences including OneHealth EJP conferences.

Overall, the COVID-19 pandemic has had also a positive impact on the TELEVIR project and many of the experiences and problems encountered during the crisis can be used or translated to the development of the TELEVIR poi-tool box, which will help in the future to control outbreaks of new emerging viruses at national, regional, European and even global levels.

5.1.4.3.10.2 Progress of the project: description of activities

WP1: Coordination and data management

JRP16-WP1-T1- Coordination and project management

Project management and coordination of the project is proceeding according to the plan. The WP1 leaders organize monthly meeting for the whole TELEVIR consortium to guarantee the best outcome

of the project. This is ongoing.

JRP16-WP1-T2- Data management

Data management plan (DMP) was submitted in the first year of the project (December 2020).

JRP16-WP1-T3- Kick-off-meeting at IZSAM, Italy

The kick off meeting of the TELEVIR project was held on January 21st – 22nd 2020 at the International Centre for Veterinary Training and Information "F. Gramenzi" (CIFIV) of Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (Via G. Caporale, Teramo, Italy). All partners were present. This task is completed.

JRP16-WP1-T4-1st TELEVIR meeting online, organized by SSI

The first meeting of the TELEVIR project was held on January 25th 2021 as an online meeting. All partners were present. This task is completed.

JRP16-WP1-T5-2nd TELEVIR meeting at SSI, DK

This meeting will be postponed to June 2022, as we want to have the project parts completed before having the 2nd TELEVIR meeting to discuss the outcome. This is possible as we applied for an extension of the project for six months.

JRP16-WP1-T6-Workshop in the use of the POI toolbox at SSI, DK

This workshop will be postponed to January - June 2022, as we want to have the project parts almost completed before we will hold the workshop. This is possible as we applied for an extension of the project for six months.

WP2: Development of a Bioinformatics tool-kit for POI data analysis

JRP16-WP2-T1- Survey and collection of databases for genotype-phenotype associations

At the kick off meeting it was agreed that the first stage was selection of important phenotypic characteristics for the chosen model virus (influenza and coronaviruses), followed by assessment of the amount and quality of data available. During M25-M36, a literature review was performed to identify coronavirus phenotypes of relevance to tropism, emergence, and clinical disease, and any data available for their prediction based on genotype. A summary was circulated to partner institutes and associated virologists, together with a survey. The aims of the survey were two-fold: (1) to obtain the views of virologists (potential end users of the TELEVIR toolkit) on coronavirus phenotype prediction and variant monitoring activities that they would like to see in a genomic surveillance toolkit; and (2) to obtain test datasets for further development of the toolkit.

During M37-M45, progress covered several fronts. The results of the coronavirus survey were reviewed and discussed in TELVIR internal meetings, and presented as a poster at the OHEJP ASM 2021. Additionally, a scoping review to identify relevant phenotypes and their feasibility of prediction was performed for influenza A virus. Finally, data linking genotypes (lineages and mutations) with phenotypic changes for both SARS-CoV-2 and influenza A virus were collected from available literature.

For SARS-CoV-2, genotype-phenotype data are expanding rapidly, and data collection will have to continue to keep the database up-to-date and relevant. Multiple curated online databases have recently come into existence, and periodic automated update using these is being considered as an approach.

JRP16-WP2-T2- Development of bioinformatics modules for third-generation sequencing analysis and pathogen identification

In the context of pathogen detection and field genome sequencing, there are multiple advantages in either using online bioinformatics tools or running the platforms locally. As such, a Docker version of the online INSaFLU platform has been built and distributed publicly (https://github.com/INSaFLU/docker) in order to facilitate the local installation process. INSaFLU users, TELEVIR partners and other stakeholders (e.g., ECDC) were notified of this novel feature. INSaFLU has been successfully installed and run locally 'offline' on partner computer servers including at UoS.

As a response to COVID-19 pandemic, both the locally installed INSaFLU version and original website (https://insaflu.insa.pt/) were adapted to better accommodate the identification and genome-based analyses of the novel coronavirus (SARS-CoV-2), as follows:

- a new module for rapid assignment of Human Betacoronavirus (BetaCoV), including the novel coronavirus (SARS-CoV-2), has been developed and implemented, and the rationale behind the classification and outputs was documented (https://insaflu.readthedocs.io/en/latest/data_analysis.html#influenza-type-and-sub-type-identification-and-human-betacoronavirus-classification-as-of-march-2020; check more details in the list of current INSaFLU genetic markers used for Influenza type and sub-type identification and Human Betacoronavirus classification);
- the publicly available SARS-CoV-2 reference genome sequence (NCBI accession number MN908947) was inserted as default in the INSaFLU reference database;
- multitasking configurations were changed, considerably speeding up the analyses, and the maximum upload file size was made more flexible;
- a new tab "Settings" was created making software parameterization more flexible and tailored to SARS-CoV-2 NGS analyses, with emphasis on including user-defined parameters for reads quality analysis, mapping and consensus generation.
- automatic masking of low coverage regions was incorporated in the platform, i.e., automatic generation of consensus sequences for incomplete locus, i.e., undefined nucleotides ("N") are automatically introduced in low coverage regions at a user-selected coverage thresholds
- an automate pipeline for Oxford Nanopore Technologies (ONT) data analysis was optimized and implemented in INSaFLU, from raw reads to quality analysis, reference-based generation/curation of consensus sequences, mutation annotation, gene/protein/genome alignments, phylogenetic tree, metadata visualization, etc (details about the pipeline, including software version, default settings, etc, can be found in: https://insaflu.readthedocs.io/en/latest/data_analysis.html#). By automatically detecting the sequencing technologies (e.g., Illumina, or ONT) and subsequently allocating the technology-specific pipeline, the platform allows flexible data analysis and sample comparison regardless the technology used. This flexibility is particularly useful, for instance, in the context of genomic epidemiology consortia with centralized data analysis, but decentralized sequencing.
- Automatic SARS-CoV-2 Pango lineages (https://pangolin.cog-uk.io/) assignment using Pangolin (https://github.com/cov-lineages/pangolin), as described by Rambaut and colleagues (Nat Microbiol; 5:1403-1407), was integrated in both the locally installable and website versions of the platform. Whenever a new Pangolin / Pangolearn version is released, a button "Update Pango lineage" is automatically made available, so that users can re-assign all samples in the project using the latest software/database versions.
- Direct links for consensus sequences analysis using Nextclade (https://clades.nextstrain.org/) were also included in the INSaFLU online, allowing an easy and rapid SARS-CoV-2 clade

classification, mutation exploration and other analyses available at the Nextclade framework.

- A first version of the module for the rapid mapping-free influenza type and subtype/lineage identification, as well as Human Betacoronavirus (BetaCov) identification, using ONT read data was also released in both platform versions (locally installable and online)

These and other updates were documented and are available at full INSaFLU documentation webpage: (https://insaflu.readthedocs.io/en/latest/) and Github (https://github.com/INSaFLU/)

Partner Sciensano shared ONT data on animal influenza A viruses, SARS-CoV-2, and locally implemented the INSaFLU pipeline, and, in a joined effort with partner INSA evaluated the assembly approach for animal DNA viruses (LSDV).

Work on this task is ongoing to upgrade the platform for automatic pathogen identification.

JRP16-WP2-T3- Development of bioinformatics modules for sequence curation and phenotypic association

This task is highly dependent on the collected databases for genotype-phenotype associations, and the design of the bioinformatics approach cannot be fully drawn at this stage. As a priority, work is currently ongoing to implement the detection of known genotype-phenotype associations (such as, amino acid site changes already linked to: antiviral resistance, resistance to neutralizing antibodies, enhanced affinity to host-receptors antibodies or enhanced transmissibility). In particular, work is being done to automatically implement a rapid screening of such mutations of interest, which will be reported together with the predicted/known impact on phenotype. Additionally, we will continue to investigate the feasibility of inferring biochemical and immunological properties, using existing models and machine learning approaches being tested at UoS. In this context (and in work aligned with JIP COVRIN), we have been exploring SARS-CoV-2 antigenic variation and its genetic determinants, using antigenic cartography analysis of published datasets. Work on this task is ongoing.

JRP16-WP2-T4- Development of bioinformatics modules for genomic and metadata integration towards enhanced surveillance

Following the kick off meeting a strategic approach has been agreed to achieve this task. First, Nextstrain (https://nextstrain.org) tools will be implemented for temporal and phylogeographical analysis. Then, we implement novel functionalities focused on fitting the needs of labs working in different sectors (vet, PH, etc.) and that can be handled by users from multidisciplinary fields. In this context, INSaFLU was also upgraded to easily display metadata on phylogenetic trees (through user-defined node colouring and metadata blocks), thus facilitating integration of relevant epidemiological and/or clinical data and pathogen genomic data (https://insaflu.readthedocs.io/en/latest/change_log.html). Other functionalities for better data navigation and interpretation were also implemented, namely two novel interactive and dynamic "expand-and-collapse" panels for enhanced report of: 1) all detected mutations (including detailed information about genome position, nucleotide change, coverage evidence, frequency, impact at protein level, etc); and, ii) the mean depth of coverage and horizontal coverage per locus for all samples using interactive color-coded buttons. Still, further developments are ongoing, dependent on tasks 2 and 3.

JRP16-WP2-T5-development of a user and surveillance oriented web-based interface

During M25-M36, an application for an STM in 2021 was successful, for UoS and INSA to align bioinformatic approaches and share knowledge of the existing INSaFLU bioinformatics pipeline, in the context of WP2-T2 and WP2-T3 and to facilitate the eventual design of the user interface. The STM has been delayed by current travel restrictions but researchers are coordinating virtually and aim to complete the STM when possible (an option to postpone to 2022 has been granted). Work on this task is ongoing. In particular, we are investigating approaches to: i) synchronize local and web instances of the platform, allowing an easier integration and sharing of data, i.e., results of local analysis ("offline") can be "communicated" to a centralized repository with web access; ii) facilitate data flow and sharing

with external resources (GISAID/NCBI/ENA…).

WP3: Development of a protocol for POI MinION sequencing

JRP16-WP3-T1- Development and validation of a S.O.P for sample handling & pre-treatment

1) Protocol to verify the inactivation of animal viruses using three diferent lysis buffers.

For propagation of selected animal viruses (myxoma virus, MYXV; canine adenovirus type-2, CAV-2; canine coronavirus, CCoV) representing different virus families, the following cell lines were used: rabbit kidney (RK13), madin-darby canine kidney (MDCK) and A-72. Viruses were grown in cell cultures to the titres of $10^{5.84}$ $TCID_{50}$ /ml for MYXV, $10^{7.47}$ $TCID_{50}$/ ml for CAV-2 and $10^{4,20}$ $TCID_{50}$ /ml for CCoV. Additionally, to increase MYXV titre in cell culture suspension a virus concentration step was performed. The virus stock suspensions were prepared for subsequent inactivation studies. The virus inactivation of MagNA Pure lysis / binding buffer (MPLB, Roche), AL (Qiagen) and AVL (Qiagen) were assessed under various temperature-time conditions. For the virus inactivation studies the following buffer concentrations were tested 40% (MPLB), 50% (AL) and 80% in the case of AVL buffer. The tested virus suspensions were treated with the particular buffer solutions and subjected to heat treatment at 20°C (MPLB, AL, AVL) and 56°C (AL) for 1 and 10 minutes respectively. The inactivation efficiency of viruses was evaluated on the basis of a CPE appearing in the infected cells. The cytotoxicity of the cells caused by buffers used was assessed by a MTT assay. The following reduction of the virus titre was achieved ≥ 6.0 log_{10} (AL, AVL) and ≥ 5.0 log_{10} (MPLB) for CAV-2, ≥ 3.0 log_{10} (AL, AVL, MPLB) for MYXV and ≥4 log_{10} for CCoV when MPLB buffer was used. All buffers in the tested concentrations and temperature-time profiles were effective in virus inactivation. Likewise, the minimal inactivating concentration of MPLB for CAV-2 was assessed. A complete reduction of the virus titre was observed in 20% MPLB , while at the lower concentrations of the tested buffer solutions it was equal to 0.33 log_{10} (5%), 1.64 log_{10} (10%), and 2.83 log_{10} (15%). The study also covered the experiments on inactivation of hepatitis A virus (HAV) in different concentrations of buffer solutions. The virus was grown on monkey cell line (Frp/3 cells) to the titre of $10^{8,28}TCID_{50}$/ml. So far the Frp/3 cell cytotoxicity caused by AL, AVL and MPLB buffers was assessed using the MTT assay. This study is ongoing.

2) A protocol to verify the inactivation activity of two buffers for Bovine Beta-CoV (model virus fo SARS-CoV-2.

MPLB (Roche) and eNAT™ (Copan) against a Bovine Beta-CoV (Bov-CoV strain 9WBL77) was developed. The eNAT buffer is a Guanidine-thiocyanate based medium that stabilizes the RNA and DNA of Viruses. Bov-CoV strain belongs to the same genus as SARS-CoV-2 but can be propagated in BSL2 facilities and thus was used as a model to validate virus inactivation using different inactivating buffers. The protocol is based on the ultracentrifugation of the virus-inactivating buffer compound in order to remove the toxic effect of the inactivating buffer on cell cultures. For inactivation protocols using MPLB and eNAT buffers, one virus titer (10 $^{5}TCID_{50}$/ml) and one contact time (30min) were used. After 30 min of contact, the three samples: 1)Virus control (Virus + MEM), 2)virus + MPLB and 3) virus + eNAT were ultracentrifuged in sucrose cushion (100.000 g / 2 h) to remove the toxic supernatant. The pellet of samples 1, 2 and 3 was resuspended in the same volume of PBS and then titrated in HRT-18 cells: Sample 1:10 $^{5}TCID_{50}$/ml, sample 2: < 10 $TCID_{50}$/ml and sample 3: < 10 TCID50/ml. In conclusion, the inactivating power of the MPLB and eNAT buffers against a beta-CoV under test conditions was highlighted.

3) Heat inactivation protocols for Bov Beta-CoV 9WBL77 as model virus.

One ml of virus suspension with an infectious titer of 10^{5} $TCID_{50}$/ml was heated at two different temperatures. In the first protocol the virus suspension was heated at 56°C for 30 min and 60 min. In the second it was heated at 60°C for 60 min. After heat treatment, the viral suspensions were inoculated into HRT18 cells for three blind passages and viral growth was tested using a Mab-based virological ELISA. Heat treatment at 56°C was shown not to completely inactivate the virus for both 30 and 60 min as evidenced by viral growth after three passages. The viral suspension was instead

completely inactivated at 60°C for 60 min.

4) Fast and easy field-deployable inactivation method for SARS-CoV-2 (and other viruses) using MPLB-buffer.

Reducing transmission risk during sample handling is paramount. Previous studies have shown virus inactivation abilities of the highly cytotoxic MagNA Pure Lysis Binding (MPLB) buffer used for nucleic acid extraction and purification (Rosenstierne et al. Journal of clinical microbiology. 2016 Oct;54(10):2521-9 ; Vinner. and Fomsgaard., 2007. *Journal of virological methods*, *146*(1-2), pp.401-404.). In this study, we show how 20 minutes of incubation in MPLB-buffer at room-temperature produce rapid inactivation of the SARS-CoV-2 using a modified published protocol by Rosenstierne et al (2016).

Two human SARS-CoV-2 isolates of 10^5 and 10^6 Tissue Culture Infectious $Dose_{50}$/ml were incubated 1:1 with MPLB-buffer or PBS (positive controls) for 20 minutes. The mixture was diluted to non-cytotoxic MPLB-buffer concentrations (determined empirically) and incubated on VeroE6 cells. Supernatant and cells were harvested at multiple time-points in biological duplicates with technical triplicates. We used reverse transcription quantitative-PCR (RT-qPCR) with a standard curve for quantification. To ensure inactivation, we serially passaged supernatant from a 144h culture and measured active replicating virus at the 24h time-point with a SARS-CoV-2 E-gene RT-qPCR

Results. MPLB-buffer incubated SARS-CoV-2 samples were non-infectious in the 10^{-4} MPLB-buffer dilution, except three samples indicated by median ct-value 39.8 (IQR=4.8) from the 10^5 $TCID_{50}$/ml stock. The control grew efficiently with median ct-values 11.8 (IQR=1.8) for cells and 16.1 (IQR=1.8) for supernatant. The sub-genomic RT-qPCR for the 24h time-point showed no evidence for virus replication, including the three exceptive samples.

We show that MPLB-buffer can reduce SARS-CoV-2 titers at least 2-log units. With this protocol, we can facilitate POI-diagnosis and promote field research without risk of infection.

JRP16-WP3-T2- Development and validation of a S.O.P for sample NA purification

Selected nasopharyngeal swabs have been processed with different lysis/inactivation buffers trying to circumvent the NA extraction. The first trials resulted in a lesser viral RNA detection by real-time PCR when compared to the established diagnostic procedure, resulting in the ultimate loss of the weak positive samples. Preliminary assays using samples collected on FTA cards have also led to lower detection of viral RNA. Experiments incorporating simple washing steps of FTA cards, with no need of NA extraction, combined with different RT-PCR reagents allowed to detect positive samples with Ct<30. This study is ongoing.

Heating of nasopharyngeal swabs has also been tested in order to circumvent NA extraction (Fomsgaard and Rosenstierne, Euro Surveill. 2020 Apr;25(14):2000398. doi: 10.2807/1560-7917). The use of MagNA Pure Lysis Binding (MPLB) buffer as a field-deployable method for viral NA extraction and purification has been proven useful for targeted diagnostics of EBOV using RT-qPCR (Rosenstierne et al. 2018. *Journal of visualized experiments: JoVE*, (136)). Same method will be tested on grown SARS-CoV-2.

As we want to detect RNA and DNA viruses applying the metagenomics approach work is also ongoing on extracting rapidly of nucleic acids from surface liquids (mucus) of fish infected by a DNA virus. Field samples were obtained after an outbreak and tested positive using conventional methods (extraction by columns and test by PCR). Starting with these samples, a simplified extraction procedure with a specific lysis buffer and magnetic beads will be tested in 2021, both in lab. and field.

In order to facilitate sample testing in field settings, a new evaluation study to circumvent NA extraction was accomplished. To this end, a panel of 71 nasopharyngeal swabs and 19 saliva samples, previously analysed for SARS-CoV-2 by the regular PCR method, were initially selected. Briefly, the so-called method “Saliva Direct” (Vogels et al, 2021, doi: 10.1016/j.medj.2020.12.010) was followed and

samples were heated at 95ºC for 5 minutes in the presence of proteinase K, to be afterwards directly incorporated to the standard PCR mix. All samples with an initial Ct value <35 were detected using the "saliva direct" protocol. The Ct values reported by the two procedures compared, standard NA extraction and "saliva direct", were mostly similar for samples with Ct<33. Even more, the "saliva direct" protocol allowed the SARS-CoV-2 detection by conventional RT-PCR and the subsequent Sanger sequencing was also successful. Whether these heated &treated samples could serve as template for NGS analysis using the MinION device still requires investigation.

Considering these promising results, additional studies were planned to evaluate the convenience of this system for the detection of other relevant viral pathogens. An initial panel of 10 samples (swabs, blood and faeces), collected at different days from a sheep experimentally infected with peste des petits ruminant virus (PPRV), was analysed. Samples were processed as mentioned above and tested with the real-time RT-PCR established in the lab for PPRV detection (using the same reagents as for SARS-CoV-2 PCR). Unfortunately, only samples with an initial Ct<30 could be detected, although reporting a Ct value much higher than using the standard NA extraction. Besides, samples with an initial Ct>30 were not detected. This preliminary study indicates that this "Saliva Direct" alternative could not be a reliable procedure for PPRV detection, at least under the assayed conditions.

JRP16-WP3-T3- Development and validation of a S.O.P for NGS library preparation & MinION sequencing (DNA & RNA)

A SOP for MinION sequencing of Avian influence virus (AIV) has been tested. Four primer PCR protocol and the PCR Barcoding Kit (SQK-PBK004) from Oxford Nanopore Technologies (ONT, Oxford,UK) was used to sequence and typing of avian influenza virus. Preliminary amplification of RNA was conducted prior to sequencing on MinION utilizing SuperScript IV One-Step RT-PCR System with Platinum Taq (Invitrogen/ThermoFisher Scientific, Waltham, MA) and universal Influenza A primers designed for the conservative ends of all AIV segments (Zhou et al., 2009, J Virol 83:10309-10313). Spot on Flow Cell, R9 version (FLO MIN 106D; ONT) and basecaller Guppy (v3.29; ONT) was used for the real-time basecalling to produce sequencing data and monitor the run. Sequencing data from two samples were analyzed using CLC Genomics Workbench (Qiahen-CLCBio). Study is ongoing.

A SOP for targeted sequencing of SARS-CoV-2 on MinION has been tested and validated and is used for comparison to a metagenomics approach on the same clinical sample material from humans and mink. The SOP is based on the ARTIC nCoV-2019 amplicon sequencing protocol (https://artic.network).

For the metagenomics approach, a sequence independent isothermal amplification step will be included before library preparation and two methods are currently being tested. Sequencing independent single primer amplification (SISPA) and Recombinase polymerase amplification (RPA). Both have the potential to amplify sample NA before sequencing and thereby increase the sensitivity of viral detection. This study is ongoing.

A SOP for MinION sequencing of bovine coronavirus (BCoV) as a model has been tested. The SOP is based on Sequence-Independent Single-Primer Amplification (SISPA) and Native barcoding genomic DNA protocol (with EXP-NBD104, EXP-NBD114, and SQK-LSK109) available from the Oxford Nanopore Technologies (ONT, Oxford, UK). SISPA approach allows to amplify total RNA in ca 9 hours, however the protocol time can be shortened by reducing the number of PCR amplification cycles. The process involved the following steps: I. Reverse transcription of RNA utilizing SuperScript IV (Invitrogen/ThermoFisher Scientific, Waltham, MA) with tag-labelled primer (Allander et al., 2005); II. Second strand synthesis using 3´-5´ exo$^-$ Klenow DNA polymerase (New England Biolabs, Ipswich, MA); III. Whole Genome Amplification of double-stranded cDNA by PCR. The study is ongoing.

The other SOP which also tested on BCoV as a model was based on Complete Whole Transcriptome Amplification Kit (WTA2-10RXN, Merk, Sigma-Aldrich) and Native barcoding genomic DNA protocol (with EXP-NBD104, EXP-NBD114, and SQK-LSK109) available from the Oxford Nanopore Technologies (ONT, Oxford, UK). WTA2 kit allows to amplify total RNA in less than 4 hours. The process involved two

steps: I. Reverse transcription of RNA with non-self-complementary primers which resulted in cDNA library, comprised of random, overlapping 100 - 1000 base fragments flanked by universal end sequence; II. Amplification of cDNA by PCR using WTA2 polymerase and a universal end primer to produce WTA2 product. The study is ongoing.

A SOP for MinION sequencing of African swine fever virus (ASFV) without DNA preamplification was tested. The SOP is Genomic DNA by ligation (SQK-LSK109) which can be used in combination with Native barcoding genomic DNA protocol (with EXP-NBD104, EXP-NBD114) available from the Oxford Nanopore Technologies (ONT, Oxford, UK). The process involved DNA repair, adapter ligation and clean-up, followed by priming and loading the flow cell and sequencing. Time for the MinION library preparation from high molecular weight genomic DNA (1.5 µg input) can be shortened to 3-3,5 hours. The study is ongoing.

Sciensano: Similar efforts were done at Sciensano with targeted MinION applications for Avian influenza, as well as the ARTIC nCOV-2019 amplicon sequencing panel. Data were shared with Insa in the frame of task WP2T2 for evaluation in the InsaFlu pipeline in comparison to other analysis options. For the metagenomics approaches, Sciensano evaluated several random amplification and library prep methods with readout on Oxford Nanopore Technologies and Illumnia platforms for several cases: (1) on a farmed seabass sample from partner ANSES (Laurent Bigarré) (cluster of cases with hemarrhagic skin lesions); (2) massive mortality of EMCV infected piglets in Belgium with suspicion of an additional viral cofactor and (3) massive mortality of piglets in Spain where EMCV was already excluded using targeted diagnostic assays. Without identification of a clear etiology in the seabass case. In Belgian pig case: near full genome of EMCV was recovered + co-infecting Torque teno sus virus and Gokushoviruses. In the Spain pig case no etiology could be identified. The 3 cases highlight the need of analysing proper control samples and of the development of good metagenomic analysis workflows.

Swabs (from lymph node and spleen) from 2 cows tested positive for malignant catarrhal feverwere used to evaluate a protocol comparing 2 high-molecular weight DNA extraction methods (Monarch vs Puregene) and different amplification protocols (REPLIg vs Picoplex vs no amplification) prior to MinION sequencing. Metagenomic analysis identified for 5 out of 8 conditions tested 1 or more Ovine Gammaherpesvirus 2 read(s) that covered at most 6.4% of the viral genome. The results highlighted that more investigations are needed.

5.1.4.3.10.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
16	D-JRP14-1.1	Kick-off meeting in Italy (IZSAM)	25	31st of January 2020		Public https://zenodo.org/record/3734134#.XwbjOSgza70 Milestone reached by M25 on time	
16	D-JRP14-1.2	1st version of the DMP	30	M30		Milestone reached by M30 on time	
16	D-JRP14-WP1.3	1st TELE-Vir meeting (online)	M37	25th of January 2021		Public https://zenodo.org/record/5506645#.YUSEcLgza70 Milestone reached by M37 on time	
16	D-JRP24-WP1.4	2nd version of the DMP	M42	46		Work is ongoing	
16	D-JRP14-3.1	1st version of a poi S.O.P for sample	36	39		Deliverable reached and published	

		handling & pre-treatment				https://zenodo.org/record/5506816#.YUSElbgza70	
16	D-JRP14-3.2	1st version of a poi S.O.P for sample NA purification	36	39		Deliverable reached and published https://zenodo.org/record/5506848#.YUSFD7gza70	
16	D-JRP14-3.3	1st version of a poi S.O.P for NGS library preparation & MinION sequencing	36	46		First experiment performed and next step initiated to find the best protocol. Work is ongoing and will be delivered M46	

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
16	M-JRP14-01	Collected and curated databases for genotype-phenotype associations	36	Yes		Database for CoV genotype phenotype associations complete.
16	M-JRP14-02	Software modules for third-generation sequencing data handling	39	Yes		Modules for automate quality control and analysis of ONT data were developed and implemented.
16	M-JRP14-03	Software modules for viral rapid detection and classification	42	Ongoing		Modules for automate classification of Beta-coronaviruses, and further lineage classification of SARS-CoV-2 were implemented. Work is ongoing to implement module for pathogen identification.
16	M-JRP#-04	Software module for rapid screening of genetic features potentially linked to specific phenotypes (e.g., antigenicity, tropism, anti-viral drug resistance)	M45	Ongoing	M54	Automated add-on to existing INSaFlu modules developed and undergoing testing. The rapidly developing Sars-CoV2 available data mean delaying this milestone to ensure the resulting module is fit for purpose is strategically important

5.1.4.3.10.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors evaluated the comments you provided last January. All recommendations have been addressed and therefore this part of the report can be closed.

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
(1) Human biological samples The beneficiaries must confirm that appropriate authorizations will be sought to collect the Human samples.	Human samples used for this project have been obtained with the due signed consent of the owner permitting the use of the collected material for research purposes. Also many of the human samples are collected for diagnostic purposes and exemption for review by the ethical committee system and informed consent has been given by the Committee on Biomedical Research Ethics - Capital region in accordance with Danish law on assay development projects.	Satisfactory reply	Closed	Closed
(2) Health and Safety The beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	The group works in a high-biosecurity (BSL-3) laboratory compliant with the biosafety and biosecurity rules of the INIA-CISA (Biosafety Reference Laboratory for FAO). Bio risk assessment of the different pathogens used in the project has been evaluated by INIA-CISA	Satisfactory reply, however if you are submitting a document as evidence of practice please ensure the reviewers can access the document (this document cannot be accessed as it is password protected) or attach it to your submission	Closed	Closed

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
	Biosafety & Biocontainment service.			
(2) Health and Safety (2) The beneficiaries must confirm that authorisations for relevant facilities (e.g. security classification of laboratory) have been obtained.	INIA-CISA contains a large high-biosecurity facilities (BSL-3 and BSL-3+) with the due authorization to work with animal and zoonotic pathogens requiring this facilities, such as foot-and-mouth disease virus, African swine fever virus, SARS-CoV and SARS-CoV-2, Rift valley fever virus, West Nile virus, Avian influenza virus, among others. Moreover, INIA-CISA is Biosafety Reference Laboratory for FAO. Sciensano has obtained the authorisations for facilities up to risk classification of 3 by the competent authorities (Decision number LABO-415117). SSI has a large high-biosecurity facilities (BSL-3) with the due authorization to work with human and animal zoonotic pathogens requiring this facilities, such as African swine fever virus, SARS-CoV, HIV,	Satisfactory reply	Closed	Closed

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
	Vaccinia, influenza virus, among others.			
(3) Animals Further details are needed on the use of animals which are legal animals and any experimentation animals (e.g. from wild boar to horses). Please clearly state the 3Rs aspects of this work. Please describe how the beneficiaries are complying with access to animal material requirements and animal welfare laws.	Animal samples used for this project have been obtained from field cases and have been provided to INIA-CISA as EU/FAO reference laboratory for African swine fever or as project collaborators. Many of the animal specimens are collected for diagnostic purposes from sick or dead animals.	Do you have confirmation of sampling procedures and recommendations on animal welfare standards for sampling or is this not know?	Samples are only acquired from available biobanks or from samples collected for diagnostic purporses (see additional information under actions) or dead animals, therefore the animal welfare standard are followed.	Closed

5.1.4.3.10.5 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
An alternative workflow for molecular detection of SARS-CoV-2 – escape from the NA extraction kitshortage, Copenhagen, Denmark, March 2020 https://doi.org/10.2807/1560-7917.ES.2020.25.14.2000398 https://zenodo.org/record/4247188#.X6QLjWhKjcc	No		

Additional output

Posters

Assessing coronavirus risks: feasibility of predicting traits of clinical and epidemiological relevance from sequence data. C. Bogaardt 1, 2 • V. Borges 3 • J. P. Gomes 3 • J. Isidro 3 • J. M. Prada 2 • D. L. Horton 1

Poster presentation, OHEJP Copenhagen June 9-11th 2021

Pinheiro M, Pais RJ, Isidro J, Pinto M, Bogaardt C, Prada JM, Horton DL, Gomes JP, Borges V (2021) INSaFLU-TELE-Vir: an open web-based bioinformatics suite for influenza and SARS-CoV-2 genome-based surveillance. ARPHA Conference Abstracts 4:e68845. https://doi.org/10.3897/aca.4.e68845

Poster presentation at One Health EJP Annual Scientific Meeting:

Fast and easy field-deployable Sars-CoV-2 virus inactivation for downstream analysis. Anna S. Fomsgaard[1,2], Graham J. Belsham[2], Ria M. Lassaunière[1] , Jannik Fonager[1] , Katja Spiess[1] 1. Virus Research & Development Laboratory, Statens Serum Institute , Copenhagen, Denmark 2. Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

5.1.4.3.10.6 Data Management Plan

The DMP was uploaded on the CDP-tool.

We included all datasets and data that are generated or will be generated in the DMP. The contact details as well as the institutional email addresses are provided to access the data.

5.1.4.3.10.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

As the national member of the ECDC EVD-LabNet (Anders Fomsgaard) we are sharing methods and results from our TELEVIR methods in detection of emerging virus.

The Statens Serum Intitute (SSI) in CHP Denmark is a one health institute and thus all findings and methods and mututal surveillance testings are shared with Veterinary networks and ECDC.

5.1.4.3.11 JRP17-R2-ET2.2-IDEMBRU

5.1.4.3.11.1 Summary of the work carried out in the Project

After very hard first year of the project, where hardly any work could be done due to still ongoing COVID-19 pandemic, at the annual workshop held in December 2020, the consortium reorganised and prioritized the tasks for this year.

The sample collection for WP1 started throughout Europe mainly from wildlife species from forest, fresh water and marine coastal habitats. The sampling is now ongoing in all partners' laboratories participating in WP-1. Additionally, to reinforce this task, the consortium contacted other scientific and surveillance projects, currently ongoing and profits from samples already collected for other research tasks. Already more than 1000 samples have been processed and analysed at least by molecular or bacteriological methods. Several environmental strains of bacteria similar to *Brucella* (such as *Ochrobactrum* spp.) have been isolated, but further genetic typing is needed to establish the links.

Within the WP-2, the network with another One Health EJP project – COHESIVE has been established regarding the evaluation of presence of *Brucella canis* in European dog and human population and determining its pathogenic characteristics. The profession exposure groups have been identified and WP-2 concentrated the sera testing on samples coming from individuals who are professionally more likely exposed to *Brucellae*. The members of two projects organised the first European workshop on *B. canis* in May 2021, and it made foundation for the joint white paper review on current epidemiological situation in Europe and recommendations for further necessary actions. Therefore, IDEMBRU project decided to focus a part of its work on *B. canis.* Since the project saw many setbacks last year, the evaluation of DNA extraction methods from complex matrices (water, soil and faeces) is still waiting the final validation. Since the accent is on *B. canis*, the DNA extraction from canine faeces has been analysed additionally. The harmonised whole genome sequencing protocols have been prepared and first ring trials will be soon performed. As many strains that are similar to *Brucella* morphologically, were isolated from environmental samples, but molecular methods did not detect them. Therefore, we constructed the primers specific for *Ochrobactrum* spp. In order to distinguish between the two genera. Further step is construction of microfluidics PCR plate, so that the diagnostic and typing of *Brucella* and *Brucella*-like bacteria can be performed even from a few bacteria. The big advantage of microfluidics system is that it can perform up to 96 primer pairs PCR on up to 96 samples at the time, and the total volume of the sample is 1µl. The protocols for RNA extraction, RNA seq and antimicrobial resistance are ready. The only producer of plates for micronaut analysis stopped the production because of the economic value. Therefore, consortium decided to replace micronaut analysis by MALDI-TOF. The cell cultures for *in vitro* infection have been chosen, and pipeline for pathogenicity classification established so that first experiments in WP-5 can start as planned this year.

The regular monthly online meetings are held within consortium, and WP communication

5.1.4.3.11.2 Progress of the project: description of activities

WP 1-Recording the situation of brucellosis in emergent wild and environmental reservoirs

JRP17-WP1-T1- Mapping of existing data on emerging Brucella spp

A common database to share information about emerging Brucella and reservoirs among the consortium partners was deliverable in August 2020 (M32) and deposited in Zenodo platform (OHEJP_JRP17_IDEMBRU_Data_management_datasheet | Zenodo).

JRP17-WP1-T2- Sampling and analytical strategy according to previous epidemiological information from the different partners

Since 2021 (M37), new biological collections (serum, tissue samples, and strains) from different

reservoirs and biotopes have been included. The sampling is still ongoing in all partners' laboratories. More than 1000 samples have been processed and analysed at least by molecular or bacteriological methods. Results already achieved were shared among the consortium partners.

Standard operating procedures for sample treatment (OHEJP_JRP19_WP1_SOP1_sample treatment), sample collection (OHEJP_JRP19_WP1_SOP3) and DNA extraction from soil, and faeces. (OHEJP_JRP19_WP1_SOP5) were prepared and final documents were deposited at the SharePoint platform (M36). New SOP for sampling the environment using boot swabs was also prepared and shared at the SharePoint platform. Serological diagnosis (OHEJP_JRP19_WP1_SOP4) and bacteriological diagnosis (OHEJP_JRP19_WP1_SOP6) procedures were prepared and shared among consortium partners for consideration (M36).

RP19-WP1-T3: Synthesis and analysis of data

The integrative analysis of the data can only begin when sufficient results are available. This task is planned for 2022.

WP2 - Recording the situation of brucellosis in human

JRP17-WP2-T1- Creation of a surveillance network dedicated to human brucellosis

The hospital and private laboratories were contacted in order to establish the strains collection and/or the biological samples from human populations suspected of Brucella infections.

The flowchart of the actions to be developed was established, with safety standards to send class A biological agents. The epidemiological questionnaire was adapted from the one used in the INSA in order to safeguard the patients privacy. Furthermore, the selection of sera was made, based on the occupational and environmental risks for humans. The selection takes into account exposure to kennels, exotic animals, forest workers, marine wildlife, anglers, and people living in the countryside, who could be more exposed to atypical Brucella or potential unknown reservoirs.

JRP17-WP2-T2- Sampling and analytical strategy according to epidemiological information from the surveillance network

According to the analysis strategy, first panel of 400 samples has be processed according to classical serological methods. Several samples (n=20) was chosen for further bacteriological culturing and molecular detection based on the antibody titters, but so far, no Brucella strain was isolated. Results were shared among the consortium partners.

Upon identification of new tools, all the samples will be retested and results compared in order to determine the best flowchart for identification of human atypical brucellosis.

WP3 - Genomic characterisation of Brucella detected from samples and selected isolates

JRP17-WP3-T1- Optimisation of methods for generating molecular typing data from complex samples

Due to all delays, this task will be finished on M48. The partners agreed on performing WGS using Illumina sequencers on five reference strains. DNAs have been extracted through boiling the pure cultures.

JRP17-WP3-T2- Harmonisation and standardisation of protocols for whole genome sequencing

Due to all delays, this task will be finished on M48. Data from sequencing will be used for comparing the different protocols to achieve the harmonization and standardization.

JRP17-WP3-T3- Identification of emerging Brucella species: adaptation of molecular tools

The activities started on time and molecular tools using WGS data have been discussed. Further

meeting will be planned for finalizing the choice of the optimal molecular tools.

JRP19-WP3-T4-Integrative analysis of genomic and epidemiological data collected in WP1 and WP2

The activities from JRP19-WP3-T4 have started planning the integrative analyses on data derived from the historical collections of atypical Brucella strains. Moreover, new strains isolated from the WP1-T2 will be assessed and compared. The emergence of *B. canis* as a new priority in the European scenario has led to the decision of including strains from surveillance for additional epidemiological analyses.

WP4 - Phenotypic characterisation of Brucella detected from samples and selected isolates

JRP17-WP4-T1- Phenotyping of novel emerging Brucella spp.
JRP19-WP4-T1-ST1

The subtask has started. First strains have been phenotyped and prepared for sending it to BfR for RNA-Sequencing.
JRP19-WP4-T1-ST2

Initial motility tests have been carried out. Suitable Brucella candidates are currently being selected for electron microscopy studies.
JRP19-WP4-T1-ST3

Unfortunately, production of the commercial test system for phenotyping Brucella has been discontinued. Therefore, the work with this particular system in this subtask has been discontinued.
JRP17-WP4-T2- Antimicrobial resistance testing of emerging Brucella spp.
JRP19-WP4-T2-ST1

A microdilution antibiotic test scheme has been tested with novel merging Brucella to adapt the procedure. A suitable antibiotic pattern has been selected. The commercial ordering process for this specific plates also for the project partners is underway.
JRP19-WP4-T2-ST2

The overall evaluation of the data can only begin when sufficient results are available.
JRP17-WP4-T3- RNA sequencing of a representative panel of classical and novel Brucella spp.
JRP19-WP4-T3-ST1

Evaluation of RNA seq kits for different Brucella species identification has been done. A suitable protocol has been developed. However, based on further work, this will also be adapted in the future, if necessary.
JRP19-WP4-T3-ST2

Lab work will begin when isolates are available from the project partners. Currently, the scientific literature is being reviewed to optimize the necessary research approaches.
JRP19-WP4-T3-ST3

Lab work will begin when isolates are available from the project partners. Currently, the scientific literature is being reviewed to optimize the necessary research approaches.
JRP19-WP4-T3-ST4
Lab work will begin when isolates are available from the project partners. Currently, the scientific literature is being reviewed to optimize the necessary research approaches.

WP5 - Zoonotic potential and virulence

JRP17-WP5-T1- Develop an in silico pipeline for preliminary assessment of overall virulence and zoonotic potential

This task has started and is still ongoing. Bioinformatic tools for identification and comparison of virulence factors have been selected (VFDB and an approach based on comparative genomics).

Relevant sequences of publicly available isolates are assembled to create a *Brucella* genomes database. With these sequences the first preliminary pipeline will be created. After that, sequences from isolates within this project will be used as input. At the moment, no delays are expected.

JRP17-WP5-T2- Develop an in vitro protocol to investigate zoonotic potential based on macrophage cell lines originated from human and animals

This task has started and is still ongoing. At the SharePoint, a folder has been created in which *in vitro* infection protocols of participating partners can be exchanged. These protocols will later be tested and, if necessary, optimized in order to develop a SOP for *in vitro* infection. Furthermore, information regarding relevant cell lines has been assembled (table on SharePoint), so cell lines that might be useful for this WP can be exchanged in order to set up the model. No delays are expected.

JRP17-WP5-T3- In vivo testing of Brucella isolates with high zoonotic potential

This task is planned for 2022.

WP7 - Coordination, management and communication

JRP17-WP7-T1- Coordination and organization

The monthly meetings have been established, on which all WPs report on their progress, encountered problems and possible solutions. Besides that, the annual project Workshop was organised in December 2020. The Workshop had to be organised online due to ongoing COVID-19 situation in all partner countries. The partners discussed the progress achieved in 2020 and detailed the solutions and provisional calendar for 2021, concerning deliverables and milestones. Additionally, separate meetings regarding the setbacks and specific problems were organised as needed, between WP leader, deputy and project coordinators. The report of each meeting was made and all documentation is shared for each WP.

The joint Workshop on *Brucella canis* in Europe: Gaps and challenges in controlling the spread, was organised with another OH EJP – JIP COHESIVE. A survey was conducted with a questionnaire distributed among various laboratories, including EU national reference labs, OIE labs and private organisations, reaching 150 participants from 46 countries. The current scenario concerning epidemiological situation, detection methods and legislation was analysed in accordance with available data. The two projects decided on preparation of White Paper document, the review of current scientific facts on *Brucella canis*, conducted surveys, diagnostic methods, epidemiological situation in Europe and group recommendations for future actions. Document will be prepared by the end of 2021.

JRP17-WP7-T2- Data management

DMPs are verified by consortium, uploaded to the CDP platform and validated.

JRP17-WP7-T3- Risk management

Consortium still believes that project can be delivered with six months extension, without sacrifying none of the deliverables and milestones. Primarily the sampling collection was impacted due to the confinement measures in all partner countries. The additional delays have been experienced regarding the WP3, due to the ongoing COVID-19 situation, specifically in the WP leader's country (UK), and additional none counted impact of BREXIT. The project coordinators are working with WP leader and deputy to assure that additional delays will be respected and deliverables finished by the end of the 2021.

JRP17-WP7-T4- Synthesis and dissemination of recommendations coming from the project outputs

WP-1 T-1 and WP-2 T-1 will produce the base for the reporting system of emerging atypical *Brucella* spp

5.1.4.3.11.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
17	D-JRP19-WP1.Del1	Creation of a common database to share information about emerging Brucella and reservoirs among the consortium partners	M31	August 2020		Public: OHEJP_JRP17_IDEMBRU_Data_management_datasheet | Zenodo https://zenodo.org/record/4572555#.YUC5OzhDtN0	4
	D-JRP19-WP2.Del1	Set-up of a human brucellosis network	M42	February 2021		Public: OHEJP JRP-17 IDEMBRU Work package 2 Deliverable 1 | Zenodo https://zenodo.org/record/4569910#.YUC5RjhDtM1 Brucella canis workshop | Zenodo	5
17	D-JRP19-WP2.Del2	Creation of human samples collections (serum, samples, strains) of emerging Brucella species and related environments	M44		February 2022	Postponed due to COVID-19 pandemic	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
17	D-JRP19-WP3.Del1	Standard operating procedures for the extraction of Brucella DNA from complex matrices	M42		December 2021	The WP3 leaders (APHA) have experienced a number of delays to the completion of this deliverable, which relies to a significant degree on the distribution of strains to partner institutes. These arise from the on-going impacts of the COVID-19 pandemic at the work-package lead institute (APHA), as well as other local factors, such as staff availability. A revised work-plan has been established, and discussed with the project consortium, which will allow the deliverable to be completed by December 2021.	
17	D-JRP19-WP3.Del2	Harmonised whole genome sequencing and bioinformatic protocols	M42		December 2021	The WP3 leaders (APHA) have experienced a number of delays to the completion of this deliverable, which relies to a significant degree on the distribution of strains to partner institutes. These arise from the on-going impacts of the COVID-19 pandemic at the work-package lead institute (APHA), as well as other local factors, such as staff availability. A revised work-plan has been established, and discussed with the project consortium, which will allow the deliverable to be completed by December 2021.	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
17	D-JRP19-WP4.Del3	Drafted RNASeq protocols to identify regulatory differences between classical species and emerging Brucella strains	M60		December 2022		
17	D-JRP19-WP7.Del1	First draft of data management plan	M36	December 2020	No	Presented to consortium for consideration	8
17	D-JRP19-WP7.Del2	Creation of a data sharing common platform	M48		December 2021	Confidential	4
17	D-JRP19-WP7.Del3	Final draft of data management plan	M36	April 2021	Yes	Sent to OH EJP for publication	8
17	D-JRP19-WP7.Del4	Report of the first annual workshop including exchanges between partners and inputs from external stakeholders	M40	June 2021	June 2021	Public: One Health EJP_JRP17 WP7, Del4 Annual Workshop Report | Zenodo https://zenodo.org/record/5389832#.YUC5fDhDtM1	8

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
17	M-JRP19-M2	Conference call of the steering committee regrouping WP leaders + deputy leaders on data management	M42	Yes October 2020		
17	M-JRP19-M3	Definition of type of data generated for each WP and structure of the data sharing platform	M36	Yes October 2020		
17	M-JRP19-M4	Conference call of the steering committee regrouping WP leaders and deputy leaders	N/A	Yes October 2020		
17	M-JRP19-M5	Creation of an epidemiological questionnaire	M37	Yes December 2020		
17	M-JRP19-M6	Information on worldwide emerging Brucellae (shared database)	M34	Yes August 2020		
17	M-JRP19-M7	Definition of sampling and testing protocols (molecular, bacteriology and serology)	M36	June 2021		Presented to consortium for consideration
17	M-JRP19-M8	Definition of sampling and testing protocols (serology, bacteriology and molecular	M38	Yes May 2021		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
		biology)				
17	M-JRP19-M9	Drafted RNASeq protocols to identify regulatory differences between classical species and emerging Brucella strains	M40	Yes December 2020		
17	M-JRP19-M10	Definition of the programme of the annual workshop	N/A	Yes December 2020		
17	M-JRP19-M11	Organisation of accommodation and logistic aspects	N/A	N/A		
17	M-JRP19-M12	Implementation of first annual workshop	M38	Yes April 2021		
17	M-JRP19-M13	Definition of criteria to be included in the notification system	M38		No March 2022	
17	M-JRP19-M14	Choice of the informatic system to implement the notification system	M38	Yes April 2021		
17	M-JRP19-M15	Harmonisation of protocols for whole genome sequencing	M35		No December 2021	

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
17	M-JRP19-M16	Optimised protocols for the extraction of Brucella DNA from complex matrices	M42		No December 2021	
17	M-JRP19-M17	Creation of *Brucella* genomes database from publicly available and emerging isolates (WP 2)	44	August 2021		
17	M-JRP19-M18	Conference call of the steering committee regrouping WP leaders and deputy leaders	42	July 2021		
17	M-JRP19-M23	Definition of the programme of the annual workshop	44	August 2021		

5.1.4.3.11.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors already accepted your comments. Therefore, this part of the report has been closed.

5.1.4.3.11.5 Publications and additional outputs

Additional output

The project leaders participated in annual Stakeholder meeting including ECDC, EFSA, EEA, EMA, FAO, OIE, WHO-Euro. Among four projects who presented the research IDEMBRU presented the impact it will have on the protection of environment, toolkit usefulness for diagnostics of Brucella spp. in various ecosystems and management of contaminated areas.

Workshops

Brucella canis workshop, 10.5281/zenodo.4748926, Brucella canis workshop | Zenodo

Posters

Natural habitats for detection of emerging Brucella species: a new strategy to identify putative threats (IDEMBRU) - One Health EJP Annual Scientific Meeting 2021
ASM2021_Posters.pdf (ohejp2021.com) /

OHEJP2021_Abstractbook_A4_finalWEB.pdf

Ponsart Claire, Ferreira A.C., Daskalov H., Garofolo G., Melzer F., Freddi L., Girault G., Ferreira Vicente A., Ashford R., Whatmore A., Al Dahouk S., Prasse D., Kydyshov K., Cavaco Gonçalves S., De Massis F., Sacchini F., Milanov M., Pelerito A., van den Esker M., Kampfraath D., Djokic Vitomir

Molecular detection and differentiation within Brucellaceae family in urban and rural wildlife - One Health EJP Annual Scientific Meeting 2021
ASM2021_Posters.pdf (ohejp2021.com)
OHEJP2021_Abstractbook_A4_finalWEB.pdf

Djokic Vitomir, Michelet Lorraine, Girault Guillaume, Lecu Alexis, Freddi Luca, Ferreira Vincente Acacia, Perrot Ludivine, Laboutiere Lisa, Boschiroli Maria Laura, Ponsart Claire

An innovative microfluidic qPCR platform for high throughput testing of Brucella sp. and Ochrobactrum in environmental samples- One Health EJP Annual Scientific Meeting 2021
Guillaume Girault, Vitomir Djokic, Luca Freddi, Acacia Ferreira Vicente, Sabine Delannoy, Patrick Fach and Claire Ponsart
ASM2021_Posters.pdf (ohejp2021.com)

OHEJP2021_Abstractbook_A4_finalWEB.pdf

5.1.4.3.11.6 Data Management Plan

All DMPs were uploaded to the CDP platform and validated.

All DMPs have the name of responsible person, usually WP leaders and deputies including the project coordinators. Each DMP is identified by the unique code and all the associated documents will have the same type of code. Many DMPs are for now confidential, but how the project develops and data get published or incorporated into the toolbox, consortium will deliberate on regular monthly meetings weather to make certain data public, safeguarding the interests of all partners on the project.

5.1.4.3.11.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

- As majority of the consortium are OIE experts for Brucellosis in domestic animals, one part of the work was dedicated to the OIE expert group and updating the Brucellosis chapter. Chapters on B. melitensis, B. abortus and B. suis were updated/rewritten, while IDEMBRU consortium also proposed the new chapter on B. canis which will be finished soon.
- On May 18th IDEMBRU consortium organised the first such Workshop on the problematics of emerging zoonosis - *Brucella canis.* Within the workshop questionnaire was distributed encompassing current experiences, knowledge and problems in controlling this emerging infection that should be addressed as soon as possible. (the link for the questionnaire: https://survey.anses.fr/SurveyServer/s/animal_health/Survey_bcanis_may2021/questionnaire.htm . The results of this survey can be used for further decision making and steering joint efforts of COHESIVE and IDEMBRU towards finding solutions for *B. canis*.
- The project coordinator presented IDEMBRU at CES SABA meeting, with especial focus on B. canis and further scientific points that will be addressed within this OH EJP project.

5.1.4.3.12 JRP18-R2-ET1.1-MEmE

5.1.4.3.12.1 Summary of the work carried out in the Project

MEME is an international multicentre collaborative project that aims to fill research gaps highlighted by international agencies for the detection and control of zoonotic parasites *Echinococcus multilocularis* (Em) and *Echinococcus granulosus s.l.* (Eg), causing alveolar echinococcosis (AE) and cystic echinococcosis (CE), respectively. MEME focuses on standardization, harmonization and validation of existing parasitological and molecular methods, and the development and comparative assessment of innovative molecular tools to detect Em and Eg in the food chain. Production of epidemiological data on the presence of Em/Eg eggs in the food chain focuses on vegetables for human consumption and on canine faeces in selected endemic countries. MEME provides a comprehensive set of integrative activities to harmonize procedures, improve the detection and produce epidemiological data on potential pathways of transmission of Em and Eg.

Current achievements generated by MEME:

- Standard Operating Procedures for the sampling of matrices were produced.
- Sampling of different matrices from naturally or experimentally infected definitive and intermediate hosts is almost finished.
- Validation of the established parasitological (Segmental Sedimentation and Counting Technique, SSCT) and molecular diagnostic (multiplex-PCRs and MC-RT-PCR assay) procedures was started to detect Em and Eg in different matrices along the food chain.
- Results on development and validation of new tools have already been published: a) Comparison of two DNA extraction methods and two PCRs for the detection of Em in stool samples; b) Bayesian Analysis of three methods for diagnosis of CE in sheep; c) Microsatellite investigations of Eg cysts; d) Species detection of Eg by novel probe-based real-time PCRs; e) Validated method based on PCR-RFLP and multiplex PCR assay for the identification of Eg species; f) Identification of Eg G1/G3 by SNPs assays.
- Multicentre studies for the production of data relevant for epidemiological assessments (contamination of vegetables for human consumption by eggs of Em/Eg; prevalence of Em/Eg in dog faeces) are ongoing.
- Dissemination of project results at different levels (general public, populations at risk, biologists, veterinarians, clinicians, health authorities, policy makers and media) is ongoing.

Scientific papers published on peer-review journals under the framework of MEME/OHEJP:

- New global targets for NTDs in the WHO roadmap 2021–2030. *PLoS Negl Trop Dis*. 2021;15(5):e0009373.
- Identification of Echinococcus granulosus Genotypes G1 and G3 by SNPs Genotyping Assays. *Pathogens.* 2021 Jan 26;10(2):125.
- Molecular analysis suggests that Namibian cheetahs (Acinonyx jubatus) are definitive hosts of a so far undescribed *Besnoitia* species. Parasit Vectors. 2021 Apr 14;14(1):201.
- Comparison of Two DNA Extraction Methods and Two PCRs for Detection of Echinococcus multilocularis in the Stool Samples of Naturally Infected Red Foxes. *Animals (Basel).* 2020;10(12):2381.
- Cystic Echinococcosis: Clinical, Immunological, and Biomolecular Evaluation of Patients from Sardinia (Italy). *Pathogens.* 2020;9(6):907.
- A validated method to identify Echinococcus granulosus sensu lato at species level. *Infection, Genetics and Evolution.* 2020;85:104575.
- Bayesian Analysis of Three Methods for Diagnosis of Cystic Echinococcosis in Sheep. *Pathogens*. 2020;9(10):796.
- Species Detection within the Echinococcus granulosus sensu lato Complex by Novel Probe-

Based Real-Time PCRs. *Pathogens.* 2020;9(10):791.
- Microsatellite Investigations of Multiple Echinococcus granulosus Sensu Stricto Cysts in Single Hosts Reveal Different Patterns of Infection Events between Livestock and Humans. *Pathogens*. 2020;9(6):444.
- Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis. *The Lancet Global Health.* 2020;8(4):e470-e471.

5.1.4.3.12.2 Progress of the project: description of activities

WP1-Sampling strategy

JRP18-WP1-T1- SOPs for sampling in matrices

Task accomplished. The following Standard Operating Procedures (SOPs) were generated:

- Sampling of faecal material in dog faeces collected from the environment (WP1-T2) and their processing for molecular analysis.
- Sampling of *E. granulosus* cysts from naturally infected sheep and pigs at abattoirs (WP1-T2) and experimentally infected sheep (WP1-T3) and their processing for molecular analyses.
- Sampling of faeces and small/large intestines from experimentally infected foxes (WP1-T3) and their processing for parasitological and molecular analyses.
- Sampling of vegetables for human consumption (WP3-T6) and their processing for molecular analysis.

JRP18-WP1-T2- Matrices collection in the field from intermediate and definitive hosts

Task accomplished, although some samples will still be collected. The aim of this task is to collect different matrices from naturally infected definitive and intermediate hosts for their use in other WPs. Involved participants organized sampling from definitive and intermediate animal hosts. Totally, 590 intestines of red foxes, 167 intestines of arctic foxes, 100 intestines of raccoon dogs form endemic areas were collected; most of them were examined with SCT/SSCT. From positive intestines, Em worms were isolated. Intestines and isolated worms will be used for tasks connected with new PCRs and SSCT validation, and PTs organization. Additionally, faeces from distal part of large intestine were collected. A total of 63 red fox intestines form areas supposed to be free of Em were collected and 300 intestines of red foxes from Em free areas (Ireland) were collected. Other parasites (Mesocestoides spp., Taenia spp.) were isolated from red foxes intestines for specificity controls. A total of 210 samples of pigs' livers and 200 sheep's livers with suggestive lesions of Echinococcus tapeworm larvae were collected and prepared for identification. Additionally, few Em and Taenia hydatigena lesions isolated from Arvicola terrestris and Eg cysts isolated from sheep and pigs were collected. A total of 1,095 facel samples from dogs for epidemiological study were collected. Additionally, hundreds dog faecal samples from environment were collected as a potential matrix for validation.

JRP18-WP1-T3- Matrices collection from experimental animal models

Concerning the fox model, four infections were realized with two foxes euthanized and the two others were dewormed after 90 dpi. Worms (training on SSCT, WP4-T1), eggs (lettuce investigations, WP3-T6) and faeces were obtained. Interruptions due to COVID-19 disrupted the production of protoscoleces in the mouse model currently delaying the possibility of infecting foxes. The experimental material currently available consists of 5,000 Em eggs, 50 faeces and 100 microtubes of frozen protoscoleces.

WP2- Validation of parasitological and molecular assays

JRP18-WP2-T1- Segmental Sedimentation and Counting Technique, SSCT

Among the 594 red fox intestines already collected by five partners (NVI, PIWET, FLI, ANSES, BIOR), 477

were already analysed independently for each of the four segments of intestines resulting in 120 positive samples. Collection and analysis will continued in order to be finished in December 2021 (M48) with the aim of obtaining more positive intestines to estimate of the sensitivity of SSCT method. The protocol of validation was also used for raccoon dogs from Poland and Latvia in order to determine a potential combination of a pair of segments providing a high sensitivity. Among the 70 intestines analysed, 5 were infected by Em. These data will be combined with those from experimental results previously obtained.

JRP18-WP2-T2- Comparison of multiplex PCRs

This task focuses on the validation of molecular assays widely used for the detection of *Echinococcus* in definitive and intermediate hosts, targeting mitochondrial and nuclear markers:

Assay 1: Boubaker et al. (PLoS Negl Trop Dis, 2013), a single-tube multiplex PCR allowing discrimination of *Echinococcus* at the level of species/genotypes.

Assay 2: Trachsel et al. (Parasitology, 2007), a single-tube multiplex PCR targeting the definitive host for the identification of eggs belonging to *E. granulosus, E. multilocularis* and *Taenia* genus.

Assay 1 was tested resulting in conclusion that it is not possible to validate this method because of the unreliability of the molecular markers used in the multiplex PCR. Validation of assay 2 is ongoing and will be finished no later than December 2021 (M48).

JRP18-WP2-T3- Magnetic Capture - Real Time PCR assay

Two magnetic capture protocols, published by Isaksson et al., 2014 (10.1186/s13071-014-0583-6), and Maas et al., 2016 (10.1016/j.vetpar.2016.10.016), respectively, were performed in parallel in two laboratories (FLI and RIVM) using the artificially spiked fox faecal samples. The reproducibility of the methods and their analytical sensitivity were tested and compared in the two laboratories. In addition, these two methods were tested with a set of field faecal samples from foxes naturally infected with Em. Thus, diagnostic sensitivity of the two methods was also tested and compared. The data obtained are currently being analyzed and written up in a manuscript.

SOP for validation of Magnetic capture – realtime PCR assay: There was a delay with the milestone in this task and the lead on the task was transferred from SVA (SVA withdrew from MEME in 2021) to NVI in M37. The methods (DNA extraction with automatic washing and manual washing protocols, as well as three realtime PCR protocols) and accompanying training video (for magnetic capture DNA extraction with manual washing) were published on Zenodo as project restricted technical notes in M41 (DOIs: 10.5281/zenodo.4897969; 10.5281/zenodo.4746190; 10.5281/zenodo.4746181; 10.5281/zenodo.4746141). If needed additional guidance, via digital meeting platforms, has been offered to the seven laboratories (BIOR, CVRL, INIAV, ISS, PIWET, SSI, VFL) that have registered for the training. So far, none have requested this. The verification and ring test protocol is being finalised and has now been shared with the nine laboratories (those participating in training plus FLI, RIVM) that have registered for participation in the ring test. Feedback from the participants will be included before the final protocol is published on Zenodo. The ring test will be carried out once the laboratories that are establishing the method have provided documentation to show that verification results were successful. The ring test will consist of 5 faecal samples per laboratory that have potentially been spiked with E. multilocularis eggs. Participating laboratories should receive the ring test samples in month M47-48 as long as verification goes to plan and there are no further coronavirus-related restrictions to laboratory access.

WP3-Development validation of new tools and production of data relevant for epidemiological assessments

JRP18-WP3-T1- New molecular markers for Em and Eg s.l.: from rapid diagnostics to source attribution

ST1: new mitochondrial markers. Recent studies have revealed an unexpectedly high genetic diversity at mitochondrial genome level for Eg (*sensu stricto*) and for genotypes G6/G7. However, these studies covered only part of the genetic diversity since many important endemic regions were missing (e.g. China, Russia). Moreover, for several species including Em, the mitogenome data are scarce. In order to develop species/genotype specific diagnostic assays, additional mitogenome data are required for all *Echinococcus* species using a larger worldwide panel of different Eg (*sensu lato*) species and genotypes (N=300), and Em (N=200). Toehold Exchange Probes based diagnostic tool will be developed to provide a reliable and rapid identification of species/genotypes of Eg (*sensu lato*) and Em.
We have finished analysis (quality control and assembly) of near-complete mitogenomes for additional 27 samples from Pakistan and 12 from India. These sequences are included to the total dataset of near-complete mitogenomes.

ST2: New microsatellite markers. The screening of the Eg genome has resulted in the identification of 15 new microsatellites targets, which are currently evaluated for their polymorphism, reproducibility, limit of detection, quality of the electrophoretic profiles and their specificity. The selected targets will be associated to EgSca6 microsatellite previously described by MEME (M'Rad et al., 2020) in order to obtain a panel with a high discriminatory power. The possibility to use these microsatellites for large phylogenetic studies was initiated for the validation of the clustering method. The collection of *E. granulosus s.s.* DNA samples from North Africa (Morocco, Tunisia) and Europe (France, Moldova) are already available and will be completed for testing polymorphism of the microsatellites.
Four new microsatellite targets were identified and needs to be evaluated.

ST3: NGS-based method. The very high variability of Eg (*sensu stricto*) at the mitogenome level provides a possibility to perform source attribution analysis. Using the mitogenome data together with microsatellite fingerprinting may constitute reliable means to track the source of infection. Previous studies have shown that samples collected from the same area can be distinguished from samples from other areas based on complete or near-complete mitogenome data. At first, a method will be developed to sequence 96 mitogenomes with a single sequencing run. For this, we have already developed primers that enable PCR-amplification of the entire mitogenome in a single PCR. We aim to use 96 different barcodes and the PacBio's Single Molecule, Real-Time (SMRT) Sequencing in the Sequel System, which allows easy and cost effective generation of highly accurate long reads (>99% single-molecule read accuracy) for DNA amplicons, such as the complete mitochondrial genomes.
We have started developing the NGS assay to sequence 96 mitogenomes with a single sequencing run. For this, we have already developed primers that enable PCR-amplification of the entire mitogenome in a single PCR and have successfully tested these. However, this assay requires samples of good quality. As the quality of parasite samples varies and often important samples are of low quality, we started to develop primers for such samples that consists of several primer pairs.

JRP18-WP3-T2- New multiplex TaqMan qPCR for detection and genotyping of Eg s.l. and Em

We established six TaqMan® probe-based qPCRs that can be used for the diagnosis of Em and Eg genotypes in five epidemiologically relevant species and subgroups, i.e. *E. granulosus sensu stricto* (G1, G3), *E. equinus* (G4), *E. ortleppi* (G5), the *E. canadensis* complex (G6 to G8 and G10) and a single genotype (G8) of the *E. canadensis* complex as a single-step genotyping technique. It also allows differentiating *E. granulosus* samples from other *Echinococcus* or *Taenia* species in samples derived from cystic or faecal material. The qPCRs show high efficiency (ranging between 99% and 106%), high analytical specificity (100%) and sensitivity (ranging between 0.6 and 1.4 copies/µl), when used with DNA obtained from cysts or from cloned PCR products. Therefore, they are suitable for a PCR-based diagnosis of CE in intermediate hosts, including humans as aberrant intermediate hosts. These qPCRs will now be combined to develop a TaqMan probe-based multiplex Real-Time qPCR as a tool for a simultaneous, rapid diagnosis and typing of Eg (*sensu lato*) and Em infections. Moreover, experiments have been initiated to develop TaqMan® probe-based qPCR assays that differentiate between individual members of the *E. canadensis* complex (G6, G7, G8 and G10).

JRP18-WP3-T3- Detection of Em/Eg in complex samples: sequencing using Regions Specific Extraction (RSE) and NGS

First step was to establish a pipeline for nanopore mtDNA sequencing and to allow sufficient data generation and for testing data analysis tools. Work started using DNA from positive controls included from the MC-DNA EmNok surveillance method, as these were spiked faecal samples isolated using magnetic capture of mtDNA from EM. The processed samples contained lower number of targets compared with regular EM-samples so in order to get nanopore protocols working on such material modifications needed to be made. As an alternative enrichment step, Illustra TempliPhi, was included as a means of generating more template DNA for sequencing. A few dilutions were carried out to accommodate for faint samples. In addition to the positive EM control from MC method, a standard prepared DNA samples from Taenia spp. were also included as controls. Samples were cleaned and DNA was measured. Further, they were prepared according to the selected nanopore sequence kit and some runs on Minion flongle flowcell was attempted on the products. All runs failed to generate data, and it was discovered later that ONT was forced to made changes to the sequence kit due to incompatibility on one of the chemicals included with the flongle flowcell. The failure of runs are likely to be due to this fault. We have not had time to rerun these samples with the new kit and reagents, as the first part of 2021 our labs were mostly inaccessible partly, due to COVID-19 pandemic, but for the most part due to packing down and moving our institute from Oslo to a new campus at Ås, finalized in June 2021.

JRP18-WP3-T4- Proteomic study on biomarker discovery in exosomes from animal plasma

We had serious technical difficulties regarding the proteomic study (at ISS, Italy) from plasma of experimentally infected (and controlled) sheep in Portugal (at INIAV, Portugal). Because of COVID-19, we were late in obtaining ethics committee approval at INIAV. Afterwards, in November 2020 we made 2 attempts with parasite protoscoleces collected from sheep in Sardinia (IZSS, Italy) to infect foxes in Nancy, France (ANSES) where ethics committee approval was also obtained for foxes. Unfortunately, in January 2021 we did not obtain Eg worms from fox experimental model (neither at flotation of faecal samples nor at PCR examination). During February/august 2021 we mobilized the international community to provide Eg eggs from experimentally or naturally infected canids. We received positive feedback from Australia, Iraqi Kurdistan, Kazakhstan and China, willing to collect and subsequently provide eggs. National lockdowns and national restrictions due to COVID-19 affected such field-collaboration since all these participants have retired. These eggs would have been used to infect sheep (animals are still housed in Portugal) and collect plasma for proteomic analyses searching for biomarkers of infection. We also discussed to modify ethical clearance at INIAV and try to infect sheep with Eg protoscoleces, instead of Eg egg. Since intraperitoneal injection of protoscoleces is not mimicking the natural infection, this would affect molecular pathways and therefore proteomic analysis outcome. Even if we found another solution, there would be no time to implement it before the end of MEME because of the long time (at least 1 year) it takes for the parasite to develop in sheep. We confirm that COVID19-pandemic has definitively discontinued task 4 of WP3.

JRP18-WP3-T5- Assessment of newly developed molecular methods against the existing techniques

The different molecular methods developed in MEME (RFLP and qPCR for Eg; see MEME publications) will be compared with already available methods regarding limit of detection of each assay but also by evaluating sensitivity and specificity using DNA samples obtained from field samples but also from experimental infection. Furthermore, faecal samples obtained during SSCT will be used to be compare with results obtained by processing the available Em copro-qPCR. This evaluation will be realized using the same DNA samples in different laboratories.

JRP18-WP3-T6- Contamination of vegetables for human consumption by Em/Eg

The filtration method (Guggisberg et al., 2020) combined to real-time PCR detection in lettuces was validated at ANSES with a probability of detection of 3 Em eggs in 95% of the cases (75% for 2 eggs and 50% for 1 egg). A first survey during the summer 2020 in France has collected 106 lettuces grouped in 35 pools corresponding to origin of samples, each constituted of 2 to 5 lettuces. Seventeen pools were from private kitchen gardens (n=44) and from 18 local markets (n=62). Em was detected in two pools from local markets and one from kitchen gardens when *Hydatigera* sp. (cat tapeworm) was detected in four and two pools from kitchen gardens and local markets, respectively. In the summer 2021, a survey is currently organized through Europe including 15 partners of MEME from 12 countries endemic for Em and/or Eg. From 50 to 100 lettuces are planned be sampled by each partner which will implement the first washing step and then the frozen pellet will be send to ANSES where the filtration method and molecular detection will be realized in order to minimize bias. We inserted in MEME a new and additional subtask on detection of *Echinococcus* eggs in strawberries and blueberries. The filtration method was also validated on strawberries with a limit of detection of 3 eggs in 200g (88% for both 2 and 1 egg) and will be done for blueberries in 2022. A survey is underway in France by collecting strawberries from local markets and is planned to be extended by including blueberries to other partners in 2022.

JRP18-WP3-T7- Prevalence of Em/Eg in dog (faecal samples) from selected geographical areas

Eight MEME partners were involved in this task (six of them have started the implementation of the study, two will start soon because of COVID-19 pandemic). Until now 1095 samples of dog faeces were collected, most of them together with filled questionnaires. About 1000 more samples are planned to be collected before the end of this study. DNA was extracted from 389 samples. In France, 109 samples have already been examined using multiplex PCR and qPCR: no positive for *Echinococcus* – therefore, 1 *Taenia krabbei*, 1 *Taenia serialis* and 1 *Mesocestoides* sp. positive samples were recorded. The expected delayed end of the task is M54.

JRP18-WP3-T8- Potential human risk factors by means of questionnaires

Due to COVID-19 impact on hospitals, we replaced this task with "Human source attribution of cerebral CE by molecular approach and its quantitative assessment in the literature". This task focuses on the first source attribution based on molecular methods of a rare case of cerebral CE in a child from Roman campagna. A literature search was also conducted on 6 databases with Boolean terms for the clinical and epidemiological quantification of cerebral CE at global level. No limitation of year of publication was envisaged. Inclusion criteria were: clinical reports including case reports, case series, clinical trials, retrospective and prospective cases of confirmed human cerebral CE. Exclusion criteria were: animal cases, AE cases and no confirmed cases of cerebral CE. The search identified 1,776 relevant articles which were selected by title and abstract. Relevant data from full text of 422 papers were currently extracted.

WP4-Training, dissemination and proficiency testing schemes

JRP18-WP4-T1- Trainings

This WP focuses on establishing the most effective methods for training scientists from institutions participating to MEME in the parasitological and molecular identification of *Echinococcus* spp. Due to COVID-19 pandemic, no physical training was conducted until now. For this reason, NVI generated online training on "Magnetic capture of *Echinococcus multilocularis* DNA from red fox (*Vulpes vulpes*) faecal samples: DNA extraction with manual washing steps". A training movie showing each of the steps can be viewed on YouTube at: https://www.youtube.com/embed/hdo3mE8oMKE?rel=0

JRP18-WP4-T2- Dissemination of project results

This task focuses on establishing the most effective methods for disseminating project results at

different levels. Due to COVID-19 pandemic non-participation to physical events prevent the dissemination, while it was done during online events ("One Health EJP Annual Scientific Meeting 2021" and "Annual meeting of the European Reference Laboratory for Parasites 2020") and publishing general scientific papers for a wide scientific audience (see papers on *Lancet Global Health* and *PLOS Neglected Tropical Diseases*).

JRP18-WP4-T3- Organization of selected PTs from WP2 and WP3

A Proficiency Testing scheme (PT) on "Segmental Sedimentation and Counting Technique (SSCT)" is planned for October 2021 (M46) with at least ten participants from MEME and potentially additional European national reference laboratory outside of MEME. The participants will received 5 segments of intestines (including negative and positive ones) in order to perform sedimentation of intestinal mucosa and worms identification. A second PT on "Verification of *Echinococcus multilocularis* magnetic capture method in red fox (*Vulpes vulpes*) faeces and proficiency test" is planned for December 2021 (M48) for around ten participants.

WP5-Scientific and administrative management

JRP18-WP5-T1- Setup of the project

Task accomplished.

JRP18-WP5-T2- Administrative and scientific management

Ongoing activity throughout the lifespan of MEME to accomplish its administrative and scientific management. Depending on epidemiological scenario on COVID-19, we are planning to organize a physical annual meeting of MEME on January 2022 at FLI, Germany.

JRP18-WP5-T3- Ethics management

Ongoing activity to accomplish all ethic needs of MEME.

5.1.4.3.12.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
18		SOPs for sampling in matrices shared within the Consortium	M28	M31		Public https://zenodo.org/record/4455196#.YAmIsxbSKgo	2
18	D-JRP22-WP1.2	Collection of samples in the field finalized	M36	M45		Public https://zenodo.org/record/5499866#.YTsvmc_OMzk	8
18	D-JRP22-WP2.1	Validation of SSCT	M36	M45	M48	Public https://zenodo.org/record/5499551#.YTr8sM_OMzk	8
18	D-JRP22-WP2.2	Validation of multiplex PCRs	M44	M45	M48	Public https://zenodo.org/record/5499803#.YTsn-M_OMzk	8
18	D-JRP22-WP2.3	Validation of Magnetic Capture Real-time PCR assay	M44	M45	M48	Public https://zenodo.org/record/5500594#.YTusOaTOMzk	8
18	D-JRP22-WP5.1	Data Management Plan (DMP)	M30	M45		Public https://zenodo.org/record/5415292#.YTITD9_OOgo	8

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
18	D-JRP22-WP5.2	Periodic technical and financial reports to EJP/Commission	M36	M45		Public https://zenodo.org/record/5413157#.YTH66d_OOgo	8
18	D-JRP22-WP5.5	Kick-off annual meeting in Malzéville (France) by ANSES	M27	M28		Public https://zenodo.org/record/4455256#.YAmKvxbSKgo	8
18	D-JRP22-WP5.6	Interim annual meeting in Greifswald (Germany) by FLI	M38		M49	Depending on epidemiological scenario on COVID-19, we are planning to organize a physical interim annual meeting of MEME on January 2022 at FLI, Germany.	8

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
18	M-JRP22-01	Tasks and responsibility allocation	M25	Yes		Kick off meeting in France used for the allocation of the tasks and responsibilities
18	M-JRP22-02	Organization of Kick-off annual meeting by ANSES	M26	Yes		
18	M-JRP22-03	Ethics approvals for the use of animal model	M28	Yes		
18	M-JRP22-04	SOP for validation of SSCT	M30	Yes		
18	M-JRP22-05	SOP for validation of multiplex PCRs	M30	yes		
18	M-JRP22-06	SOP for Validation of Magnetic Capture - Real Time PCR assay	M41	yes		
18	M-JRP22-07	Protocols for New molecular methods, NGS included	M30	yes for T1/2; draft for T3	M48	
18	M-JRP22-08	Protocol for proteomic analysis in animals plasma	M30	no		COVID19: serious concerns on feasibility of this task. See WP3-T4
18	M-JRP22-09	Protocol for contamination of vegetables by Em/Eg	M30	yes		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
18	M-JRP22-10	Protocol for prevalence of Em/Eg in dog	M30	yes		
18	M-JRP22-11	(Questionnaire scheme for potential human risk factors) Tak replaced with literature search for "Human source attribution of cerebral CE".	M30	yes		
18	M-JRP22-12	Interim evaluation of collection of samples in the filed	M32	yes		
18	M-JRP22-13	Preparation of technical and financial reports to EJP/Commission	M35	yes		
18	M-JRP22-14	Organization of training periods	M36	no	M54	Due to national lock-downs.
18	M-JRP22-15	Interim evaluation of collection of samples from experimental animal model	M42	yes		
18	M-JRP22-16	Selection of new/old methods to be compared	M44	no	M48	
18	M-JRP22-17	Organization of interim annual meeting by FLI	M37	no	M49	Due to national lock-downs. Planned for January 2021.

5.1.4.3.12.4 Follow-up of the recommendations and comments by the Ethics Advisors

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021	Comments PL, June 2021
(1) Human In case of Human participation, the beneficiaries must confirm that relevant authorisations have been obtained.	Authorizations for the submission of questionnaires will be requested to the ethics committee of participating centres.	This is not a complete response. The beneficiaries must confirm that the ethical oversight has been obtained and that they will be kept on file and will be presented upon request.	Referral hospitals and medical doctors that were willing to participate to this multicentre European study by means of questionnaire are now overwhelmed by COVID-19 pandemic and they do not have access to patients affected by cystic and alveolar echinococcosis. It should be stressed that most of these hospitals were converted to COVID-Hospitals. For the above mentioned reasons, these centres are currently not even capable of dealing with local ethics committees.	The beneficiaries may find a way to get an ethical permission through other ethics bodies.	As anticipated, task replaced by "Human source attribution of cerebral CE by molecular approach and its quantitative assessment in the literature". Ethic clearence approval obtained from local ethic committee at "Bambino Gesu` Children's Hospital" for the cerebral CE case in a child from Roman campagna.
(2) personal data processing The beneficiaries must confirm that the personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of	It will be taken into account.	This is not a complete response. The beneficiaries must confirm that GDPR details when the work has ethical oversight	Data protection aspects are carefully considered in MEME. In particular, we focus on limiting personal data collection to what is necessary in relation to the purpose. Since the human questionnaire on potential risk factors was paused (see previous point), we	Satisfactory reply	Closed

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021	Comments PL, June 2021
processing the data obtained must be provided.			currently do not have personal data to be processed according to to GDPR (EU 2016/679). DPO will be immediately identified in case we will further proceed with this task.		
(3) Animals Further details are needed on the use of animals which are legal animals and / or experimentation animals (e.g. red foxes). Please clearly state the 3Rs aspects of this work. Please describe how the beneficiaries are complying with access to animal material requirements and animal welfare laws.	ANSES: According to the animal welfare and the experimentation rule, we have the authorization to work on wildlife in our animals facilities in experimentation (decree n° 2013-118 dated 1 February 2013 relating to the protection of animals used for scientific purpose; Order of April 18, 2016 setting the general operating rules for animal breeding facilities for non-domestic animals D54-431-1). Concerning the infection of mice and foxes by Echinococcus (Em and Eg), we have obtained the authorization for experimental infection from the from the ethics committee (N° du Dossier: 16-073 N° APAFiS:	Thank you for the fuller details and the authorisation but please can you clarify the comment on the Reduction on Mice numbers. It is not clear from what you have submitted.	We follow the 3Rs rules: Replacement: it is not possible since we need the parasite (eggs and worms) as reference materials and in vitro models doe's not work at this aim. Reduction: The number of mice to be used for maintaining the strain was reduced to 10 mice every 2 months. Protoscoleces successfully develop in only 60% of mice, the other mice develop only unfertile parasitic mass that can not be used for reinfections. For as concern foxes, when the protocol does not need euthanasia they are kept in specific animal facility for the collection of infected faeces and reused after deworming. Refinement: To increase	No comment	Closed ?

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021	Comments PL, June 2021
	2016091313348095 N° de l'Avis : 11/10/16-3). Concerning the rule of 3R the number of mice to use for maintaining the strain is reduced to 10 mice every 2 months. For as concern foxes, when the protocol do not need euthanasia they are kept in animal facility for the collection of infected faeces and reused after deworming.		welfare of mice that stay in experimental box from 4 to 6 months, we enrich their environment by add mouse house, igloos and paper tubes and wooden pieces. For the welfare of foxes and the enrichment of their environment, we add in the fox cages, cattle bone, balls and cardboard boxes.		
(4) Health and Safety The beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	All participants are complying on health and safety procedures according to EU legislation.	Satisfactory reply	Closed	Closed	Closed
(5) non EU countries (China) The beneficiaries must confirm that the research conducted outside the EU is in compliance with H2020 rules.	Authorization for fox and mice model obtained from ANSES, France. Authorization for sheep model pending for INIAV, Portugal.	Please update your ethics submission on the status or approval of the INIAV application in due course	The ethical authorization for the experimental infection of sheep with Echinococcus granulosus have been obtained from the Portuguese Veterinary Authority (DGAV – Direção Geral de Alimentação e Veterinária) on 14th December 2020, signed by	Satisfactory reply	Closed

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021	Comments PL, June 2021
			the General Director Susana Pombo (ref N 0421/000/000/2020). A selection of 20 one year old female sheep has been kept in adapted facilities with increased biosafety, located in Santarém, Portugal, at INIAV research centre.		
(5) non EU countries (China) (2) For the human participation in this project, the beneficiaries must confirm that relevant authorisations have been obtained.	Not applicable. China will no longer participate to MEME OHEJP. Any collaboration during the project will be in compliance with H2020 rules.	Satisfactory reply	Closed	Closed	Closed

5.1.4.3.12.5 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
New global targets for NTDs in the WHO roadmap 2021–2030. https://doi.org/10.1371/journal.pntd.0009373 https://zenodo.org/record/4771738#.YKTFCqHOOgp	YES		Yes, 0 € (free of charge; invited editorial)
Cystic Echinococcosis: Clinical, Immunological, and Biomolecular Evaluation of Patients from Sardinia (Italy). https://doi.org/10.3390/pathogens9110907 https://zenodo.org/record/4159681	YES		Yes, 1.389 €
Molecular analysis suggests that Namibian cheetahs (Acinonyx jubatus) are definitive hosts of a so far undescribed *Besnoitia* species. https://doi.org/10.3390/pathogens10020125 https://zenodo.org/record/4694200#.YTDH7t_OOgo	YES		Yes, 2.190 €
Comparison of Two DNA Extraction Methods and Two PCRs for Detection of Echinococcus multilocularis in the Stool Samples of Naturally Infected Red Foxes. https://doi.org/10.3390/ani10122381 https://zenodo.org/record/4384600	YES		Yes, 1.481 €
Species detection within the Echinococcus granulosus sensu lato complex by novel probe based Real-Time PCRs. https://doi.org/10.3390/pathogens9100791 https://zenodo.org/record/4061718#.X6PY3WhKjcc	YES		Yes, 1.258,81 €
A validated method to identify Echinococcus granulosus sensu lato at species level.	YES		Yes, 2.300 €

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
https://doi.org/10.1016/j.meegid.2020.104575 https://zenodo.org/record/4066416#.X6UQMWhKjcc			
Identification of Echinococcus granulosus Genotypes G1 and G3 by SNPs Genotyping Assays. https://doi.org/10.3390/pathogens10020125 https://zenodo.org/record/4471215#.YGLkiq8zZM0	YES		Yes, 696,38
Bayesian analysis to evaluate three diagnostic methods for Cystic Echinococcosis in sheep. https://doi.org/10.3390/pathogens9100796 https://zenodo.org/record/4061823#.X6PZl2hKjcc	YES		Yes, 1.389,79 €
Microsatellite Investigations of Multiple Echinococcus Granulosus Sensu Stricto Cysts in Single Hosts Reveal Different Patterns of Infection Events between Livestock and Humans. https://doi.org/10.3390/pathogens9060444 https://zenodo.org/record/3894117#.XvyQ6ygzZM0	YES		Yes, 654,56 €
Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis. https://doi.org/10.1016/S2214-109X(20)30066-8 https://zenodo.org/record/3730559#.Xn3FaIhKi70	YES		Yes, 0 € (free of charge; invited comment)

Additional output

Presentation of MEME project at:

- OH-EJP Summer School 2021. In: The neglected zoonoses cystic and alveolar echinococcosis. 26 July - 6 August 2021. Held online. Rome, Italy.

- Keynote Lecture. Echinococcus parasites: from correct identification to global phylogeography. 9th Conference of the Scandinavian-Baltic Society for Parasitology: Parasites in a Changing World. 21-23 April 2021. Virtual conference; Organised by Nature Research Centre and Young Academy of the Lithuanian Academy of Sciences. Vilnius, Lithuania.
- One Health EJP Annual Scientific meeting. Held online. 9-11 June 2021. Copenhagen, Denmark.
- Internal dissemination online event 'OHEJP at SSI. 4 May 2021. Copenhagen, Denmark.
- 15th Workshop of the National Reference Laboratories for Parasites. 15 December 2020. Rome, Italy.
- One Health EJP Annual Scientific meeting, held online. 27-29 May 2020. Prague, Czech Republic.
- Kick off meeting of MEME. 5-6 February 2020. Nancy, France.
- XXVIII World Congress on Echinococcosis. 29-31 October 2019. Lima, Peru.

5.1.4.3.12.6 Data Management Plan

DMP comprising the descriptions of all the data generated in the project was uploaded in December 2020. Next update planned no later than December 2021.

5.1.4.3.12.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

Ongoing and planned collaborations with the following entities:

- the other parasitology-JRPs: PARADISE and TOXOSOURCES;
- the European Reference Laboratory for Parasites (EURLP);
- the WHO Collaborating Centre WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis (in humans and animals);
- PERITAS project (molecular ePidemiological studiEs on pathways of tRansmIssion and long lasTing cApacity building to prevent cyStic echinococcosis infection). 2018-2021. Funded by the EC under EULAC-Health.

5.1.4.3.13 JRP19-R2-ET1.1-PARADISE

5.1.4.3.13.1 Summary of the work carried out in the Project

The most relevant progresses for the research-orientated activities are summarized below:

- WP2: A large effort from the Consortium allowed collecting *C. parvum* and *G. duodenalis* isolates for whole genome sequencing experiments across Europe. This resulted in the generation of >100 novel *C. parvum* genomes from both humans and ruminants collected across Europe, and of >40 novel *G. duodenalis* (assemblage B) genomes, mostly of human origin. The *in silico* metagenomics approach demonstrated the presence of parasite sequences in metagenomes from various matrices (water, calf gut microbiota). A manuscript with a focus on *Cryptosporidium* and a description of a methodology that can increase the specificity of detection has been published. The amplicon-based sequencing approach was further tested, but detection of flagellates (including *Giardia*) based on 18SrDNA remained problematic. Therefore, to overcome this problem, different detection markers will be identified based on the FLUIDIGM technology. A manuscript describing the application of the platform to zoonotic protozoa present in pig faeces has been published. Reference material (parasite cysts and genomic DNA) has been produced and used to spike plant DNA at different concentrations. The NGS experiments, planned for September 2021, will allow comparing the limit of detection of amplicon-based and shotgun metagenomics approaches.
- WP3: The first selection of 28 *C. parvum* regions endowed with high genetic variability was completed based on a comparative analysis of >140 genomes. In the case of *G. duodenalis* assemblage B, 20 regions with high genetic variability were identified by comparing 18 available genomes with a newly developed analytical pipeline. Laboratory tests by PCR and Sanger sequencing have been performed for all candidates and each parasite. For further validation of the markers, about 500 parasite samples (genomic DNA, faeces) have been made available from the Consortium.
- WP4: After immunization of two new world camelids with extracts from *Giardia duodenalis* cysts or *Cryptosporidium parvum* oocysts, PBMCs were isolated and stored in TRIZOL for later RNA preparation. Reactivity of the animal immune sera against the respective antigens was verified by ELISA and IFA. RNA from PBMCs was extracted and cDNA libraries of variable domains of heavy-chain only antibodies (VHH) were produced. Subsequent screening of *G. duodenalis* library identified several VHH clones reacting against cysts. Screening of cDNA library against *C. parvum* oocysts is still in progress. For the aptamers, a new strategy, based on high-throughput sequencing (HTS) of DNA sequences from each SELEX round and each replica, was applied. After data analysis with specific bioinformatics tools, a core of sequences progressively enriched throughout the SELEX process were identified for both *C. parvum* and *G. duodenalis*. Experiments to evaluate the binding properties and affinity of the potential binders are under way. For DNA fishing, two capture systems were designed for *Cryptosporidium* (18S rDNA and gp60 loci) and one for *Giardia* (beta-giardin). Additional capture systems will be designed based on the novel markers identified by WP2/WP3.

In short, delay in some activities has occurred, but the project is proceeding as planned.

5.1.4.3.13.2 Progress of the project: description of activities

WP1-Coordination and impact

JRP19-WP-1-T1- Management, coordination and communication

In Year 4, the restrictions caused by the Covid-19 pandemics have continued, and communication has mostly relied upon virtual platforms. WP and Task leaders did meet regularly to follow the project's activities, planning experiments and discuss new strategies. All partners have been kept informed of the project's progress by e-mail and have been consulted before important deadlines (e.g., at the time

of reports). The members of the Steering committee have been nominated, while the composition of the Advisory Board needs to be completed.

The Data Management Plan (DMP) is updated regularly. Outcomes are made available following FAIR principle. Dissemination of the outcomes has included presentations at conferences, workshops and scientific publications. Further dissemination includes presentation at the European Multicolloquium of Parasitology (EMOP, Belgrade, Serbia, 12-16 October 2021).

The Annual meeting of Year 4, originally scheduled for M42 has been postponed to M45, and will be organized as a virtual meeting, as the ongoing pandemics does not allow a meeting in presence. In the first half of Year4, the Consortium has published two manuscripts.

WP2-NGS-based genomics and metagenomics

JRP19-WP2-T1- NGS-based genome study of selected isolates of C. parvum and G. duodenalis

The goal of this task is to generate new whole genome data of isolates of *Cryptosporidium parvum* and *Giardia duodenalis*, and to ensure that isolates from different European countries and different hosts are included. Many partner institutes (ANSES, INIAV, ISS, NVI, OKI, PIWET, RKI, SSI and SVA) and associated partners (BIOR) have been able to collect faecal samples positive for *Cryptosporidium* and *Giardia*. Samples were shipped to SVA, RKI and ISS, where high quality genomic DNA was prepared, checked for the presence of bacterial contaminants by PCR, submitted (for *Cryptosporidium*) to whole genome amplification (WGA), purified and finally sent to ANSES for NGS-based whole genome sequencing experiments. All NGS experiments were based on Illumina technology (short reads, 2x150 paired ends), and scaled to achieve an average 50X coverage.

Up to now, >100 *C. parvum* isolates from humans and ruminants, collected in Denmark, Finland, France, Germany, Hungary, Italy, Norway, Poland, Portugal and Sweden, have been sequenced. To this dataset, 40 additional genomes from previous projects and collaborations, mostly from isolates collected in Italy and the UK, have been made available and included in downstream analyses.

For *Giardia*, 40 genomes of isolates of Assemblage B have been sequenced, mostly of human origin and after isolation of the parasite by *in vitro* culturing. Many additional genomes generated during previous projects and collaborations were included in downstream analyses, and also represented isolates of Assemblage A. A cloud storage space for sharing raw sequence data has been set up by SVA, and a password-protected access has been granted to all partners involved in the bioinformatics analyses. SVA has further implemented the *Cryptosporidium* pipeline developed by a previous EU Horizon 2020 project (COMPARE), whereas RKI has developed a new analytical pipeline to process *Giardia* NGS sequence data.

JRP19-WP2-T2- In silico analyses of metagenomes for detection of foodborne parasites (protozoa and helminths)

The goal of this task is to demonstrate that parasite sequences present in complex metagenomics datasets can be detected with high specificity and to evaluate the limit of detection. Indeed, specificity and sensitivity are essential parameters to consider when exploring the applicability of metagenomics as a platform for foodborne parasites detection. Genome information is available for many protozoa and helminths, and a reference database can be constructed, against which metagenomics reads can be queried. Such a reference database is close to being completed, but the deadline for the associated milestone (M-JRP-PARADISE-5, Referenced database of foodborne parasite genomes established) has been moved to M48.
During 2021, the pipeline code has been optimized to speed up the processing time. Moreover, the scalability of the pipeline was investigated in view of the huge size of the metagenomics datasets to interrogate. A manuscript presenting several test cases and focusing on bioinformatics strategies to increase the specificity of detection has been published in Frontiers in Microbiology.

A test case of particular interest was a metagenome dataset (2.5 billion reads) derived from lettuce; the lettuce was a suspected vehicle of infection in an outbreak of human cryptosporidiosis. Using kraken2 for classification of the reads, 4090 reads of *Cryptosporidium* were identified, of which 3436 as *C. parvum*. Other parasites were also identified, including *Toxoplasma* (1932 reads), *Plasmodium yoelli* (2877 reads) and *Theileria* spp. (75 reads). However, using more stringent pipeline settings, no reads from these latter parasite species were retrieved. This suggests that alignment-based tools, such as Kma and/or BWA-MEM, are best suited for taxonomical classification of the reads, but are more demanding in terms of computing time. In order to speed up the process with alignment-based tools, target specific signature sequences can be used. A set of such signature sequences has been created for *C. parvum* by comparing its whole genome sequence with those of *C. hominis*, *C. muris*, *C. ubiquitum*, *C. andersoni*, *C. baylei*, *C. meleagridis* and the chipmunk genotype. Finally, a metagenomics dataset containing 210,000 reads, free of target parasites, has been spiked *in silico* with 10 highly specific reads from *C. parvum* and *G. duodenalis*, respectively. The 10 spiked reads for the two parasite species were correctly identified using the metagenomics pipeline with kraken2 as classification tool.

JRP19-WP2-T3- Experimental amplicon-based and shotgun metagenomics for detection of foodborne parasites

One goal of this task is to optimise a platform based on amplicon-based Next Generation Sequencing for detecting parasites, with special emphasis on foodborne parasites. The principle of the method relies on amplification of the 18S ribosomal DNA gene (18S rRNA) from eukaryotic organisms. The method is part of a 16S/18S platform developed at SSI. The method has already been used in various studies and has been applied to genomic DNA extracted from various matrices such as faeces, other clinical sample types (EDTA blood, spinal fluid, skin/cornea biopsies/scrapings, etc), food products and environmental samples. The deliverable D-JRP-PARADISE-WP2.1 (Protocol for 18S rDNA-based amplicon sequencing for detection of relevant FBPs) has been submitted at M37. A manuscript describing the application of this platform to the detection of various zoonotic protozoa in pig faeces has been published. The platform still shows low sensitivity for the detection of microsporidia and some flagellates, including *Giardia*, and this may be due to problems of the Illumina sequencing technology with very GC-rich stretches (as the ribosomal sequence of *Giardia*). Therefore, the use of different detection markers can overcome this problem, and this is being investigated based on the FLUIDIGM technology.

A second goal is to compare the performance of the amplicon-based Next Generation Sequencing with that of shotgun metagenomics, an untargeted sequencing approach used to profile the taxonomic composition of communities. In particular, we intend to compare the limit of detection (LOD) of the two methodologies. To this end, genomic DNA from a plant matrix (ready-to-eat mixed salad) has been extracted at VRI and will be spiked with different amounts of genomic DNA from three parasites (*Cryptosporidium*, *Giardia* and *Toxoplasma*) provided by ISS. This will simulate the presence of 10, 50 and 100 (oo)cysts in a large excess of plant genomic DNA, mimicking the low contamination level observed in naturally contaminated foodstuff. The anonymized spiked samples have been sent to SSI and ISS for amplicon-based and shotgun experiments, respectively. Bioinformatics analyses will be performed to identify parasite-specific sequences, and the samples will be also tested by in-house *Cryptosporidium* and *Giardia* real-time PCR assays for comparison.

WP3-Design, implementation and validation of multi-locus typing schemes

JRP19-WP3-T1- In silico selection of informative loci from comparative genomics data

The goal of this task is to compare whole genome data for the identification of regions of high genetic variability in both *C. parvum* and *G. duodenalis* (Assemblage B). The following selection criteria were used: (i) the variable regions should be located on all chromosomes or (ii) at a sufficient physical distance to minimize genetic linkage; (iii) a high genetic variability across the isolates should be present; and (iv) specificity for *C. parvum* or *G. duodenalis*, determined in *silico*, should be demonstrated.

Whole genome comparison of >100 *C. parvum* isolates revealed a relatively low genetic diversity, with about 24.000 SNPs identified. To identify regions with high genetic variability, two approaches were used. First, the number of SNPs was calculated in each gene, and then in 500 bp windows. Candidates (n=28) were further selected after calculation of Simpson's diversity index. In the second approach, physical intervals containing a predefined number of SNPs (e.g., 10) were identified in the genome. Next, intervals of approximately 500-700 bp were selected and ranked in terms of their information content. Candidates from both approaches were retained for laboratory validation.

For the Assemblage B isolates of *G. duodenalis*, whole genome comparison revealed greater variability compared to *C. parvum*. A dedicated analytical pipeline developed at RKI was used to identify variable regions. At present, 20 candidates have been selected, having high genetic variability among the tested isolates and being located on the five different chromosomes.

More details on the selection procedures can be found in the report on the deliverable D-JRP-PARADISE-WP3.1 (Report on the *in silico* selection of highly polymorphic sequences in *C. parvum* and *G. duodenalis* genomes).

JRP19-WP3-T2- Development of MLST schemes for C. parvum and G. duodenalis

In the first half of Year 4, primers were designed for all *C. parvum* and *G. duodenalis* candidates. Reference genomic DNA and DNA from faecal samples were used for PCR and Sanger sequencing experiments. Different combination of candidates have been evaluated for their resolution power before inclusion in the final typing schemes. For *C. parvum* eight different markers (one per chromosome) have been selected while for *G. duodenalis*, which has 5 chromosomes, 6 markers have been selected. .

When developing new tying schemes, it is of paramount importance to test candidate markers on a large collection of samples from different hosts and geographical origin. These tests are under way at the four partner Institutes in charge (ISS, RKI, SVA and UoS). Besides samples already available in these Institutes, additional *C. parvum* and *G. duodenalis* DNA sample from the Consortium have been shipped during spring 2021. This includes 124 samples now available at ISS, 47 at RKI, 118 at SVA and 247 at UoS.

In parallel to the selection and testing of SNP-based markers, a different typing scheme for *C. parvum* developed by the associate partner CRU will be evaluated. This system is based on seven markers containing Variable Number of Tandem Repeats (VNTR), which are amplified in two multiplex PCR reactions and analyzed on a capillary sequencer to determine fragment size. SVA already applied the VNTR typing scheme to *C. parvum* samples from humans (origin, the Netherlands) and calf (origin, Sweden) with promising results.

The associated deliverable D-JRP-PARADISE-WP3.2 (Report on the identification of markers for multi-locus sequence typing of *Cryptosporidium parvum* and *Giardia duodenalis*) has been completed.

JRP19-WP3-T3-Interlaboratory comparison of typing schemes

In the first half of Year 4, collection of information on the parasites isolates, and the associated metadata, available at each partner Institute has been completed. Part of these samples were shipped and used for laboratory validation of candidate markers. The inter-laboratory comparison aimed at assessing the robustness and reproducibility of the typing schemes will be organized towards the end of Year 4. WP4-Parasite enrichment strategies

The objectives of WP4 are the development and evaluation by inter-laboratory comparison of: i) two pre-DNA extraction protocols based on two alternative affinity reagents, nanobodies (single-chain variable fragment antibodies) and aptamers (oligonucleotides that bind to a specific target molecule), and their application for magnetic capture of *C. parvum* and *G. duodenalis* (oo)cysts in different

matrices; ii) a protocol for post-DNA enrichment to concentrate parasite DNA based on hybridization of target-specific biotinylated probes.

JRP19-WP4-T1- Development of pre-DNA extraction enrichment strategies

Nanobodies: Two new world camelids were immunized with extracts from *Giardia duodenalis* cysts or *Cryptosporidium parvum* oocysts, respectively, and peripheral blood mononuclear cells (PBMCs) were isolated and stored in TRIZOL for later RNA preparation. Immune sera of the animals were tested by ELISA and IFA to ensure sufficient antibody production, and thus reactive B cells, against *Giardia* and *Cryptosporidium* antigens. The results showed that both animals react to the respective antigens. RNA from PBMCs was extracted and cDNA libraries of variable domains of heavy-chain only antibodies (VHH) were produced. Subsequent screening of *G. duodenalis* library identified several VHH clones reacting against cysts. Screening of cDNA library against *C. parvum* oocysts is still in progress. Positively selected VHH clones will be used for expression and purification by affinity chromatography for further characterization. The associated deliverable D-JRP-PARADISE-WP4.2 (Report on the selection of highly affine nanobodies specific for C. parvum and G. duodenalis) has been completed.

Aptamers: ANSES accomplished ten rounds of SELEX for five aptamer pools for both *C. parvum* (IOWA strain) and *G. duodenalis* (assemblage B). The PCR products of each round and each replica were subjected to high-throughput sequencing (HTS) and the data analysed by bioinformatics tools (Patternity suite). A core of sequences progressively enriched throughout the SELEX process were identified for both *C. parvum* and *G. duodenalis*. Similarly, but using a different SELEX protocol, ISS has completed 14 round of selection (in triplicate) against *G. duodenalis* assemblage B cysts. Sequence pools were also analyzed by HTS and Patternity suite, and enriched sequences selected. Screening of the aptamers candidates is in progress to assess if they are true “binders” and evaluate their binding properties and affinity. The associated deliverable D-JRP-PARADISE-WP4.1 (Report on the cloning and sequencing of aptamers specific for *C. parvum* and *G. duodenalis*) has been completed.

JRP19-WP4-T2- Development of post-DNA extraction enrichment strategies

In Year 3, SVA designed a magnetic capture (MC) system for *Cryptosporidium* (18S ribosomal DNA gene) and one for *Giardia* (beta-giardin gene), whereas RIVM designed a different capture probe for *Cryptosporidium* (gp60 gene). Rigorous testing of these capture systems on FACS-purified sorted oocysts (SVA) and on fecal samples (RIVM) demonstrated high sensitivity (10 purified oocysts and 125 oocysts in 2g fecal samples, respectively). Further testing to better define the detection limit in different matrices are under way.
In Year 4, novel capture system will be designed based on the new, informative markers selected for *C. parvum* and *G. duodenalis* by WP2/WP3. The associated D-JRP-PARADISE-WP4.3 deliverable (Report on the probes designed to targets developed in WP3 for *C. parvum* and *G. duodenalis*) has been completed.

5.1.4.3.13.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
19	D-JRP-PARADISE-WP1.1	Report of the kickoff meeting	M26	April 2020		Public OHEJP: available https://zenodo.org/record/4452807#.YAhQRDmg9dg	8 (Meting report)
19	D-JRP-PARADISE-WP1.2	Report of Annual meeting	M42		M46	Organization of a physical meeting still impossible. Due to some delays in research-oriented WPs, athe virtual Annual meeting will be organized in October 2021.	8
19	D-JRP-PARADISE-WP2.1	Protocol for 18S rDNA-based amplicon sequencing for detection of relevant FBPs	M30	M37		Public OHEJP: available https://doi.org/10.5281/zenodo.4478998	2
19	D-JRP-PARADISE-WP3.1	Report on the *in silico* selection of highly polymorphic sequences in *C. parvum* and *G. duodenalis* genomes	M30	M36		Public OHEJP: available https://doi.org/10.5281/zenodo.4452771	2
19	D-JRP-PARADISE-WP3.2	Report on the identification of markers	M42		M45	Public OHEJP: available https://doi.org/10.5281/zenodo.5494475	2

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		for MLST of *C. parvum* and *G. duodenalis*					
19	D-JRP-PARADISE-WP4.1	Report on the cloning and sequencing of aptamers specific for *C. parvum* and *G. duodenalis*	M36		M45	Public OHEJP: available https://doi.org/10.5281/zenodo.5495542	2
19	D-JRP-PARADISE-WP4.2	Report on the selection of highly affine nanobodies specific for *C. parvum* and *G. duodenalis*	M36		M45	Public OHEJP: available https://doi.org/10.5281/zenodo.5495560	2
19	D-JRP-PARADISE-WP4.3	Report on the probes designed to targets developed in WP3 for *C. parvum* and *G. duodenalis*	M44		M45	Public OHEJP: available https://doi.org/10.5281/zenodo.5495592	2

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
19	M-JRP-PARADISE-1	Kick-off Meeting (WP1)	M26	Yes		
19	M-JRP-PARADISE-2	Study visit (ISS-ANSES) for optmizing aptamer selection strategy	M28	No	No longer planned	Study visit impossible due to the COVID-19 epidemics
19	M-JRP-PARADISE-3	Key isolates of *C. parvum* collected	M30	No	M45	Collection now completed. Delays in this activity were due to the COVID-19 epidemics
19	M-JRP-PARADISE-4	Key isolates of *G. duodenalis* collected	M30	No	M45	Collection now completed. Delays in this activity were due to the COVID-19 epidemics
19	M-JRP-PARADISE-5	Referenced database of foodborne parasite genomes established	M30	No	M45	Database now completed. Delays in this activity were due to the COVID-19 epidemics
19	M-JRP-PARADISE-6	Pipeline for metagenome data analysis for FBPs optimized	M30	No	M45	Pipeline now completed. Delays in this activity were due to the COVID-19 epidemics
19	M-JRP-PARADISE-7	First set of candidate markers for MLST development available	M30	Yes		
19	M-JRP-PARADISE-8	Animal immunization and cDNA library of nanobody sequences for *C. parvum* and for *G. duodenalis* completed	M36	Yes		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
19	M-JRP-PARADISE-9	Sumbission of genome sequences of *C. parvum* and *G. dudenalis* to public repository	M36	No	M45	The last round of genome sequencing has been completed, and data will then be submitted to a public repository
19	M-JRP-PARADISE-10	Aptamers sequences selected	M36	No	M58	Laboratory work delayed due to Covid-19 restrictions in Year 3. Change in the experimental strategy decided at the beginning of Year 4.
19	M-JRP-PARADISE-11	Highly affine nanobodies selected	M36	No	M45	Laboratory work delayed due to Covid-19 restrictions in Year 3. Technical challenges to solve for *Cryptosporidium*
19	M-JRP-PARADISE-12	Pre-evaluation of probes directed towards markers commonly used for *C. parvum*	M36	Yes		
19	M-JRP-PARADISE-13	Pre-evaluation of probes directed towards markers commonly used for *G. duodenalis*.	M36	Yes		
19	M-JRP-PARADISE-14	Annual meeting (WP1)	M42	No	M46	The Annual meeting will be held, virtually or as a hybrid event, on October 2021.
19	M-JRP-PARADISE-15	Technical Workshop on on enrichment strategies (WP4)	M42	No	M48	Due to the Covid-19 pandemics, organization of this technical workshop was impossible.
19	M-JRP-PARADISE-16	Final set of markers for MLST of *C. parvum* available	M42	No	M45	Selection of markers impacted by delay in generation and analyses of whole genome data

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
19	M-JRP-PARADISE-17	Final set of markers for MLST of *G. duodenalis* available	M42	No	M45	Selection of markers impacted by delay in generation and analyses of whole genome data

Follow-up of the recommendations and comments by the Ethics Advisors

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
(1) Human Participation For the human participation in this project, the beneficiaries must confirm that relevant authorisations have been obtained.	Partners involved in the collection of human samples will provide evidence to the project coordinator that the relevant authorisations have been requested and obtained	Satisfactory reply	Closed	Closed
(2) personal data processing The beneficiaries must confirm that any personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.	Partners involved in handling personal data will provide evidence to the project coordinator of adherence to the GDPR (EU 2016/679), as well as the contact address of the Data Protection Officer, if available.	Satisfactory reply	Closed	Closed
(3) Health and Safety The beneficiaries must confirm that authorisations for relevant facilities	The project involves handling/processing of class 2 pathogens. Partners will provide evidence to the project coordinator that their facilities	Satisfactory reply. This partner has confirmed they will provide evidence but can they confirm when. Before the work starts?	The Coordinator has received and archived documents from the partners involved in the manipulation of material that may contain class 2 pathogens,	Satisfactory reply

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
(e.g. security classification of laboratory) have been obtained.	are authorised for the specific use.		stating that the facilities are appropriate for this purpose.	
(4) Animals Further details are need on the use of animals which are legal animals although not experimentation animals. Please describe how the beneficiaries are complying with access to animal material requirements and animal welfare laws.	Animal work, i.e., immunization and blood sampling of new world camelids, outsourced to a dedicated company (Preclinics, Potsdam, Germany). The work has been approved by the local authorities (licence 17A210).	Please provide the name of the authorising body.	The work has been approved by the local authorities (licence 17A210) at the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) and is compliant with the Directive 2010/63/EU on animal welfare	Satisfactory reply
(5) non EU countries (China, Australia, Middle-East) The beneficiaries must confirm that the research conducted outside the EU is in compliance with H2020 rules.	It is unclear whether non-EU countries are still to be considered associate partners of the project.	Further clarification is needed. When any non-EU country participates in a EU-funded project (even when participating without receiving direct funding), EU regulations must be applied. Please confirm how you are handling this aspect	No research actively is planned with the potential non-EU external partners; any collaboration during the project will be in compliance with H2020 rules.	Satisfactory reply
(5) non EU countries (China, Australia, Middle-East) (2) The beneficiaries must confirm that the adequate authorisations have been obtained to import/export materials.	It is unclear whether non-EU countries are still to be considered associate partners of the project.	Further clarification is needed. When any non-EU country participates in a EU-funded project (even when participating without receiving direct funding), EU regulations must be applied. Please confirm how you are handling this aspect	No research actively planned with the potential non-EU external partners; any collaboration during the project that will imply import/export of materials will be handled in compliance with EU rules.	Satisfactory reply

5.1.4.3.13.4 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Molecular Characterization of *Giardia duodenalis* in Children and Adults Sampled in Algeria https://doi.org/10.3390/microorganisms9010054 https://zenodo.org/record/4422456#.YArbG-hKjcc	YES		Yes 2730.00 $ = 2437.96 €
Veterinary Students Have a Higher Risk of Contracting Cryptosporidiosis when Calves with High Fecal Cryptosporidium Loads Are Used for Fetotomy Exercises 10.1128/AEM.01250-20 https://zenodo.org/record/4129990#.X6PX8WhKjcc	YES		Yes 1475.33 €
Parasitic Intestinal Protists of Zoonotic Relevance Detected in Pigs by Metabarcoding and Real-Time PCR https://www.mdpi.com/2076-2607/9/6/1189 https://zenodo.org/record/4898752#.YTnBA50zaUk	Yes		Yes Free of cost
Mining public metagenomes for environmental surveillance of parasites: a proof of principle. https://zenodo.org/record/5494583	Yes		Yes 1580.00 $ = 2176.47 €

Additional output

A general description of the project has been published (in Danish) by partner 13 (SSI) in the Danish Veterinary Journal (https://ipaper.ipapercms.dk/fsek/dvt/2020/dvt102020/?Page=36)

A presentation on the metagenomics work in WP2 was given at the meeting of the Dutch Society for Parasitology on 26th of May, 2021 (NVP - Evenementen (parasitologie.nl)

Two posters were presented at the OHEJP 2021 Annual meeting (9th-11th June 2021, Copenhagen, Denmark), namely P020 “A brief excursion into PARADISE”, and P021 “Comparative genomics of *Cryptosporidium parvum* and *Giardia duodenalis* isolates from Europe: towards the development of novel genotyping scheme” (https://onehealthejp.eu/annual-scientific-meeting-2021/)

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

A major outcome of the project is the generation of a large number of whole genome data for both *Cryptosporidium parvum* and *Giardia duodenalis*. Exploitation of these data will provide an unprecedented opportunity to study the biology of these parasites and to shed light on the mechanisms that have influenced their evolution, virulence, host adaptation, and population genetic structure. Remarkably, the OHEJP will be at the forefront of genomic research for these two pathogens.

5.1.4.3.13.5 Data Management Plan

The DMP of the project has been submitted, using the CDP tool, at the end of September 2020 (M33). The DMP comprises descriptions of all the data generated in the project, and will be updated regularly.

5.1.4.3.13.6 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

- Complementarities with the OHEJP research project "Toxosources". Specifically, collaboration on bioinformatics approaches for genome comparison and development of new typing schemes. Further interactions foreseen in relation to new methodologies for enrichment and detection of parasites in specific food matrices (ready-to-eat salads).
- Complementarities with the OHEJP integrative project "Harmony-CAP", in which *Cryptosporidium* has been selected as a model organism for evaluation of the current and best practices and the development of harmonised protocols.
- Link with the objectives of the **European Union Reference Laboratory for Parasites** in terms of new typing schemes for FBP that, in perspective, may become official, validated methods.
- Complementarities with the **EFSA-funded research project "IMPACT"** for the definition of optimized protocols for the molecular detection and characterization of FBPs on selected food matrices.

5.1.4.3.14 JRP20-R2-FBZSH3-DISCoVeR

5.1.4.3.14.1 Summary of the work carried out in the Project

Data inventories for all DISCOVER target pathogens have been finalised and data collection and data gathering is ongoing. Some partners have met delays in collecting new data from non-livestock and non-food sources, so we have extended the completion of project datasets to February 2022. Developed source attribution models will be updated when new data become available.

For *Salmonella* and *Campylobacter*, several partners are developing WGS-based attribution models. Preliminary results indicates that the outputs are very much in line with previous subtyping approaches (phenotypic and MLST based), although the WGS-based models appear to have a higher predicting accuracies due to the increased discriminatory power. Conventional subtyping approaches are also being applied for all the target pathogens, and we expect at least for *Salmonella* to be able to make a multi-country model.

A systematic literature review of recently published (2011-2021) case-control studies of sporadic *Salmonella* and *Campylobacter* infections has been completed and will together with data from previous reviews form the basis for an overall meta-analysis using Bayesian evidence synthesis methods.

Multi-Country Comparative Exposure Assessments (CEA) of target pathogens in pets have been completed. The results are currently being reviewed by partners. The output is the relative exposure of humans to pathogenic and antimicrobial resistant bacteria due to (in)direct contact with cats or dogs. The General Exposure Indicator, which is the ratio of the number of dogs or cats and the number of humans in a country, showed clear variation and differences between countries. However, the uncertainty introduced by limited data availability on pathogen prevalences in dogs and cats implied that only a limited number of differences between countries could be found for the Bacterium Exposure Indicator, which is the ratio of the number of bacterium-specific infected dogs or cats and the number of humans in a country.

An online stakeholder workshop was held on January 22, 2021. Around 20 participants from EFSA, ECDC, EURLs, other relevant EJP projects, as well as DISCOVER WP-leads met to discuss how DISCOVER results could support the work of the stakeholders and vice versa. A mapping of the currently existing control programmes of the target pathogens and AMR in humans, animals and the environment at the EU and national level is almost completed. This task is addressed by i) a scientific literature review (completed), ii) a review of grey literature (completed), and iii) a survey aimed at relevant national experts (ongoing). The survey will be distributed within the next 1-2 months through the OHEJP network and EFSA's zoonosis network.

On the project management side, we have since the project start had one face-to-face meeting and six web meeting for all partners, and nine web meetings for the project management team. We have created a share-site for partners to share documents and data. For sharing of genomic sequences, we have set up a secure space on the data platform sciencedata.dk. Finally, all partners have agreed on a Material Transfer Agreement, which is currently being signed.

5.1.4.3.14.2 Progress of the project: description of activities

WP1: Project coordination and administration (M25-60)

JRP20-WP1-T1- Project management (M25-60) - ongoing

Since the start of the project, we have had one face-to-face kick-off meeting hosted by DTU on February 10-12, 2020, and 6 web meetings for all partners and 9 web meetings for the project management team (PMT) consisting of all work package leaders, their deputies and task leaders.
A share-site hosted by DTU has been set up, where project partners can share documents and data, including minutes of meetings and the developed data inventories.
Partners have agreed on a Material Transfer Agreement (MTA), which is currently being signed by all partners.

JRP20-WP1-T2- Mapping of existing knowledge gaps (M25-28) - completed

For the mapping of existing knowledge gaps a systematic literature search by means of a rapid review was performed. Based on predefined search queries publications relevant for source attribution of the bacterial species *Salmonella*, *Campylobacter*, VTEC/STEC and for antimicrobial resistant (AMR) bacteria (*Salmonella*, *Campylobacter*, *E. coli*) were identified in the two databases Scopus and Web of Science. Each identified publication was tagged according to categories like the organisms and sources they considered or the methods they employed. Using the data of the tagged publications knowledge maps were created, which showed how much publications were found for each method-source combination for each of the above-mentioned bacterial species and AMR. The methodology and results of this mapping together with suggestions how to fill the identified knowledge gaps were put together in a report and submitted as deliverable D-JRP FBZ-1-WP1.1 to the project coordination. The results were also presented at the OHEJP ASM in Copenhagen June 9-11, 2021.

JRP20-WP1-T3- Data Management Plan (DMP) (M25-60) - ongoing

The first draft of the DMP was prepared in Nov 20, but its development is a continuing process. Considering the structure of the new DMP tool, we plan to upload information about our selected datasets, whenever we have a 'finalised' dataset ready for data analysis. Next update is planned for M48, when all WGS data have been uploaded to sciencedata.dk.

WP2: Data: Coordination of data collection (M25-M50)

JRP20-WP2-T1- Mapping of existing data/database est. (M25-36) - completed

A data inventory form in Excel was adapted to each pathogen subgroup. The purpose of the inventory was to get a quick overview of strains and sequences available in order to pinpoint areas with limited data availability to i) direct further sampling, and ii) identify for which pathogens and models we can expect to have sufficient data for source attribution. The data inventories have been filled-in by partners and hazard-specific web meetings have been organized to present the data available and agree on further sampling of mainly non-animal food reservoirs and/or isolate characterization (mainly sequencing) and transmission to the other WPs for source attribution analysis.

The data inventories and expected new data have provided the basis for selection of appropriate datasets for source attribution for the focus hazard and attribution approaches (see WP4 description for the source attribution approaches selected for each organism).

For sharing WGS data among participants, we created a space on the data platform sciencedata.dk and made a plan for uploading data. A meeting to demonstrate the sciencedata platform was held May 27, 2021. All data will be uploaded by the October 2021 (M46).

Partners are either uploading i) raw sequence data, which are then run through the FoodQC pipeline at DTU, or ii) assembled sequences, which should then adhere to a set of agreed quality criteria, which are uploaded to the share-site.

JRP20-WP2-T2- Data collection: Salmonella (M25-48) - ongoing

Overall, ten participants from nine countries (Belgium, Czech Republic, Denmark, France, Ireland, Netherlands, Poland, Portugal and Spain) delivered data about *Salmonella* serovars in their collections. Seven institutes showed data from animals, food and environment, five concerning humans.

Overall, the most numerous *Salmonella* serovars are *S.* Enteritidis (>19100 isolates/> 800 sequences), Typhimurium (>9600 isolates/> 270 sequences), monophasic variant of *S.* Typhimurium (>6500 isolates/> 300 sequences) and S. Infantis (>2560 isolates/ 190 sequences). Those serovars occur in all main sources. SE and ST were found as a top 1 and 2 in humans and animals. In food *S.* Infantis and *S.* Typhimurium are dominant. The environment is represented by the least number of isolates of different *Salmonella* serovars (>300 isolates/ 0 sequences). Some countries declared ongoing sampling or being ready to sequence selected isolates if needed.

For WGS-based source attribution, focus is on the following serovars: Typhimurium, monophasic Typhimurium, Enteritidis, Infantis, Derby, and Newport. For these serovars it was possible for a handful of countries to contribute with 25 sequences per source per serovar and country during the past 5-10 years.

For a multi-country attribution model based on phenotypic information only, available subtyping data for *Salmonella* were submitted from several partners and formatted for source attribution analyses in WP4.

JRP20-WP2-T3- Data collection: Campylobacter (M25-48) - ongoing

The *Campylobacter* specific questionnaire was filled-in by 10 institutes from 7 countries. A total of >5000 whole genome sequenced strains were reported to be available for the project. This includes genomes of human clinical strains, food, animal and environmental strains.
Fairly good data sets are available already and the modelers expect to be able to use the data, despite the incompleteness when it comes to full coverage of countries, sources, years. New sampling should primarily focus on environmental samples and other sources that are not well covered already. Focus is on *C. jejuni* and *C. coli*.

For WGS-based source attribution, focus is on collecting data from recent years, 2015-2020, with around 100 human genomes and 50 genomes from each source. To increase the amount of available data, 7-loci MLST data were also collected to construct a 'conventional' multi-country source attribution model as well. For the modeling work, see WP3 and WP4.

JRP20-WP2-T4- Data collection: VTEC (M25-50) - ongoing

The initial STEC specific questionnaire was filled in by 13 partners from 11 countries. Five partners from five countries shared information regarding human STEC isolates including more than 2400 isolates whole genome sequenced of different serotypes. Information regarding STEC isolates from animal, food and environmental were shared by 11 partners from 10 countries. More than 2800 STEC isolates from animal, food and environmental sources were described, not all isolates were whole genome sequenced. The number of isolates from sources other than food-producing animals was sparse. The main serogroups in the combined dataset are *E. coli* O157 and O26.

A new, more detailed isolate inventory was created and this was filled in by 16 partners from 11 countries. More than 3000 human STEC isolates with WGS data will be available for the project. For the animal, food and environmental STEC isolates almost 1000 isolates with WGS data will be available. This is one of the more comprehensive datasets for STEC available. For now, serotype and virulence gene content (*stx*$_1$, *stx*$_2$ and *eae*) have been shared together with metadata regarding each isolate. For the WGS data, a pipeline for analysis has been decided upon and will be established in a Galaxy environment as well as a pipeline to be installed and run on the command line on a high computing cluster for bioinformatics.
The need for further sampling of other sources than food-producing animals has been highlighted and sampling is planned. Some sampling have been performed, however, due to COVID-19 the some sampling has been postponed or cancelled for several partners.

JRP20-WP2-T5- Data collection: AMR (M25-48) - ongoing

Overall, eight countries (Czech Republic, Denmark, Germany, Ireland, Netherlands, Poland, Portugal and Spain) have in their strain collections clinical/indicator *E. coli* (including ESBL-*E. coli*) gathered for task 4. Number of available isolates/sequences ranges from less than 100 (Ireland) to 500-700 (Czech Republic, Poland) to > 1.000 (remaining countries). Most of the isolates, however, come from animals/food products, so that only Denmark and the Netherlands report a large number (>100) of isolates of human origin.

It was decided to focus on ESBL-EC from years with overlapping data from multiple reservoirs, and thus the period 2013-2020 was considered the best period. It was proposed to collect additional information for countries with available strains/sequences (possibly at the strain level), including: 1) availability of phenotypic AMR information, and 2) specifics on the genotypic information available (for non-sequenced strains): gene families/specific genes. A strain-level database has been built containing currently information on ~5,600 ESBL-EC (pending addition of strain-level data from Germany and Denmark), with most isolates up to now originating from the Netherlands (~2,700 isolates) and Spain (~2,200), mostly from animal sources.

WP3: Methods: Assessment/improvement (M25-54)

In this WP, monthly webmeetings are held to discuss progress and preliminary results.
Deliverable (D-JRPFBZ-1-WP3.2) on evaluation of SA methods is due M48 and work on this ongoing. We are applying the so-called TRACE framework to evaluate the various models identified through a scoping review. The 1st draft of the deliverable is expected for M44.

JRP20-WP3-T1- Assessing and developing source attribution methods based on microbial subtyping (M25-54)–- ongoing

JRP20-WP3-T2- Assessing and developing source attribution methods based on phylogenetic data (M25-54) - ongoing
As it turned out, there is a considerably overlap between the objectives and activities of T1 and T2, and we have consequently merged the two task, which will therefore be reported together below.
A working group has been assembled and is meeting bi-monthly. The research objectives has been defined: Using WGS/phylogeny of surveillance data to apply weights in source attribution models to move from reservoir attribution towards source attribution that is directly actionable by public health and food authorities, by better reflecting exposure evidence. Focus will be on *Campylobacter* and *Salmonella*.

A Danish dataset of *Campylobacter* sequences collected from humans, animals incl. pets, foods and environments from 2015-2017 is currently being processed through different bioinformatics pipelines to obtain cgMLST, wgMLST, SNPs and Kmer data. Also analysing accessory genes in data to measure associations between presence or absence of these genes and sources is investigated. All these kinds of genomic output data will be explored in different source attribution models using machine learning (ML) to identify any host-associated genetic (groups of) markers. Kmer analysis has had challenges to process the data due to large matrix analysis and the sparse matrix approach doesn't work because the analysis requires a full matrix to find kmers. Activities to solve these issues are ongoing. The different models will be assessed with regard to accuracy and number of human cases that they would be able to predict. The best method will be applied to other partners' *Campylobacter* data as well as to more recent data from Denmark (2019-2020). A MSc student at DTU contributing to the development of the above-mentioned bioinformatics and machine-learning approaches for *Campylobacter,* will hand in her thesis by the end of June 2021.

A comparison of source attribution methods is ongoing using a sample of 280 *Campylobacter* isolates from human cases and animal/environmental sources in the Netherlands using STRUCTURE, BEAST and a Random Forest methods on core-genome SNPs, gene presence absence data and cgMLST data. The three methods show slightly different attribution estimates, with the Random Forest method showing a noticeable lower attribution to the environment using both genetic sources, cgMLST representing the core genome and gene presence absence representing the accessory genome. Although there is no golden standard, the output of each method was compared with the majority vote. In this dataset, it seems that STRUCTURE based on cgMLST data slightly outperforms the other

methods as it most often agrees with the majority vote. More analyses will be done to confirm this finding.

A ML approach using random forest (RF) to perform source attribution of 420 human UK and DK sequences of *S*. Typhimurium and monophasic variants of *S.* Typhimurium was applied. 482 animal sequences from nine primary source classes were used for model training and testing. The cgMLST profiles (3002 core genomes) and accessory genome (1230 genomes incl. plasmids, phages) of all 902 sequences were retrieved and used as model inputs (features) after imputing missing data. Preliminary data of the RF model using cgMLST as input suggest that the major source of human *S*. Typhimurium infections in UK and DK is pig meat (≈66% of cases) followed by 'other non-livestock cases (≈10%). However, further refinement of the models are ongoing. The same dataset were also analysed using a Bayesian model and using the accessory genes present/absence as input.

JRP20-WP3-T3- Evaluation of microbial subtyping source attribution by infectious disease modelling (M25-54) - ongoing

This task sets out to develop a method for measuring the quality of source attribution based on subtyping. The work was planned to result in a milestone in M33, but this has been postponed to allow for evaluating also the models coming out of T1 and T2. The approach taken is based on simulating bacterial population using the software: Bacmita https://doi.org/10.1093/bioinformatics/bty093

JRP20-WP3-T4- Assessing and developing approaches for source attribution of antimicrobial resistance based on metagenomics (M25-54)–- ongoin.g

Nothing to report specifically for the project, as we are still identifying and collecting relevant metagenomic data. In particular, we are in need of data from the general population to further develop the approach. So far, such data seems only available in the Netherlands. A paper (Duarte et al., 2021) describing the methodology was recently published by one of the partners (but related to another EU financed project).

JRP20-WP3-T5- Assessing and developing source attribution approaches based on case-control study results (M25-48) - ongoing

A systematic literature review of recently published (2011-2021) case-control studies of sporadic *Salmonella* and *Campylobacter* infections has been conducted. The merging of the this new systematic review with previous reviews is ongoing. When all data have been compiled, overall meta-analysis using Bayesian evidence synthesis methods will be conducted to obtain updated source attribution estimates in WP4.

A methodological paper "A statistical modelling approach for source attribution of foodborne pathogens", presenting the approach used in WP4 to combine attribution estimates from different sources, will be submitted soon for publication by one of the partners.

JRP20-WP3-T6- Assessing and developing source attribution approaches based on data from reported outbreak investigations (M25-48) - ongoing

Milestone in M33 (M-JRPFBZ-1-06): Methods for source attribution based on outbreak data evaluated was met. Outbreak data for this task was requested from EFSA, which released them in M40. Modeling for *Salmonella* is ongoing in WP4.

JRP20-WP3-T7- Assessing and developing source attribution approaches based on Risk-assessment (M25-45) - ongoing

A review of existing comparative exposure assessment (CEA) models was completed in M38.

For the CEA approach, data for all DISCOVER partner countries are incomplete or non-existent. A comparative exposure assessment, to be "comparative" needs to include different sources or to be

performed in different regions/countries, which would also be in line with the spirit of OHEJP projects. It was therefore decided that a reasonable approach would be to perform an exposure assessment for a specific source for which we expected to find only few typing data, i.e. pets like dogs and cats. The pet-CEA were performed for all three target pathogens and AMR in different countries that have the necessary data. The resulting exposure estimates were compared between the countries, and it is investigated whether possible differences are also reflected in the attribution estimates obtained by the other methods. The first draft of the results from the CEA models are under review by project participants. The models are including available data on national pathogen prevalences in dogs and cats received from partners.

WP4. Results – Quantifying the contribution of various sources of foodborne zoonoses and AMR (M30-56)

In WP4, data collected in WP2 and the methods assessed/developed in WP3 are used to quantify the contributions of the main sources of the three target pathogens and AMR. Results will be presented per pathogen, attribution method, type of data and, when/if applicable, geographical region/country. Particular attention will be given to environmental and non-livestock (pets and wildlife) sources besides the 'traditional' livestock/food sources. The results of applied methods for each pathogen will be compared in the light of data availability and robustness, underlying uncertainties, the point in the food production chain where source attribution takes place, and the usefulness of different methods to answer different One Health questions. Before performing any attribution, it has become evident in the past months that it is necessary to compare the typing data between countries using, e.g. PCA and/or similarity metrics like PSI. In this way, geographical regions could be identified as the "epidemiological units" for the attribution analyses. Moreover, such an analysis would already be very informative in itself as it would provide information on the distribution of relevant subtypes among the DISCOVER partner countries and it would also offer the opportunity to identify potential sources of surrogate data for the attribution analysis.

Overall, a plan for which models to apply for each pathogens was agreed upon at a meeting on February 15, 2021. At the same meeting, persons/groups responsible for data management and modeling, respectively, for each pathogen-model combination were identified.
As an activity of interest for all tasks within WP4 and in collaboration with WP2 and WP3, we worked together to define a minimum set of (meta)data to be collected to perform source attribution in a meaningful way. A document was prepared and shared with all partners. This document was integrated in the milestone M-JRP FBZ-1-07 (Format for results presentation (standard structure) for all pathogens and AMR).

JRP20-WP4-T1- Salmonella source attribution and comparison of results from different approaches (M30-54) - ongoing

The specific activities of this task are structured as follows: Attributions based on microbial subtyping, which will include frequency-based models based on phenotypic subtyping data and machine-learning approaches based on WGS data. The data for running the frequency-based models are being made available and exploratory analyses are ongoing: this specific analysis based on serotyping data will target four countries (the Netherlands, Portugal, Czech Republic and Denmark, as examples of respectively Western, Southern, Eastern and Northern Europe). The analyses based on the genomic data will start as soon as the data will be made available by WP2.

1) Analysis of outbreak data based on national data reported to EFSA's outbreak database. The database has been cleaned and an overall preliminary analysis has been performed, in which it appears that eggs are the main source of the salmonellosis outbreaks observed in Europe between 2015 and 2019. Further analyses are ongoing to stratify the results by geographical

region (Western, Southern, Eastern and Northern Europe) and serotype. A trend analysis will also be attempted.

2) Meta-analysis of case control data to update previous systematic reviews. The systematic review of articles published between 2011 and 2021 has been performed using a standardized process to review literature and selecting relevant articles using the key-terms: *Campylobacter* (campylobacteriosis) or *Salmonella* (salmonellosis), Case-control, Sporadic and Risk Factors. Studies were included when passing the relevance screening, quality assessment and criteria for eligibility. From a total of 2084 identified references, 9 articles (5 on campylobacteriosis and 4 on salmonellosis) were deemed fit for data-extraction and were included in this review. Information on the frequency of mentioning and estimated odds ratios were collected. The most often mentioned risk factors for campylobacteriosis were: the consumption of chicken (5-times), owning a dog and/or cat (4-times), consumption of raw/undercooked meat (2-times) and contact with live poultry and/or birds (2-times). For Salmonellosis these were: the consumption of raw pork (4-times) and chicken (2-times), owning a cat and/or dog (2-times) and travelling abroad (2-times). The previous use of gastric anti-acidic drugs was mentioned often for both campylobacteriosis and salmonellosis, as well. The next step will be to perform a meta-analysis of these studies together with those already meta-analyzed in the period prior 2011.

Exposure assessment (pets only, but all target pathogens). The results of this analysis are available and are currently being reviewed by the project participants. The goal of this analysis was to estimate the relative exposure of humans to pathogenic and antimicrobial resistant bacteria due to (in)direct contact with cats or dogs, as compared between countries, using two approaches. The General Exposure Indicator, which is the ratio of the number of dogs or cats and the number of humans in a country, gives a relative, bacterium a-specific, quantitative exposure estimate which proves to show clear variation and differences between countries. The Bacterium Exposure Indicator is the ratio of the number of bacterium-specific infected dogs or cats and the number of humans in a country, and this gives a relative, bacterium-specific, quantitative exposure estimate. The uncertainty introduced by the dog/cat prevalence data, and its availability, implies that only a limited number of differences between countries could be found for the Bacterium Exposure Indicator.

The task is awaiting input from WP2 and WP3.

JRP20-WP4-T2- Campylobacter source attribution and comparison of results from different approaches (M30-54) - ongoing

1) Like in the previous task, specific activities have been defined: Attribution based on microbial subtyping, which will include frequency-based models based on 7-loci MLST data, machine-learning approaches based on WGS data, and population genetic models. These analyses will start as soon as the data will be made available by WP2.
2) Meta-analysis of case-control data to update systematic reviews (please see corresponding point in task JRP20-WP4-T1)
3) Exposure assessment (pets only). (Please, see corresponding point in task JRP20-WP4-T1)
Analysis of outbreak data is not included because the scarcity of documented *Campylobacter*iosis outbreaks would make such an analysis not very useful.

The task is awaiting input from WP2 and WP3.

JRP20-WP4-T3- VTEC source attribution and comparison of results from different approaches (M30-56) - ongoing

1) Specific activities include: Attribution based on microbial subtyping, which will include frequency-based models based on phenotypic data (virulence genes, etc.), machine-learning

approaches based on WGS data (if data allows), and population genetic models. These analyses will start as soon as the data will be made available by WP2.

2) Exposure assessment (pets only). (Please, see corresponding point in task JRP20-WP4-T1)

Meta-analysis of case-control studies and outbreak is not included, because there are already recent work available through a piece of WHO work led by one of the partners.
The task is awaiting input from WP2 and WP3.

JRP20-WP4-T4- AMR source attribution results presented regionally and by region/country, for each applied method and integrated (M30-56) – ongoing

1) Specific activities include: Attributions based on microbial subtyping, more specifically frequency-based models of ESBL based on the distribution of AMR determinants. The database including ESBL data for source attribution using the frequency-based models is completed and analyses are ongoing.
2) Attribution based on a ML approach comparing the resistomes in human and various sources based on available metagenomic data.

A conceptual model for bidirectionality of ESBL transmission has been developed and is currently being parameterized with data for the Netherlands.
The task is awaiting results from WP3.

WP5: Conclusions and policy translation (M32-60)

JRP20-WP5-T1- Technical expert evaluation (M41-57) - ongoing
It is considered as part of this task to turn the deliverable (D-JRPFBZ-1-WP3.2) into a document/report for decision makers/risk managers for an overview of the advantages, limitation, and data requirements for the various attribution models.

JRP20-WP5-T2- Translating source attribution estimates into options for control policies (M33-58) - ongoing
An online stakeholder workshop was held on January 22, 2021. Around 20 participants from EFSA, ECDC, EURLs, other relevant EJP projects, as well as Discover WP-leads joined the meeting.
A Master student enrolled at DTU has in collaboration with ISS addressed the deliverable D-JRPFBZ-1-WP5.1: Map of the current existing control programme and intervention strategies to mitigate the risk of transmission of *Salmonella*, *Campylobacter*, VTEC, and antimicrobial resistance to human at the EU and national level. This task is addressed by i) a scientific literature review (completed), ii) a review of grey literature (completed), and iii) a survey aimed at relevant national experts (ongoing). The survey will be distributed through the OHEJP network and EFSA's zoonosis network. DG SANTÈ and EFSA have been informed about the survey and provided their endorsement as well as their help to distribute the survey. The survey tool is almost complete and will be distributed within the next 1-2 months. The MSc student handed in her thesis on June 7. Finally, one abstracts was selected for oral presentation at the OHEJP ASM on June 9-11 in Copenhagen.
JRP20-WP5-T3- Final recommendations in a OH frame (M49-60) – not started yet

5.1.4.3.14.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
20	D-JRP16-1.1	Mapping of knowledge gaps and recommendations for new data generation and method development	28	August 20th 2020		Confidential until it has been published as a scientific article.	1/5
20	D-JRP16-1.2	Data Management Plan	30	35		The DMP is public	8
20	D-JRP16-2.5	Database/sharing platform solution established	36	38		Confidential – only partners has access to the WGS data selected for the project. By the end of the project, all data used for peer-review papers will be uploaded to publicly available genomic archives such as ENA. Some of the data used are already at ENA.	3
20	D-JRP16-4.1	*Salmonella* source attribution results presented regionally and by region/country, for each applied method	40		54	Public. Delay is not really a delay, but rather a mistake of the initially determined end date of tasks and deliverables in WP4. WP4 is dependent on getting data from WP2 and methods and results from WP3, so WP4 tasks cannot be completed before WP3 tasks are completed.	

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
20	D-JRP16-4.2	*Campylobacter* source attribution results presented regionally and by region/country, for each applied method and integrated	42		54	Public. See comment above.	
20	D-JRP16-5.1	Map of the current existing control programme and intervention strategies to mitigate the risk of transmission of *Salmonella*, *Campylobacter*, VTEC, and antimicrobial resistance to human at the EU and national level.	42		48	Public. Distribution of the survey has been delayed due to COVID-19 activities by the responsible task leader.	1/5

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
20	M-JRP16-01	Identification of Project Management Team	25	Yes		
20	M-JRP16-02	First annual project meeting	27	Yes		
20	M-JRP16-03	Completion of mapping of knowledge gaps and recommendations for new data generation and method development	28	Yes		
20	M-JRP16-04	Identification of types of samples to investigate and for which species to include in the sampling	29	Yes		Achieved M32
20	M-JRP16-05	Framework for evaluation of Microbial subtyping methods	33	Yes		
20	M-JRP16-06	Methods for source attribution based on outbreak data evaluated	33	Yes		
20	M-JRP16-07	Format for results presentation (standard structure) for all pathogens and AMR	33	Yes		
20	M-JRP16-08	Mapping of data available for *Salmonella*	34	Yes		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
20	M-JRP16-09	Mapping of data available for *Campylobacter*	34	Yes		
20	M-JRP16-10	Mapping of data available for VTEC	34	Yes		
20	M-JRP16-11	Mapping of data available for AMR	34	Yes		
20	M-JRP16-12	Protocol for presentation of results for *Salmonella*, *Campylobacter*, VTEC and AMR	34	Yes		
20	M-JRP16-13	Framework for using phylogenetic data for source attribution	36	No	48	The milestone more difficult to achieve than expected. Need other WGS modelling tasks to be completed first, as the results will feed into the phylogenetic approach.
20	M-JRP16-14	Comparison of data and methods for each pathogen and AMR	36	Yes		Achieved M38
20	M-JRP16-15	Framework for using metagenomic data for attribution of antimicrobial resistant	39	Yes		
20	M-JRP16-16	Results from evaluation of microbial subtyping methods using artificial data	40	No	54	Task has been delayed due to prioritisation of other activities.

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
20	M-JRP16-17	Description of method of source attribution using existing case-control	42	Yes		
20	M-JRP16-18	Method for source attribution by risk assessment evaluated	42	Yes		
20	M-JRP16-19	Second annual project meeting hosted by ANSES	41	No		No physical meeting due to COVID-19
20	M-JRP16-20	Completion of control programme and intervention strategies mapping	41	No	48	Survey delayed as explained above
20	M-JRP16-21	Update of Data Management Plan	41	No	48	Postponed to end of Y4, as it makes more sense according to our scheduled plan for partners to upload data.
20	M-JRP16-22	Completion of data collection	42	No	50	Data collection has been delayed due to COVID-19. We expect almost all data to be uploaded to our data-sharing platform by M48, although a few additional data may still roll in in the beginning of Y5.

5.1.4.3.14.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors evaluated the comments you provided last January. All recommendations have been addressed and therefore this part of the report can be closed.

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
(1) Human biological samples The beneficiaries must confirm that appropriate authorizations will be sought to collect the Human samples.	This project is not collecting human samples, but uses available results from the partners' countries' laboratory surveillance. Results of human samples e.g. diagnoses and WGS information are obtained from the participating countries' reference laboratories, which the project partners are either a part of or collaborating with. This means that all necessary authorizations are in place and is irrelevant do apply for these specifically for this project.	Satisfactory reply	Closed	Closed
(2) non EU countries (African countries) The beneficiaries must confirm that the research conducted outside the EU is in compliance with H2020 rules.	The non-EU obtained data that we plan to use in DiSCoVeR originates from another project (FOCAL) co-funded by BMGF and FCDO. This project has obtained all ethical approvals necessary from all involved countries and since the project's PI is of DTU, the project is conducted also in compliance with H2020 rules. We plan only to use a small proportion of the samples (sewage samples) collected in this project for DiSCoVeR.	Satisfactory reply. However the PI should clearly state that the terms of the consent for the original collection is also appropriate for this use. The ethical approval codes should be referred to in any reporting and the ethical approvals should be available upon request	We do not need to obtain specific consent to collect and use sewage samples. They are regarded as publicly available data.	Satisfactory reply

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
(2) non EU countries (African countries) The beneficiaries must confirm that the adequate authorisations have been obtained to import/export materials / appropriate Material Transfer Agreements. More specifically, the beneficiary must provide details on the material which will be imported to/exported from EU and confirm that the adequate authorisations have been obtained.	As mentioned above, the activities involving using sample results from African countries originates from another project. All necessary material and data transfer agreements between partners/countries in this project have been obtained and is available from the PI, who is also PI of DiSCoVeR. It is irrelevant to obtain separate authorisations for DiSCoVeR.	It is not clear from the statement but can the PI explicitly confirm that the terms of the consent for the original human samples collection is also appropriate for this use. The ethical approval codes should be referred to in any reporting and the ethical approvals should be available upon request	We will not reuse the individual human samples from the FOCAL project in Discover. Only the sewage samples, which are obviously not identifiable to the individual level, will be used in Discover.	Satisfactory reply
(2) non EU countries (African countries) (2) 'As low / middle income countries are participating in the study, the beneficiaries must confirm that fair benefit-sharing arrangements with local stakeholders are ensured during the project (cf the Global Code of Conduct for research in resource-poor settings – www.globalcodeofconduct.org). The research team needs to state and submit their policies and practice for ensuring good practice when doing research in resource-poor countries.	Again, we will only use a subset of the sample results from the FOCAL project in DiSCoVeR, and the FOCAL project, co-funded by BMGF and FCDO, adheres to the policies of these two foundations, which also include a policy for conducting research in LMICs. To this end, any research output in DiSCoVeR based on samples from the FOCAL project will also be a FOCAL output (co-financing DiSCoVeR) and therefore comply with this policy. Practically, this means that any LMIC data collector and/or data analyst will contribute to the outputs and be included as co-	Satisfactory reply, however please remember that EU rules also apply so if co-financed, research practice must conform to all funders requirements.	Goes without saying.	Closed

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
	author etc. in relevant publications and deliverables.			
(3) Health and Safety The beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	This is confirmed by the fact that all involved partners are employed by public governmental institutions (universities or research institutions), which must be expected to conform to all relevant local/national guidelines/legislation.	Satisfactory reply	Closed	Closed
(4) Other – Overall Ethics Management Considering the nature of the ethical issue raised, we would encourage the beneficiaries to include an Ethics element as part of the project management to ensure that the various ethics issues, raised by this work as it is done, are properly handled.	An ethic element, more specifically a project-level ethical committee, is included in the FOCAL project, and is, therefore, considered irrelevant for DiSCoVeR. In addition, it can be mentioned that the FOCAL project is conducted in accordance with the Danish Act on scientific ethical treatment of health research, as administrated and confirmed by the Research Ethics Committees of the Capital Region of Denmark (www.regionh.dk), Journal nr.: H-14013582. and fulfils the requirements of the Nagoya Protocol.	Satisfactory reply. We advise you not to use the work irrelevant but rather not required as other processes are in place or are available. We are assuming you can access the FOCAL Committee if needed.	Closed	Closed

5.1.4.3.14.5 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
V. Lopez-Chavarrias, M. Ugarte-Ruiz, M. C. B. Asensio, A. Olarra, M. Garcia, J.L. Saez, C. De Frutos, T. Serrano, I. Perez, M.A. Moreno, L. Domínguez, J. Alvarez Monitoring of Antimicrobial Resistance to Aminoglycosides and Macrolides in Campylobacter coli and Campylobacter jejuni from healthy livestock in Spain (2002-2018) doi: 10.3389/fmicb.2021.689262	yes		Yes

Additional output

Abstracts from the ASM One Health EJP 2021, 9-11 June, 2021:

- Sara Perestrelo, Guido Correia Carreira, Lars Valentin, Jennie Fischer, Yvonne Pfeifer, Guido Werner, Judith Schmiedel, Linda Falgenhauer, Annemarie Käsbohrer. "Source Attribution of ESBL-producing Escherichia coli in Germany" Poster presentation at the ASM One Health EJP 2021, 9-11 June, Denmark and online.
- Guido Correia Carreira, Annemarie Käsbohrer. "Mapping knowledge gaps of source attribution methods and sources for zoonoses and resistant bacteria using a rapid review approach". Oral presentation held at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.
- Rebekka Sørensen, Tine Hald, Gaia Scavia, Michele Luca D'errico. "MAPPING THE CURRENT EXISTING HAZARDS' CONTROL PROGRAM AND STRATEGIES IN THE VARIOUS SECTORS AND TRACTS OF THE ANIMAL-ENVIRONMENTFOOD-HUMAN CHAIN IN EU". Oral presentation held at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.
- Mónica Oleastro, Maria Leonor Lemos, Alexandra Nunes, Paulo Martins da Costa. "A PRELIMINARY STUDY OF CAMPYLOBACTER SPP. IN DOGS IN PORTUGAL – A ONE HEALTH PERSPECTIVE". Poster presentation at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.

- Ana Amaro, Célia Leão, Patrícia Themudo, Lurdes Clemente. “EMERGENCE OF MULTIDRUG RESISTANT SALMONELLA INFANTIS ST32 IN THE POULTRY INDUSTRY, PORTUGAL 2016-2020”. Poster presentation at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.
- Leonor Silveira, Sofia Ribeiro, Iúri Lopes, Mariana Fontes, Rita Castro, Frederico Lemos, Angela Pista. “Prevalence and characterization of Salmonella spp. and pathogenic Escherichia coli in food-producing animals in Portugal”. Poster presentation at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.
- Pista A., Ribeiro S., Fontes M., Batista R., Coelho A., Furtado R., Lopes T., Moura I., Saraiva M., Maia C., Belo-Correia C., Lopes I., Silveira L. “Prevalence and characterization of pathogenic Escherichia coli and Salmonella spp. in wild animals in mainland Portugal”. Poster presentation at the ASM One Health EJP Annual Scientific Meeting 2021, 9-11 June in Copenhagen, Denmark and online.

Other abstracts:

- Sara Perestrelo, Guido Correia Carreira, Lars Valentin, Jennie Fischer, Yvonne Pfeifer, Guido Werner, Judith Schmiedel, Linda Falgenhauer, Annemarie Käsbohrer. "To which extend do humans and animals share the same reservoirs of ESBL producing Escherichia coli?" Poster presentation at the Junior Scientific Zoonosis Meeting 2021, 3-4 June, online.

Scientific reports:

Eric Evers (RIVM, The Netherlands), Annemarie Käsbohrer (BfR, Germany). “Assessing and developing source attribution approaches based on risk assessment”, OHEJP Discover WP3-T7, milestone M-JRPFBZ-1-18, 10 February 2021.

5.1.4.3.14.6 Data Management Plan

Our DMP in the CDP tool is being updated annually as more and more data are collected. All data will be made publicly available by the end of the project through Zenodo, except for the sequence data, which will be available through ENA. Some of the sequence data are already at ENA.

5.1.4.3.14.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

As part of WP5, we held a stakeholder webmeeting on January 22, 2021 inviting EFSA, ECDC and the relevant EURLs to discuss how DISCOVER results can contribute the work of the stakeholders and vice versa. In addition, one of our partners is also part of OHEJP WP5, and she will inform about DISCOVER's activities in relevant fora (Stakeholder committee, Scientific Steering Board (SSB) and Program Owner Committee (POC)), and potentially asking DISCOVER to give a presentation at a later stage. We are also linking in with projects ADONIS, ORION and MATRIX to coordinate potentially overlapping activities.

5.1.4.3.15 JRP21-R2-FBZ3.1-BIOPIGEE

5.1.4.3.15.1 Summary of the work carried out in the Project

The BIOPIGEE project is successfully running since January 1st 2020.

WP1: The BIOPIGEE Mid-term meeting took place as a virtual 2-days-event in March 2020. Collaborations with other projects were initiated. A new task defining 'biosecurity measures' was initiated to harmonise understanding across project tasks. Several changes in the project plan were coordinated to cope with consequences of the COVID-19 pandemic. A 6-month-extension of the project was requested in agreement with all partner institutes. Our data management plan was updated following recent information from the DMP group.

WP2: A BIOPIGEE biosecurity questionnaire was already applied in farms of several partner countries, but this task is half a year delayed due to the pandemic situation. Additionally, a slaughterhouse online survey on best biosecurity practice was designed and carried out in six participating countries (DE, CZ, EE, IT, AT and NL). Sampling methods for visits to slaughterhouses were agreed and bacteriological samples were collected in two slaughterhouses (in CZ, IT) so far. Additional field studies are heavily affected by the pandemic but were initiated in UK, IT and NL. A new focus on internal biosecurity and spread of HEV between pens was included.

WP3: The comparison of methods and planning of how to test effectiveness of disinfectants is ongoing. *Salmonella* isolates for testing were selected. The method for testing persistence of infectious HEV in surface microlayers was established.

WP4: Data collection for the transmission modelling began. Pig movement data was obtained from UK and France. Due to data privacy issues, some data (e.g. information to identify individual farms) could not be shared, but geographical data could be used instead. It was not possible to obtain movement data from Italy, but similar data from Germany could recently be obtained. Work began on the individual transmission models but delays due to staff resourcing and COVID-19 befell. However, the integration of data and models was designed. A network analysis with French data was carried out.

WP5: The catalogue of biosecurity measures to reduce HEV and *Salmonella* prevalence was revised to ensure consistent data and safe updates. A systematic literature review about the effectiveness of biosecurity measures against *Salmonella* and HEV in pigs farms is finished. Estimates of the effectiveness of the identified biosecurity measures were summarised by meta-analyses. The expert panel from T2.1 (scientists) was expanded to incorporate knowledge of other stakeholders as e.g. practitioners, advisors. The panel was asked to assess biosecurity measures to reduce *Salmonella* and/or HEV in pig herds in an online questionnaire.

WP6: Illustrations of examples for good biosecurity were collected together with WP2, and uploaded to our website. Planning of workshops was amended due to the pandemic and physical conferences were cancelled. For the first workshop, presumably national events will be used to report and discuss results of the project with stakeholders. For the second workshop, the expert panel, recruited for T5.4, will be invited for a web-meeting to discuss BIOPIGEE results. Relevant conferences for hosting the third workshop are currently discussed and will be re-assessed when the pandemic situation allows.

5.1.4.3.15.2 Progress of the project: description of activities

WP1 Project coordination and integration of results

The project management, development of data management and provision of project deliverables and reports are ongoing tasks until the end of the project.

JRP21-WP1-T1- Project management and meeting organization (M25-60)

The BIOPIGEE Mid-term meeting took place virtually on 2./3. March 2021 (organised with APHA). Two quarterly WP-leader meetings and collaborations with other projects and institutions were arranged. The BIOPIGEE website, shared documents (uploaded to the BfR cloud server) and data reports from a survey-tool for T2.2 are continuously managed. The change of leadership in WP4 was accompanied to guarantee a smooth hand over of coordinating tasks. Since several tasks of the project are affected by the pandemic situation (farm visits, lab work, workshops), changes in the timeline of tasks and consequences for links between Tasks/WPs were coordinated. The Gantt chart and list of due dates for deliverables/milestones was modified accordingly. In agreement with all project partners, despite budgetary consequences, a 6-month-extension of the project was requested (April '21) in order to enable fulfilment of planned tasks.

The new subtask "Definition of 'biosecurity measures' across WPs/Tasks in BIOPIGEE" (T1.1.2) was identified and initiated, and the group is working towards a common understanding of 'biosecurity measures' across project tasks based on discussed inclusion/exclusion criteria from BIOPIGEE task groups and a literature review. A publication is planned.

JRP21-WP1-T2- Development of data management plan (M25-60)

Our draft of the data management plan (developed in 2020, stored in the CDP-tool) was updated in June and August 2021. New options to describe complex datasets with multiple choice for describing species and matrix were considered together with the option to split data sets as was informed in a training session by the DMP group in March 2021. Also contact information for each data set was updated. The DMP will be updated repeatedly until the end of the project.

JRP21-WP1-T3- Provision of project deliverables and reports (M25-60)

The publication plan for the project, arranged in a shared document, was modified to indicate the status of publications (intended, submitted, accepted) for WP2-5 and is being continuously managed. Deliverables are getting continuously collected and distributed (uploaded to the private web-group and to Zenodo). Reporting is a repeated task. The AWP-Y5 was amended together with the WP-leaders, considering a 6-months-extension, was submitted to OHEJP coordination team (9th June 2021) and is currently being revised. The first draft of 9M-report was worked on with the WP-leaders.

WP2 Biosecurity effectiveness studies

JRP21-WP2-T1- Development of biosecurity protocol (M25-28)

This objective was completed.

JRP21-WP2-T2- Application of the biosecurity protocol (M27-M48)

Although COVID-19 restrictions had a large impact on the progress of the task, as of 1st September 2021, 78% of the planned farm visits (203) had taken place and questionnaires uploaded onto the electronic data capture system. Five countries (DE, NL, CZ, PL, BG) have completed all of their visits. Photos are being collected from farm visits to show case examples of good and bad biosecurity practice, to add to project documentation in work package 6.

JRP21-WP2-T3 Slaughterhouse biosecurity practices (M37-48)

An online survey was designed to collect information on best biosecurity practice in slaughterhouses. The survey took place in six countries (DE, CZ, EE, IT, AT and NL). The survey includes 20 questions organised into the sections general, transportation, lairage, scalding, singeing, evisceration, carcase splitter, decontamination and chilling. The content was based on information about biosecurity measures' effectiveness presented in peer-reviewed literature and on opinions from slaughterhouse

experts within the partner institutes.

Sampling methods for visits to slaughterhouses were agreed. Bacteriological samples were collected in two slaughterhouses, one in CZ and one in IT. The samples were collected along the slaughter line. Another visit and sampling is planned for one slaughterhouse in IT. Hereby, validating information on the effectiveness of biosecurity practices is aimed for.

JRP21-WP2-T4 Field studies (M25-M54)

This task has been largely affected by COVID-19 lockdowns in the participating counties. However, longitudinal studies are planned to start soon in the UK and in IT. In NL, the research focus for this task has been updated and will now focus on internal biosecurity and spread of HEV between pens. A pilot study on four farms has been completed, the results of which are currently being analysed and will inform the final study protocol (choice of farm and age groups to sample) for the longitudinal study, which is expected to start in the autumn.

WP3 Impact of disinfection on persistence of pathogens in biofilm

WP3 and its labwork is partly delayed due to COVID-19 pandemic and consequences.

JRP21-WP3-T1 Comparison of methods for testing the effect of disinfectants (M25-46)

Parameters to compare methods for testing disinfectants effectivity were described. Reference strains to be used by all participating laboratories have been defined. Comparison of biofilm methods between labs and selection of methods to continue with in Task 3.2 are ongoing.

The deliverable WP3.7 “Assessed methods for testing disinfectants on *Salmonella* in biofilm” was produced.

JRP21-WP3-T2 Effect of disinfectants on biofilm-associated wild type Salmonella (M25-50)

This task is ongoing. It is dependent on the outcome of JRP21WP3-T1. Discussions and planning are ongoing among the participants in JRP21WP3 participants. Method establishment has started.

JRP21-WP3-T2-ST1: Selection of wild type Salmonella isolates (M25-M36)

A panel of *Salmonella* isolated from UK pig farms have been screened for their biofilm abilities using two methodologies. From this panel, five biofilm-forming *Salmonella* isolates, from serovars Derby and Typhimurium, have been selected for the use in T2-ST2.

JRP21-WP3-T2-ST2 Assessing the effect of disinfectants (M37-M46)

Task 2 depends on results of Task1. Lab work has been started, initial testing is ongoing.

JRP21-WP3-T3- Study of HEV stability in relation to disinfection approaches (M25-M54)

HEV stability in microfilms will be studied using appropriate HEV infectivity assays which have been developed in JRP21-WP3-T3-ST1, and will be further implemented in year 2 (and 3) of the project.

Deliverable WP3.6 “HEV infectivity assay is available” (M36) and is implemented further. It has been tested on field samples and will be further validated using other HEV bioassays.

The deliverable WP3.5 “Method for testing persistence of infectious HEV in surface microlayers” was completed in June 2021.

JRP21-WP3-T3-ST1 Adaptation and implementation of HEV infectivity assay for testing biofilms (M25-M36)

The HEV infectivity assay was completed (deliverable 3.6). In this assay, primary hepatocytes are isolated from liver tissue of (HEV free) piglets using a collagenase treatment. The obtained primary hepatocytes are aliquoted and stored at -180 Celsius until use. For the actual assay, the hepatocytes are seeded onto plates and inoculated with different samples potentially contaminated with HEV. A second method for testing of HEV infectivity, precision-cut liver slices (PCLS) has been developed also and will be used further for validation of the hepatocytes assay (see above). Adaptations of the bioassay will be made as appropriate, to make it suitable for testing biofilms.

WP4 Modelling of the cost and effectiveness of biosecurity measures

The lead of WP4 changed. The former leader Beate Pinior left AGES/Vetmeduni Vienna and the BIOPIGEE project. The former deputy Robin Simons (APHA) overtook the lead and Mathieu Andraud (ANSES) is new deputy.

JRP21-WP4-T1- Development of questionnaire on biosecurity costs (M25-M27)

This task has been completed

JRP21-WP4-T2- Stochastic simulations on the effectiveness of biosecurity measures (M33-M56)

JRP21-WP4-T2-ST1- Data collection for the transmission models

Data collection has begun. Pig movement data has been obtained from France and the United Kingdom and a process of anonymisation has allowed them to be shared between countries for the use in the model. Unfortunately, due to data privacy issues some data (such as information that could identify individual farms) could not be shared, but geographical data could be used instead. It was not possible to obtain movement data from Italy, but similar data from Germany could recently be obtained. Thereby, we can work with movement data from three case study countries. Work began on the individual transmission models. A network analysis with French data was carried out and is in publication process.

JRP21-WP4-T3- Merge of models into one QMRA (M33-M56)

JRP21-WP4-T3-ST1- Different transmission models will be matched to one QMRA zoonotic pathogen model

Work has begun on the individual models but delays due to staff resourcing and COVID-19 has meant that integration has not begun in earnest yet. However, work has been done to understand how the integration will occur; a poster on this process has been presented at the 2021 OHEJP annual meeting.

WP5 Benchmark of biosecurity practice

JRP21-WP5-T1- Data integration from WP2-4 in catalogue of biosecurity measures (M27-56)

The catalogue of biosecurity measures to reduce HEV and/or *Salmonella* prevalence was revised to ensure consistent data and safe updates. Procedures for inputs and updates as well as backups were defined. Data was updated by the first results from T5.2. All BIOPIGEE participants were invited to supplement the BIOPIGEE catalogue of biosecurity measures. The catalogue is getting updated continuously with identified biosecurity measures and estimates of their effectiveness. This task is ongoing.

JRP21-WP5-T2- Literature review/meta-analysis (M27-50)

Ongoing. A systematic literature review about the effectiveness of biosecurity measures specifically against *Salmonella* and HEV in pigs farms is finished. The articles found in literature databases were evaluated and relevant data was extracted. Estimates of the effectiveness of the identified biosecurity measures were summarised by meta-analyses. The risk of bias of the included articles was assessed. First effect estimates were offered to WP4 at the end of M38 and entered in the catalogue of biosecurity measures (T5.1). The systematic literature and meta-analyses are being updated and expanded including biosecurity measures to reduce the occurrence of pathogenic *E. coli* in pig herds.

JRP21-WP5-T3-Machine learning approaches (M53-58)

This task has not started yet.

JRP21-WP5-T4- Expert panel to add estimations on effectiveness/ weights (M33-52)

This task is ongoing. The expert panel set up and surveyed in T2.1 (scientists) was expanded to additionally incorporate knowledge of other stakeholders like practitioners, advisors, etc.. We found marked differences between the veterinary and consulting systems within the pig sectors of the different partner states. These differences in the systems considerably influence the understanding of the roles within the pig sector of different professions. Experts from participating countries were recruited for the panel. Due to the COVID-19 pandemic, the experts were asked to assess biosecurity measures to reduce *Salmonella* and/or HEV in pig herds in an online questionnaire. The questionnaire was based on the biosecurity measures included in T2.2 and on the results of T5.2. The experts' answers to the questionnaire are currently summarised and analysed.

JRP21-WP5-T5 Benchmark system for effectiveness of biosecurity (M51-58)

This task has not started yet.

WP6 Dissemination

JRP21-WP6-T1- Assembly and development of biosecurity information (M27-60)

JRP21-WP6-T1-ST1- Identification of appropriate websites or other online channels

So far, 21 web sites in 6 countries have been identified for dissemination and are listed in a shared document. The link is accessible for the consortium via the OHEJP BIOPIGEE website. This task is carried out on an ongoing basis and the list is amended and updated throughout the project.

JRP21- WP6-T1-ST2a Provision of information for farming schools and websites

Illustrations of examples for good biosecurity (pictures), which are planned to be used in this task, have been initiated to be collected in WP2. First pictures have been uploaded to an assembly at our website. This task is ongoing.

JRP21-WP6-T1-ST2b Provision of slaughterhouse protocol to slaughter industry/related associations

This task has not started yet.

Data to be used in this task is currently being collected in JRP21-WP2-T3 Slaughterhouse biosecurity practices.

JRP21-WP6-T2- Development of a support tool to calculate cost effectiveness (M55-60)

This task has not yet begun.

JRP21-WP6-T3- Organisation of a workshop-series (M25-58)

JRP21-WP6-T3-ST1 Identification of relevant stakeholders

Ongoing. For the second workshop, an expert panel, recruited for T5.4, will be invited for a web-meeting to discuss BIOPIGEE results. The list is updated as necessary.

JRP21-WP6-T3-ST2 Identification of relevant conferences

Due to the COVID-19 pandemic, physical conferences have been cancelled. Relevant conferences for hosting the third workshop in conjunction with a conference, are currently been listed in our publication list, options have been discussed in the WP-leader meeting in June 2021 and will be re-assessed when the pandemic situation allows it.

JRP21-WP6-T3-ST3 Organisation of Workshop 1 (M25-52)

Due to the Covid pandemic, the first workshop was cancelled. National information events are planned instead. In Germany, it is planned to participate in an information day of an animal health service and to inform and discuss results from BIOPIGEE there (autumn 2021).

JRP21-WP6-T3-ST4 Organisation of Workshop 2 (M40-48)

The second workshop is currently planned as an online event to be held during late autumn 2021. Listed experts for JRP21-WP5-T4 will be invited to this workshop where preliminary results from the BIOPIGEE project will be disseminated. Relevant conferences for hosting the third workshop are currently discussed, including smaller national events, and will be re-assessed when the pandemic situation allows.

JRP21-WP6-T4- preparation of a BIOPIGEE Flyer (M25-32)

Completed in 2020

Additionally, a press release about activities in the project has been drafted between partners and published in some countries.

5.1.4.3.15.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
21	D-JRP21-WP2.1	Biosecurity protocol (addressing measures to control *Salmonella* and HEV in primary production) designed for data collection in the field	28	01.05.2020		Public as short version since 31.08.2021, 10.5281/zenodo.5336900 https://zenodo.org/record/5336900	2
21	D-JRP21-WP6.4	BIOPIGEE flyer prepared	32	21.08.2020		Public,10.5281/zenodo.4009015 https://zenodo.org/record/4009015, additional deliverable, produced to quickly inform about the project (dissemination, WP6)	8 (project publicity)
21	D-JRP21-WP1.2	First draft of data management plan finished	30	18.10.2020	34	Confidential (OHEJP DMP group recommends to upload only the final DMP to Zenodo), is entered into CDP, uploaded to project website, the DMP gets continuously updated in a shared document and changes transferred to CDP	8 (reporting)

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
21	D-JRP21-WP6.3	Workshop 1 completed	30	No		Cancelled due to pandemic situation. It may be replaced by local national workshops to disseminate results.	5
21	D-JRP21-WP1.4	Project report 1st year submitted	36	17.12.2020		Public, 10.5281/zenodo.4361090 https://zenodo.org/record/4361090	8 (reporting)
21	D-JRP21-WP3.6	HEV infectivity assay available	36	09.12.2020		Public as short version, 10.5281/zenodo.5242819 https://zenodo.org/record/5242819	2
21	D-JRP21-WP3.5	Method for testing persistence of infectious HEV in surface microlayers	36	06.06.2021	42	Public, 10.5281/zenodo.4940091 https://zenodo.org/record/4940091; was delayed due to COVID-19 pandemic and consequences	2
21	D-JRP21-WP3.7	Assessed methods for testing disinfectants on *Salmonella* in biofilm	40	17.08.2021	44	Public as short version, 10.5281/zenodo.5211242 https://zenodo.org/record/5211242	2
21	D-JRP21-WP6.14	Workshop 2 completed	42	No	48	Delayed due to COVID-19 pandemic, postponed to December 2021 as virtual workshop	5

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
21	M-JRP21-01	Kick-off meeting successfully organised	26	Yes (M25)		
21	M-JRP21-02	Questionnaire on biosecurity costs	26	Yes (M27)		Farm performance questions on costs were included instead of biosecurity cost questions (not feasible; data will be gathered from other sources).
21	M-JRP21-03	Relevant conferences for workshops to be held are identified	26	No	48	Delayed due to pandemic situation. It is planned to disseminate findings in an online-workshop and at national, if possible international workshops at the end of 2021 and late 2022 instead.
21	M-JRP21-04	Biosecurity protocol designed for *Salmonella* and HEV	28	Yes		
21	M-JRP21-05	Relevant stakeholders identified	28	Yes (M36)		Was postponed due to the current COVID-19 situation (no workshops took place yet); Instead of stakeholders, a list of experts for a panel in T5.4 has been expanded (online table, link in BIOPIGEE private webgroup). These experts will be invited for an online workshop
21	M-JRP21-06	Appropriate websites or other online channels for dissemination identified	30	Yes		List of web sites is been filled in an online table (link in BIOPIGEE private webgroup); Content will be continuously updated throughout the project

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
21	M-JRP21-07	Workshop 1 completed	30	No	Not possible under COVID-19 circumstances	WP-leader decision: The series of workshops is cancelled due to COVID-19-outbreak, instead national information events in 2021/22 are planned
21	M-JRP21-08	Design of field study protocols	32	Yes		
21	M-JRP21-09	First part of meta-analysis finished	36	Yes (M38)		A literature review and meta-analysis was carried out. Was delayed due to partners involved in covid-testing and missing personnel for 4 months at BfR
21	M-JRP21-10	*Salmonella* strains for testing are collected	36	Yes		
21	M-JRP21-11	HEV infectivity assay available	36	Yes		
21	M-JRP21-12	Mid-term meeting successfully organized	39	Yes		
21	M-JRP21-13	Application of protocols across countries	40	No	46	Delayed due to pandemic situation and restrictions for visiting farms. More than half of the countries have finished their visits incl. survey and sampling, the other countries have restarted their visits.
21	M-JRP21-14	Relevant stakeholders re-identified	36	Yes	41	As stated for M-JRP21-05, an expert panel is used instead. This was re-identified in M41.
21	M-JRP21-15	Transmission model adaptation based on available data	39	No	45	First versions include data from 2 countries, hope to include data from a third country, for

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
						which recently obtained data need to be checked.
21	M-JRP21-16	Test methods to be used in JRP21-WP3-T2-ST2 are selected	40	No	46	Milestone delivery date postponed to M46 due to delay in completion of JRP21-WP3-T1 on which completion this milestone is based.
21	M-JRP21-17	Produce summary of evidence of slaughterhouse biosecurity effectiveness	42	No	47	Development of questionnaire took longer than planned (review of peer-reviewed papers; consideration of technique in all countries; common understanding of relevant biosecurity measures and formulation of questions; translations; implementation into survey tool; test runs; data protection issues). The questionnaire is prepared and the survey was started in June.
21	M-JRP21-18	Workshop 2 completed	40	No	48	Postponed to second half of 2021, due to pandemic situation, changed to a virtual workshop with the expert panel from T5.4.
21	M-JRP21-19	Completion of farm visits and lab work	44	No	54	Postponed, due to pandemic situation and restrictions on visiting farms in some countries.
21	M-JRP21-20	Application of appropriate economic methods based on available data	45		51	Postponed to beginning of 2022 as this task is depending on delayed tasks.

5.1.4.3.15.4 Follow-up of the recommendations and comments by the Ethics Advisors

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
(1) Human biological samples In case of use of human cells/tissues available commercially, details on cells/tissues type and provider must be provided.	There was an error in the ethics form. The project does not use any human biological samples. A new form is being prepared and sent.	Satisfactory reply	Closed	Closed
(1) Human biological samples In case human cells/tissues are obtained within the project, details on cells/tissues type must be provided. The beneficiary must also confirm that relevant approval has been obtained.	See above.	Satisfactory reply	Closed	Closed
(2) Environmental Harms The ethical checklist has not been completed, please tick all boxes. You have ticked the "harm to environment" box. Please confirm the types of environmental harms and that the work will compliant with good practice and EU legislation	Has been done and form is available.	Concern remains regarding how the beneficiaries are handling the potential harm to humans and to research staff, as pointed by the researchers	Collection and processing of fecal samples from pigs can cause spread of pathogens and infections in humans (research staff). In order to minimise the risk in the procedures 1) on farm, 2) in the transport/ shipment of samples and 3) in/around lab analyses, samples are a) collected by skilled personal following consistent instructions on biosafety (incl. protective clothing, equipment and handling), b) transported and shipped firmly sealed	Satisfactory reply

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
			according to national safety regulations and c) handled and processed under safety measure conditions required for the individual pathogen, respectively (e.g. Germany: German Ordinance on Safety and Health Protection at Workplaces Involving Biological Agents). Analyses take place under quality control in accredited, certified laboratories (DIN EN ISO/IEC 17025; DIN ISO 9001). By that we follow institutional, national and European legislation/ guidelines and management systems which address biosecurity measures.	

5.1.4.3.15.5 Publications and additional outputs

No publications yet.

Additional output

Flyers

The Flyer (D-JRP21-WP6.4) is repeatedly used to e.g. inform participating farms in T2.2

Posters

- Ivana Koláčková, Renáta Karpíšková, Tereza Gelbíčová, Zdenka Vacková, Jonaáš Vaňhara, Daniel Sperling (2021). *Salmonella* spp. in pig farms – still an issue?. Poster at ESPHM. Online.
- Gergana Krumova-Valcheva, Albena Dimitrova, Eva Gyurova, Gergana Mateva, Mihail Milanov, Hristo Daskalov (2021). Hepatitis E in humans and pigs in Bulgaria (REVIEW). Poster at OHEJP ASM. Copenhagen/Online. https://doi.org/10.5281/zenodo.4926180
- Pachka Hammami, Nicolas Rose, Vladimir Grosbois, Stefan Widgren, Andrea Apolloni and Mathieu Andraud (2021). Live animal movements - Understand trade partners choices to predict chains of contact. Poster at SVEPM. Online. https://doi.org/10.5281/zenodo.5005733
- Tarmo Niine, Arvo Viltrop, Imbi Nurmoja, Richard Smith, Elke Burow (2021). Salmonella in slaughterhouse!? What would be the right questions in that situation?. Society for Veterinary Epidemiology and Preventive Medicine (SVEPM) annual conference 2021. Online. Zenodo. https://doi.org/10.5281/zenodo.5496890
- Smith, Richard P., M. J. Vilar, Hannah Jones, Elke Burow (2021). Preliminary description of biosecurity practices related to importing pig and semen onto European pig farms. Poster at OHEJP ASM 2021. Copenhagen. https://doi.org/10.5281/zenodo.4906860
- Tarmo Niine, Arvo Viltrop, Imbi Nurmoja, Richard Smith, Elke Burow (2021). Identification of the best biosecurity practices in slaughterhouse in regards to Salmonella and hepatitis E virus. The One Health EJP Annual Scientific Meeting. OHEJP ASM 2021. Copenhagen/Online. Zenodo. https://doi.org/10.5281/zenodo.5496902
- Claire Oastler, Mardjan Arvand, Katharina Konrat, Ane M. Osland, Vanessa Pfiffer, Lene Vestby, Becky Gosling (2021). Assessment of the biofilm forming capability of *Salmonella* isolates sourced from pig farms in Great Britain. Poster at OHEJP ASM 2021. Copenhagen/Online. https://doi.org/10.5281/zenodo.5497688
- Nikolaus Huber, Elena L. Sassu, Gergana Krumova-Valcheva, Marina Meester, Ivana Kolackova, Petra Vasickova, Elisabeth Waller, Giuseppe Aprea, Annemarie Käsbohrer, Veit Zoche-Golob, Elke Burow (2021). Biosecurity measures reducing *Salmonella* ssp. andhepatitis E virus prevalence in pig farms: A systematic Review and Meta-analysis. Poster at OHEJP ASM 2021. Copenhagen/Online. https://doi.org/10.5281/zenodo.4905616
- Catherine M^{c}Carthy, Robin Simons, Pachka Hammami, Mathieu Andraud, Stefan Widgren, Beate Conrady (2021). BIOPIGEE: Modelling of the cost and effectiveness of biosecurity measures. Poster at OHEJP ASM 2021. Copenhagen/Online.

Press release

A press release about the project has been developed for creating awareness. Partners have translated it to local languages. It has been used as a template to spread information in Sweden , Austria and Germany (https://www.sva.se/forskning/internationellt-samarbete/europeisk-samverkan-kring-livsmedelsburna-smittor/biopigee-ett-one-health-ejp-projekt/

https://www.bfr.bund.de/cm/343/gefahr-im-schweinestall-ein-forschungsvorhaben-soll-die-ausbreitung-von-salmonellen-und-hepatitis-e-viren-begrenzen.pdf). German farming magazines like topagrar and Rind&Schwein published a project description as well.

Social media

The annual BIOPIGEE progress meeting was advertised on the OHEJP twitter feed.

Websites

- The Bfr website and BfR twitter account informed about the project to increase its awareness - https://www.bfr.bund.de/en/biosecurity_practises_for_pig_farming_across_europe__ejp_biopigee_-252636.html
  https://twitter.com/bfrde/status/1356619760625201152?lang=bg
- The cross-sectional study within work package 2 task 2 was advertised on the British National Pig Association website - http://www.npa-uk.org.uk/Participants_wanted_for_biosecurity_study.html

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

In the BIOPIGEE project, biosecurity protocols and questionnaires, tailored to identify measures against the specific and different pathogens *Samonella* and HEV on farm (indoor and outdoor system) and at slaughterhouse, have been developed and harmonised among European countries. This is an important base for controlling occurrence of these zoonotic pathogens in the food chain. The listed measures will be available for use beyond the project period. The protocol development approach could serve as orientation for the adaptation of protocols to target other pathogens.

A common and deeper understanding of the meaning of biosecurity practice through the definition of 'biosecurity measure' was developed between the partners through a scope review and discussion sessions in the project group. The definition may facilitate future communication on biosecurity practice.

A procedure was developed for a complex literature search on effective biosecurity measures against different pathogens with a group of several reviewers.

Within the framework of work package 3 (impact of disinfection on persistence of pathogens in biofilm), harmonisation of methods among the participating laboratories was achieved with regard to the definition of reference strains and the selection of test methods for the effect of disinfectants on *Salmonella* and HEV in biofilms, and is still in progress. This is new and important basic knowledge for the control of HEV and *Salmonella* occurrence.

Data Management Plan

A first draft of a Data Management Plan (DMP) for the BIOPIGEE project was developed with WP-leaders and is entered into the CDP-tool, from which it is available since 18th Oct 2020. From the CDP-tool, an excel sheet of the DMP was downloaded, uploaded to the BIOPIGEE website and transferred to a shared document hosted at the BfR-server (link in FORUM at the BIOPIGEE website). Updates in the shared document get continuously transferred to CDP.

The DMP includes 16 different kinds of datasets which are closely related to deliverables of the project. It is research data and methodologies, mainly related to pigs as focused in the project. Most of the data are intended to be published in scientific journals and are therefore confidential until publication. Location, where to find the data (e.g. website, Zenodo) is listed together with information whom to

contact for each dataset (names, institutions and email addresses), which have a unique identifier in the plan. There is little to consider for ethical aspects as data will be anonymised and no personal or identifiable data will be included. Data related keywords from ORION were selected on a drop down list in the CDP tool and included. Thereby, the plan takes into account the following aspects: data summary, making data findable, making data openly accessible, making data interoperable, increase data re-use (FAIR data principles), data dissemination, data security and ethical aspects.

The feedback from the DMP-group (received 22nd Dec 2021) was that the DMP is well developed, and that several draft fields in CDP would be normal at the stage of the project.

5.1.4.3.15.6 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

A collaboration with the **OHEJP projects ORION/Matrix** was initiated. A number of terms related to biosecurity measures in primary pig production were defined in BIOPIGEE tasks. For these definitions, common understanding for these terms were developed during task meetings. It was agreed between ORION/Matrix and BIOPIGEE to include the defined terms from BIOPIGEE into the OHEJP Glossary, which had been set up in ORION, and thereby to make the definitions used in BIOPIGEE findable, accessible and reusable.

Dr Carla Gomes of **Animal Health Ireland** was engaged to see if there is potential for collaboration with a study that she is conducting on the effectiveness of biosecurity measures in Irish pig herds. There is the potential to add their data into the analyses of this project, although the Irish data will only be available towards the end of the project.

BIOPIGEE is in contact with the EU/global project **HealthyLivestock**. In a web-meeting between both projects on May 4th 2021, overlap between several parts in the projects was identified and we agreed that sharing insights is valuable. In particular, the key factors associated with disease prevention to be identified in HealthyLivestock and the key biosecurity measures associated with reduction of *Salmonella*/HEV prevalence to be identified in BIOPIGEE appear valuable to compare. It is intended to present this comparison in a joint paper. Further planning of the collaboration is agreed for autumn when relevant tasks are further along in their process.

The **German Federal Ministry of Food and Agriculture** indicated interest in the T2.3 survey on biosecurity measures in the pig slaughter line. We forwarded a link to the German version of the questionnaire to the Ministry.

Thanks to a collaboration with the **German Meat Industry Association** (Verband der Fleischwirtschaft e.V.) and its support of our study, the survey on biosecurity measures at slaughter was sent out to more than 90 German slaughterhouses via the association's newsletter.

The project clearly benefits from **national collaborations with animal health services/veterinary services and practicing veterinarians**, which partly have already existed before (in Germany, we could build on a proven cooperation with animal health services after the project ORRES) and partly are being built during the BIOPIGEE project, are of high importance and support for the project. These services/vets can recruit farms based on their client pool and are less restricted in accessing farms for sample collection/survey in the covid-19 situation. They are also involved in our expert panel and will play an important role in the dissemination part as having a special interest in our findings and to disseminate them. In the expert panel, also staff with agricultural and teaching background (chambers of agriculture), scientists from different national (research) institutes (e.g. FLI in Germany) and universities (e.g. Vetmeduni Vienna/Leipzig, University of Rostock, Utrecht University), quality controllers of the pig production chain are included. Additionally, the cooperation with universities also makes it possible to obtain data and information from on-going national projects to fill any data gaps in our WPs. For instance, cooperation with Austrian swine clinics enables to recruit farms to

participate in questionnaires and to obtain information about existing data sources. Thanks to these collaborations, we can build on a strong network of varied experts between practice and science in Europe.

To enable consideration of pig movement information for simulation studies in WP4, we intensified a contact to the **Chamber of Agriculture** in the German federal state North Rhine-Westphalia and initiated their collaboration with ANSES and AGES. The Chamber and institutes are exchanging information and ideas.

5.1.4.3.16 JRP22-R2-FBZ4.1-TOXOSOURCES

5.1.4.3.16.1 Summary of the work carried out in the Project

TOXOSOURCES is a Joint Research Project of the One Health EJP that focuses on *Toxoplasma gondii* at the interface between humans, animals, food, and the environment.

Toxoplasma gondii is a foodborne parasite that causes a high disease burden. The infection can be acquired by ingesting oocysts (environmental pathway) or tissue cysts (meatborne pathway). The relative contributions of the two pathways to the infection and disease in humans remain unknown, partly due a to lack of appropriate methods.

TOXOSOURCES investigates the relative contributions of the different transmission routes and sources of *T. gondii* infection using multidisciplinary approaches. The project is developing new methods and yielding new knowledge, and it has both ambition and potential to advance science in the field.

The TOXOSOURCES consortium has collected data for a multicentre quantitative microbiological risk assessment for *T. gondii*: a multicentre quantitative exposure survey was conducted, and systematic literature reviews yielded estimates of prevalence of the infection in a high number of animal host species as well as in fresh produce and the environment. After an extensive literature review was conducted to support the selection of a method to detect *T. gondii* oocysts in fresh produce, a Standard Operating Procedure was developed and is being implemented and validated, while the sampling strategy for a multicentre pilot survey on ready-to-eat salads has been finalised. The project has selected promising antigens for exploring serology for detecting *T. gondii* infections caused by oocysts, and an unprecedented effort of whole genome sequencing of *T. gondii* isolates was used to identify polymorphic marker regions for the establishment of a new typing method to detect within-genotype variation.

The outcomes of TOXOSOURCES will include quantitative and comparable estimates of the contribution of the main sources and transmission routes to *T. gondii* infections across Europe. The project fills several relevant data gaps. A major outcome already reached is the collection and whole genome sequencing of a large number of *T. gondii* isolates across Europe. Moreover, the consortium itself is a major achievement - exemplifying a successful cross-sectoral, international One Health collaboration, which is needed to address this zoonotic parasite.

The TOXOSOURCES Consortium comprises 21 One Health EJP partners and several external partners. The TOXOSOURCES Consortium has shown impressive resourcefulness and adaptability, and while timelines have needed adapting, all Milestones have been reached on time and all Deliverables and reports have been submitted by their planned deadlines. Dissemination of outcomes has been active and included scientific publications and presentations at conferences, workshops and webinars, and several collaborations with other projects and networks have been established.

5.1.4.3.16.2 Progress of the project: description of activities

Progress made/expected to be made during M37-M45 for all tasks is in line with the planned timeline, with no delays in reaching milestones or deliverables for Y4 (2021) expected. The period started well, as all milestones and deliverables of Y3 (2020) were reached on time.

Due to the COVID-19-pandemic there has been a need to considerably adapt internal timelines, in particular those related to laboratory work due to lockdowns. TOXOSOURCES applied for a 6-month no-cost extension until the end of the year 2022, to ensure 1) that the project can fully achieve all the planned aims; 2) that all partners have the opportunity to benefit from implementation of the new methods and knowledge; 3) that the studies are able to cover all the aspects planned; 4) that the outputs scheduled for 2022 will be of high quality and we have time for their efficient dissemination; and as a result of all the previous points: 5) that TOXOSOURCES will have the impact it has the potential

to have.

The work is organized into 5 work packages (WPs) with tasks (T) and some subtasks (sT). The project spans over three Annual Periods (Y3-Y5, 2020-2022).

Key outputs are available via the project homepage: https://onehealthejp.eu/jrp-toxosources/

Kick-off Meeting of TOXOSOURCES, February 2020, at Statens Serum Institut, Copenhagen, Denmark.

WP1 Coordination and impact

TOXOSOURCES-WP1 manages the project and is responsible for its progress, and integrates all the results of the project to achieve the goals. TOXOSOURCES-WP1 ensures the project adheres to H2020 rules regarding e.g. ethics, IRP, dissemination and publication. Moreover, TOXOSOURCES-WP1 is responsible for the Data Management Plan of the project. TOXOSOURCES-WP1 coordinates the compiling of deliverables and reports and their timely submission, as well as the organizing of project meetings and communication. Science-to-policy translation and efficient dissemination are emphasized to maximize the impact. Interest Group facilitates targeted dissemination to stakeholders.

The main focus of TOXOSOURCES-WP1 during Y4 is ensuring that the project progresses efficiently as planned, and organizing the Annual Meeting (divided into two parts, Part I, held in June, and Part II, planned for October). These aims are being reached. All Milestones have been reached on time and all Deliverables and reports have been submitted by their deadlines. The Annual Meeting is organised in two parts to take benefit of synergies with both the Annual Scientific Meeting of One Health EJP and a major event in parasitology in Europe, EMOP. Dissemination has been active and included scientific publications and presentations at conferences, workshops and webinars - including several invited presentations. TOXOSOURCES was invited to present its results at the Stakeholders Committee Meeting of One Health EJP on June 11, 2021, and was highlighted in a Thematic Report focusing on environmental aspects addressed by One Health EJP projects. Several collaborations with other projects and networks have been established. Of specific note is the collaboration with SafeConsume project in taking benefit of Horizon Results Booster services. TOXOSOURCES-WP1 integrates all the results of the project to make them useful, in particular to contribute to developing interventions.

The impact of the COVID-19 situation is followed closely, and challenges have been managed well by planning and resourcefulness, deputies and replacements. The effect of delays caused by the COVID-19-pandemic is evident, and there has been a need to considerably adapt internal timelines, in particular those related to laboratory work due to lockdowns. The project applied for a 6-month extension until the end of the year 2022, to ensure 1) that the project can fully achieve all the planned aims; 2) that all partners have opportunity to benefit from implementation of the new methods and knowledge; 3) that the studies are able to cover all the aspects planned; 4) that the outputs scheduled for 2022 will be of high quality and we have time for their efficient dissemination; and as a result of all the previous points: 5) that TOXOSOURCES will have the impact it has the potential to have. The

extension request was solely due to the challenges caused by the COVID-19-pandemic.

JRP22-WP1-T1- Management, coordination and communication

The Kick-off Meeting was held February 3–4, 2020, at SSI, Copenhagen, Denmark, with a possibility to participate remotely. Online meetings with the whole consortium have been held on October 20, 2020 and on June 8, 2021 (Annual Meeting 2021, Part I). Next Consortium meeting (Annual Meeting 2021, Part II) is planned to be held in connection to EMOP conference.

The established key structures for management of the project include monthly online meeting with TOXOSOURCES WP-Leaders and Co-Leaders, Consortium emails, use of the online group for sharing and storage of relevant documents, and WP-level online meetings.

The Data Management Plan (DMP) is updated regularly, and the project leader participates in the work of the One Health EJP DMP Committee. Outcomes are made available following FAIR principle. Dissemination of the outcomes has been active, and included presentations to relevant audiences at conferences, workshops and webinars, and scientific publications.

The collaborations with the Interest Group and other collaborators has started by establishing key contacts. Invited by SafeConsume, TOXOSOURCES joined a Horizon Results Booster project group called “DISH - Towards healthy and safe diet” that includes SafeConsume, TOXOSOURCES, Stance4Health, EAT2beNICE and FOODSAFETY4EU. TOXOSOURCES suggested OHEJP Cogwheel Workshop with SafeConsume, and it was organized on November 25, 2020.

Challenged by the COVID-19-pandemic, the TOXOSOURCES Consortium has showed impressive resourcefulness and adaptability, and the careful risks-and-dependencies planning has proved useful. All Milestones, Deliverables and reports have been reached and submitted by their planned deadlines. The consortium is highly motivated and the general atmosphere is positive and supportive.

JRP22-WP1-T2 Integration of all results to contribute to developing interventions, dissemination

Efficient dissemination and science-to-policy activities at national, European and global levels include facilitating the use of the new knowledge, linking the providers and users of the knowledge, and advocating multidisciplinary approaches.

TOXOSOURCES is part of a Horizon Results Booster project group “DISH - Towards healthy and safe diet” that includes SafeConsume, TOXOSOURCES, Stance4Health, EAT2beNICE and FOODSAFETY4EU. Module A was finished at the end of 2020, and module B services are launched, mainly scheduled to take place during the second half of 2021.

TOXOSOURCES was invited to present its results at the Stakeholders Committee Meeting of One Health EJP on June 11, 2021, and was highlighted in a Thematic Report focusing on environmental aspects addressed by One Health EJP projects.

Outcomes of TOXOSOURCES are made available following FAIR principle. Dissemination of the outcomes has been active, and included presentations to relevant audiences at conferences, workshops and webinars, and scientific publications. Early-career colleagues involved in the work are particularly encouraged to present the results. Another special focus in the dissemination is integration of results from the different TOXOSOURCES-WPs.

Screenshot from an online meeting of TOXOSOURCES-WP-Leaders and WP-Deputy-Leaders.

WP2 Multicentre quantitative microbiological risk assessment for T. gondii infections

TOXOSOURCES-WP2 aims to quantify the relative contribution of sources of *T. gondii* infection, including meat products, fresh produce and environmental pathways, in all EU regions by quantitative microbiological risk assessment (QMRA). TOXOSOURCES-WP2 develops QMRA models for infection via tissue cysts (meat) and oocysts (environmental pathways), and applies the models in a multi-country study covering all four EU regions. Input data for the QMRA have been collected with contributions/support by all partners and TOXOSOURCES-WP3. An overview of the prevalence of *T. gondii* infection in humans and animals used for human consumption as well as cats was obtained by review of available literature, including grey literature. Exposure data were collected in a harmonised way using a survey specifically designed for QMRA purposes. Region- or country-specific products, dishes or eating habits were identified and the associated key processing parameters collected by the partners. The literature review of human infections also covers risk factor studies, to compare QMRA outcomes and epidemiological data.

During Y4, the main focus is finalising the data collection and running the QMRA model. The exposure survey was conducted. Meat processing information is collected and literature reviews are finalized.

The work has progressed following the plans. All milestones have been reached on time, and Deliverable about the review of prevalence of *T. gondii* in animals was submitted on time.

JRP22-WP2-T1- QMRA modelling for human T. gondii infections

The Consortium members involved in QMRA modelling met at RIVM, The Netherlands, to discuss plans in 2020. The development of the structure for the QMRA model for environmental transmission of *T. gondii* was finished, and expansion of both the meatborne and environmental QMRA models to include multiple countries was started. The work builds on previous work, in particular on an existing meatborne QMRA model, as well as on ongoing work in a PhD project of Huifang Deng, which was successfully defended on December 08, 2020.

During Y4, the main focus is checking input data as preparation for running the QMRA model. In addition, sensitivity analyses (to identify the most influential input parameters) and scenario analyses (in case of large uncertainty regarding input parameters) that will be initiated are being planned.

JRP22-WP2-T2- Review of prevalence of T. gondii infection in animals

A list of European countries and a list of key animal species raised or hunted for human consumption in Europe were collated. These were used to develop a search strategy for data on prevalence of *T. gondii* in animals. Experiences from systematic reviews performed by EFSA and in the Baltic-Nordic region were taken into account in the process.

The search, screening of retrieved records, and data extraction were finished in 2020, and the data were checked and cleaned. The process and general results were summarised in a Deliverable. Selected primary results were submitted as abstracts and selected for presentation at scientific events.

The results are prepared for scientific publication.

This task is an example of a task that has included collaboration with early-career colleagues, and cross-project collaboration with OHEJP PhD project.

Related to this task, TOXOSOURCES members are also participating in another review together with the ORION project, focusing on risk factors for *T. gondii* infections in animals. While that information is not directly needed for the QMRA, together with the prevalence estimates it will provide important input for planning interventions.

JRP22-WP2-T3- Quantitative exposure survey

In 2020 a general questionnaire was developed by expanding an existing questionnaire and taking into account experiences from the Dutch National Food Consumption Survey, risk factor information from a prospective case-control study in the Netherlands, and applying categorisation of the EFSA FoodEx system, which enables comparison with existing surveys. The questions were adapted to the different countries by including region/country-specific products. Decisions on sample size (respondents per each country) were finalized.

The questionnaires were distributed via a market research agency to collect harmonised data on food consumption and handling and exposure to oocysts from the countries included in the QMRA. The data were provided for the QMRA, and plans for making the data publically available to be used for QMRA modelling of other foodborne pathogens were initiated.

JRP22-WP2-T4- Overview of processing parameters for relevant meat products

Information on food consumption in the different countries was collected from the EFSA FoodEx2 database. Information on region/country-specific relevant products was also collected from consortium members. The list of products that need to be included in the exposure survey was harmonised across the countries. Product-specific processing parameters were discussed in the process of planning the survey questions, in particular regarding local products.

The data from the exposure survey inform which meat products are included in the QMRA model. Next, product-specific parameter values for freezing, salting and heating (e.g. time, temperature, concentration) are being collated. The information are obtained using meat processing handbooks, communication with meat producers, or from package information in retail, and will be delivered as input data to the QMRA.

JRP22-WP2-T5- Review of prevalence and risk factors for human T. gondii infection

This task has been among those most affected by COVID-19 pandemic due to involvement of several key consortium members in the COVID-19 response. Efficient replacements and synergy with TOXOSOURCES-WP2-T2 ensured that this task has also progressed well. The experiences from TOXOSOURCES-WP2-T2 work were incorporated into the planning of the systematic review protocol under this task. The search was undertaken, and screening progresses. During 2021, the main focus is to finalize data extraction, analyse the results and deliver data for the QMRA modelling team. The data will be analysed by Bayesian hierarchical modelling to estimate the prevalence by country.

Results from the literature review on *T. gondii* source attribution (including risk factor analyses) for COST-action Euro-FBP were presented at OHEJPASM2020, emphasizing the continuation of the work in TOXOSOURCES. The screening and extracting protocol was aligned with that of a review done in collaboration with ORION project.

WP3 Multicentre survey to fill the key existing gap: role of fresh produce (i.e. Ready-to-Eat salads)

TOXOSOURCES-WP3 aims to fill the knowledge gap concerning the relevance of fresh produce contamination by *T. gondii* oocysts as an infection source for humans. TOXOSOURCES-WP3 selects the most reliable methods for the molecular detection of *T. gondii* oocysts in fresh produce using a literature review, expert experiences, experimental evaluation and inter-laboratory comparison. Harmonised detection is implemented among the partners. TOXOSOURCES-WP3 collects existing data on *T. gondii* oocyst prevalence in fresh produce and the environment, together with information on fresh produce (e.g. RTE production, trading and consumption) in Europe. The data are used to design a risk-based sampling strategy for a multicentre pilot survey to detect *T. gondii* in fresh produce, applying the defined SOP. The multicentre pilot study, spanning all four European regions, will be the first of its kind and will deliver valuable input for the TOXOSOURCES-WP2 and future QMRAs.

In 2021, the focus is to: i) implement the molecular detection method(s) among partners and perform a ring trial to assess the implementation and complete SOP validation; ii) to complete the collection through a literature review of existing data for *T. gondii* oocysts in environment to be provided to WP2; iii) to start sampling and tests for the multicentre survey.

All these aims are being reached. The work has progressed following the plans. The Deliverable describing the organisation of the ring trial was submitted on time, and the milestone of finalizing the sampling strategy was reached. The ring trial timeline was adjusted to allow all interested laboratories to implement the method. The final sampling strategy was presented to the consortium at the Annual Meeting, Part I.

JRP22-WP3-T1- Selection, evaluation and implementation of detection procedure for T. gondii oocysts in fresh produce

An extensive literature review and multi-attribute assessment of the different steps (oocyst recovery, DNA extraction and DNA amplification) was performed and complemented by a survey of expert opinions, current practices and experiences on molecular detection of *T. gondii* (DNA), to select the most suitable molecular method for *T. gondii* oocyst detection in fresh produce. The Deliverable D-JRP-TOXOSOURCES-WP3.1 summarized this process, which provided a good starting point for developing a standard operating procedure (SOP) for the multicentre survey of *T. gondii* oocysts in fresh produce. The results were published as a scientific article in early 2021.

The comparative experimental work was completed in 2020, and instead of the planned physical technical workshops, video tutorials were produced. The work towards the SOP was reported in Deliverable D-JRP-TOXOSOURCES-WP3.2 and presented to relevant audiences in 2021.

The deliverable describing the organisation of the ring trial was submitted on time. The ring trial timeline was adjusted to allow all interested laboratories (higher number of laboratories than originally planned) to implement the method, and thus this task continues some months longer than originally planned. The main work was finished on time and all the outcomes (Deliverables and in addition a scientific article) have been delivered on time.

JRP22-WP3-T2- Design of a risk-based sampling strategy

An extensive review of peer-reviewed literature on the prevalence of *T. gondii* oocysts in fresh produce was performed, as well as a review of literature on *T. gondii* prevalence in the environment (soil and water) and bivalves. These were complemented by an online questionnaire to consortium partners to collate grey literature on the topic. A data summary on literature-based prevalence of *T. gondii* oocysts in fresh produce was provided to TOXOSOURCES-WP2, and the results are being prepared for scientific publication.

The design of a risk-based sampling strategy for the multicentre survey of *T. gondii* oocysts in fresh produce was started by preparing an online questionnaire to gather relevant information on trade and consumption of ready-to-eat salads from local and international industry. In addition, a request for information was sent via the EFSA focal point and a survey on RTE salads sold locally at supermarkets was conducted. Data were combined to design a sampling plan, taking into account sample size calculations, feasibility and costs, and the sampling strategy for WP3-T3 was defined.

The milestone of finalizing the sampling strategy was reached. Final sampling strategy was presented to the consortium at the Annual Meeting, Part I.

JRP22-WP3-T3- Multicentre pilot survey on T. gondii in fresh produce in Europe

The sampling strategy and plan for the multicentre pilot survey was finalized. A multicentre pilot survey for the molecular detection of *T. gondii* in selected fresh produce in Europe will be performed based on outputs of WP3-T1 and WP3-T2. Samples are collected, over one year, in countries representative of the four EU regions according to the strategy developed in WP3-T2. Fresh produce samples are dispatched refrigerated, within 24h-48h, to partner institutions to be analysed using the SOP defined in WP3-T1. Some partners may perform only the oocyst recovery step of the SOP, store frozen the processed samples and deliver them to partners that have implemented the full SOP for the molecular testing. This approach will ensure faster processing of the perishable fresh produce thus increasing the overall amount of tested samples.

WP4 Serology method based on novel antigens to discriminate T. gondii infections acquired from oocysts

TOXOSOURCES-WP4 aims to develop a source-attributing serological method. TOXOSOURCES-WP4 identifies novel oocyst/sporozoite-specific antigens of *T. gondii* that have source-attributing potential and explores serological methods able to discriminate between oocyst- versus tissue cyst-driven infections. Finally, the methodology is applied to estimate the proportion of oocyst-driven infections in humans and animals used for human consumption.

The bioinformatic selection of promising protein candidates was finalized in 2020, and although laboratory work has been affected by the COVID-19-related restrictions, the work has progressed following the overall plans in 2021. The recombinant expression of proteins of interest is followed by assessing them using suitable sera.

JRP22-WP4-T1- Identification and production of T. gondii stage-specific antigens for source attribution

The predicted proteome of the *T. gondii* oocyst/sporozoite was analysed bioinformatically to identify the best stage-specific and antigenically relevant protein candidates. A list of 96 proteins with source-attributing potential was defined. Main selection criteria were exclusive expression in oocysts, evidence of secretion, and a high score in linear epitope prediction.

The bioinformatic selection of promising protein candidates was finalized in 2020, and the work on the next steps continues in 2021 and further in 2022. The recombinant expression of proteins of interest is followed by assessing them using suitable sera. A selection of known stage-specific proteins are tested in parallel to the novel candidates.

JRP22-WP4-T2- Development of a novel stage-specific antigen-based ELISA to diagnose oocyst- and bradyzoite-driven T. gondii infections

Key sera have been characterized using a selection of widely employed serological tests, yielding interesting comparative data.

The plan and experimental design for the development work were finalized in 2020 for the screening of the proteins using a step-by-step validation process.

JRP22-WP4-T2-sT1 Standardization of a POI-based ELISA to diagnose oocyst- and/or bradyzoite driven T. gondii infections using reference pig sera

A suitable panel of sera from pigs experimentally infected with either oocysts or tissue cysts, first characterized using commercially available and routinely used serological tests for the selection of the best secondary antibody for the novel stage-specific antigen-based ELISA, is then tested using the approaches developed.

JRP22-WP4-T2-sT2 Validation of a novel stage-specific antigen based ELISA to diagnose oocysts-and/or bradyzoite-driven T. gondii infections using reference sera from several relevant host species including humans

A suitable panel of sera from sheep experimentally infected with oocysts was characterized using commercially available and routinely used serological tests and the best secondary antibody for the novel stage-specific antigen-based ELISA was selected. International collaboration identified suitable sera from humans that would be useful for the work. The reference sera are then tested using the approaches developed.

JRP22-WP4-T3- Exploring the prevalence of oocyst-derived T. gondii infections in animals and in humans

Using the POI-ELISA validated in WP4-T2, an inter-laboratory ring trial will be conducted, followed by pilot studies. The method will also be offered to other Consortium partners for testing existing collections of positive sera. The planning of this work has started.

JRP22-WP4-T3-sT1 Inter-laboratory validation of the POI-based ELISA

POI-based ELISA will be adapted to the participating laboratories to obtain comparable results. Inter-laboratory reproducibility and analytical sensitivity will be evaluated using positive and negative reference sera (experimental *T. gondii* infection) identified in WP4-T2-sT1. The planning of this work has started.

JRP22-WP4-T3-sT2 Exploring the prevalence of oocyst-driven infections in domestic animals (pigs, small ruminants) and wildlife (wild-boar, wild ungulates) used for human consumption

To reveal differences in the relative prevalence of oocyst-driven infections between animal species, regions and management systems, positive sera from naturally infected animals from different European regions will be tested using the POI-based ELISA. The planning of this work has started.

JRP22-WP4-T3-sT3 Exploring the prevalence of oocyst-driven infections in humans

Well-defined positive sera from *T. gondii*-infected humans available from different European regions will be tested using the POI-based ELISA by pilot studies, that may include, based on availability, sera from congenitally-infected children aged <13 months and from older children >2 years; sera from recently infected adults; as well as sera from any suspected *T. gondii* outbreaks in Europe. The planning of this work has started.

WP5 Novel T. gondii typing method to detect within-genotype variation

TOXOSOURCES-WP5 aims to identify highly polymorphic regions in genomes of very closely related *T. gondii* strains across Europe, which are made available by partners and collaborators. Preliminary NGS data on European clonal type II *T. gondii* isolates has revealed substantial variation between isolates and relative to reference strains. Using the panel of strains from various parts of Europe, regions in the genome with an optimal SNP density are identified and used to establish a novel typing method.

The work in year 2020 was successful for both retrieval of relevant isolates and their whole genome sequencing (WGS). The work has progressed following the plans in 2021, with focus on disseminating the results by presentations to relevant audiences.

JRP22-WP5-T1- Retrieval of relevant T. gondii isolates or NGS-quality DNAs for NGS and NGS-MST

T. gondii isolates, WGS-quality DNAs or WGS-data on isolates were collected from across Europe. Isolates were expanded *in vitro*, and DNA was extracted for WGS. The focus was on *T. gondii* Type II isolates, while other isolates were included as well. This retrieval of key *T. gondii* isolates or DNAs was successful and the total number of isolates retrieved for the work is markedly higher than the original target. Isolates from northern and eastern European regions were first underrepresented on the list, but further efforts were successful in gathering more isolates from these regions.

During 2021, further isolates are collected to establish a panel for inter-laboratory comparison and pilot studies (WP5-T3). A request for contributions was sent out to networks including NRL network. For particular regions, we aim at a larger number of further isolates (about n=10) to show that resolution of typing is high enough to trace differences of local isolates.

All DNAs are also characterised based on polymorphism of fewer markers using existing standard techniques (PCR-RFLP and microsatellite (MS) typing).

JRP22-WP5-T2- Novel, standardized high-throughput direct NGS-MLST T. gondii genotyping method

Based on sequence information obtained by WP5-T1 a novel, standardized high-throughput targeted NGS-MLST genotyping method is established and validated to distinguish between closely related *T. gondii* strains. Timely dissemination is emphasized, and results of this work are presented to relevant audiences.

WP5-T2-sT1 Whole genome sequencing (WGS) of key T. gondii isolates and WGS-quality DNAs

A large number of whole genome sequences have been generated. The sequences of key *T. gondii* isolates have been provided to TOXOSOURCES-WP5-T2-sT2.

WP5-T2-sT2 Establishment, validation and refinement of a novel, standardized high-throughput targeted NGS-MLST T. gondii genotyping method

Based on the sequences from WP5-T2-sT1, highly polymorphic marker regions (partially focusing on introns and/or particular gene regions of e.g. virulence associated genes) were identified and are being evaluated for suitability for the establishment of a new typing method. The higher number of sequences than originally planned has proven highly beneficial for the work.

For validation, the novel NGS-MLST results were compared with results of conventional techniques (PCR-RFLP, MS typing) (i). Similarities and differences in the capability of the novel NGS-MLST method and the PCR-RFLP- and MS-based techniques for the *T. gondii* differentiation are established. (ii) The analytical sensitivity will be determined using different sample matrices, spiked with different levels of parasites. Plans are being made for this work.

A standardized workflow for the collection and preliminary analysis of NGS-MLST data on *T. gondii* is established. Finally, the new technology will distributed to interested partners, if possible by a workshop at FLI, prior to inter-laboratory comparison and pilot studies (WP5-T3).

JRP22-WP5-T3- Inter-laboratory comparison and NGS-MLST pilot studies

The plans for this work are being made. First, an inter-laboratory comparison of the established novel NGS-MLST genotyping method is performed. In Y5 the method will be applied to "real/unknown" *T. gondii* positive samples from the field, i.e. samples from intermediate hosts (including humans), definitive hosts and environment by laboratories that implemented the method.

Plans are being made for three pilot studies, based on availability of suitable isolates/DNA/samples. Aim is to apply the method to different types of samples.

JRP22- WP5-T3-sT1 Inter-laboratory comparison on the novel NGS-MLST **method**

The plans for this work are being made. An inter-laboratory comparison of the established novel NGS-MLST genotyping method will be based on a defined set of samples sent out to participating laboratories across Europe, to confirm that typing results are in accord between different laboratories and to obtain information about the robustness and applicability

5.1.4.3.16.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
22	D-JRP-TOXOSOURCES-WP1.1	Data Management Plan	M30	M30		Public. 10.5281/zenodo.3924450 OHEJP: available	8
22	D-JRP-TOXOSOURCES-WP2.1	Report on prevalence of *T. gondii* infection in animals for human consumption and cats within Europe	M40	M40		Public. https://doi.org/10.5281/zenodo.4730705 OHEJP: available	5
22	D-JRP-TOXOSOURCES-WP3.1	Report on available analytical procedures for detection of *T. gondii* in fresh produce and list of promising analytical procedures	M28	M28		Public. 10.5281/zenodo.3778719 OHEJP: available	2
22	D-JRP-TOXOSOURCES-WP3.2	SOP on detection of *T. gondii* in selected fresh produce matrix	M36	M36		Public. 10.5281/zenodo.4405242 OHEJP: available	2

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
22	D-JRP-TOXOSOURCES-WP3.3	Report on the ring trial of WP3	M40	M40		Public. https://doi.org/10.5281/zenodo.4730717 OHEJP: available	2

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
22	M-JRP-TOXOSOURCES-01	Kick-off Meeting held by WP1	M26	Yes		Kick-off Meeting held on 3.-4.2.2020 at SSI, Copenhagen, Denmark, with possibility to attend online. Milestone reached by M26 (on time).
22	M-JRP-TOXOSOURCES-02	Bioinformatic selection of oocyst/sporozoite-specific antigens completed in WP4	M28	Yes		Selection done. Milestone reached by M28 (on time).
22	M-JRP-TOXOXOURCES-03	Key isolates summarized in WP5	M30	Yes		Summary list of key isolates compiled. Milestone reached by M28 (2 months ahead of time).
22	M-JRP-TOXOSOURCES-04	List of meat products or dishes that need to be included in exposure survey is provided by WP2-T4 to WP2-T3	M34	Yes		List provided. Milestone reached by M34 (on time).
22	M-JRP-TOXOSOURCES-05	Experimental selection of the appropriate methods for samples analysis in WP3	M36	Yes		Experimental selection done. Milestone reached by M36 (on time).
22	M-JRP-TOXOSOURCES-06	Delivery of data summary on literature-based prevalence of *T. gondii* oocysts in fresh produce from WP3 to WP2	M36	Yes		Data summary delivered. Milestone reached by M36 (on time).
22	M-JRP-TOXOSOURCES-07	Production of the first sets of purified soluble recombinant	M36	Yes		Production started. Milestone reached by M36 (on time).

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
		proteins (up to 100) for WP4-T2 serological assays				
22	M-JRP-TOXOSOURCES-08	Evaluation of the source attributing ability of the first sets of stage-specific antigens produced in WP4-T1	M36	Yes		Evaluation started. Milestone reached by M36 (on time).
22	M-JRP-TOXOSOURCES-09	Finalization of risk-based sampling strategy of WP3	M38	Yes		Strategy ready by the deadline of the milestone. The sampling plan was presented to the consortium at Annual Meeting 2021, Part I. Milestone reached by M38 (on time).
22	M-JRP-TOXOSOURCES-10	Country-specific estimates of prevalence in animals provided as input for QMRA	M38	Yes		Input data provided. Milestone reached by M38 (on time).
22	M-JRP-TOXOSOURCES-11	Questionnaires for exposure survey ready to be rolled out by WP2	M38	Yes		Questionnaires finalised. Milestone reached by M38 (on time).
22	M-JRP-TOXOSOURCES-12	List of meat products for which processing parameters need to be collected is provided to WP2-T4	M40	Yes		Information provided, with focus on local products included in the survey. Milestone reached by M40 (on time).
22	M-JRP-TOXOSOURCES-13	Exposure data from survey are provided as input for QMRA	M43	Yes		Data available in M41. Milestone reached by M41 (2 months ahead of time).

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
22	M-JRP-TOXOSOURCES-14	Processing parameters for relevant meat products are provided as input for QMRA	M45	Yes, will be		Reaching this milestone is supported by the work done to identify relevant local products (synergy). Milestone is being reached on time, by the end of M45.
22	M-JRP-TOXOSOURCES-15	Country-specific estimates for prevalence of human *T. gondii* infection provided as input data for QMRA	M45	Yes, will be		Reaching this milestone is supported by the work done in animal prevalence review (synergy). The papers included in systematic review contain the information. The final format of how the information is provided as input data is being tailored for the purpose, based on experiences from the animal prevalence review. Milestone is being reached on time, by the end of M45.
22	M-JRP-TOXOSOURCES- 16	Delivery of data summary on fresh produce consumption in EU from WP3 to WP2	M45	Yes		Data delivered. Milestone reached by M45 (on time).
22	M-JRP-TOXOSOURCES-17	Delivery of data summary on literature-based prevalence of *T. gondii* oocysts in environment from WP3 to WP2	M45	Yes		Data delivered. Milestone reached by M45 (on time).
22	M-JRP-TOXOSOURCES-18	Antigens for serological assays	M48			
22	M-JRP-TOXOSOURCES-19	Annual Meeting held by WP1	M48	Yes		Part I held in M42, part II planned for M46.

5.1.4.3.16.4 Follow-up of the recommendations and comments by the Ethics Advisors The

Ethic Advisors already accepted your comments. Therefore, this part of the report has been closed.

5.1.4.3.16.5 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
A real-time quantitative polymerase chain reaction for the specific detection of *Hammondia hammondi* and its differentiation from *Toxoplasma gondii* https://doi.org/10.1186/s13071-020-04571-8 https://zenodo.org/record/4647866#.YGNbWK8zaUk	YES	NO	YES, 2190 €
Expanding the Known Repertoire of C-Type Lectin Receptors Binding to *Toxoplasma gondii* Oocysts Using a Modified High-Resolution Immunofluorescence Assay https://doi.org/10.1128/mSphere.01341-20 https://zenodo.org/record/4657064#.YGXUY68zaUk	YES	NO	YES, 2130 €
Experimental infection with *Toxoplasma gondii* in broiler chickens (*Gallus domesticus*): seroconversion, tissue cyst distribution, and prophylaxis https://doi.org/10.1007/s00436-020-06984-x https://zenodo.org/record/4647895#.YGNbDT9JGUl Shareable link: https://rdcu.be/chKFi	YES OHEJP is not mentioned under 'Funding', as the main authors are from external partners, participating at their own funding. OHEJP is acknowledged under 'Acknowledgments', stating: "AG, RB and	NO	NO But as FAIR as possible: a "shareable link" is provided by the Springer Nature SharedIt content-sharing initiative: https://rdcu.be/chKFi "Authors of original research articles: // Can post shareable links to view-only versions of their peer-reviewed research paper anywhere, including via social channels, institutional repositories and authors' own websites as well as scholarly collaborative networks"

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
	GS are part of the TOXOSOURCES consortium, supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme."		The metadata are available via Zenodo, together with the shareable link.
Expression of in vivo biotinylated recombinant antigens SAG1 and SAG2A from *Toxoplasma gondii* for improved seroepidemiological bead-based multiplex assays 10.1186/s12896-020-00646-7 https://zenodo.org/record/4129949#.X6Ldr4hKjcc	YES	NO	YES, 1.890 €
Fluorescent bead-based serological detection of *Toxoplasma gondii* infection in chickens https://doi.org/10.1186/s13071-020-04244-6 https://zenodo.org/record/3974390#.X6PV0WhKjcc	YES	NO	YES, 1990 €
Infection prevention and control practices of ambulatory veterinarians: A questionnaire study in Finland https://doi.org/10.1002/vms3.464 https://zenodo.org/record/4647937#.YGNfea8zaUk	YES	NO	YES, 0 €
Isolation and genetic characterization of *Toxoplasma gondii* in Spanish sheep flocks https://doi.org/10.1186/s13071-020-04275-z https://zenodo.org/record/3974417#.X6PWsWhKjcc	YES	NO	YES, 1990 €

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Molecular Methods for the Detection of Toxoplasma gondii Oocysts in Fresh Produce: An Extensive Review https://doi.org/10.3390/microorganisms9010167 https://zenodo.org/record/4647840#.YGNQk68zaUk	YES	NO	YES, 738.89 €
Molecular analysis suggests that Namibian cheetahs (*Acinonyx jubatus*) are definitive hosts of a so far undescribed *Besnoitia* species https://doi.org/10.1186/s13071-021-04697-3 https://zenodo.org/record/4694200#.YHg5Z-gzaUk	YES, both TOXOSOURCES and MEME	NO	YES, 2190 € Open Access funding enabled and organized by project DEAL.
Isolation, Genotyping, and Mouse Virulence Characterization of *Toxoplasma gondii* From Free Ranging Iberian Pigs. https://doi.org/10.3389/fvets.2020.604782 https://zenodo.org/record/5516873#.YUg5qOxxe70	YES		YES, ND (= no data on fee)
In vivo and in vitro models show unexpected degrees of virulence among Toxoplasma gondii type II and III isolates from sheep. https://doi.org/10.1186/s13567-021-00953-7 https://zenodo.org/record/5516897#.YUg7QOxxe70	YES		YES, ND
Comparison of Direct and Indirect Toxoplasma gondii Detection and Genotyping in Game: Relationship and Challenges. https://doi.org/10.3390/microorganisms9081663 https://zenodo.org/record/5516907#.YUg8qOxxe70	YES		YES, ND

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Zoonotic pathogens in wild muskoxen (*Ovibos moschatus*) and domestic sheep (*Ovis aries*) from Greenland https://doi.org/10.1002/vms3.599 https://zenodo.org/record/5516925#.YUhCMexxe70	YES		YES, 1600 €
A longitudinal study of *Toxoplasma gondii* seroconversion on four large Danish sow farms. https://doi.org/10.1016/j.vetpar.2021.109460 https://zenodo.org/record/5516957#.YUhCDexxe70	YES		YES, ND
Veterinarians as a risk group for zoonoses: exposure, knowledge and protective practices in Finland *submitted manuscript*	YES		

Additional output

TOXOSOURCES is active in disseminating its outputs, with presentations to relevant audiences.

Invited talk at Mandagsmøde, Department of Infectious Disease Epidemiology & Prevention, SSI, March 2, 2020:

How and why to prevent *Toxoplasma gondii* infections

Pikka Jokelainen, SSI, Denmark

Oral presentation at OHEJPASM2020, May 27-29, 2020:

Source attribution for *Toxoplasma gondii* infections in Europe

Marieke Opsteegh (1), Hannah Morgan (1), Huifang Deng (1), Gereon Schares (2), Sandra Stelzer (2) Sara Monteiro Pires (3), Helga Waap (5), Jacek Sroka (6), Heidi Enemark (7), Jelena Srbljanovic (8), Olgica Djurkovic-Djakovic (8), Chiara Trevisan (9), Agnetha Hofhuis (1), Lasse S. Vestergaard (4), Pikka Jokelainen (4), Joke van der Giessen (1), Euro-FBP (COST Action FA1408), TOXOSOURCES Consortium

RIVM, The Netherlands (1); FLI, Germany (2); DTU, Denmark (3); SSI, Denmark (4); INIAV, Portugal (5); PIWET, Poland (6); NVI, Norway (7); UoB, Serbia (8); ITG, Belgium (9)

Poster at OHEJPASM2020, May 27-29, 2020:

TOXOSOURCES – *Toxoplasma gondii* sources quantified

Pikka Jokelainen (1), Marieke Opsteegh (2), Marco Lalle (3), Furio Spano (3), Gereon Schares (4), Sara Monteiro Pires (5), Anne Mayer-Scholl (6), Frank Seeber (7), Simone M. Cacciò (3), Joke van der Giessen (2), TOXOSOURCES Consortium (Joint Research Project of the One Health European Joint Programme)

SSI, Denmark (1); RIVM, The Netherlands (2); ISS, Italy (3); FLI, Germany (4); DTU Food, Denmark (5); BfR, Germany (6); RKI, Germany (7)

10.5281/zenodo.3924467

Poster and talk at 3-Minute-Thesis competition at OHEJPASM2020, May 27-29, 2020:

Tropism and persistence of *Toxoplasma gondii*: from pork carcass to sausage and dry ham, a quantitative risk assessment

Filip DAMEK1, Bastien FREMAUX2, Dominique AUBERT3, Marieke OPSTEEGH4, Sandra VUILLERMET1, Pikka JOKELAINEN5, Joke VAN DER GIESSEN4, Pascal BOIREAU1, Isabelle VILLENA3, Radu BLAGA1

1 UMR BIPAR, Ecole Nationale Vétérinaire d'Alfort, ANSES, France 2 IFIP - Institut du Porc, France 3 National Reference Center on Toxoplasmosis, Toxoplasma Biological Resources Center, CHU Reims and EA7510, SFR CAP-Santé, University of Reims Champagne-Ardenne, USC EpiToxo ANSES, France 4 National Institute for Public Health and the Environment, The Netherlands 5 Statens Serum Institut, Denmark

Short oral presentation at One Health EJP Cogwheel workshop with JPIAMR, April 28, 2020:

#TOXOSOURCES *Toxoplasma gondii* sources quantified

Pikka Jokelainen, SSI, Denmark

Poster and short oral presentation in a webinar 'Toxoplasma gondii e toxoplasmosis in una prorspettiva One Health' organized by Italian Society of Parasitology (SOIPA), June 30, 2020:

TOXOSOURCES – TOXOplasma gondii SOURCES quantified

P JOKELAINEN1, M OPSTEEGH2, M LALLE3, F SPANO3, G SCHARES4, S MONTEIRO PIRES5, A MAYER-SCHOLL6, F SEEBER7, S M CACCIÒ3, J VAN DER GIESSEN2, TOXOSOURCES CONSORTIUM (JOINT RESEARCH PROJECT OF THE ONE HEALTH EUROPEAN JOINT PROGRAMME)

1 Statens Serum Institut, Copenhagen, Denmark, 2 National Institute for Public Health and the Environment, Bilthoven, The Netherlands, 3 Istituto Superiore di Sanità, Rome, Italy, 4 Friedrich Loeffler Institute, Insel Riems, Germany, 5 Technical University of Denmark, Kongens Lyngby, Denmark, 6 German Federal Institute for Risk Assessment, Berlin, Germany, 7 Robert Koch Institute, Berlin, Germany

Two lectures at One Health EJP Summer School August 17-28, 2020:

Parasites in the food chain: global One Health risks

Wildlife and Public Health

Joke van der Giessen, RIVM, The Netherlands

Oral presentation at PhDay, October 14, 2020:

Desarrollo de un nuevo ELISA para la detección de anticuerpos frente a *Toxoplasma gondii* en el ganado porcino

Nadia María López Ureña, UCM, Spain

Invited talk at International One Health Webinar Series of School of Public Health and Zoonoses, Guru Angad Dev Veterinary and Animal Sciences University, India, November 5, 2020:

Relative contributions of the different sources of *Toxoplasma gondii*, a globally important pathogen of major public health concern

Pikka Jokelainen, SSI, Denmark

Oral presentation at International Pathology Day, November 11, 2020:

Endemic pathogens and international research projects during a pandemic: *Toxoplasma gondii* and international research project TOXOSOURCES as an example

Pikka Jokelainen, SSI, Denmark and Martha Betson, UoS, UK

Roundtable, International Pathology Day, November 11, 2020:

Discussion topic: Why international knowledge sharing is a winner

Pikka Jokelainen, SSI, Denmark

Oral presentation at ApicoWplexa virtual meeting series, November 12, 2020:

Toxoplasma gondii in Spanish farm animals: opening new avenues from genotype to phenotype

Mercedes Fernández-Escobar, UCM, Spain

Short oral presentation at One Health EJP Cogwheel workshop with SafeConsume, November 25, 2020:

TOXOSOURCES - *Toxoplasma gondii* sources quantified

Pikka Jokelainen, SSI, Denmark

Short oral presentation at 15th Workshop of the National Reference Laboratories for Parasites, December 15, 2020:

#TOXOSOURCES *Toxoplasma gondii* sources quantified

Pikka Jokelainen, SSI, Denmark

Oral presentations at TOXO-21 webinar, 2021,
by Rafael Calero-Bernal and Pikka Jokelainen

Oral presentations at ApicoWplexa virtual meeting series, February 18, 2021:
Selection, validation and SOP development of a molecular detection method for identification of Toxoplasma gondii oocysts in leafy-green vegetables
Iva Slana1, Nadja Bier2, Borbara Bartosova1, Gianluca Marucci3, Anne Mayer-Scholl2, Pikka Jokelainen4, Marco Lalle3

Oral presentation at ApicoWplexa virtual meeting series, February 18, 2021: (audience n 120, scientists)
A comparative study of the most widely used serological tests in the diagnosis of Toxoplasma gondii infection in small ruminants
López-Ureña, Nadia María1; Calero-Bernal, Rafael1; Pazmiño-Bonilla, Elvis Daniel1; Vázquez-Calvo, Ángela 2; Sánchez-Sánchez, Roberto1; Ortega-Mora, Luis Miguel1; Álvarez-García, Gema1

Invited oral presentation at RSU conference, virtually in Riga, Latvia
Pikka Jokelainen

Poster presentation at CSBSP conference, virtually in Vilnius, Lithuania
Pikka Jokelainen

Presentation at internal dissemination event 'OHEJP at SSI' 4.5.2021 (audience n=55, scientists)
Pikka Jokelainen

OHEJPASM 2021, June 9-11, 2021 (audience n= 530, scientist, policy makers)
Two oral presentations and several posters.

Poster presentation at conference 'Responsible Use of Antibiotics in Animals'
Infection prevention and control practices among ambulatory livestock and equine veterinarians
Marie Verkola[1], Terhi Järvelä[1], Asko Järvinen[2], Pikka Jokelainen[3,4], Anna-Maija Virtala[4], Paula M. Kinnunen[4], Annamari Heikinheimo[1,5]

Keynote presentation at ApicoWplexa virtual meeting series, June 24, 2021
Pikka Jokelainen

Oral presentation at ApicoWplexa virtual meeting series, June 24, 2021
Environmental contamination with Toxoplasma gondii oocysts: a systematic review

López-Ureña, Nadia María1‡; Chaudhry, Umer2‡; Calero-Bernal, Rafael1; Messina, Davide2; Evangelista, Francisco2; Betson, Martha2; Lalle, Marco3; Jokelainen, Pikka4; Ortega-Mora, Luis Miguel1; Álvarez-García, Gema1

Poster presentation at annual conference of the German Veterinary Medical Society, section "Parasitology and Parasitic Diseases", 28th to 30th June 2021
Development of a next-generation sequencing-based typing method to detect within-genotype variation in *Toxoplasma gondii*
M. Joeres[1], P. Maksimov[1], M. Tuschy[1], A. Bärwald[1], F. J. Conraths[1], B. Koudela[2,3], R. Blaga[4], S. Caccio[5], M. Fernández-Escobar[6], R. Calero-Bernal[6], L. M. Ortega-Mora[6], P. Jokelainen[7], G. Schares[1]

Poster presentations at WAAVP, Dublin, Ireland, July 2021
Tropism and persistence of *Toxoplasma gondii*: from pork carcass to dry sausage
Filip Dámek1, Bastien Fremaux2, Dominique Aubert3, Marieke Opsteegh4, Sandra Thoumire1, Sandra Vuillermet1, Pikka Jokelainen5, Joke van der Giessen4, Pascal Boireau6, Isabelle Villena3, Radu Blaga1

Toxoplasma gondii prevalence in animals in Europe: a systematic review
Filip Dámek1, Helga Waap2, Marieke Opsteegh3, Pikka Jokelainen4, Delphine Le Roux1, Gunita Deksne5, Huifang Deng3, Gereon Schares6, Arno Swart3, Anna Lundén7, Gema Álvarez-García8, Martha Betson9, Rebecca Davidson10, Adriana Gyorke11, Sanne Stokman3, Daniela Antolová12, Zuzana Hurníková12, Henk Wisselink13, Jacek Sroka14, Siv Klevar15, Rob van Spronsen3, Radu Blaga1

Endoparasites of feral cats in Denmark
Stine Thorsø Nielsen1, Ida Sofie Thuesen1, Søren Saxmose Nielsen1, Peter Sandøe1, Pikka Jokelainen2, Christen Rune Stensvold2, Michelle Bitsch Hardy1, Nikoline Johansson1, Heidi Huus Petersen3, Stig Milan Tamsborg1, Helena Mejer1

International workshop on molecular methods for *T. gondii* during summer 2021 teachers and participation from TOXOSOURCES.

One Health session of Project Review Module of ECDC's EPIET and EUPHEM programmes, August 25, 2021
Project presentation, chairing, participation in round table.

Examples of other activities:

#TOXOSOURCES has been used on social media:
https://twitter.com/hashtag/toxosources?f=live

Participation in WomenInScience tweet of OHEJP 11.2.2021
Pikka Jokelainen, Joke van der Giessen

Interviewed to Pathologist-journal
Pikka Jokelainen

Interview for article in Finnish Cat Association on feline toxoplasmosis
Pikka Jokelainen

Artistic collaboration:
Pikka Jokelainen
Anni Puolakka: From the Heart (2021)

Single channel video, duration 14:14
The work has been / will be shown at Jyväskylä Art Museum, Tampere Film Festival, Baltic Triennial (Vilnius), HAM gallery / Helsinki Art Museum
http://annipuolakka.com/fromtheheart/

TOXOSOURCES partner institutes have also mentioned TOXOSOURCES in their communications, e.g.:
https://www.ssi.dk/aktuelt/nyhedsbreve/epi-nyt/2020/uge-4---2020
https://www.rki.de/DE/Content/Forsch/EJP_OH2020.html
https://www.sva.se/forskning/internationellt-samarbete/europeisk-samverkan-kring-livsmedelsburna-smittor/toxosources-ett-one-health-ejp-projekt/
https://www.bfr.bund.de/en/toxoplasma_gondii_sources_quantified__ejp_toxosource_-249310.html
https://www.iss.it/en/web/iss-en/-/news-1

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

- Molecular detection of *T. gondii* oocysts in leafy vegetables: SOP
- Data from exposure survey
- Whole genome sequences of high number of *T. gondii* strains from Europe
-

5.1.4.3.16.6 Data Management Plan

TOXOSOURCES DMP is updated regularly. TOXOSOURCES uses the CDP-tool provided. Project Leader Pikka Jokelainen participates in DMP Committee of One Health EJP.

5.1.4.3.16.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

TOXOSOURCES builds largely on previous work and is actively looking for collaborations and synergies.

- An example of building on previous work is the building on the work performed within **COST-Action Euro-FBP**.
- Another example of building on previous work is that the TOXOSOURCES-WP3 work builds on work done in **project IMPACT** (Standardising molecular detection methods to IMprove risk assessment capacity for foodborne protozoan Parasites, using *Cryptosporidium* in ready-to-eat salad as a model organism"; Partnering Grant Project Grant Agreement Number GP/EFSA/ENCO/2018/03 – GA03).
- TOXOSOURCES addresses several key gaps identified in **DISCONTOOLS** work - https://www.discontools.eu/database/110-toxoplasmosis.html and collaborates in the updating of the database.
- TOXOSOURCES collaborates with **SafeConsume project** (http://safeconsume.eu/). Both projects are interested in the relevance of *Toxoplasma gondii* contamination in fresh produce, with different focus: SafeConsume focuses on consumer behaviour on safe handling of fresh produce at home, whereas TOXOSOURCES WP3 focuses on fresh produce from harvest to packaging. TOXOSOURCES suggested OHEJP Cogwheel Workshop with SafeConsume, and it was organized on November 25, 2020. SafeConsume invited TOXOSOURCES to join a Horizon Results Booster group. Via this collaboration, TOXOSOURCES also established links with projects **Stance4Health**, **EAT2beNICE**, and **FOODSAFETY4EU**.

- Collaboration with **International network for environmental Toxoplasma studies (INETS)** is another important established collaboration. INETS is a global network that organizes e.g. workshops. TOXOSOURCES will be presented at INETS workshop 2022.
- Collaboration with international colleagues on workshop on molecular methods during summer 2021.
- TOXOSOURCES has links to several PhD projects. For example, work in WP2 builds on the PhD work by Dr. Huifang Deng 'Source attribution of human toxoplasmosis, A quantitative microbiological risk assessment approach'. **One Health EJP PhD project ToxSauQMRA** (PhD candidate Filip Damek) is closely linked to TOXOSOURCES. At RKI a recently started PhD project (David Warschkau) aims at establishing an *in vitro* model of oocyst generation in intestinal organoids by which antigens identified in WP4 will be further analysed for function/localization.
- There are collaborations with **other One Health EJP projects**, including **MEmE, PARADISE, OH-Harmony-CAP, MATRIX, COHESIVE and ORION**. Synergies and complementary approaches have been identified.
- To enable and encourage collaborations, the QMRA models will be made available via a repository (https://foodrisklabs.bfr.bund.de/rakip/)
- **European Reference Laboratory of Parasites** and network of **National Reference Laboratories** are well represented in the consortium, and used for e.g. distributing request for sample collaboration.
- The German EFSA Focal Point encouraged TOXOSOURCES to reach out to the **Focal Point network** to distribute the link to the survey gathering information on production and trade of fresh produce in Europe (TOXOSOURCES-WP3). Consumption of fresh produce is one of the possible routes of *Toxoplasma gondii* transmission to humans, which has been little explored to date. Thus, one aspect of the project is to investigate the presence of *T. gondii* in ready-to-eat fresh produce. The data will be used to evaluate the possible role of fresh produce as a source of *T. gondii* infection (quantitative microbiological risk assessment), and to design a sampling strategy for a multi-centre study investigating selected fresh produce for presence of *T. gondii* oocysts. Action on this was taken in early 2021, and the EFSA-contact of OHEJP was informed via OHEJP-WP5.
- TOXOSOURCES collaborates with and builds on the results of several national and regional projects.

5.1.4.3.17 JRP23-R2-FBZSH5-ADONIS

Summary of the work carried out in the ProjectSalmonellosis remains the second most common zoonosis in humans in the EU despite a significantly long-term decreasing trend in human cases since 2008. Signals at EU level that the decreasing trend has levelled-off in some MSs are confirmed in the ADONIS project by detailed statistical analysis of epidemiological surveillance data. An exposure assessment regarding S. Enteritidis contamination eggs revealed the absence of a correlation of total exposure (product of prevalence and microbiological load) with clinical incidence, indicating that might changes in exposure are unlikely to explain observed changes in the S. Enteritidis epidemiology. On primary production level the project evaluated the national control programmes (NCPs) of some MSs in order to elucidate factors that determine the outcome of the Salmonella status. In addition, audit reports of the NCPs were extensively analysed in order to rank the type of flaws identified and type of recommendations made. Preliminary analyses suggest that the sampling process (by whom and how) and supervision on the programmes are critical points and a potential confounder is assessing trends. The project made a start with the genomics part by constructing a sequence inventory and a shared database from which analysis will be performed in order to assess potential changes in the population biology of S. Enteritidis on EU scale. Preliminary results suggests a role of plasmid content. Finally, a framework for the multi-criteria decision analysis (MCDA) has been made, the criteria for the assessment of the possible explaining determinants for the changing *Salmonella* epidemiology have been defined, and a first survey for weighting these criteria according to their importance has been completed by the panel of experts. A subsequent survey for scoring the alternatives according to the (weighted) criteria is also ready for piloting and will be distributed to the experts in July 2021.

5.1.4.3.17.1 Progress of the project: description of activitiesWP1 Project management

JRP23-WP1-T1- Coordination

Although challenging due to the COVID19 situation the project is running so far without major problems. Regular TCs within the project management team and within WPs were/are conducted.

JRP23-WP1-T2- Aligning and communication

JRP23- WP1-T2-ST1 Reporting

Draft 9M report over 2021 is being made (this document) and 12M report is prepared.

JRP23- WP1-T2-ST2 Alignment and communication

The ADONIS project was shortly discussed at the ECDC food-and waterborne disease theme coordination meeting.

WP2 Salmonella controls at the primary production level

JRP23-WP2-T1- Comparative analysis of Salmonella controls and management measures at MSs level

Comparative analysis of *Salmonella* national control programs (NCP) and management measures at MS level has been based on three activities: 1) an evaluation of historic data from controls performed by competent authorities and food business operators in laying hen flocks in one MS (Spain), 2) a questionnaire assessing differences in the NCPs performed at the country level filled in by partners participating in this task (France, the Netherlands, Poland, Spain and United Kingdom), and 3) a review of the audit reports on NCPs performed by the European Commission. An update on the status of each of these activities is provided below:

1) Analysis of historic data from NCP: Data on the surveillance activities carried out in laying hen flocks as part of the NCP in Spain between 2013 and 2019 were shared by the national veterinary official services (Ministry of Agriculture, Food and Fisheries). The database included information on the date, type and number of samples collected in all laying hen flocks, the

agent conducting the sampling (Competent Authorities – CA – or Food Business Operators – FBO) and the result regarding Salmonella detection (negative, positive for a target serovar – Enteritidis or Typhimurium – or positive for other serovars). Overall, approximately 66,000 samples were collected during 48,000 sampling events over the seven-year period, with only 4% of the samplings resulting in the detection of Salmonella (and 0.8% positive for a target serovar). Significant variations in the proportion of positive events were observed in terms of the location of the farm, year, time of the year, sampler (CA or FBO), type of sample (dust, feces, boot swabs), flock type (cage, barn, free-range and organic) and age of the animals. Results were analyzed using a Bayesian hierarchical mixed logistic regression model using the result in the sampling (positive or negative to Salmonella isolation) as the response variable and the available covariables at the farm (location), flock (flock size, production type) and sampling (date of sampling, age of the animals, sample type, sampler) levels as potential explanatory variables.

Results revealed a major effect of the sampler (FBO vs. CA) even after the effect of all the other known predictors was considered. Furthermore, a significant effect of the year and the time of sampling were also found. Additional details will be provided in the deliverable report.

2) Questionnaire on NCP at the country level: a questionnaire with open and closed questions (multiple-choice and dichotomous) was generated tobe filled in by interested partners in WP2. The document was divided into four main sections scope of the program and population assessed, auto control checks performed by FBO, official controls performed by CA, and biosecurity measures. Questionnaires were filled in by partner members/associated institutions with experience on the NCP in each country.

   The answers revealed many similarities between countries (as expected since all NCPs are based on European Regulation (EC) No 1177/2006) but some differences were found in all four sections. The laying hen populations assessed differed largely on predominant production systems, and small farms (outside of the scope of the NCPs) were defined differently depending on the country. Target serotypes included in NCPs also differed in certain cases, and funding mechanisms for compensation to producers were also different (public vs. Private insurance systems). Similarly, NCPs differend slightly in terms of biosecurity requirements/vaccination policies, although lack of precise information on vaccination was in fact identified as a limitation in several countries Additional details will be provided in the deliverable report).

3) The main conclusions from reviewing of audit reports:

a. Member States put quite some effort and resources on the implementation of NSCP, so does the EC to verify it
b. Although the quality and precision of recommendations seem to improve, the key for auditing countries is unclear. It is neither
c. human population since Bulgaria was audited 3 times, France – 1, and Sweden – 0
d. nor animal production (Germany and Malta – 2 each
e. Numerous legislation quoted in the recommendations shows multiplicity and complexity of requirements
f. As a result, although NSCPs have been implemented more than a decade ago, and they are being improved ever since, almost none NSCP is prefect.
g. Deficiencies concern sampling, laboratory testing, reporting, restrictions and measures taken along the food chain (including outbreak analyses), as well as evaluation of NSCPs progress.

Anses contributed to the development of the questionnaire on *Salmonella* controls at MSs level. We provided data for France, along with the National Laboratory for *Salmonella* in poultry production and the French Ministry of Agriculture. Regarding analysis on temporal trends of Salmonella in poultry, we are presently in discussion with the Ministry of Agriculture to obtain data on the results of the national control programs from 2013 to 2020 in France.

JRP23-WP2-T2- Comparative analysis of Salmonella controls and management measures at farm level

JRP23- WP2-T2-ST1 Field studies during outbreaks and sensitivity testing

A harmonized protocol for the investigation of Salmonella outbreaks in laying farms was developed by APHA and Anses in 2020. Shortly, the protocol includes a study to assess the sensitivity of several sampling methods to detect *Salmonella* in the environment of a positive flock (swabs, boot swabs, dust samples) and to estimate the intra-flock prevalence of birds excreting *Salmonella* in droppings. The results obtained in the sensitivity study will make possible to update the sensitivity models previously developed in the UK and France in 2000's. The field investigation also includes a study on the efficacy of cleaning and disinfection (C&D) measures taken in *Salmonella* positive layer farms. More than thirty environmental samples are performed before C&D for *Salmonella* detection in order to identify the most contaminated surfaces in the farm. Samples are taken at the same points after C&D to evaluate the efficacy of C&D practices. We focus on the study of outbreaks occurring on aviary or barn laying hen farms, as those types of housing systems are less studied than the furnished cage system. Field studies were planned to begin during spring 2020. The objective was to study five outbreaks In the UK and five outbreaks in France. We had to postpone the study due to the COVID-19 crisis (no farm visit was allowed in 2020). The first visit was carried in April 2021 in France.
As mentioned above, opportunities for field work were severely curtailed by the pandemic. APHA managed to visit two farms in 2020 in between national lock-downs. Four post- cleaning and disinfection visits were carried out on layer farms linked with ongoing human outbreaks of *Salmonella* Enteritidis. Sampling followed the agreed protocol (see above). At one premises, no S. Enteritidis was found within the bird area of the sampled poultry houses, but it was isolated from a sample collected in an adjoining service area and from two dead mice. Multiple samples collected outside one of the houses were positive, primarily from puddles in various locations. At the second premises, samples collected from both inside and outside of the poultry houses had *S*. Enteritidis isolated from them. No positive samples were found after cleaning and disinfection a further two follow-up visits. Advice was given to farms on *Salmonella* control, which included recommendations for cleaning and disinfection, egg handling, and enhanced biosecurity. No *S*. Enteritidis has been reported in subsequent flocks from any of the farms

WBVR works on systematic literature review that aims at checking the scientific literature on available data on sampling protocols. In this review information is collected to answer two questions. First one aims at reviewing what is the lowest prevalence (limit of detection) of *Salmonella* Enteritidis in laying hens, which can be detected in the environmental samples with a high confidentiality. This is a comparison of the prevalence in laying hens with the prevalence in environmental samples. The requirements here are that it is known what is the *S.* Enteritidis prevalence in laying hens as detected with culture on samples of individual laying hens.
The second research question aims at reviewing the diagnostic sensitivity of environmental sampling methods to detect *S.* Enteritidis in laying hens. It is a comparison of the detection ability of different environmental sample procedures. It does not matter what the *S.* Enteritidis prevalence is. The only requirement is that the flock is infected with *S.* Enteritidis. The databased searches resulted in identifying 1175 publications. Following removal of duplicates and screening of title and abstract resulted in determination of 118 full texts. They are currently assessed for eligibility by two researchers. This screening will be followed by extraction of data from identified publications, its analysis and reporting.

JRP23- WP2-T2-ST2 On farm surveys

PIWet is involved in the development of questionnaires on welfare, biosecurity, and other preventive vaccinations carried out on farms concerning other pathogens and in the context of *Salmonella spp.* Infections on poultry farms located throughout the country. The questionnaires were prepared and sent almost all over the country. We are currently awaiting the results of the sent out questionnaires

containing detailed information on the poultry flocks under management. Unfortunately, the collection of the survey is significantly delayed than previously thought.
We are still waiting for the results of the farm surveys.

WP3 Surveillance, epidemiology and source attribution

JRP23-WP3-T1- Evaluation of surveillance systems in humans

In the previous 12M report of 2020, we described that key elements and attributes were identified for evaluation, as well as the countries of which the *Salmonella* surveillance system would be evaluated. These countries were Spain, UK, Netherlands, Belgium, and Norway. Due to the COVID-19 pandemic, resources were limited and we decided to focus on the Netherlands, Belgium and Spain first. If time allows, we would also evaluate the *Salmonella* surveillance system for the UK. Because Norway is not part of the ADONIS project, we decided to not evaluate their surveillance system.
Currently, the key elements for the surveillance system of the Netherlands and Belgium have been described, including e.g. objectives of the surveillance system, case definition etc. Key attributes, such as internal completeness and geographic coverage of the surveillance system, have been partly described. We expect this do be done at the end of June/beginning of July 2021. After that, we will evaluate the Spanish surveillance system. However, because they are not part of the ADONIS project, depending on their time availability, we might evaluate the UK surveillance system. We expect that the full report of the evaluation will be finalized by the end of 2021, in line with the initial planning.

JRP23-WP3-T2- Assessment of changes in the epidemiology of human S. Enteritidis cases and other relevant serovars

Time series analyses to identify factors that might explain the stagnating trend of S. Enteritidis have been finalized for the Netherlands and Belgium, and the analyses for Spain is ongoing. We plan to perform the same time series analyses for the UK. So far, analyses have not yet revealed factors that can explain the stagnation of the decreasing S. Enteritidis trend. At the same time, we will liaise with the EJP DISCOVER project to perform source attribution analyses of the period before and after the stagnating S. Enteritidis trend. The deliverable for the full report of this task is due in September 2021 but will be delayed by an expected 6 months.

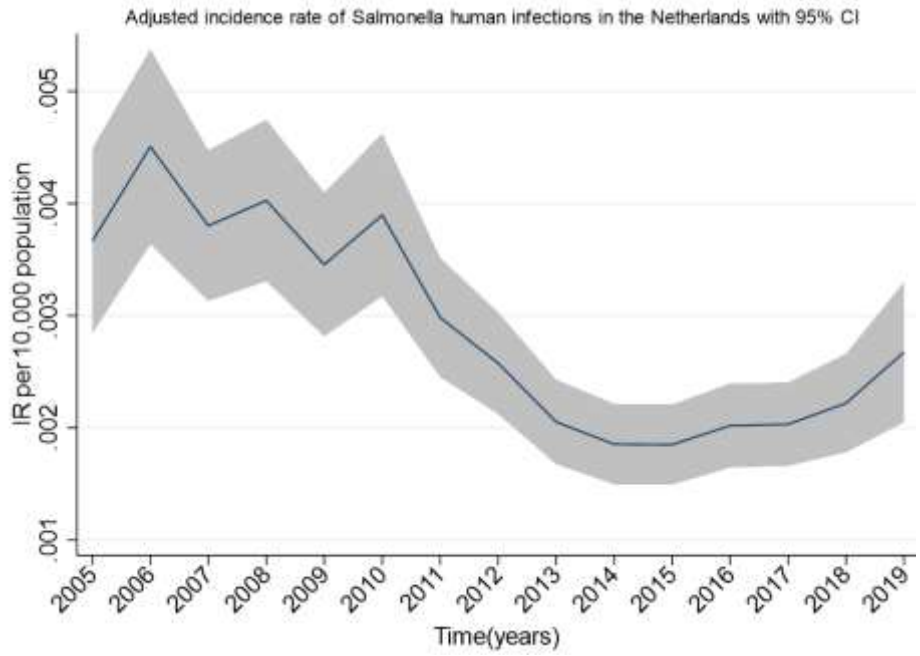


Fig. 1. *Time-trend of adjusted incidence rate of S. Enteritidis in* ***The Netherlands****. Clearly, a declining trend is visible from around 2007 to 2015 after which the decline levels off and even increases slightly.*

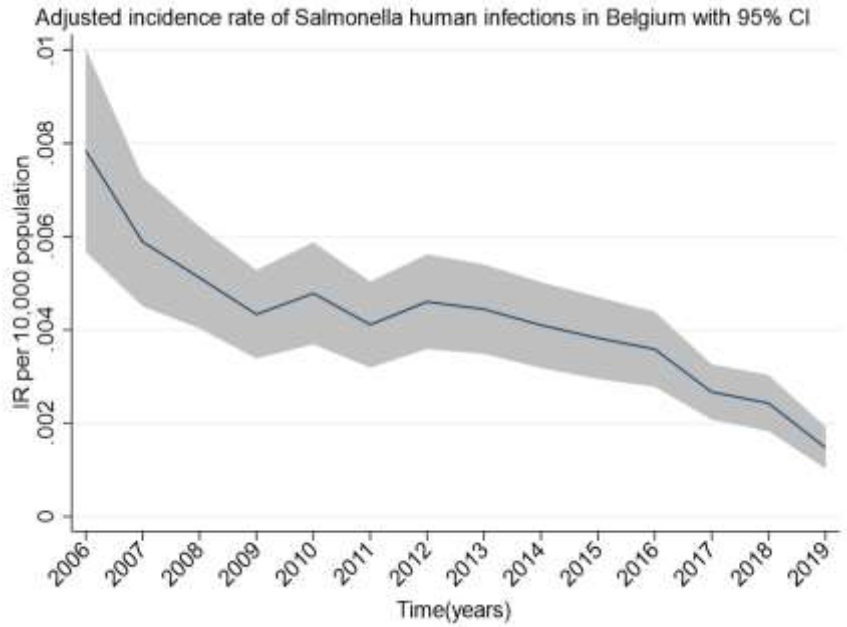


Fig. 1. *Time-trend of adjusted incidence rate of S. Enteritidis in* ***Belgium****. Clearly, a continuous declining trend is visible without a levelling-off.*

JRP23-WP3-T3- Assessment of human exposure to S. Enteritidis

The exposure assessment has been performed and finalized. For the exposure assessment the simplified approach of the pre-treatment load was used, which was calculated as the product of egg SE prevalence and egg consumption rate. The main challenge was data availability, because data on S. Enteritidis in eggs (used as input for the exposure assessment model) was only available for Spain. For the other countries for which the exposure assessment would be performed, namely the Netherlands, Belgium and the UK, these data were not available or could not be obtained.

Results for Spain suggest a poor correlation between the number of human S. Enteritidis and the pre-treatment S. Enteritidis cases was poor. This indicates that changes in human exposure to S. Enteritidis through consumption of eggs, which is the most important source of human S. Enteritidis infections, cannot explain the stagnating S. Enteritidis trend. Furthermore, the significance of import of eggs played a minor role in Spain. However, it is important to note that these conclusions are based on data from Spain only.

WP4 Salmonella Genomics

JRP23-WP4-T1- Collection overview

All partners are involved in WP4, especially in the collection of sequences. An inventory was created to get an overview of the available strains and sequences at each partner institution. The inventories were collated and presented at a videoconference held on 17 February 2020, where a decision was made on the final selection of strains to be sequenced for the genomic analyses.

The need of an MTA for sharing the sequences in order to analyse them in different partner laboratories was brought to attention in a meeting (Videoconference) on 30 October 2020, and this, in the context of the SARS-CoV-2 pandemic and the bureaucratic consequences of BREXIT, has significantly delayed the completion of the sequence sharing.

At present we are waiting for the last institution to sign the MTA and two institutes to share their sequences.

JRP23-WP4-T2- Population structure and comparative genomics

This task is awaiting the collection of sequences in order to begin. Small pilot studies on limited datasets have been initiated on Mobile Genetic Element (MGEs) detection and analysis.

JRP23-WP4-T3- Phylodynamics and Phylogeography

The first analyses have been performed on the available sequences. Further work will be done once all the sequences are available for all partners implied in the genomic analyses.

JRP23-WP4-T4- Mutant creation and testing including GWAS studies

A pilot GWAS study was performed on a limited dataset of 120 genomes. The study detected 6 plasmid related genes slightly associated with the *S*. Enteritidis increase since 2014. More analysis is needed to confirm this pilot study.

WP5 MCDA model to support priority setting

JRP23-WP5-T1- Framework building

This task is completed. A framework for the MCDA, based on the AHP method, has been made and agreed upon with the project partners.

JRP23-WP5-T2- T2 MCDA modelling

The criteria for the assessment of the alternatives (i.e. determinants of the stagnating trend of Salmonella and the intervention to reverse it) have been defined and a first survey for weighting these criteria according to their importance has been completed by the panel of experts. A subsequent survey for scoring the alternatives according to the (weighted) criteria is also ready for piloting and will be distributed to the experts in July 2021.

5.1.4.3.17.2 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
23	D-JRP#-1.2	Y3 report delivered	39	39			
23	D-JRP#-2.1	Report on the evaluation of the questionnaires	43			To be delivered in Month 43 (July 2021)	4
23	D-JRP#-2.2	MS's specific analysis of temporal trend of Salmonella in poultry flocks	43			To be delivered in Month 43 (July 2021)	4
23	D-JRP#-2.3	Recommendations for improving Salmonella surveillance in poultry flocks	48			Field work bit delayed due to COVID19 but hopefully to be delivered in M48 (end of 2021).	
23	D-JRP#-3.1	Description of surveillance systems regarding their key elements	36			Completed for NL. Not done for all countries yet. Spain and possibly UK will follow. Expected to be completely done by week 48.	
23	D-JRP#-3.2	Description of basic epidemiological characteristics of human S. Enteritidis cases and other relevant serovars	36			Completed for NL. Not done for all countries yet. Spain and possibly UK will follow. Expected to be completely done by week 48.	

23	D-JRP#-3.4	Extended the report on epidemiological characteristics with results from the rarefaction analysis the refined source attribution models, but also with data on food consumption patterns	45			This work is integrated into the work with D3.2 and will be reported in one joined deliverable (see D-JRP#-3.2).	
23	D-JRP#-3.5	Report on human S. enteritidis exposure in different countries and time periods	42	42		Finished. Not yet uploaded, will be done this month.	
23	D-JRP#-4.1	Sequence inventory report	31	38		Brexit and COVID-19 have significantly affected the sequence sharing.	3

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
23	M-JRP26-1	Kick-off meeting organised	26	Y		
23	M-JRP26-2	Communication partners mapped	28	Y		EFSA and ECDC participate in the MCDA

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
23	M-JRP26-3	Inventory of documents available for the comparative analyses	30	Y		
23	M-JRP26-4	Farm survey planed and questionnaires developed	30	partly		COVID19 delayed field work
23	M-JRP26-5	Selection of three countries of which their national S. Enteritidis surveillance systems will be evaluated	27	Y		
23	M-JRP26-6	Obtained national surveillance data on S. Enteritidis and other relevant serovars	30	Y		
23	M-JRP26-7	Sequence collection to be shared amongst partners	31	dealyed	44	One partner missing in MTA signature and 2 partners sequence sharing. Brexit and COVID-19 have significantly affected the sequence sharing.
23	M-JRP26-8	Phylogenetic trees describing the sequence collection (D-JRP#-WP4.1)	32	Delayed	44	One partner missing in MTA signature and 2 partners sequence sharing. Brexit and COVID-19 have significantly affected the sequence sharing.
23	M-JRP26-9	Inventory of MCDA methods suited to the data being collected.	30	Y		
23	M-JRP26-10	Annual meeting organised	40	Y		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
23	M-JRP26-11	Outline of the assessment framework (transmission chain)	42	Y		
23	M-JRP26-12	Meeting on aligning epidemiological and QMRA approach	45	Y		
23	M-JRP26-13	List of candidate strains for phenotype testing	37	delayed	60	We expect to have the complete sequence collection in Month 44, before the first genomic analyses suggest a list of candidates for phenotypic testing
23	M-JRP26-14	Identification of the experts to participate in the weighting exercise of the MCDA	42	Y		
23	M-JRP26-16	Final meeting organised	54			
23	M-JRP26-17	Data finalized for manuscript on Phylodynamics and Phylogeography	51			
23	M-JRP26-18	Data finalized for manuscript on phenotypes	54			
23	M-JRP26-19	Publication of sequence collection on European Nucleotide Archive (ENA)	54			

5.1.4.3.17.3 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors already accepted your comments. Therefore, this part of the report has been closed.

5.1.4.3.17.4 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Assessing variability of IncX1 plasmids from S. Enteritidis isolates in Spain (OHEJP ASM, 2021)	Yes	NA (oral presentation at OHEJP ASM)	NA (oral presentation at OHEJP ASM)
Factors associated with Salmonella detection in the frame of national control programs in breeding and laying hen flocks in Spain	Yes	NA (poster presentation at OHEJP ASM)	NA (poster presentation at OHEJP ASM)
Trend Reversal in Human Infections with *Salmonella enteritidis* in the Netherlands (2005-2019)	Yes	NA (oral presentation at OHEJP ASM)	NA (oral presentation at OHEJP ASM)

5.1.4.3.17.5 Data Management Plan

5.1.4.3.17.6 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

Experts of ECDC and EFSA are participating in the multi-criteria decision analysis modelling approach to operationalize an assessment framework for the identified determinants. This will the cumulative results of this project and will deliver stakeholders and policy makers with anchor points to at least prevent a continued stagnation or even an re-establish a decreasing trend in Salmonella incidence in humans and poultry.

5.1.4.3.18 JRP24-R2-FBZSH9-BeONE

5.1.4.3.18.1 Summary of the work carried out in the Project

The work has been significantly delayed during the last year. There has still been significant progress in the project.

The State of the art delivery (D1.1) was completed in collaboration with the ORION project, and a Dataset collection and curation is ongoing. An automated system for congruence analysis has been set up, and is awaiting the completion of the dataset.

Likewise a draft manuscript for a conceptual model on genomic epidemiology has been written (D2.1).

The core of a database model has been developed, that supports comparison of data from different data supplying components. Domain specific areas are still under development, and crucially epidemiological data (person/animal) is still missing. The collected metadata (M3.4) will help the finalization of this last stage of the data model development.

The backend development is complete with regard to strain characterization, including antimicrobial resistance (AMR) profiles. SNP and cgMLST data is still missing, and not implementation in the data model is not completed.

The dashboard in its current state is focused on a searchable and sortable data table. It can merge and display data from multiple sources, however development is still ongoing.

The dashboard and backend development has been closely linked to a Danish project, where food authorities, DTU (as expert dataprocessor of food and livestock samples) and SSI (public health institute), collaborate on a common platform for integrated analysis of food and patient samples for outbreak detection. This project has pressure tested the security, risk assessment and GDPR compliance requirements for a system like BeONE, which will work with personal health data across networks and with multiple actors.

5.1.4.3.18.2 Progress of the project: description of activities

WP1: Typing comparability and nomenclature

JRP24-WP1-T1 Establishment of state of the art

Status: Completed

This task was completed in collaboration with the ORION project (deliverable available at https://doi.org/10.5281/zenodo.5155570). In particular, BeONE contributions were mainly focused on describing start-to-end workflows for routine genome-based surveillance of the four foodborne bacterial species under study, with emphasis on identifying challenges and opportunities for harnessing WGS as a key tool for One Health Surveillance. The final output is an online handbook covering the main technical and practical aspects associated with the aplication of WGS for foodborne diseases surveillance. By following a simple and straightforward writing and structure, this document can be easily understandable by laboratory staff starting in the field, thus helping national and local labs to build capacity and competence on the use of NGS methods for surveillance purposes. The One Health Sequencing for Surveillance HandBook is available through the link https://oh-sfs-handbook.readthedocs.io/en/latest/.

JRP24-WP1-T2 Dataset selection and curation
Sub-Task: WP1-T2-ST1 Dataset selection and collection

Status: Ongoing

An anonymized dataset of reads and metadata has been assembled.

Sub-Task: WP1-T2-ST2 Quality check and assurance, and genome assembly

Status: Ongoing

The pipelines to be subjected to cluster congruence analysis will be implemented and run for the assembled dataset (from reads QA/QC to clustering analysis). We expect thus to finalize this task by the end of this year (M48), with the associated milestones (M1.3) and deliverables (D1.2) achieved by M48.

JRP24-WP1-T3 Clustering congruence and thresholds

Sub-Task: WP1-T3-ST1 Selection of WGS-based typing methods to be evaluated

Status: Completed

Sub-Task: WP1-T3-ST2 Assessing clustering congruence between different methods at different hierarchical levels

Status: Ongoing.

The completion of this task is dependent on the completion of the dataset collection and curation that is currently ongoing. Still, we have already developed bioinformatics pipelines to automatize the clustering congruence analysis, i.e., the developed scripts are able to obtain clusters at every partition levels for each method, which is the basis to: i) identify threshold levels of clustering stability for each method; ii) compare the congruence between methods and ultimately define threshold ranges showing clustering convergence.

Sub-Task: -WP1-T3-ST3 Correlating clustering congruence with existing nomenclature schemes

Status: Ongoing.

The completion of this task is dependent on the completion of the dataset collection and curation that is currently ongoing. As described in WP1-T3-ST2, the developed scripts are also adjusted to allow a rapid comparison of the clustering profiles obtained at different threshold levels and the strains grouping defined by existing nomenclatures.

WP2: Joining molecular and epidemiological methods

JRP24-WP2-T1: Conceptualization of epidemiological and biological factors impacting on fine resolution clustering and outbreak detection

A conceptual model of the factors impacting fine resolution clustering and outbreak detection has been made and explained in detail in a draft manuscript which is now undergoing the last revision round with the co-authors. The abstract hereof was submitted as deliverable to Zenodo and the OHEJP online platform.

JRP24- WP2-T2: Integrating genomics with epidemiology

Status: Ongoing

The development of algorithms for cluster refinement in view of outbreak detection has suffered delays due to lack of personnel caused by the SARS-COV2 crisis. We expect thus to finalize this task by the end of this year (M48), with the associated milestones (M2.2) and deliverables (D2.2) achieved by M46 and M48, respectively. We have identified several suitable datasets to be used in the process of algorithm validation. Furthermore, for specific microorganisms, we have developed part of the algorithms where the input data is in the form of single nucleotide polymorphisms. Further efforts will be put into accommodating allelic profiles data.

JRP24- WP2-T2-ST1 Comparison of the epidemiologic clusters with the phylogenetic tree of the Campylobacter jejuni isolates

Progress has been made in this task by partner SSI (13) with identification of several clusters/outbreaks and evidence that a successful surveillance programme can be based on sampling a relatively small fraction (10%) of the cases (DOI: 10.3201/eid2603.190947).

JRP24- WP2-T2-ST2 Comparison of the epidemiologic clusters with the phylogenetic tree of the Shiga toxin producing Escherichia coli (STEC) isolates

We have identified at least one dataset of epidemiologically validated outbreak with available WGS and detailed epidemiological metadata; this will be used in the validation process.

JRP24- WP2-T2-ST3 Comparison of the epidemiologic clusters with the phylogenetic tree of the Salmonella enterica isolates

We have identified several datasets, corresponding to outbreaks of various Salmonella enterica serovars, that will be used in the validation process. Furthermore, we have identified a couple of evolutionary mechanisms that are at play in the maintenance of natural populations of Salmonella, and that might have to be taken into account in improved cluster/outbreak detection algorithms.

JRP24- WP2-T2-ST4 Comparison of the epidemiologic clusters with the phylogenetic tree of the Listeria monocytogenes isolates

We have identified at least one dataset of an epidemiologically validated outbreak, where WGS and detailed metadata are available. We have built a large part of the cluster/outbreak detection algorithm and are now working on refinements. The first analyses indicate that inclusion of WG sequences from other sectors (i.e. food safety) is highly desirable if not mandatory.

JRP24- WP2-T3: Definition of guidelines for cluster investigation

Status: Delayed

As consequence of the delays in the previous tasks this task will have to be postponed to M52, with milestone M2.3 forecasted to be achieved by M50 and the deliverables D2.3 and D2.4 by M52.

WP3: Storage, management, and sharing for meta- and molecular data

JRP24- WP3-T1: Evaluate national level data sharing

Status: Completed (see 12M REPORT 2020)

JRP24- WP3-T2: Metadata acquisition and standardisation

JRP24- WP3-T2-ST1 Metadata acquisition

Status: Completed

The metadata acquisition form was distributed among data submitters for WP1-T2-ST1. The data extraction from the returning forms was automated and information is casted into JSON data structures that are ready for import into the BeONE database via an API (see WP3-T3-ST2). For each sample-related information, where data collection is restricted to controlled vocabularies from the EFSA Data Collection Framework, the superordinate hierarchy of each term is complemented, thus allowing information queries in a non-uniform dataset with varying levels of information provided by data submitters without the need to process DCF catalogues.

JRP24- WP3-T2-ST2 Standardisation using ontologies

Status: Delayed

Epidemiological metadata fields, e.g. those acquired in WP3-T2-ST1, are well covered by many ontologies that claim farm-to-fork coverage of terms. In contrast, bioinformatic data analysis result description is still in a draft state. The GenEpiO project combines several ontologies covering surveillance, food, antimicrobial resistance and genetic mobile element and was identified as the ontology with the best coverage for sequencing parameters (GENEPIO_0001681 and subordinates). Standardization of result data by GenEpiO will be evaluated when the database model is finalized.

JRP24- WP3-T3: Database design and implementation

JRP24- WP3-T3-ST1 Determine and implement data structure for database

Status: Delayed

Database schemas have been established for WGS data though some details need to be finalized. Database design regarding epidata has also begun but still needs to be established. This section has been impacted due by work priorities related to covid-19 from related developers at SSI, and further by personnel changes.

JRP24- WP3-T3-ST2 Implement API for import of data

Status: Delayed

The codebase for the API in Bifrost has been set up. However, since the API depends on the underlying database structure, the finalization of the API is delayed.

JRP24- WP3-T3-ST3 Data porting from an existing pipeline

Status: Delayed

The WGS assembly and quality control pipeline AQUAMIS (https://doi.org/10.3390/genes12050644) as well as the cgMLST-based clustering pipeline chewieSnake (https://doi.org/10.3389/fmicb.2021.649517) were adapted to output analysis results in a BeONE compatible JSON data structure. This is a prerequisite to port information via the API. Both publications acknowledge funding from JRP27-R2-FBZ-BeONE. This subtask was worked on ahead of schedule and will be resumed upon implementation of the API for import of data (WP3-T3-ST2) due to delays caused by the COVID-19 crisis and constraints in available personnel.

JRP24- WP3-T3-ST4 Data porting from further pipelines

Status: Not starting until M41

The data structures and schemas are being designed with compatibility to a number of different pipelines in mind. The data porting frameworks are not yet in development.

JRP24- WP3-T3-ST5 Expansion of the API for referencing/exporting of entries in other reference databases

Status: Not starting until M41

JRP24- WP3-T3-ST6 Expansion of the API for queries from the dashboard WP4

Status: Not starting until M47

JRP24- WP3-T4: National data sharing pilot

WP4: Development of a user-oriented interface (dashboard) for analysis and sharing of epi and molecular data

The work in WP4 has been strongly affected by COVID-19 as well as changes in personnel at SSI, where the WP leader and two developers found other employment during the last year. The positions have been filled with new competent staff, however some rescoping is necessary. Additionally, significant work has gone into making the system GDPR compliant.

JRP24- WP4-T1 Dashboard

Status: ongoing

JRP24- WP4-T1-ST1 Core display components

Currently a data display table of data, as well as comparison by phylogeny is close to completion.

JRP24- WP4-T1-ST2 Component integration

Status: Delayed

This subtask is awaiting further development in *JRP24- WP4-T1-ST1.*

JRP24- WP4-T2 Back end analysis implementation

Status: Ongoing

Back end analysis implementation is based on the Bifrost framework being developed at SSI, as well as tools from Center for Genomic Epidemiology at DTU. This framework employs Dockerized Snakemake workflow management pipelines, the non-relational database MongoDB (https://www.mongodb.com/) and a Python-based set of tools to handle data flow from bioinformatic tools to database storage. This is complete except for the cgMLST implementation based on chewbbaca, which is still ongoing.

JRP24- WP4-T3 Cluster analysis and collaboration tool

Status: Ongoing

Other projects that we have recently been working on have revealed that data sharing and collaboration in the area of genetic data is extremely complicated, even within the EU, due to GDPR and other regulatives. This means that systems that handle personally identifiable health data need to be designed from the ground up to be 'aware' of these regulatives. As a part of WP4, a security model has to be developed that deals with these issues, meaning that the scope of WP4 will grow, and as a consequence the scope of other areas will have to be adjusted.

JRP24- WP4-T4 Data sharing front end

Status: Delayed

Due to personnel changes, as well as the experiences described above, we are rescoping the data sharing system.

Web based input of fastq files and associated metadata is completed.

WP5: Dissemination, Testing, Evaluation and Sustainability

JRP24- WP5-T1 Dissemination

Status: Ongoing

A documentation effort of the backend system is prioritized. Code organization, containerization and strict observation of separate development, testing and production environments, has the goal of stable and in turn portable code.

JRP24- WP5-T2 Continuous Testing and Feedback

Status: Ongoing

A testing plan has been made.

JRP24- WP5-T3 Final evaluation

Status: Delayed

Final evaluation is postponed.

JRP24- WP5-T4 Sustainability

Status: Ongoing

Key requirements for sustainability of the dashboard and analysis system have been identified as good documentation of the system, and an open source organisation around the system to carry on the collaborative work after the project ends. Documentation has been prioritized, and the initial steps have been taken in terms of how continued collaboration will be organized when project funding runs out. Parts of the BeONE system is currently being implemented in Denmark for collaboration between the Danish food authorities (FVST) and public health institute (SSI).

WP6: Management

JRP24- WP6-T1 Management

There have been monthly meetings in the core group. Since late november 2020 the deputy lead had to fill in while the Project leader was on sick leave.

JRP24- WP6-T2 Communication

JRP24- WP6-T2-ST1 Kickoff meeting

Status: Completed

JRP24- WP6-T2-ST2 2nd Annual Meeting

Status: Delayed

The second annual meeting has been postponed until the autumn in the hope that the Covid situation improves sufficiently, so that a physical meeting is possible.

JRP24- WP6-T2-ST3 3rd Annual Meeting

Status: Pending

It is proposed to postpone the third annual meeting until early autumn 2022, supposing the project extension is approved.

JRP24- WP6-T2-ST4 Development hackathon

Status: Cancelled due to Covid-19 crisis

JRP24- WP6-T3 Data Management

5.1.4.3.18.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
24	D-BeONE.1.1	Report on the state-of-art	30		39	Public (D-BeONE.1.1 Report on the state of the art | Zenodo). https://zenodo.org/record/5155571#.YUC8GzhDtM3 Scope changed to avoid duplication of work with ORION, and mitigate delays due to COVID-19. Delivery date changed to wait for ORION delivery fall 2020.	10
24	D-BeONE.1.2	Finalized BeONE dataset	32		48	Confidential, Delays due to COVID-19	3
24	D-BeONE.2.1	Draft manuscript on the conceptual model	34	36	36	Public (D-BeONE.2.1 Draft conceptual model of factors impacting genomic clustering | Zenodo) https://zenodo.org/record/4476394#.YUC8MThDtM2	4
24	D-BeOne.2.2	Final Outbreak detection algorithm	46		48		4

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
24	D-BeONE.2.3	Final Guidelines	48		52		4
24	D-BeONE.2.4	Draft manuscript on outbreak detection algorithm and guidelines	48		52		4
24	D-BeONE.3.1	Report on national data sharing pilot	54		60	Delayed due to COVID-19	8 (Report)
24	D-BeONE.4.1	Back-end analysis pipeline	36		46	Delayed due to COVID-19	4
24	D-BeONE.4.2	Web input system	36		42	Delayed due to COVID-19	4
24	D-BeONE.4.3	Final BeONE system	54		60	Delayed due to COVID-19	4
24	D-BeONE.5.1	Instruction for local installation of dashboard	54		60	Delayed due to COVID-19	8 (Documentation)
24	D-BeONE.5.2	Software list	54		60	Delayed due to COVID-19	8 (Documentation)
24	D-BeONE.5.3	Tutorial	50		54	Delayed due to COVID-19	8 (Documentation)

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
24	D-BeONE.5.4	Final evaluation report	54		60	Delayed due to COVID-19	8 (Report)
24	D-BeONE.5.5	Final sustainability document	54		60	Delayed due to COVID-19	8 (Report)
24	D-BeONE.6.1	Initial data management plan	30	33	39	Public https://zenodo.org/record/5497853#.YToRjxlxdaQ	8

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
24	M-BeONE.1.1	State-of-the-art completed	30	Yes	39	Delayed due to COVID-19 crisis and constraints in hiring personnel
24	M-BeONE.1.2	Collected dataset	30	no	48	Delayed due to COVID-19 crisis and constraints in hiring personnel
24	M-BeONE.1.3	Curated WGS dataset completed	32	no	48	Delayed due to COVID-19 crisis and constraints in hiring personnel
24	M-BeONE.1.5	Clustering congruence analysis completed	48		48	
24	M-BeONE.2.2	Outbreak detection algorithm -provisional version	42	no	46	Delayed due to COVID-19 crisis and constraints in available personnel
24	M-BeONE.2.3	Guideline proposal	42	no	50	Delayed due to COVID-19 crisis and constraints in available personnel
24	M-BeONE.3.1	Agreement on minimal and optimal set of metadata	28	yes		
24	M-BeONE.3.2	Finalized evaluation of data sharing experiences	34	yes		
24	M-BeONE.3.3	Implementation of data structure	34	no	48	Delayed due to database development
24	M-BeONE.3.4	Finished implementation of controlled data entry	36	yes		

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
24	M-BeONE.3.5	Plan for ontology implementation	36	no	44	Delayed due to database development
24	M-BeONE.3.6	Implementation of API for import of data	36	no	48	Delayed due to database development
24	M-BeONE.3.7	Data porting from an existing pipeline	42	yes		
24	M-BeONE.3.8	Application of the selected ontology system(s)	48	no	48	
24	M-BeONE.3.9	Data porting from at least one further pipeline	48	no	48	
24	M-BeONE.3.10	Expansion of the API for export functionality	48	no	48	
24	M-BeONE.3.11	Expansion of the API for queries from the dashboard WP4	54	no	54	
24	M-BeONE.4.1	Initial requirement list from workshop at kick-off meeting	26	yes		
24	M-BeONE.4.2	Implementation plan	28	yes		
24	M-BeONE.4.3	Prototype dashboard	32	no	46	Being redone due to personnel changes
24	M-BeONE.4.4	Plan for data sharing system	34	no	48	Delayed due to COVID-19

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
24	M-BeONE.4.5	Basic system for display component integration	36	no	52	Delayed due to coordinated development with other project on data integration between authorities in Denmark to avoid duplication of work.
24	M-BeONE.4.6	Cluster analysis and collaboration tool ready for testing	42	no	52	Delayed due to coordinated development with other project on data integration between authorities in Denmark to avoid duplication of work.
24	M-BeONE.4.7	Import/Export front end for reference databases implemented	48	no	52	Delayed due to coordinated development with other project on data integration between authorities in Denmark to avoid duplication of work.
24	M-BeONE.4.8	Dashboard in workable condition for Final Evaluation workshop	48	no	54	Delayed due to coordinated development with other project on data integration between authorities in Denmark to avoid duplication of work.
24	M-BeONE.4.9	BeONE data exchange system implemented	54	no	60	Project extension
24	M-BeONE.5.1	Simulated international outbreak at 3rd annual meeting	52	no	54	Project extension
24	M-BeONE.5.2	Yearly sustainability document	36	no	48	Will be combined with yearly sustainability document for Y5. Not prioritized due to delays in other activities.
24	M-BeONE.5.3	Initial test plan	28	yes	31	Delayed but achieved by M31

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
24	M-BeONE.5.4	Overall evaluation at 2nd annual meeting (workshop)	40	no	46	Delayed due to COVID-19
24	M-BeONE.6.1	2nd annual meeting	40	no	46	Delayed due to COVID-19
24	M-BeONE.6.2	3nd annual meeting	52	no	54	Project extension

5.1.4.3.18.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors already accepted your comments. Therefore, this part of the report has been closed.

5.1.4.3.18.5 Publications and additional outputs

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Prediction of antimicrobial resistance in clinical Campylobacter jejuni isolates from whole-genome sequencing data 10.1007/s10096-020-04043-y https://zenodo.org/record/4249355#.X6UnVWhKjcc	YES	TBD	TBD

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

Work in progress code is available on github as described in the DMP.

5.1.4.3.18.6 Data Management Plan

The Data Management plan can be found in the CDP-tool

The data are Findable, in that the data locations are listed in the DMP, and data are stored in public data hubs (e.g. ENA, github, zenodo). In terms of accessibility, data is kept as public as possible, while maintaining necessary confidentiality. We were aiming for an anonymous dataset, allowing for full public disclosure, however, there may be discretization issues, which may hinder the full publication of all metadata.

We use standard data formats to ensure interoperability and reusability.

5.1.4.3.18.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

BeONE is in contact with EFSA on streamlining cgMLST implementation, exploring if/how the BeONE implementation can be in accordance with the joint EFSA/ECDC database.

There is close cooperation between BeONE (particularly WP4) and the SOFI project. The SOFI project is a collaboration on a multi-actor data sharing platform between the Danish Food Authorities (Fødevarestyrelsen, not an OH-EJP partner), DTU and SSI.

5.1.5 Task 3.4: Organisation of annual scientific meetings (ASM)

The third Annual Scientific Meeting was organised as a hybrid event, in Copenhagen and online, hosted by SSI and DTU-FOOD. The event included 6 keynote talks, 30 oral presentations and more than 160 poster presentations, and there were about 500 participants.

5.2 Deliverables and Milestones

5.2.1 Deliverables

Del. Ref.	Deliverable title	Expected Submission	Notification
D3.15	3rd periodic report on JRP	M39	Timely submitted
D3.16	Abstract book for 3rd Annual Scientific Meeting (ASM)	M41	Timely submitted
D3.17	Report n°1 on evaluation of finalised JRP	M48	Under preparation

5.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS40	Final reports n°2	M45	Acheived
MS41	Third Annual Scientific Meeting	M41	Acheived

6 WP4 - Joint integrative projects

6.1 Work carried out to date

6.1.1 Task 4.1: Development of procedures and guidelines for submission and selection of JIP proposals, and for reporting and evaluation

N/A

6.1.2 Task 4.2: Supervision of JIP

The structure with continuous follow-up meetings with all JIP Project Leaders (PLs) works very well. In between there are frequent consultations between WP4 and the PLs. The projects are reminded by the WP4 support team well in advance of expected deliverables and support is given how to upload the completed deliverables. All projects are also encouraged to follow the updated procedures for scientific publishing, dissemination, and communication. In collaboration with WP3 templates for reporting have been prepared, partly prefilled, and sent out to projects. To harmonize the work between work packages 3, 4 and 6, there are recurring meetings, where questions concerning the supervision and monitoring of JRPs, JIPs and PhD projects are discussed. WP4 representatives participate in the project consortium meetings, whenever possible. In M39, WP4 submitted the 3rd periodic report on JIPs (D4.22).
Actions resulting from the follow up of milestones and deliverables include:

- Continued meetings regularly between the WP4 support team and the JIP Project leaders (PLs) to inform about upcoming events, reports to be written etc., but also to share experiences.
- The PLs are reminded about upcoming deliverables about one month before each date for delivery.
- The WP4 team checks the delivery of each project deliverable and that it is uploaded correctly. WP4 have provided guidance on how to do this.
- WP4 takes care of the dissemination of deliverables to stakeholders as indicated by the PLs.
- All PLs have been asked to make plans for how the effects of the pandemic will be handled.
- The Scientific Publication Policy has been updated to make the scope clearer and the routines easier to follow.

In March there was a second shift of PL for JIP-5 MATRIX. The new leader was nicely introduced to the project and has taken on the duty very well. The JIP-6 COVRIN started in March and had a digital kick-off meeting at the end of March. All JIPs have asked for and been approved an extension, providing the extension for the full OHEJP is granted. According to the time schedule, ORION will end in M42, COHESIVE in M48, CARE, OH-HARMONY CAP and MATRIX in M60 and COVRIN in M63. The recruitment of external evaluators for the two JIPs ending during Y4 has been conducted.
During the ASM 2021 there was a PLs Forum, where updates from the OHEJP project management team were provided and the PLs got an opportunity to reflect and discuss on different aspects. BfR with ORION as a co-organizer hosted a satellite workshop linked to the ASM. Nine software tools, developed or adapted within OHEJP projects, were presented, making this workshop an important dissemination event. In three sessions the tools were further explained for interested potential users.

6.1.2.1 *Detail of the JIPs activity reported in 9M reports*

6.1.2.1.1 JIP01-R1-IA1.1-ORION – Final Report

6.1.2.1.1.1 Summary of the work carried out in the Project

The ORION project finalized the project work as planned in June 2021. As outlined in the original project plan the ORION project developed and optimized One Health resources that were evaluated in a number of national One Health (OH) pilots as well as in a supra-national pilot with EFSA and ECDC.

One of the main ORION project outcomes is the so-called "One Health Surveillance (OHS) Codex", which is a framework supporting the implementation of the One Health paradigm in areas linked to the harmonization of OHS data and solutions. This framework was implemented as a continuously updateable online resource (https://oh-surveillance-codex.readthedocs.io). It is founded on the ORION requirement analysis where ORION partners jointly concluded that surveillance data integration in a One Health context requires a number of specific actions on different problem types by various stakeholders along the full surveillance pathway. This finding led to the design of an extendable OHS Codex structure that covers four main principles. Each principle represents an area, where resources to support cross-sector understanding and information exchange will help stakeholders to adopt the One Health paradigm. This structure enabled ORION partners to develop and integrate a broad spectrum of innovative solutions, resources and findings into a consistent overarching framework, including lessons learned from national pilots. Among others, the OHS Codex encompass the "One Health Report Annotation Checklist" (OH-CRAC), National OHS Report Templates, the One Health EJP Glossary, a OHS Inspiration catalogue, the OH Surveillance Pathway Visualization, the OH Knowledge Base – Surveillance systems, the Sequencing for Surveillance (SfS) Handbook and the **Health Surveillance Ontology.**

In several national OH pilots country specific solutions were established that created direct impact on cross-sector communication, surveillance data exchange and interpretation. For example, in the WP2-NGS pilot the first cross-sector IRIDA (https://www.irida.ca/NGS data analysis platform was established at the Norwegian Research and Education Cloud (https://www.nrec.no/). As a result, there is an increased collaboration and information exchange between the veterinary and public health sectors in Norway. The Danish pilots contributed to improved OH reporting, e.g. the *Salmonella* and *Campylobacter* chapter in DANMAP 2019, and the Danish national surveillance system for AMR and AMU (https://www.danmap.org/). They also established a sequencing based *Campylobacter* surveillance system that will be extended in the future into a platform for real-time surveillance data and result sharing. The Swedish pilot led to the implementation of new work procedures in the process of generating the annual surveillance reports on zoonotic diseases with an improved collaboration among the national OH sectors. The revised processes helped to identify and address bottlenecks in cross-sectoral interoperability and FAIRness of surveillance results. Innovative data interoperability solutions supporting data interoperability were developed and tested.
On international level, the ORION project performed a number of activities to share knowledge with international stakeholders like EFSA and ECDC, for example in a dedicated pilot project. In addition, a number of ORION solutions were integrated into the Surveillance and Information Sharing Operational Tool (SISOT) of WHO/FAO/OIE. Members of the ORION project presented research results at various international conferences, e.g. ASM2019, ASM2020, ASM2021, the 6th World One Health Congress etc., conducted several webinars and organized international workshops with other research projects and institutes, e.g. the NGS workshop 2020, the CPD2021 module, the ASM2021 Satellite Workshop Software Fair. ORION partners published eight scientific publications in peer-reviewed journals and several other publications are still in preparation.

The project coordination organized trimonthly web meetings for the whole ORION consortium

including stakeholders and interested EJP members, and monthly calls for the WP leaders & deputy leaders. The project contributed to the EJP DMP committee, initiated a number of collaborations and information exchange with other EJP projects (e.g. MATRIX, COHESIVE, RADAR, NOVA, BeONE), initiatives (e.g. RAKIP, SISOT, IRIDA, PAHO-WHO workshops) as well as with national and international research and development projects (SafeConsume, SEQ-TECH, SIGMA, Vinnova, FORMAS, COMBACTE-MAGNET EPI-Net network).

6.1.2.1.1.2 Work carried out in the JRP/JIP, scientific results and integrative outcomes

WP1: "OH Surveillance Codex"

WP1-T1: Inventories and requirement analysis for "OH Surveillance Codex" (M1-M12).
The requirement analysis was performed in the first year of the ORION project and laid the foundation for the development of the OHS Codex. The OHS Codex was envisioned as a high level framework for harmonised, cross-sectional description and categorisation of surveillance data (SD) covering all surveillance phases and all knowledge types. To identify the needs of the community/stakeholders, WP1 carried out literature reviews, online surveys and individual interviews with different domain experts.

The collected feedback outlined the lack of resources supporting mutual understanding and interpretation of data from the different OHS domains, which is a major bottleneck for meaningful OHS data analysis. In order to facilitate and contribute to mutual understanding between OH sectors, it was decided that the WP1 should work on two different solutions:

a) a community driven glossary collecting OH related terms that helps to identify terms with different or shared interpretation across sectors helping to overcome communication hurdles in the future;
b) a guidance document for harmonized categorization of metadata from different OHS domains providing recommendations on how to structure metadata in SD reports.

Further details about the methods and results of this requirement analysis can be found in the deliverable D-JIP1-1.1.

WP1-T2: Development of "OH Surveillance Codex" (M13-M24)
In the second year of the project, the outcomes from the requirement analysis phase collected by the different ORION WPs were brought together to identify current best practices and needs within the OHS community. The collected feedback confirmed that cross-sectoral and multi-disciplinary communication, collaboration and knowledge exchange are still significant challenges for the OHS community. To address these challenges, the conceptual design of the OHS Codex evolved into a high-level framework that comprises four high-level "action" principles (Collaboration, Knowledge, Data and Dissemination) that match well with priority areas identified in the "Tripartite Guide to Addressing Zoonotic Diseases in Countries" published by WHO, FAO and OIE. Within each of the four principles, the OHS Codex provides a collection of useful resources developed by the different ORION WPs, as well as pointers to success stories for the application of these resources. The OHS Codex was envisioned as a "living" framework that is continuously reviewed and updated as the project and the developed resources evolved adapting to the needs of the OH community in the future. From a technical perspective, the OHS Codex was implemented as an open source community resource that is available via: https://oh-surveillance-codex.readthedocs.io/en/latest/index.html.

In this phase, the conceptual design of the tools developed in WP1 to support communication and OHS harmonization were also updated. The OHEJP Glossary was further developed and maintained as a collaborative effort of three EJP projects, ORION, NOVA and COHESIVE, with support from OH experts of EJP stakeholders. An essential part of the work was the extensive curation and review of the terms and definitions by OH experts from each target sector (animal health, public health and food safety). The first draft of the OH Consensus Report Annotation Checklist (OH-CRAC) was also outlined in this

phase. The OH-CRAC aims at providing best practice annotation of SD in reports from different OH sectors.

WP1-T3: One Health pilot (M7-M39)

WP1 pilots had the goal of demonstrating that the resources developed by WP1 (OHEJP Glossary and OH-CRAC) and the supporting standards and tools developed by WP3 are practically applicable and generate the expected added value. WP1 performed the following pilots:

WP1 One Health Pilot - For this pilot, the EJP project "Antibiotic Resistance Dynamics" (ARDIG) and "German One Health Initiative" (GOHI) were chosen as use cases to validate the WP1 solutions in the AMR and AMU domains. The WP1 OH pilot specifically tested and improved the OH-CRAC, OHEJP Glossary and Glossaryfication Web Service. This web service was developed to add value to the OHEJP Glossary by generating automatically glossaries for user provided documents (reports, protocols, manuscripts, etc.).

EJP ORION WP1 & WP3 supra-national pilot with EFSA & ECDC - In addition to the originally planned national WP1 OH pilot, the ORION WP1 and WP3 agreed to pursue together a pilot to test the uptake-potential of WP1 and WP3 solutions by EFSA and ECDC. Under this pilot, we evaluated the potential benefits and usability of the following tools: OH-CRAC, OHEJP Glossary and the Health Surveillance Ontology (WP3) facilitating its accessibility and providing use cases via the One Health Linked Data Toolbox (OHLDT, https://foodrisklabs.bfr.bund.de/one-health-linked-data-toolbox/).

During the execution of the WP1 pilots, the feedback collected from both pilots was used to make the necessary adjustments and improve the different resources. A detailed description of the performed activities, results and lessons learned during the implementation of the pilots for each of the tools can be found in the final pilot reports provided as appendices to the deliverable D-JIP1-1.3.

WP1-T4: Evaluation and Recommendations (M31-M42)

- During the course of the project, the OHS Codex was significantly improved, updated and a new version was implemented as an open source community resource that is available via https://oh-surveillance-codex.readthedocs.io/en/latest/index.html. Furthermore, the OHS Codex was further extended to include the summaries and lessons learned from the pilot studies performed in the context of ORION. A collaboration plan was established with the EJP MATRIX project to further extend and maintain the OHS Codex once the ORION project ends.

- The development of the OHEJP Glossary (available at https://foodrisklabs.bfr.bund.de/ohejp-glossary/) required a collaborative effort involving actors from all OH sectors, disciplines and countries. This collaborative work implies the continuous extension, curation and validation of the glossary content. This process was supported by the implementation of a new backend software infrastructure, which provides efficient technical and operational resources to curate and manage the glossary content in a collaborative manner.

- The Glossaryfication Web Service extended the use of the OHEJP Glossary as end users can automatically search within any user-provided text document for terms that are contained in the OHEJP Glossary. Based on the feedback collected in the pilots, other glossaries from national and international institutions were added to the service (available at: https://foodrisklabs.bfr.bund.de/ohejp-glossary/. The inclusion of glossaries within OH reports using this service can improve communication and collaboration across OH sectors in a long term perspective.

- The OH-CRAC was also made available as a web-based interactive annotation tool

(https://aflex.vrac.iastate.edu/checklist/?t=OH-CRAC) for functional meta-information extraction in reports/documents uploaded as pdf files. The application of OH-CRAC in the WP1 pilots proofed that the adoption of OH-CRAC could improve the completeness and transparency of the SD reports and facilitates the cross-sector mapping of meta-information on SD.

- The OHLDT is a platform combining different tools developed based on the Health Surveillance Ontology (HSO). The joint work between ORION WP1 and WP3 demonstrated the potential of using HSO to join and enrich different datasets and link them to other sources (e.g. EFSA, ECDC or Wikipedia data).

WP2 Epi

Multiple surveillance systems are carried out annually by Member States (MS) of the European Union in the sectors of public health, animal health and food & feed. Some results of these surveillance systems are reported to EFSA, ECDC, and/or other reporting systems of the EU. However, several MS carry out additional national or regional surveillance related to zoonoses that are not reported to higher EU reporting bodies. The aim of this work package was to develop a knowledge base for surveillance systems including an accessible search platform with references to data sources.

To understand the surveillance systems in different sectors and to get an overview of surveillance systems in existence across EU MS, within this WP we first reviewed the literature and ran a questionnaire between WP2Epi partners (T1). The results showed a large difference in terminology and definitions between sectors. Hence, we supported the setup of a glossary in WP1. Altogether, these differences were too large to create a common inventory for all three sectors (public health, animal health, and feed & food), and therefore, we decided to develop three different inventory tables that were as similar as possible yet maintained the integrity of the different terminology between the sectors. We discussed our tables with EFSA and ECDC and based on their comments, analysed the reporting tool metadata of EFSA and ECDC to align our terminology with theirs wherever possible.

As a result, we developed a knowledge base to easily access and search for information on zoonotic and foodborne diseases under surveillance in EU MS within a single platform, tested it and improved it (T2). We used a scientific software within R to develop an application (shiny-app). The advantage being that R was free and the language widely used by scientists making it easy to proceed with the work and maintain the knowledge hub.

Close to 500 surveillance systems are currently in the inventory (https://shiny.fli.de/ife-apps/EJPOrion_WP2Epi/), and the collection of surveillance systems is expected to increase given the support by other EJP projects, such as EJP MATRIX.

To test the knowledge base on suitability for adding all kinds of surveillance systems and other scientific studies, we tested the knowledge hub within the pilot studies (T3).

Another objective of this WP was the analysis of methods for planning and assessment of surveillance systems with respect to the latent trait One Health-ness (OH-ness). By OH-ness, we mean the willingness of public and private bodies to follow the OH mindset during the planning and the implementation of surveillance and monitoring measures. The methodology of the Rasch model was adopted and implemented as an analysis script in Python to investigate OH-ness.

Additionally, a web UI to a database collection of statistical methods and software tools used in surveillance is set up at FLI (M30) using a public shiny interface. The web application was published in M33 at https://shiny.fli.de/ife-apps/toolsdatabase/. The databases will continue to be maintained in the upcoming years.

Even if the pilot studies are designed to test and support the general aim of WP2, in most cases the scope was much broader and gave additional impact in the analysis of surveillance systems in Europe.

In the pilot study 1 (ST1), carried out by FLI and BfR we analysed in detail the role of Toxoplasma gondii as a zoonotic agent. Therefore, a literature research and a systematic review was carried out. Despite staff turnover and the Covid-19 pandemic, the vast majority of goals were achieved. The results of this pilot study were directly added to the knowledge hub.

Other pilot studies analysed the role of *Salmonella*, Hepatitis E, and AMR. These pilot studies were not only supporting WP2Epi but other WPs as well. For example, in the Dutch pilot a more in-depth collaboration between Dutch ORION partner institutes concerning hepatitis E surveillance was set up. Overview of involved institutes, collaborations and their projects were visualized in a country map. Both institutes (WBVR and RIVM) performed an evaluation of the current status of HEV surveillance within the institutes. A start has been made for joint analysis, by making a data template to organize HEV data in a FAIR manner, including a codebook with names and descriptions of relevant variables.

In the UK pilot study carried out by PHE and APHA on *Salmonella* surveillance data exchange it was explored how the data flow and data sharing between different sectors, including gene sequences, could be improved. This pilot established a framework for the sharing of *Salmonella* sequence data between human, food safety and animal health sectors (including associated isolate metadata at level of granularity/sensitivity determined according to one of six data sharing objectives). Data sharing protocol and a Memorandum of Understanding were developed. Hence, this pilot was relevant for WP1, WP2Epi, WP2NGS, and WP3. In the pilot study carried out by Sciensano, different aspects of AMR reporting with an OH vision in Belgium were tested. This has a strong link to the OHEJP glossary (WP1), to WP2Int, and to WP3.

From a scientific perspective, the overall result of this WP, a comprehensive knowledge base, will support scientists to better understand the surveillance systems in different sectors and to include this information in future publications. From a management perspective, the knowledge hub is an important source of information as it provides an overview on surveillance systems beyond the current available databases (e.g. EFSA, ECDC). Furthermore, the development of the inventories identified some important obstacles to collaboration and exchange between different sectors. E.g., it was not possible to create a common table to collect surveillance systems for all sectors due to different terms. The glossary developed in WP1 is an important step to harmonize terminology, and for mutual understanding.

Multiple collaborations and information exchange within and outside the EJP consortium were part of this WP. Important partners are EJP COHESIVE, EJP MATRIX, EFSA and ECDC.

WP2 - NGS

JIP1-WP2-T4: Inventories and requirement analysis for OH Knowledge Base – NGS (M1-M12)

This task was completed in M12. The main target for this task was to figure out what information was needed to be able to use sequencing for surveillance purposes. The work in this task was completed through several means. We first performed a literature review, both of peer-reviewed papers and grey literature, to get a view of the landscape. This review included documents produced by both the COMPARE project and the ECDC and EFSA regarding the state of the art. Based on this, we created a questionnaire that was sent out to project partners. We also conducted several site visits and meetings with domain experts at various veterinary and public health labs. Our main focus for these meetings was on exploring the work processes and the best practices that these labs had gathered in their work with establishing sequencing as a tool at their institutions. The labs we visited in person were Public Health England (PHE), Statens Veterinærmedicinska Anstalt (SVA) and Folkhalsomyndigheten (FOHM), both in Sweden, and also Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (ISZAM, Teramo, Italy). Additionally, we had an online meeting with Statens Serumsinstitut (SSI) in Denmark. This work formed the basis for the Knowledge base, which for WP2-NGS has resulted in the handbook described in the next task.

JIP1-WP2-T5: Improving OH Knowledge Base – NGS (M13-M24)

Bioinformatics is a rapidly developing field, and thus the work with the handbook (https://oh-sfs-handbook.readthedocs.io/) that forms the basis of the WP2-NGS knowledge base has continued to the end of the project.
Through the mapping work done in the first year of the project, it was established that there were especially four areas in which it would be particularly useful to catalogue knowledge for new practitioners within the field. These areas were infrastructure, pipelines, typing methods and how to perform surveillance with such results. Thus the handbook was developed to cover these areas. The technical framework that was chosen to host the platform was Github in combination with Readthedocs. This technical solution provides a reasonably low threshold for people to contribute to the handbook, and also helps the sustainability of the project.

Within the scope of this task we also set up a cogwheel workshop together with WP3. This workshop was organized in conjunction with the ECCMID conference in Dubrovnik in 2019. For this cogwheel, we identified and invited initiatives that provide tools for WGS-based surveillance. This workshop saw participation from IRIDA, INNUENDO and COMPARE projects, as well as several OHEJP projects. In addition, the EFSA and ECDC were present. This workshop helped foster communication and understanding within the groups present.

JIP1-WP2-T6: NGS OH pilot studies (M7-M39)
There were two pilots associated with this WP: one pilot in Norway, focusing on infrastructure and joint analysis of *Listeria monocytogenes* between the NIPH and the NVI, and one in Denmark, focusing on using sequencing data to do surveillance of *Campylobacter*.

The work done in the Norwegian pilot has resulted in new infrastructure set up at the NVI, and thus the work done in this task is still ongoing. The aim for this pilot was to set up facilities for joint analysis between the NIPH and the NVI. We explored the two data management and analysis platforms (DMAPs) that at the time were free and open and available for local installation, these were the IRIDA and the INNUENDO platforms. Through evaluation of the platforms, we chose to use the IRIDA platform, primarily for two reasons: the platform allows for differentiated access to data and it is receiving continued support from Canadian funding agencies.
We subsequently proceeded with installing the DMAP in the Norwegian Research and Education Cloud (NREC), where it is currently being tested out by users at the NVI and the NIPH. The focus pathogen for this project has been *Listeria monocytogenes*, and we have in that connection developed an IRIDA plugin which specifically aims at analysing this pathogen.

A Danish pilot study was set up to promote collaboration among all agencies involved in surveillance of *Campylobacter.* An OH working group was set up for this project. The group includes stakeholders from all sectors that have met with regular intervals to discuss all aspects of the project. This includes the results from a retrospective WGS study, to set up the real-time study for 2019 and further implement routine OH surveillance system in 2020, based on the evaluation of the first year (2019) of real-time surveillance. Data on Campylobacter has been analysed using cgMLST derived from WGS and used to detect genetic clusters among patients. Furthermore, an OH approach was used to detect possible links between food/animal sources and human clusters.
This work resulted in a publication from 2020, “Whole-genome sequencing to detect numerous *Campylobacter jejuni* outbreaks and match patient isolates to sources, Denmark, 2015-2017”.

JIP1-WP2-T7: Evaluation and Recommendations (M31-M42)
The work done on the handbook and in the associated for this WP have gone hand in hand. The experiences collected in the pilots have been collected into the handbook, and information collected in the handbook was used to inform the work done in the pilot. In this way the handbook now contains the knowledge the project has uncovered with regards to using sequencing for surveillance.

WP2 - Integration

The WP2-integration work package has collated and developed proof-of-principles that support following statements:

- One Health Surveillance initiatives already exist and are implemented in EU countries
- Integration of surveillance data, analyses, interpretation and outcome communication a) are feasible, and b) can improve our understanding to control risks and zoonotic diseases
- Development and implementation of One Health Surveillance Initiatives are complicated and collaboration is the foundation for successful One Health Surveillance.

JIP1-WP2-T7: Inventories and requirement analysis for OH Knowledge Base- integration (M1-M12)

A survey was initially conducted to identify existing and implemented One Health Surveillance Initiatives (OHSIs) in the EU and beyond. The OHSIs were identified using a two-step approach with a screening questionnaire and then approaching a selected subset of responders for semi-structured interviews (for detailed description of methods see Deliverable-JIP1-D2.3 Report on requirement analysis for an "OH Knowledge Base – Integration" (ORION) | Zenodo.

The initial screening questionnaire identified 78 OHSI in 20 EU countries, of which 38 OHSIs matched the project definition of an OHSI, i.e. concern foodborne pathogens, was a surveillance activity and included active collaboration between at least two sectors. A total of 15 OHSIs were included in the final report: *Inspiration and ideas: One Health Integration in Surveillance* (Ellis-Iversen, 2019 Report on OH Surveillance Initiatives). In the report, each OHSI was described by a one-pager explaining the main objectives, the work carried out, actors, future developments and challenges. It also provided a contact institute for further information.

The 15 examples provide proof-of-principle that OHSI are possible for almost every step in the surveillance pathway except for 'sample collection', which no OHSI in our survey addressed. The cross-sector laboratory initiatives were implemented either for rare diseases or when applying a novel/advanced analysis, so all strains of one species may be sent for typing or sequencing in one sectoral laboratory specialized in the methodologies. Multi-sectorial expert groups used to discuss and interpret surveillance result were also reported from several countries. In two countries, these were established groups to deal with new, emerging or changing pathogens, and to generate prioritized advice on response and control; these groups included risk-managers and decision-makers as well as scientific experts. Other countries reported cross-sector groups that meet regularly and discuss the current situation, outbreaks and interpret or explain changes together.

Publishing a joint report on zoonoses from multiple sectors was common in the screening questionnaires. Some variation was reported between countries on how much the sectors shared data, collaborated on result interpretation and on the collation of the report. A cross-sector governance structure for zoonotic or AMR surveillance was also reported from several countries. The governance structures were all at national level and were responsible for varying levels of strategy, optimization, communication and prioritization of response.

The survey was not comprehensive nor randomised to provide representativeness, but it showed clearly that OH surveillance is feasible and already implemented throughout the EU and beyond. It provides ideas and inspiration to other countries, and specific examples are now available as proof-of-principle, when advocating for a new OHSI in a country. The catalogue of OHSIs can be found here: Report on OH Surveillance Initiatives

JIP1-WP2-T8: Improving OH Knowledge Base – Integration (M13-M24)

WP2-integration hosted three pilot project workshops between June 2019 and March 2021 to support the pilot project leaders, to ensure planning and structured outputs and to facilitate collaboration and knowledge sharing between the pilots. WP2-integrations pivotal role in anchoring the pilot projects within ORION allowed us to improve the OH knowledge base with additional OHSIs, but also to understand what challenges OHSIs face between the initial idea and implementation. The three workshops were used ensure coherence between the pilots, identify integration opportunities, avoid overlaps and share ideas between the studies.

The first workshop in June 2019, co-hosted with WP1, focussed on finding commonalities and potentials for collaboration between pilot projects. The need for a generic way to document the planned pilot and expected outcomes was also identified, and a template for pilot project description was developed and agreed. Furthermore, the OHS Codex was introduced, and its potential to become a central outcome of ORION was established (Filter et al, 2021).

The next pilot project workshop was co-organised with WP3 in January 2020. The pilots were now progressing. The project leads presented progress and preliminary outcomes, and we discussed how the outcomes of the different pilot projects would fit into the OHS Codex, and thereby, become anchored within the ORION project. A discussion on how to evaluate the outcome of pilot studies was also initiated, and WP3 and WP2integration developed frameworks for OH progress evaluation for each pilot study to apply.

The last workshop in March 2021 focussed on the lessons learned from each pilot project, and how these could be incorporated into the OHS Codex. The ORION leads for each pillar in the OHS Codex collated the relevant lessons and chaired the discussion on lessons within their pillar. It was decided that each pilot project delivered a report using a template developed by the Coordination Team and WP2-integration to ensure capture of lessons learned.

Twelve pilot projects were completed during the time of ORION, and most of them can be considered either newly developed OHSIs, improvements to current OHSIs or as tools that can used by OHSIs to improve cross-sector working. They all improved the knowledge base on OHSIs and provided proof-of-principles in multiple countries. Details on all the pilots projects can be found in the ORION Knowledge Hub

JIP1-WP2-T9: Integration OH pilot studies (M7-M39)

Integration between all the ORION pilots projects was achieved by the workshops and templates already described in the previous section (JIP1-WP2-T8). WP2-integration also contributed to the knowledge base with two pilot studies, with great success and large impact. One of these was a considerable OH improvement to an existing OHSI and the other has the potential to become a new OHSIs providing proof-of-principle for the additional value of surveillance data integration. The third pilot was commenced and planned, but terminated mid-way due to COVID-19 demands on resources. The remaining resources at DTU were used in pilot 2 instead.

WP2-integration pilot 1: Increasing the One Health interpretation of AMR and AMU surveillance.

In 2019, this DTU and SSI pilot generated a One Health-focussed way of reporting AMR for *Campylobacter* by integrating data from the five relevant data streams of: AMU for treatment in humans and animals and AMR in animals, food and people. In 2019 the group worked on the *Campylobacter* section and in 2020, the work focussed on reporting AMR for *Salmonella*. Each year, the project group met early in the year to agree the objectives, plan the flow in the chapter and in each section. Over the summers, data was analysed and the text written and the reports were published in October 2019 and October 2020, respectively.

In short, this pilot study aimed to explore, whether the zoonoses chapter of the DANMAP report could report to One Health objectives, and whether further integrated analyses and interpretations

would enhance the One Health conclusions for decision-makers and other users of the report. The OH integration consisted of several steps: 1) Identify the antimicrobial drug classes relevant for treating human patients with acute gastroenteritis; 2) present the resistance levels to the relevant drug(s) for all populations in one figure; and 3) discuss the use the relevant antimicrobial drug(s) in animals and their OH implications.

The new OH focused structure of the DANMAP chapter was well-received by the users the Danish Veterinary and Food Administration expressed: *"The overall target of our efforts on AMU and AMR in the food production is to support human health and ensure that it is and will be possible to treat human illnesses with antimicrobials. To achieve this target, we aim to reduce or maintain a very low level of AMR throughout the food production, focusing on the critically important antimicrobials. When reporting the status of AMU and AMR surveillance it is important, that the impact and trends concerning the risk posed to human health, by foodborne AMR is addressed and if possible assessed. The enhanced One Health focus in the zoonoses chapter of DANMAP 2019 supports this agenda."*

The outcomes of the pilot project can be found in:

- DANMAP 2019 on DANMAP.org
- The ORION Knowledge Hub as ORION_WP2_INT_PILOT_ 1_DK
- The final deliverable from WP2 Integration D2.9 (https://zenodo.org/record/5062452)
- As a resource in the OH Codex as "Guidance on reporting antimicrobial resistance in a One Health perspective" (https://oh-surveillance-codex.readthedocs.io/en/latest/5-the-dissemination-principle.html#national-ohs-report-templates)

WP2- integration pilot 2. Does One Health integration of surveillance data improve surveillance and disease control? - Integrating national Campylobacter surveillance data to establish proof-of-principle

This pilot project was developed to investigate the usefulness of integrating surveillance data across sectors, and to explore whether we could establish a proof-of-principle that could be used by others. At the same time, we wanted to support the Danish National Campylobacter Action Plan with any potentially useful outputs. The pilot project used data from two *Campylobacter* surveillance data streams: animal health and food safety, and used a model to estimate the effect of changes in public health. This was investigated through three studies:

- Study 1 explored surveillance outcomes across data streams by comparison of *Campylobacter* prevalence at flock and carcass levels and assess whether adjustments are needed.
- Study 2 investigated whether any new information on public health risk arises from data stream integration by testing the agreement of surveillance outcomes for individual flocks and carcasses, and how this information could feed into the National Campylobacter Action Plan
- Study 3 designed a risk-based control programme by using information from the data streams and assessed the value of risk-based control by combining it with a public health risk model to measure the effect on public health.

The outcomes showed that it was possible integrate the data and analyse it across sectors and populations, but that adjustments of the estimates increased the precision and interpretability. Furthermore, we learned that when you integrate data, you can improve the understanding of the risk and may find new relevant surveillance points. The last outcome of the project demonstrated the

additional value of data integration, and proposed a more cost-effective way of controlling *Campylobacter* in poultry in Denmark. The outcomes are currently under discussion between the authorities and the poultry industry for implementation in the Danish National Campylobacter Action Plan. A detailed description of the project and its outcomes can be found in the final deliverable from WP2 Integration D2.9 (https://zenodo.org/record/5062452) and two papers are being prepared for peer-reviewed publication.

Pilot 3. Descriptive surveillance framework farm-to-patient

The aim of this study was to develop at template for describing national OH surveillance systems. The project was abandoned due to COVID-19 resource drain and staff changes.

JIP1-WP2-T10: Evaluation and Recommendations- Integration (M30-M42)

The 3rd pilot project workshop had focus on evaluating, what we learned from all the ORION pilots. The main advantage of conducting pilot studies within the ORION project instead of only gathering information from literature or interviews, was the opportunity of learning through the process, and learning lessons about the applicability of the OH approach in practice. This acquired knowledge may guide others in developing OHSIs in the future and is included in the OHS Codex and in the individual pilot project reports in the final deliverable from WP2 Integration D2.9 (https://zenodo.org/record/5062452)

Already by the time of the 2nd workshop, many pilot projects experienced that lack of tools, methodology or technical solutions were often not what hindered or complicated integration across sectors. It was often 'individuals', 'the human factor' or 'sensitivities' that slowed things down or hindered implementation of novel solutions. This emphasised the importance of understanding and facilitating a collaborative culture and nurturing positive attitudes for successful OH surveillance initiatives.

WP2-integration was the lead on gathering lessons learned about collaboration from all the ORION pilot projects. Positive lessons and tools that facilitated collaboration and characterised successful outcomes were: frequent meetings/workshop between partners, mutual and clear definitions and goals from the beginning of the project agreed between all partners, templates/check lists/schematic drawings, data-sharing agreements in place and that the project addressed a mutual need/interest. Other things that motivated good collaborations were piggy-backing on existing partnerships and previously established trust; when political interest or pressure existed, and if equal priority/interest/buy-in/enthusiasm from the participating organisations was present. Clear areas of responsibilities and a continuous focus on the outcomes and goals rather than on detailed process and resources, were also experienced as positive in building collaborations.

Some pilot projects saw collaboration grow after having 'planted the seed' a while ago. However, this approach is not well-suited for a project with a specific timeframe such as funded (research-type) projects. In general, it was recognised that an OHSI take time to develop and establish itself, which does not always fit well with academic project funding streams and deadlines. Some pilot projects experienced that success in starting up an OHSI could be very person-dependent and convincing individuals to integrate their expert topics with others could be difficult and occasionally a barrier.

Inability to enlist leadership support both internally in the organisations and externally was highlighted as problem. For some of the OHSI-developing pilot projects, it could be difficult to get buy-in from or within organisations without proof-of-principle. Interestingly, in contrast our tool-developing pilot projects found that, despite offering and demonstrating an actual tool, it was also difficult to obtain adoption in existing OHSI.

The lack of concept-of-principle to facilitate advocacy and agreement within and between agencies and up the management ladder is the problem addressed by this work package. We have gathered a

catalogue of pre-existing initiatives to inspire and provide proof-of-principle. We have further nurtured collaboration and knowledge-sharing between the ORION pilot projects, and anchored them within the ORION project, whilst ensuring some level of standardisation allowing lessons to be shared in an effective way. These pilot projects and the lesson learned has updated and improved our knowledge base on One Health in Surveillance. WP2-integration have further conducted two pilot studies improving the OHS in Denmark, whilst providing proof-of-principle of the additional value of data integration and OH-focussed dissemination.

WP3: OH Surveillance Harmonisation Infrastructure

WP3-T1: Inventories and requirement analysis for OH Harmonisation Infrastructure (M1-M12)

This task provided a detailed overview of the needs and requirements in OH data interoperability through three main tasks:

- a literature review, which is currently under peer-review.
- A joint ORION "Requirement Analysis" workshop (hosted by WP4). In that workshop a decision was made to focus this WP on the development of a "knowledge base" - an ontology for surveillance, plus a surveillance dataset instantiated following the ontology model.
- A written survey and interviews with surveillance data experts from within the consortium, which were summarized in the first deliverable JIP1-3.1[4]

The results of these three sub-tasks were used to, in the fourth subtask, construct a vision for an Harmonisation Hub that addressed the main gaps in data interoperability in OHS. It was defined in year 1 that WP3 should fulfil these gaps by working in the following tasks to deliver three main outputs : an ontology,

1) the ontology itself ;
2) a dataset of public surveillance data published as linked-open data (LOD), marked up following the ontological model developed;
3) a proof-of-concept workflow to adopt the ontology in practice, which can be used by any other country to expose, as LOD, their surveillance data already shared with other MS

To fulfil this vision, the WP was split into 3 main tracks: the ontology track, concerned with the development of the ontology to be used; the practice of surveillance track, which was responsible for the OH pilot task described further below; and a technical track, responsible for building the technical architecture needed to achieve this use of an ontology in the practice of surveillance.

WP3-T2: Improving OH Surveillance Harmonisation Infrastructure (M13-M24)

During year 2, within Task 2, we reviewed and aligned terminology from the ECDC, EFSA and previous key projects in animal health surveillance design (RISKSUR and AHSURED), as well as current EFSA projects on surveillance reporting (SIGMA). All relevant concepts were added to the ontology developed, which was named Health Surveillance Ontology, and made publicly available in various locations:

- a globally unique and eternally persistent identifier: https://w3id.org/hso. This address is subjected to content negotiation: humans accessing this link via browser will be referred to a page listing all ontology documentation and additional resources, such training materials. Software agents pointed to the same address will find the machine-readable codes for the knowledge model (written using the Web Ontology Language - OWL).

[4] https://zenodo.org/record/3754615#.YLiW2vkzYkV

- Bioportal: https://bioportal.bioontology.org/ontologies/HSO
- A persistent uniform resource locator (PURL) under the Open Biomedical Ontologies Foundry: http://purl.obolibrary.org/obo/hso.owl

An Excel plug-in was developed, so that surveillance data can be annotated with the ontology using simple Excel templates. The Excel plug-in is free and open source, and it was developed in conjunction with the RealEstateCore project in Sweden. Codes for developers, as well as a guide to install the plug-in for users are available at https://github.com/RealEstateCore/ExcelRDF. ExcelRDF is a Visual Studio Tools for Office (VSTO) plugin.

WP3-T3: One Health pilot (M7-M39)

In Year 1, active discussions have led to the decision of focusing the OH-pilot in three main chapters of the Swedish surveillance report: *Campylobacter, Salmonella*, and VTEC/EHEC. During months 13-18, the surveillance practice followed closely the production of the surveillance report for these three zoonotic agents, conducting workshops that gathered AH, PH and FS surveillance officials to discuss opportunities for data sharing, joint data analysis, and publication of main findings that highlighted the OH aspects of the activities and findings of the previous year.

During year 2 the OH-work with the report was extended to include all zoonotic chapters in the report. The work during year one was evaluated and the experiences were incorporated into the following year. As a result, a new process was introduced, and new instructions were produced to clarify the role of the main responsible author of each chapter. To improve the OH work in all zoonotic chapters it was mandatory for the main authors to contact the other authors for a meeting to discuss the results of the previous year's surveillance and what to highlight in the report. The report was published in June and in this issue several of the zoonotic chapters had special In-focus sections, highlighting an unusual finding, an outbreak or a more detailed description of a method.

WP3-T4: Evaluation and Recommendations (M25-42)

This task was extended to M42, following ORION's extension. During the last project year (and the 6 months extension) we focused on improving the outputs based on the lessons learned from the pilot in Sweden, and across the ORION project; and in disseminating these outputs and making them publicly available in sustainable online locations.

For the second main output planned for this WP, a "dataset of public surveillance data published as linked-open data (LOD), marked up following the ontological model developed", we used data gathered during the pilot to publish a dataset with results of 10 years of Campylobacter surveillance in Sweden (animal and public health) as a full FAIR (findable, accessible, interoperable and reusable) resource. The dataset has its own URI (https://data.sva.se/dcat/surveillancereport/campylobacter_surveillance_sweden.rdf) and it is also published in the Swedish data portal for public sector information: https://www.dataportal.se/en/datasets/59_1684/campylobacter-surveillance-in-sweden.

While the ExcelRDF plugin had been delivered in year 2, in year 3 we further improved the third main deliverable, "a proof-of-concept workflow to adopt the ontology in practice" by creating automated workflows using the KNIME Web Server infrastructure provided by ORION's coordinator institute, BfR. The Linked Data Toolbox workflows are available at https://foodrisklabs.bfr.bund.de/one-health-linked-data-toolbox/.

WP4 - Coordination, Communication, Training and Sustainability

JIP1-WP4-T1: Internal project coordination (M1-M42)

- The project coordination accomplished the project coordination tasks with great support from all project partners. As technical infrastructure for project management the project

used in the beginning the ORION Virtual Research Environment (VRE) and towards the end of the project the web-based infrastructure provided by EJP.

- Over the whole duration of the project the coordination held trimonthly web meetings for the whole ORION consortium (including EFSA, ECDC, EJP WPs and interested other EJP project leads) and a monthly call for the WP leaders & deputy leaders.

JIP1-WP4-T2: External project integration (synchronized with EJP WP5) (M1-M42)

- The project coordination contributed to relevant overarching EJP activities and continued to extend collaboration and information exchange specifically with the new EJP projects.
- EFSA / ECDC were actively informed on project results via the "EJP ORION WP1 & WP3 supra-national pilot with EFSA & ECDC"
- ORION successfully applied and conducted the 2021 CPD Module that took place from 15th to 19th February 2021. ORION also supported the ASM2021 Satellite workshop on 7th June 2021
- ORION kept close collaboration with a number of other EJP projects, specifically JIP COHESIVE and JIP MATRIX, and exchanged experiences through dedicated meetings. ORION also kept close collaboration with the JRP NOVA, focused on surveillance.

JIP1-WP4-T3: Sustainability roadmap (M7-M42)

- For the majority of ORION solutions first activities were undertaken to guarantee the short-to-medium term sustainability. This included a number of dissemination and publication activities as well as the adoption of technical infrastructure that supports long-term maintenance and collaboration. A Sustainability roadmap, describing the planned steps towards such long-term sustainability, was provided as a dedicated deliverable in M42.

JIP1-WP4-T4: Training and Dissemination (M1-M42)
JIP1-WP4-T4-ST2: Knowledge integration (web portal, Wiki, curricula, tutorials, videos, sample data) (M7-M42)

- Continuous updates performed on ORION's main online platform "OHS Codex" as well as on individual web resources that each partner created for their specific solution, e.g. for OHEJP Glossary linked resources the website https://foodrisklabs.bfr.bund.de/ohejp-glossary/ was created and updated regularly.

JIP1-WP4-T4-ST3: Training and support for other EJP projects & partners (M7-M42)

- See list of Dissemination and communication activities provided in this report.

6.1.2.1.1.3 Project self-assessment

The ORION project has fully accomplished its project objective to "establishing and strengthening inter-institutional collaboration and transdisciplinary knowledge transfer in the area of surveillance data integration and interpretation, along the One Health (OH) objective of improving health and well-being". As planned in the project proposal the ORION project developed a framework that supports the implementation of the One Health paradigm in all areas linked to the harmonization of One Health Surveillance (OHS) data and solutions. This framework has been named the "OHS Codex" and was

implemented as a continuously updateable online resource (https://oh-surveillance-codex.readthedocs.io). It builds on the joined understanding that surveillance data integration in a One Health context requires a number of individual actions by various stakeholders along the full surveillance pathway. This finding also opened up the way to integrate a broad spectrum of ORION solutions, resources and findings into a consistent overarching framework. Other specific project objectives outlined in the ORION project plan were accomplished as well, like the development of "a high level framework for harmonised, cross-sectional description and categorisation of surveillance data covering all surveillance phases and all knowledge types" which was finally named "One Health Report Annotation Checklist" (OH-CRAC). In WP2 "a cross-domain inventory of currently available data sources, methods / algorithms / tools, that support OH surveillance data generation, data analysis, modelling and decision support" was developed and integrated into the OHS Codex as the "Knowledge principle" (https://oh-surveillance-codex.readthedocs.io/en/latest/3-knowledge-principle.html). In WP3 a number of "practical, infrastructural resources forming the basis for successful harmonization and integration of surveillance data and methods" were developed and integrated into the OHS Codex under the "Data principle" (https://oh-surveillance-codex.readthedocs.io/en/latest/4-the-data-principle.html).

Over the course of the ORION project it turned out, that the originally designed project structure with a number of well-defined work packages and several, flexible national pilots were a very good choice. In this way, the project could adapt to the specific needs in each project member country and the involved national stakeholders giving all project partners the opportunity to contribute with specific and relevant solutions to the overarching ORION framework. This flexibility also helped to overcome a number of challenges that the ORION project had to face, e.g. frequent changes of project staff, delays in the hiring process of new staff, and the COVID pandemic. Even though for some pilots and some sub-tasks not all of the initially planned activities could be accomplished as planned, the overall number of solutions and resources developed by the ORION project overcompensated this. In sum, the ORION project accomplished its core objective to create impact on national and international level in improving One Health surveillance data harmonization and integration.

6.1.2.1.1.4 Progress of the project: milestones and deliverables

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered on Project Group (month)	If deliverable not submitted: Forecast delivery date (month)	Comments *Please mention: **public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID)**, other comments*	Proposed categories* *(1 to 8) (several categories may be applicable)*
ORION	D-JIP1-1.1	Report on requirement analysis for "OH Surveillance Codex"	M12	M12		https://zenodo.org/record/3754468	1,2
ORION	D-JIP1-1.2	Draft on OH Surveillance Codex	M24	M24		https://zenodo.org/record/3754474	1,2
ORION	D-JIP1-1.3	Revised OH Surveillance Codex, including lessons learned from the OH pilots	M42	M42		https://zenodo.org/record/5062641	1,2
ORION	D-JIP1-2.1	Report on requirement analysis for an "OH Knowledge Base – Epi"	M12	M13		https://zenodo.org/record/3754572	2,5
ORION	D-JIP1-2.2	Report on requirement analysis for an "OH Knowledge Base - NGS"	M12	M14		https://zenodo.org/record/3754649	2,5
ORION	D-JIP1-2.3	Report on requirement analysis for an "OH Knowledge Base – Integration"	M12	M14		https://zenodo.org/record/3754596	2,5

ORION	D-JIP1-2.4	Status report on OH Knowledge Base – Epi	M24	M24		https://zenodo.org/record/3754600	2,5
ORION	D-JIP1-2.5	Status report on OH Knowledge Base – NGS	M24	M24		https://zenodo.org/record/3754604	2,5
ORION	D-JIP1-2.6	Status report on OH Knowledge Base – Integration	M24	M24		https://zenodo.org/record/3754609	2,5
ORION	D-JIP1-2.7	Revised OH Knowledge Base - Epi, including lessons learned from the OH pilots	M42	M42		https://zenodo.org/record/5062653	2,5
ORION	D-JIP1-2.8	Revised OH Knowledge Base -NGS, including lessons learned from the OH pilots	M42	M42		https://zenodo.org/record/5062329	2,5
ORION	D-JIP1-2.9	Revised OH Knowledge Base - Integration, including lessons learned from the OH pilots	M42	M39		https://zenodo.org/record/5062452	2,5
ORION	D-JIP1-3.1	Report on requirement analysis for an “OH Harmonisation Infrastructure Hub”	M12	M12		https://zenodo.org/record/3754615	4
ORION	D-JIP1-3.2	Status report on OH Harmonisation Infrastructure Hub	M24	M24		https://zenodo.org/record/3754621	4
ORION	D-JIP1-3.3	Revised OH Harmonisation Infrastructure Hub, including lessons learned from the OH pilots	M42	M42		https://zenodo.org/record/5062410	4
ORION	D-JIP1-4.1	Two internal training workshops for ORION	M12	M9		https://zenodo.org/record/5062531	8 (training)

		partners					
ORION	D-JIP1-4.2	Draft on Sustainability Roadmap	M18	M18		https://zenodo.org/record/5062531	8 (sustainability)
ORION	D-JIP1-4.3	Two training workshops for other EJP: Training workshop "Nextflow" for EJP, NVI ORION Webinar	M24	M24		https://zenodo.org/record/3754627	8 (training)
ORION	D-JIP1-4.4	Revised Sustainability Roadmap	M42	M42		https://zenodo.org/record/5062617	8 (sustainability)
ORION	D-JIP1-4.5	OHS Knowledge Hub populated with resources from WP1, WP2 and WP3	M42	M42		https://zenodo.org/record/5062623	4,7
ORION	D-JIP1-4.6	Two additional training workshops and two webinars	M42	M42		https://zenodo.org/record/5062493	8 (training)

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP/JIP Code	Milestone number	Milestone name	Delivery date from AWPs	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
ORION	M-JIP1-1	Requirement analysis synchronization workshop	M4	Yes		Workshop held in Berlin, 18th-20th April 2018.
ORION	M-JIP1-2	Prioritization workshop	M15	Yes		Workshop held in Uppsala on 16th- 18th January 2019
ORION	M-JIP1-3	ORION evaluation workshop	M39	Yes		Workshop held online from 9th-11th March 2021

6.1.2.1.1.5 Follow-up of the recommendations and comments by the Ethics Advisors

Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020	Comments of Ethics Advisors, November 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021	Comments Projects Leaders June 2021
The applicants must confirm the compliance with GDPR	In WP1, no data from individuals will be collected or used. We see no risk of infringing the GDPR. In WP2Epi, no data from individuals will be collected or used. We see no risk of infringing the GDPR. In WP2-NGS, we might utilize some human related pseudonymized metadata for sequences that we might seek to analyze in collaboration with other EJP projects, however, these will if so be stored on an e-infrastructure approved for use for human sensitive data set up in Norway. For WP2int 2.3 - Requirement analysis for an "OH Knowledge Base – Integration", professional email addresses of individuals	As regard to the proposed interviews with volunteering key personnel (WP2int 2.3), an informed consent procedure should have been established and proposed to the research participants. It must mention the rights to participants as described in GDPR, among which the contact address of	Informed consent was established with reference to GDPR by the Data Protection Officer and according to the existing rules. In the end, contact persons emails were omitted from the report, only publishing the organisational contact point emails already available on the web.	As a reminder of one important part of GRPD, the beneficiaries must provide the contact address of the Data Protector Officer of the institution in charge of processing the data obtained.	BfR: dsb@bfr.bund.de SVA: jerker.plobeck@sva.se DTU: Joell@food.dtu.dk for data from ORION The overarching DPO of DTU is: anesa@dtu.dk FLI: Martina.Rychly@fli.de NVI: Siv.Gunhild.Boe-Tondevold@vetinst.no Sciensano: dpo@sciensano.be NIPH: Erlend.Bakken@fhi.no FOHM:	Could you confirm that the other partners, e.g. niph, ssi, fohm, coda-cerva, wbvr, rivm, apha, phe, are not collecting any personal data?	Sciensano: No personal, private or confidential information were collected. SSI: We store all Danish human metadata used in the project on servers approved for use for human sensitive data in-house at SSI. RIVM, WBVR, APHA, PHE:	Satisfactory answers for Sciensano, RIVM, WBVR, APHA, PHE, Univ. Of Surrey and regarding the i-bird cohort; for SSI, need of a confirmation from SSI's DPO that data are processed in accordance to GDPR	SSI confirms now that they were not collecting personal data for the ORION project.

Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020	Comments of Ethics Advisors, November 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021	Comments Projects Leaders June 2021
	were volunteered in the screening questionnaires. Interviews with volunteering key personnel of some initiatives was recorded and the original sound files will be kept in a restricted folder complying with ORION data management plans, DTU data management plans, DTUs Policy of the Retention of Primary Materials and Data, GDPR, until deletion on the last day of the ORION project. In WP3, no data from individual cases or laboratory tests will be used, only data already aggregated at the surveillance level, and already made public by the owner institution. We see no risk of infringing the GDPR	the Data Protector Officer of the institution in charge of processing the data obtained through the interviews. It is not clear that the above documents were used for the questionnaire work			dataskyddsombud@folkhalsomyndigheten.se SSI: compliance@ssi.dk		confirmed that they are not collecting personal data for the ORION project All other ORION partners: now provided the contact details of the responsible data protection officer		

Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020	Comments of Ethics Advisors, November 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021	Comments Projects Leaders June 2021
The applicants must specify whether human genome will also be sequenced in the pilot study. In case of whole genome analysis a procedure to address any incidental / adverse findings must be prepared and available.	The human genome will not be sequenced in any of ORION's pilot studies	Satisfactory reply to the genome analysis issue		**Nothing Pending - Closed**	N/A	N/A	N/A		

6.1.2.1.1.6 Publications and additional outputs

Publications

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Data interoperability in health surveillance: a literature review to support the development of One Health frameworks (SUBMITTED, Zenodo link not yet available)	yes		planned as gold access in the Journal of Biomedical Semantics - JBSM-D-20-00030
Stelzer S, Basso W, Benavides Silván J, Ortega-Morad LM, Maksimov P, Gethmann J, Conraths FJ, Schares G. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact, DOI: https://doi.org/10.1016/j.fawpar.2019.e00037 Zenodo : https://zenodo.org/record/3693772	yes		yes (1580 €)
Filter M, Buschhardt T, Dorea F, Lopez de Abechuco E, Gunther T, Sundermann EM, et al. One Health Surveillance Codex: promoting the adoption of One Health solutions within and across European countries. One Health. 2021;12:100233.https://zenodo.org/record/4709670	yes		Yes (3330€)
Houe, H., Nielsen, SS., Nielsen, LR., Ethelberg, S., Mølbak, K. (2020). Opportunities for Improved Disease Surveillance and Control by Use of Integrated Data on Animal and Human Health. *Frontiers in Veterinary Medicine,* 6: 301, pp. 1-8. DOI: https://doi.org/10.3389/fvets.2019.00301	yes		yes

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
https://zenodo.org/record/4269579#.YVxdaDh7mMo			
Joensen, KG., Kiil, K., Gantzhorn, MR.,Nauerby, B., Engberg, J., Holt, HM.,Nielsen, HL.,Petersen, AM., Kuhn, KG.,Sandø, G.,Ethelberg, S., Nielsen, EM. Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries (2020). Whole-Genome Sequencing to Detect Numerous *Campylobacter jejuni* Outbreaks and Match Patient Isolates to Sources, Denmark, 2015–2017. *Emerging Infectious Diseases*, 26(3), 523-532. DOI: https://dx.doi.org/10.3201/eid2603.190947	yes		yes
Jeevan Karloss Antony-Samy, Georgios Marselis, Eve Zeyl Fiskebeck, Taran Skjerdal, Camilla Sekse, Karin Lagesen (2021). Practical aspects of implementing the IRIDA system as a solution for One Health bioinformatics analyses. ARPHA Conference Abstracts, 2021 http://doi.org/10.3897/aca.4.e68913	Yes		Yes (no cost)
Joensen KG, Schjørring S, Gantzhorn MR , Vester CT, Nielsen HL , Engberg JH , Holt HM , Ethelberg S, Müller L , Sandø G , Nielsen EM. Whole genome sequencing data used for surveillance of **Campylobacter** infections: detection of a large continuous outbreak, Denmark, 2019. Eurosurveillance - Volume 26, Issue 22, 03 June 2021**.** doi: 10.2807/1560-7917.ES.2021.26.22.2001396	Yes		Yes

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Buschhardt T et al. A One Health glossary to support communication and information exchange between the human health, animal health and food safety sectors. DOI: https://doi.org/10.1016/j.onehlt.2021.100263 https://zenodo.org/record/4769914	yes		Yes (3330€)
Lopez de Abechuco E et al. One Health Consensus Report Annotation Checklist (OH-CRAC): a cross-sector checklist to support harmonized annotation of surveillance data in reports (SUBMITTED, Zenodo link not yet available)	yes		planned as gold access (3500€) in the Zoonoses and Public Health journal
Scaccia N, Günther T, Lopez de Abechuco E, Filter M (2021). The Glossaryfication Web Service: an automated glossary creation tool to support One Health community. Research Ideas and Outcomes 7: e70183. https://doi.org/10.3897/rio.7.e70183 https://zenodo.org/record/5176156#.YVwR2-c6-Mo	yes		Yes (535€)
Inspiration and ideas: One Health Integration in Surveillance. Technical University of Denmark. Report on OH Surveillance Initiatives https://www.food.dtu.dk/-/media/Institutter/Foedevareinstituttet/Publikationer/Pub-2019/Rapport-One-Health-Integration-in-Surveillance.ashx?la=da&hash=7E90900F125AF0EFAB8E6EEA415AAB82EF465CF3	yes		Yes (no cost)

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
DANMAP 2018, chapter 6. Resistance in zoonotic bacteria and animal pathogens. www.DANMAP.org https://www.food.dtu.dk/-/media/Institutter/Foedevareinstituttet/Publikationer/Pub-2019/Rapport-DANMAP-2018.ashx?la=da&hash=1E02D03F74A26651CF1B53BB280626DA5666A080	verbally		Freely available online
Foddai, Nao Takeuchi-Storm, Johanne Ellis-Iversen. Integrating Danish Campylobacter surveillance data streams to investigate broiler flock prevalence and potential cross-contaminations at slaughterhouse. **In preparation for submission to peer reviewed journal**	yes		Planned
Foddai, Maarten Nauta, and Johanne Ellis-Iversen. Risk-based control of Campylobacter spp broiler farms and slaughtered flocks to mitigate risk of human campylobacteriosis - A One Health approach. **In preparation for submission to peer reviewed journal-**	yes		Planned
Korsgaard, H. B., Ellis-Iversen, J., Hendriksen, R. S., Borck Høg, B., Ronco, T., Attauabi, M., Boel, J., Dalby, T., Hammerum, A. M., Hansen, F., Hasman, H., Henius, A. E., Hoffmann, S., Ilan, M. B., Kaya, H., Kjerulf, A., Kristensen, B., Kähler, J., Rhod Larsen, A., ... Laursen, M. (2020). DANMAP 2019 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Statens Serum Institut og Technical University of Denmark. WWW.DANMAP.ORG	yes		Freely available online (no cost)

Additional output

- OHS Codex – an online resource collecting various ORION resources, tools and lessons learned: https://oh-surveillance-codex.readthedocs.io/en/latest/index.html
- ORION Pilot reports – available via the public EJP project group ORION Knowledge Hub (and as attachments to corresponding the ORION deliverables)
- WBVR/RIVM: Surveillance of a disease/pathogen often includes several institutes and/or departments. Within the national ORION pilot study on Hepatitis E surveillance, a template was made, named "Country Map", to describe and visualize institutions, projects and data flows. This template consists of a MS Visio document and a Excel document. With the Country Map the existing but also currently missing links between institutes, data sources and data streams are visualized. The Country map can be found on Zenodo (https://doi.org/10.5281/zenodo.4767400)
- PHE/APHA: as part of the national ORION pilot project on Salmonella data sharing across the OH domain in the UK a "data sharing protocol template" and exemplar "case studies" detailing the approaches to and utility of real time One Health surveillance data sharing were developed and made available in the public EJP project group ORION Knowledge Hub
- The *Listeria* plugin created for IRIDA: https://github.com/NorwegianVeterinaryInstitute/irida-plugin-listeria-typing
- The ORION WP2-NGS handbook: https://oh-sfs-handbook.readthedocs.io/
- OHEJP Glossary and Glossaryfication Web Service: https://foodrisklabs.bfr.bund.de/ohejp-glossary/
- One Health Consensus Report Annotation Checklist (OH-CRAC) web service: https://aflex.vrac.iastate.edu/checklist/?t=OH-CRAC
- One Health Linked Data Toolbox (OH-LOD) – a set of prototypic web services that facilitates the semi-automatic mapping of concepts from the EFSA´s SSD catalogues into the new HSO, to convert Excel-based surveillance data into the data exchange format RDF by exploiting HSO and to make those RDF files accessible for semantic search technologies and services: https://foodrisklabs.bfr.bund.de/one-health-linked-data-toolbox/
- All available Swedish reports on "Surveillance of infectious diseases in animals and humans" (2006 to 2020) were published with unique identifiers in the Swedish data portal (www.dataportal.se) for open public information. For a full list of all individual URL's, please visit: http://datadrivensurveillance.org/campylobacter-surveillance-in-sweden/
- One dataset compiling all surveillance data available in the reports, including surveillance

methods annotated according to the OH-CRAC principles[5] developed in ORION (consensus report annotation checklist), was also published in the Swedish data portal in both human readable format (comma separated values, CSV) and machine readable format (RDF). The latter is explicitly annotated with machine readable surveillance concepts from the Health Surveillance Ontology. All permanent unique identifiers created are listed at: http://datadrivensurveillance.org/campylobacter-surveillance-in-sweden

- 'Surveillance inventory' - publicly accessible in a user-friendly, purposefully set-up, web application: https://shiny.fli.de/ife-apps/EJPOrion_WP2Epi/.
- Web-based database on tools and methods to plan and analyse surveillance systems: https://shiny.fli.de/ife-apps/toolsdatabase/

Webinars:

- Webinar: first version of ORION glossary. 6 November 2018. Recording available here: https://goo.gl/sUWj2c.
- Webinar WP1 OHEJP Glossary. 4 September 2019. Recording available here: https://svasweden.adobeconnect.com/pm7lzofjjogo/.
- ORION KnowledgeHub Webinar. 6 December 2019. Recording available here: https://svasweden.adobeconnect.com/p90575ar1eht/.
- ORION Cogwheel workshop. 5 October 2020. Recording available here: https://svasweden.adobeconnect.com/p1wuf07ym9pu/.
- The OHEJP Glossary - A Tool to Support Cross-sectoral and Transdisciplinary Communication in One Health Surveillance. One Health EJP Continuing Professional Development Module 2021: 15-19 February 2021, online workshop. https://bfr-elearning.de.
- The Glossaryfication web service: an automated glossary creation tool to support the One Health community. One Health EJP Continuing Professional Development Module 2021: 15-19 February 2021, online workshop. https://bfr-elearning.de.
- Linked Open Data. One Health EJP Continuing Professional Development Module 2021: 15-19 February 2021, online workshop. https://bfr-elearning.de.

Conference and poster presentations:

- T. Buschhardt, T. Guenther, F. Dorea, M. Filter .The One Health Surveillance Codex. A high level framework to facilitate efficient information exchange across One Health sectors. ICPMF11 conference (poster presentation): 17-20 September 2019. Braganza, Portugal. http://esa.ipb.pt/icpmf11/
- T. Buschhardt, T. Günther, F. Dorea, V.H.S Oliveira, V. De Waele, M.E. Filippitz, S. Stelzer, J.

[5] https://oh-surveillance-codex.readthedocs.io/en/latest/5-the-dissemination-principle.html

Gethmann, M. Torpdahl, T. Skjerdal, L. Larkin, S. Aabo, I. Boone, M. Filter. The ORION Glossary - An Essential Tool to Support Cross-sectoral and Transdisciplinary Communication in One Health Surveillance. ASM Scientific conference 2019 (oral presentation): 21-24 May 2019, Dublin.

- T. Buschhardt. Das One Health EJP Glossar - eine web-basierte Lösung zur Unterstützung der sektor-übergreifenden Kommunikation im „One Health" Bereich. Symposium „Zoonosen und Lebensmittelsicherheit (oral presentation). 4-5 November 2019, Berlin.

- E. Lopez de Abechuco (BfR): Introduction to ORION project. Cogwheel workshop, 28th April 2020. Link to presentation: https://onehealthejp.eu/wp-json/pm/v2/projects/128/files/20376/users/1051/download

- M. Filter, T. Buschhardt, T. Günther, E. Lopez de Abechuco, E. M. Sundermann, J. Ellis-Iversen, J. Gethmann, K. Lagesen, V. De Waele, G. Boseret, F. Dórea. The OH Surveillance (OHS) Codex - a high level framework supporting mutual understanding and information exchange between One Health sectors. OHEJP ASM 2020, 27th-29th May; Link to presentation: https://onehealthejp.eu/wp-json/pm/v2/projects/128/files/20372/users/1051/download

- E. Lopez de Abechuco, F. Dorea, T. Buschhardt, T. Günther, E. Sundermann, N. Scaccia, A. Foddai, M. Dispas, M. Umaer, M. Holmberg, J. Gethmann, M. Filter. One Health Consensus Report Annotation Checklist (OH-CRAC): a generic checklist to support harmonization of surveillance data reports. OHEJP ASM 2020, 27th-29th May (Oral presentation). Link to presentation: https://onehealthejp.eu/wp-json/pm/v2/projects/128/files/20371/users/1051/download

- N. Scaccia, T. Guenther, T. Buschhardt, L. Valentin, M. Filter. Text mining technology as added-value infrastructure to the One Health EJP Glossary. OHEJP ASM 2020, 27th-29th May (Poster). Link to poster: https://onehealthejp.eu/wp-json/pm/v2/projects/128/files/20373/users/1051/download

- T. Günther, E. Lopez de Abechuco, N. Scaccia. BfR internal colloquium (oral presentation): "Der "Glossaryfication" Webdienst - automatisierte Glossarerstellung zur Unterstützung des One Health Ansatzes". 20 October 2020, Berlin.

- N. Scaccia, E. Lopez de Abechuco, T. Günther, F. Dórea, M. Filter. 38th meeting of the Scientific Network for Zoonoses monitoring data, EFSA, (oral presentation). 19th – 20th October 2020. "Join Integrative Project ORION EFSA-ECDC supra-national pilot", online event. Link to presentation: https://onehealthejp.eu/wp-json/pm/v2/projects/88/files/20651/users/1898/download.

- E. Lopez de Abechuco, F. Dorea, T. Buschhardt, T. Günther, E. Sundermann, N. Scaccia, A. Foddai, M. Dispas, M. Umaer, M. Holmberg, J. Gethmann, M. Filter. One Health Consensus Report Annotation Checklist (OH-CRAC): a framework for harmonization of surveillance data reports. World One Health Congress (poster presentation): 30th October-3rd November 2020, online event.

- N. Scaccia, T. Guenther, T. Buschhardt, L. Valentin, M. Filter. The Glossaryfication web service: an automated glossary creation tool to support the One Health community. World One Health Congress (poster presentation). 30th October-3rd November 2020, online event.

- E. Lopez de Abechuco, N. Scaccia, T. Günther, M. Filter. The Glossaryfication Web Service – an automated glossary creation tool to support One Health communication. One Health EJP Annual Scientific Meeting (ASM) Satellite Workshop 2021. 7th June (Oral presentation). Link to abstract: https://aca.pensoft.net/article/68843/

- T. Günther, M. Filter, F. Dórea. Making Linked Data accessible for One Health Surveillance with the "One Health Linked Data Toolbox". One Health EJP Annual Scientific Meeting (ASM) Satellite Workshop 2021. 7th June (Oral presentation). Link to abstract: https://aca.pensoft.net/article/68821/

- J. Gethmann et al. Poster Presentations during the ASM Scientific conference 2020: The ORION project – OH knowledge base "surveillance systems" OHEJP ASM 2020, 27th-29th May (Poster).

- F. Dorea et al. Presentation in the Integrated Food Ontology Workshop (IFOW), part of the 11th International Conference on Biomedical Ontologies (ICBO 2020). Proceedings paper and video recording freely available in this link https://foodon.org/icbo-2020-food-workshop/ . Title: Health Surveillance Ontology: supporting semantic interoperability in One-Health.

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

- One Health Surveillance Codex: https://oh-surveillance-codex.readthedocs.io/en/latest/

- Ontology and Linked-Open-Data instructional and dissemination materials: http://datadrivensurveillance.org/ontology/

- A One Health glossary to support communication and information exchange between the human health, animal health and food safety sectors. DOI: https://doi.org/10.1016/j.onehlt.2021.100263

Are there any outcomes of this project that are already discussed or even implemented and in use at any institute of the project consortium, at stakeholders' organisations (ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), or at the level of national authorities?

All ORION project results were at least discussed in one of the involved national authorities, as all project partners used their national ORION pilots for that. The ORION solutions that were already integrated into operational work at national or international level are described in the One Health Impact section of this report.

6.1.2.1.1.7 One Health impact

The following ORION solutions created impact on the national and/or international level:

- The OHEJP Glossary has been integrated into the OHEJP Data Management Software, is used by the BfR to improve the own internal glossary and by Sciensano to establish a national OH glossary.

- In Norway, the first cross-sector IRIDA (https://www.irida.ca/) NGS data analysis platform could be implemented at the Norwegian Research and Education Cloud (https://www.nrec.no/) including a working pipeline for *L. monocytogenes*. As a result, there is an increased collaboration and information exchange between the veterinary and public health sectors in Norway.
- In Norway, ORION work promoted increased collaboration between NVI and the ARIES EU reference lab with regards to the development of pipelines, and also with EFSA regarding the harmonization of pipeline outputs. The project has also facilitated extensive collaboration with other IRIDA users, including the IRIDA developers themselves, and other users in South Africa and in the UK.
- In Denmark, the Danish Campylobacter pilot project has had a significant impact on the OH-approach for surveillance of Campylobacter. The Danish surveillance system has undergone real-time changes during the lifetime of this project, as new insights were gained as the project developed. The project showed that it is possible to detect clusters, outbreaks and link to sources based on comparison of sequences in an OH setting. During the project, the source of several outbreaks was found and control measurements implemented, not only at slaughterhouse level but also in the broiler production. The study resulted in national and international recognition of sequencing based *Campylobacter* surveillance being of high value, also compared to ”older” methods used. The project will be continued with the development of a platform for real-time sharing of data and surveillance. An OH working group was set up in connection with the project and the working group will continue after the project has ended. Results from this study has been presented nationally as well as at internationally and data used for annual reporting as well as published in a peer-reviewed journal for international dissemination. A seminar on control of *Campylobacter* in Broilers was set-up to improve knowledge sharing with industry, universities and agencies. Funding has also been granted for continuous use of WGS to examine the diversity and transmission within/between chicken flocks, farms, etc.
- In Denmark, improved integrated, OH reporting was implemented, e.g. in the *Salmonella* and *Campylobacter* chapter in DANMAP 2019, and the Danish national surveillance system for AMR and AMU (https://www.danmap.org/). The enhanced OH-focus contributed to a better understanding of the OH perspectives in AMR among all report users and encouraged OH policy actions.
- In Sweden, a OH-driven process has been developed and implemented for the annual reporting on surveillance of zoonotic diseases. The report is now produced in close collaboration with the public health-, animal health- and food safety sector. The food safety sector in Sweden was not a part of the ORION project but has been involved in the ORION work with the report. The new process is already implemented in the work with the annual surveillance report and is no longer dependent on resources from the ORION project to continue. The revised approach resulted in significantly improved understanding between OH sectors regarding surveillance activities and results.
- In Sweden, the revision of the surveillance reporting process helped to address bottlenecks in cross-sectoral interoperability and FAIRness of the surveillance output data. Innovative data interoperability solutions as well as the new instructions and guidelines to facilitate future reporting practices contributed to the improvements. All tools developed, as well as the workflows for their application, can be reused.
- In the Netherlands, the two Dutch ORION partners strengthened their collaboration regarding HEV surveillance. Several meetings took place, and first steps for joint analysis of human and veterinary

HEV data were made. Furthermore, both institutes evaluated their surveillance programmes leading to recommendations for improvement. The steps already taken will also help to build and strengthen contacts with other institutes in the field of hepatitis E.

- In Belgium, an interactive online tool has been developed that maps the main stakeholders in antimicrobial resistance (AMR) and consumption (AMU) covering the veterinarian, human, food and environment sectors in Belgium, as well as their activities on these topics. Activities helped to implement and promote One Health multi- and transdisciplinary approaches in the process of developing the national OH AMR report to increase the acceptability and feasibility of interventions. In parallel, the OHEJP Glossary was used to support the creation of a national OH glossary.
- In UK, a new Data Sharing Protocol was implemented between APHA and PHE that facilitates data sharing on *Salmonella* isolate/sequence and associated metadata to defined levels of granularity/ sensitivity as per the Data Sharing Protocol.
- In Germany, the OH-CRAC was presented to the German Federal Office of Consumer Protection and Food Safety (BVL) as a potential solution to further improve the interpretation of surveillance data on a national level. The OHEJP Glossary and the Glossaryfication Web Service was extended to include German glossaries relevant for German agencies (BAUA, BfR, BVL, EFSA, RKI, BfArM and Schülke glossaries)
- On international level, there were an extensive knowledge exchange with EFSA and ECDC during the WP1-WP3 supra-national pilot with 10+ web meetings leading to a better understanding on potential application scenarios for a number of ORION solutions. In addition, NVI collaborated with EFSA regarding the tool set for pipelines for WGS analysis of *L. monocytogenes.* Additionally, a number of ORION solutions were also integrated into the Surveillance and Information Sharing Operational Tool (SISOT) of WHO/FAO/OIE.
- Several ORION partners were able to synchronize with national or international projects outside OHEJP to create synergies, e.g. BfR (SafeConsume, SISOT team from WHO/FAO/OIE, COMBACTE-MAGNET EPI-Net network), NVI (IRIDA, SEQ-TECH), SVA (IRIDA, SIGMA, Vinnova, FORMAS), DTU (PAHO-WHO workshops, Fleming Fund Fellows in establishing integrated AMR surveillance in Africa and in requested consultancy to policy and scientists in South East Asia).

6.1.2.1.1.8 Data Management Plan

The ORION DMP has been uploaded to the EJP portal group ORION. It has been under final review by the DMP committee.

6.1.2.1.2 JIP02-R1-IA1.2-COHESIVE

6.1.2.1.2.1 Summary of the work carried out in the Project

Physical meetings are key to an integrative project such as COHESIVE. Unfortunately, this was impossible due to the COVID-19 epidemic. We continued the series of virtual meetings and workshops that were started in 2020. COHESIVE was granted an extension until December 2021 to finalise the delayed tasks.

For Task 2.1 the main goal is to develop guidelines for national One Health systems or other ways to strengthen human-veterinary-food collaborations, with the aim to improve signalling, risk assessment and response (risk analysis) to zoonoses. The framework of the web-based guideline is ready. All webpages are written in concept and will be checked in a review process. The website will eventually be linked to the MedVetNet association website to be able to maintain the guideline also after OHEJP ends.

Within Task 2.3 several pilots will be performed, in which Belgium, Portugal, Norway and Italy will go through the first steps of the implementation guidelines. After postponing the workshop on systems mapping (step 3 of the guidelines) several times in 2020, the workshops of Norway and Belgium are planned in September and October respectively.

The online decision support tool to help the user decide on the most appropriate method to use when tasked with conducting a risk assessment for a specific situation is available via http://cohesive.onehealthejp.eu/ and a scientific paper is published https://doi.org/10.1016/j.onehlt.2021.100266

For Task 3.3 the aim is to learn from past experiences with respect to zoonotic outbreaks. In this respect a systems analysis of publications relating to Q-fever in the UK has been performed and completed. A signalling pathway has also been produced showing the route for reporting Q-fever cases in the UK. Analysis and validation of EU-level Q-fever systems analysis with input from other countries is ongoing.

For Task 3.4 one aim is to facilitate low threshold sharing of interesting signals on zoonoses across multiple countries within the consortium. A highly successful low-level signals workshop was conducted to share some low-level threats and to establish a working group of experts for continued collaboration in the future. A spin-off workshop in collaboration with the OHEJP project IDEMBRU consortium was also conducted on Brucella canis attracting over 150 participants from a range of countries within and outside Europe.

For Task 3.5 the aim was to build on previous experiences of cost-benefit analysis (CBA) to produce a tool and/or method that is applicable to zoonotic pathogens and to make data comparable across countries. However, delays and resource issues due to COVID-19 have limited the scope of this task to a systematic literature search and collaborative data extraction. Write-up has started on the paper and is ongoing.

For WP 4.1 the aim is to develop a prototype information system at the national level allowing different databases to be interoperable. The feasibility studies in Italy and The Netherlands are finished, while in Norway it is still ongoing. Demo versions of the COHESIVE prototype information system (CIS) are published and available for the three involved member states.

With regard to WP 4.2, in 2021, the development of the tracing web portal - FoodChain-Lab Web – (FCL Web) advanced further. A new release of the software including a highlighting function as well as a performance optimisation became available in August 2021. In the second half of 2021, a prediction tool from EJP NOVA and Whole Genome Sequencing results will be integrated into FCL Web.

In WP 4.3 the development of the shiny app is complete. However, the deployment of the app on a suitable server and the writing of a manual are delayed. A security check must take place at the BfR

before deployment and is now in progress. Because of the delay in the deployment, the external validation of the program is also delayed.

In WP4.4 many dissemination activities for COHESIVE took place in 2021. WP4 tools were presented at the One Health EJP CPD module "Digital Innovation for One Health Practitioners", the Online Advanced Course on "Innovative tools and methods for ensuring seafood authenticity", the ASM Satellite Workshop "Online Software Fair" and at the One Health EJP Annual Scientific Meeting.

6.1.2.1.2.2 Work carried out in the JRP/JIP, scientific results and integrative outcomes

WP1: Coordination, communication and sustainability

JIP2-WP1-T1: Coordination

The progression of COHESIVE was hampered strongly by the COVID-19 pandemic. An extra extension has therefore been granted until December 31, 2021. The steering group is meeting online every 3 weeks. In these meetings all tasks are discussed shortly, including (possible) problems. Also, general tasks are discussed and decided upon, such as annual meetings and reports. There are regular video-conferences organised between the contact persons of EFSA and ECDC and the coordinator of COHESIVE. There are links with other OHEJP projects such as ORION, NOVA, IDEMBRU, BEONE and MATRIX. ORION, NOVA and COHESIVE have been working together at a glossary including terms used within the different projects, which has resulted in a publication (https://doi.org/10.1016/j.onehlt.2021.100263).

JIP2-WP1-T2: Communication/dissemination

The workshop during the IAFP European Symposium on Food Safety conference was postponed until 2021 and has now taken place. COHESIVE was presented, with focus on WP2.1. Several dissemination activities are in preparation, such as the end symposium. This will take place from November 8 until November 10 in Bilthoven, The Netherlands. Also, the workshop on systems thinking, originally planned as a satellite workshop before the ASM in Prague, is in preparation and will take place virtually on October 8. There are also regular meetings with project leaders from all joint integrative projects, led by the OHEJP WP4 leader. Other dissemination activities that did take place, related to the other work packages, are indicated below, such as publications and presentations during the ASM of OHEJP. Also, the relation with the Tripartite OIE, FAO and WHO will be extended, i.e. by being involved in the pilot of the SISOT tool in Romania. This is a possibility to disseminate products of COHESIVE but also other OHEJP projects.

WP2. Integrated risk-analysis at the national level

JIP2-WP2-T1: Development of guidelines for national One Health structures

The main goal of this task is the development of guidelines in order to support countries to set up/strengthen the collaboration between the human-vet-food domains with respect to signalling, risk assessment, response and control of zoonoses. The framework of the website is ready. All webpages are written in concept and will be checked in an internal review process within COHESIVE followed by an external review process. This includes guidance on 4 barriers, gaining political will, obtaining trust, sharing information and communication. Also an interactive tool has been developed to support the designing of the One Health Risk Analysis System (OHRAS). The progression has been extremely hampered by the COVID-19 pandemic. After the OHEJP ends, the guideline-website will be transferred to the MedVetNet Association (MVNA) and linked to their website, to secure long-term sustainability. There is regular contact with the FAO with respect to the Tripartite Zoonoses Guide (TZG) of OIE, WHO and FAO, specifically in relation to the SISOT work group, and now also contacts are made with the WHO in relation to the governance part of the TZG. We will be involved in the pilot of the SISOT tool organized by the Tripartite, giving the opportunity to promote the guidelines. The European pilot country will be Romania.

JIP2-WP2-T2: Development of structured decision making

Work on this task has now been completed. The tool has been finalised and the results have been written as a manuscript which has been accepted into the One Health journal (Rob Dewar, Christine Gavin, Catherine McCarthy, Rachel A. Taylor, Charlotte Cook, Robin R.L. Simons, A user-friendly decision support tool to assist one-health risk assessors (2021). *One Health*, 100266, ISSN 2352-7714, https://doi.org/10.1016/j.onehlt.2021.100266).

JIP2-WP2-T3: Knowledge transfer and dissemination

Different dissemination activities of WP2 have taken place during the OHEJP ASM; two oral presentations and a poster. Also, an oral presentation on the guidelines during the IAFP Conference was given. An article on the decision support tool was published (https://doi.org/10.1016/j.onehlt.2021.100266). An important element of this WP is the actual use of the guidelines developed in WP2.1. The guidelines focus on implementation and operationalisation. The idea is to have pilots in several European countries to start with implementing or strengthening the collaboration between the human-veterinary-food domain in the area of risk analysis of zoonoses. However, due to COVID-19 the progression, which was on scheme, was stopped abruptly. In the beginning of 2020, Belgium and Norway were ready for step 3 in the implementation process, a systems mapping workshop. The planned workshops were cancelled again in the beginning of 2021 and are now postponed until September and October, which is possible due to the extension. A successful physical workshop took place in Norway on September 9-10. It was crucial that the workshop took place because the experiences within the pilots including the workshop will provide important feedback to further improve the guidelines of WP2.1. Unfortunately, not much time will be left for the following steps, reducing the impact of these show cases for other countries. Possibly, MATRIX will be able to progress where COHESIVE stops.

WP3: Towards an EU zoonoses structure

JIP2-WP3-T1: Explore current ways for exchanging signals between countries and cross disciplines – pathway analysis

The task focuses on identifying factors that contribute to well-functioning systems or ways to share signals of zoonotic events within and between countries. The report (D-JIP2-3.3) has been finalised and submitted. Work on a manuscript (in addition to deliverable) is ongoing but delayed due to the task leader being involved in management of a HPAI-outbreak.

JIP2-WP3-T3: Retrospective systems analysis of detection of outbreaks

The aim of this task is to learn from past experiences with respect to zoonotic outbreaks. A systems analysis of publications relating to Q-fever in the UK is complete. A signalling pathway has also been produced showing the route for reporting Q-fever cases in the UK. Analysis and validation of EU-level Q-fever systems analysis is ongoing and is still open for feedback from COHESIVE partners. Work is progressing on writing up this deliverable (D-JIP2-3.5), but progress has been slow because of resource issues due to COVID-19. This deliverable is on track to be submitted by the revised deliverable date of October 2021. A systems analysis of publications relating to Q-fever in the UK is complete.

JIP2-WP3-T4: Pilot One-Health Early Warning System

A highly successful low-level signals workshop was conducted on February 3, 2021, to share experiences on some low-level threats in Europe and to establish a working group of experts for continued collaboration in the future. A spin-off workshop in collaboration with the OHEJP project IDEMBRU was conducted on May 18, 2021 on *Brucella canis* attracting over 150 participants from a range of countries within and outside Europe. Work continues on summarising the outputs of this workshops and establishing the clear data-gaps for continued international collaboration in this area. This deliverable (D-JIP2-3.6) is on track to meet the revised delivery date of November 2021.

JIP2-WP3-T5: Development of a tool for systematic cost-benefit analysis (CBA)

COVID-19 has significantly impacted the original scope of this deliverable (D-JIP2-3.8) due to both resource issues and complications working across institutes during lockdown. Nevertheless, APHA and AGES have been working together to produce a review of economic analyses in the area of foodborne

zoonoses. A systematic literature search has been conducted and collaborative data extraction has been conducted. Write-up has started on the paper and is ongoing. This review is on track to meet the revised deliverable date of November 2021.

WP4: Data platform to facilitate risk-analysis and outbreak control

JIP2-WP4-T1: Molecular typing data and metadata – database creation

The feasibility studies on Italy and The Netherlands are finished. Norway is still on delay. Delays are somehow related to COVID-19 *pandemic*.

Three prototypes information systems (cis-holland, cis-italy, cis-norway) are online and have been filled with information provided by the involved member states.

JIP2-WP4-T1-ST5: Filling of DBs

In the new T4.1 description, the sub-task title is "*Linking of the national databases with the COHESIVE prototype information system and the epidemiological analysis tools*".

- The activity of integration among the three information systems of the WP4 is started
  - In order to test interoperability between CIS and the two BfR softwares (FoodchainLab and RRisk), a demo version is available for BfR at address https://cohesive.izs.it/cis_bfr/
  - Covid crisis delayed this point
  - The new plan is to finish the activity after summer 2021.

JIP2-WP4-T2: Development of a platform-independent tracing framework

JIP2-WP4-T2-ST2

The overall status and progress of the whole FCL project can be inspected at https://foodrisklabs.bfr.bund.de/foodchain-lab. The specific status and new software versions of the FCL Web are deployed automatically to a test server where new features of the tool can be seen live. Since November 2020, FCL Web is in production mode which is accessible at https://fcl-portal.bfr.berlin.

In 2021, the development of the tracing web portal - FoodChain-Lab Web – (FCL Web) advanced further. A second release of the software including a function to highlight specific properties of companies using colors and shapes as well as a performance optimisation became available in August 2021.

JIP02-WP4-T2-ST3

Due to staff issues, this WP did not advance as expected in the first half on 2021 but work will be continued from July 2021 with the integration of a predition tool from EJP NOVA and the integration of Whole Genome Sequencing results. Hence, this subtask again needs to be prolongued until M47.

JIP2-WP4-T3: Development of a platform-independent risk modeling framework

JIP2-WP4-T3-ST2 Implementation

ST2 includes

a) the implementation of standards,
b) the GUI development and
c) the documentation and creation of the user manual.

The documentation and the creation of the user manual are in progress and will be completed close to the end of the project (M47). The other items have been completed.

JIP2-WP4-T3-ST3 Validation of the risk modelling framework

ST3 includes

a) test scenario creation,

b) program verification and validation (internal), and
c) external validation.

Items a) and b) have been processed. Item c) could not be processed because the implementation of the program on the BfR's own server could not be done yet. For this, the program must be security-checked. This is currently being conducted. Once the security check has been completed, the program can be deployed immediately. Delivery date is extended to M47.

JIP2-WP4-T3-ST4 Deployment of the risk modelling framework

The activation could not yet be carried out. Reasons for this are given in the previous subtask (JIP2-WP4-T3-ST3c). All preparatory work for the deployment is already being done. Delivery date is extended to M47.

JIP2-WP4-T4: Dissemination

In WP4.4 many dissemination activities for COHESIVE took place in 2021. WP4 tools were presented at the One Health EJP CPD module "Digital Innovation for One Health Practitioners" (https://onehealthejp.eu/cpd-2021/). In the framework of the OHEJP CPD module, FCL Web (WP4.2) was presented in an interactive virtual event (presentations, interactive live demo, interactive traceability exercise) and in an e-learning course developed in a project with EFSA. rrisk from WP4.3 was presented during the digital innovation sharing forum of the CPD module. In the framework of the Online Advanced Course on "Innovative tools and methods for ensuring seafood authenticity" (https://edu.iamz.ciheam.org/SeafoodAuthenticity/en/), FCL Web was introduced to the participants and an interactive live demo as well as an interactive traceability exercise using FCL Web was conducted. During the One Health EJP Annual Scientific Meeting Satellite Workshop "Online Software Fair" (https://onehealthejp.eu/asm-satellite-workshop-2021/) a pitch presentation and a dedicated session (presentation and interactive live demo) on FCL Web was conducted. Furthermore, FCL Web was shown as a poster during the 2021 One Health EJP Annual Scientific Meeting in an online format. The work done within COHESIVE is also highlighted in the final report of a project with EFSA which will be published soon. Results and impact of COHESIVE in general and particularly of WP4.2 (FCL Web) and WP4.3 (rrisk) were presented at the German OH EJP Mirror Group meeting for stakeholders like e.g. the German Federal Ministry of Food and Agriculture. In addition, the results and impact of COHESIVE were presented at the Swedish OH EJP Mirror Group meeting for national stakeholders.

In 2021, a joint interactive workshop on WP4 tools is envisioned as a satellite event for the COHESIVE final meeting which is open for the COHESIVE community and other interested users.

6.1.2.1.2.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
COHESIVE	D- JIP2-1.7	Closing symposium	M42		M47	An extension of 6M months has been granted. Therefore, this closing symposium will be postponed to month 47.	4,8
COHESIVE	D-JIP2-1.8	Annual report	M42		M48	An extension of 6M months has been granted. Therefore, this report will be postponed to month 48.	4,8
COHESIVE	D- JIP2-2.5	Development of common procedures and tools	M 42		M46	Delayed due to COVID-19.	8
COHESIVE	D- JIP2-2.6	Knowledge transfer and dissemination	M42		M48	Delayed due to COVID-19.	8
COHESIVE	D- JIP2-3.5	System analysis of detection of outbreaks	M42		M46	Delayed due to COVID-19.	4, 7, 8
COHESIVE	D- JIP2-3.6	Knowledge transfer and dissemination	M42		M47	Delayed due to COVID-19.	8
COHESIVE	D- JIP2-3.8	Tool for systematic cost-benefit analysis	M42		M47	Delayed due to COVID-19.	2, 8
COHESIVE	D- JIP2-4.1.4	Description of the pipelines implemented to feed the	M40		M47	Delayed due to COVID-19.	4

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
		analysis tools with the data stored in the three databases of this project					
COHESIVE	D- JIP2-4.2.3	Integration and analysis of epi-data from 4.1 → WGS-Epi module and selected features from IA-1, WP2, WP3	M42		M47	Some epi-data (case information, sample data) already integrated into FCL Web. However, for this deliverable i.e. the WGS data and the NOVA tool need to be integrated in FCL Web. Due to staff issues, the deliverable needs to be postponed to M47.	4, 8
COHESIVE	D- JIP2-4.3.2	Populating a model project model repository Harmonised graphical user interface prototypes Technical documentation and user guidance	M41		M47	Delayed due to COVID-19.	4, 8
COHESIVE	D- JIP2-4.3.3	Final testing design elaborated Report section about testing results (internal standards) Report section about testing results using external comparative models) Final release (open access)	M42		M47	Delayed due to COVID. Deploy program to a web server, this includes security concept for the web application for BfR IT. Including Documentation & Manual	4, 8

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
COHESIVE	D- JIP2-4.4.1	Each subtask conducts workshops, provides documentation and tutorials	M42		M48	Dissemination is ongoing and will be continued until the end of the project	4, 8

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities; 7. Prevention: aligned use of facilities and models; 8. Other (please specify); Co-creation of common procedures, tools and information to support One Health risk-analysis of zoonoses (national and international)*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
COHESIVE	M-AI2.COHESIVE.1.4	Closing symposium	42		M47	An extension of 6M months has been granted. Therefore, this closing symposium will be postponed to month 47.
COHESIVE	M-AI2.COHESIVE.1.5	Annual report	42		M48	An extension of 6M months has been granted. Therefore, this report will be postponed to month 48.
COHESIVE	M-AI2.COHESIVE.2.2	Development of common procedures and tools	40		M46	Delayed due to COVID-19.

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
COHESIVE	M-AI2.COHESIVE.2.3	Knowledge transfer and dissemination	42		M48	Delayed due to COVID-19.
COHESIVE	M-AI2.COHESIVE.3.3	Local dissemination	42		M48	Delayed due to COVID-19.
COHESIVE	M-A2.COHESIVE.4.9	Availability of the pipelines from the databases to the analysis tools	40		M47	Delayed due to COVID-19.
COHESIVE	M-A2. COHESIVE.4.10	Analysis of epi-data into tracing framework enclosed	40		M47	Some epi-data (case information, sample data) already integrated into FCL Web. However, for this milestone i.e. the WGS data and the NOVA tool need to be integrated in FCL Web. Due to staff issues, the milestone needs to be postponed to M47.
COHESIVE	M-A2. COHESIVE.4.11	Final release of risk modeling framework	40		M48	Delayed due to COVID the final release MUST include the manual and not only the program.

6.1.2.1.2.4 Follow-up of the recommendations and comments by the Ethics Advisors

N.a.

6.1.2.1.2.5 Publications and additional outputs

Publications

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Rob Dewar, Christine Gavin, Catherine McCarthy, Rachel A. Taylor, Charlotte Cook, Robin R.L. Simons, A user-friendly decision support tool to assist one-health risk assessors (2021). *One Health*, 100266, ISSN 2352-7714, https://doi.org/10.1016/j.onehlt.2021.100266 Zenodo: https://zenodo.org/record/5215753#.YR0iAI5KjIV Tool: http://cohesive.onehealthejp.eu/	Yes		Yes £1330
Buschhardt T, Günther T, Skjerdal T, Torpdahl M, Gethmann J, Filippitzi ME, Maassen C, Jore S, Ellis-Iversen J, Filter M; OHEJP Glossary Team. A one health glossary to support communication and information exchange between the human health, animal health and food safety sectors. One Health. 2021 May 8;13:100263. doi: 10.1016/j.onehlt.2021.100263. PMID: 34041347; PMCID: PMC8141924. A one health glossary to support communication and information exchange between the human health, animal health and food safety sectors | Zenodo	Yes		Yes. Processing charges covered by ORION.

Additional output

WP2 Presentations at the OHEJP annual meeting in Copenhagen 9-11 June, 2021
Oral presentation:
One Health: Setting up a risk analysis system for zoonoses
Sandra Cavaco Gonçalves, Zuzana Nordeng, Solveig Jore, Charlotte Cook, Rob Dewar, Carmen Marco Castillo, Elina Lahti, Karin Nyberg Malin Jonsson, Cecilia Wolff, Ed van Klink, Geraldine Boseret, Renata Karpiskova, Gaia Scavia, Rosangela Tozzoli, Tineke Kramer, Mathilde Uiterwijk, Frits Vlaanderen, Kitty Maassen on behalf of the WP2.1 team of COHESIVE

Oral presentation:
A user-friendly decision support tool to assist one-health risk assessors
Rob Dewar, Christine Gavin, Catherine McCarthy, Rachel A. Taylor, Charlotte Cook, Robin R.L. Simons

Poster presentation:
Building political will for One Health Risk Analysis System
GIK Mogami-Asselin, Claire Hilgenga, Ludovico Sepe, Zuzana Nordeng, Kaylee Myhre Errecaborde, Frits Vlaanderen, Mathilde Uiterwijk, Kitty Maassen

WP4 Presentations at the OHEJP annual meeting in Copenhagen
Oral presentation:
FoodChain-Lab Web: An integrative modular software to visualise and analyse complex global food supply chain networks during foodborne incidents. Gottschald M, Lewicki B, Falenski A, Rügen M, Gerber I, Fusiak J, Tölle D, Käsbohrer A, Weiser A (2021) ARPHA Conference Abstracts 4: e68835.
Presentation and interactive live demo in the framework of the One Health EJP Annual Scientific Meeting Satellite Workshop "Online Software Fair"
Abstract accessible via https://onehealthejp.eu/wp-content/uploads/2021/06/Abstract-Book-ASM-Satellite-Workshop.pdf; doi: 10.3897/aca.4.e68835

Poster presentation:
The FoodChain-Lab web application as integrative tool to trace food along complex global food supply chains in foodborne crises. Gottschald M, Lewicki B, Falenski A, Rügen M, Gerber I, Fusiak J, Tölle D, Käsbohrer A, Weiser A (2021).
Accessible via https://epostersonline.com/ohejp2021/node/487?view=true

Abstracts can be accessed from the proceedings of the 3nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. https://onehealthejp.eu/

WP2 Presentations at the IAFP 27-28 April 2021
Oral presentation:
Cohesive: Organizing Risk Analysis for (re-) Emerging Zoonoses: A One Health Approach
Sandra Cavaco Gonçalves, Zuzana Nordeng, Solveig Jore, Charlotte Cook, Rob Dewar, Carmen Marco Castillo, Elina Lahti, Karin Nyberg Malin Jonsson, Cecilia Wolff, Ed van Klink, Geraldine Boseret, Renata Karpiskova, Gaia Scavia, Rosangela Tozzoli, Tineke Kramer, Mathilde Uiterwijk, Frits Vlaanderen, Kitty Maassen on behalf of the WP2.1 team of COHESIVE

WP4 presentations at the One Health EJP CPD module "Digital Innovation for One Health Practitioners" (https://onehealthejp.eu/cpd-2021/).
Oral presentation:

FoodChain-Lab: an innovative tool to increase food safety through supply chain analyses. Gottschald M, Lewicki B, Falenski A, Rügen M, Gerber I, Fusiak J, Tölle D, Käsbohrer A, Weiser A (2021). Interactive virtual event (presentations, interactive live demo, interactive traceability exercise) and e-learning course (developed in a project with EFSA) on FCL Web

Oral presentation:

Rrisk: Presentation during the digital innovation sharing forum of the CPD module by Thomas Selhorst.

WP4 presentation at the Online Advanced Course on "Innovative tools and methods for ensuring seafood authenticity" (https://edu.iamz.ciheam.org/SeafoodAuthenticity/en/).

Oral presentation:

FoodChain-Lab: an innovative tool to increase food safety through supply chain analyses. Gottschald M, Lewicki B, Falenski A, Rügen M, Gerber I, Fusiak J, Tölle D, Käsbohrer A, Weiser A (2021). Interactive virtual event (presentations, interactive live demo, interactive traceability exercise) on FCL Web

Conference proceedings at IFOW 2021 Integrated Food Ontology Workshop, September 15-18, Bolzano, Italy

Oral presentation:

Refinement of the COHESIVE Information System towards a unified ontology of food terms for the public health organizations. Iolanda Mangone, Nicolas Radomski, Adriano Di Pasquale, Andrea Santurbano, Paolo Calistri, Cesare Cammà, Kitty Maassen

Other:

Infographics to promote the web-based guidelines to support countries in setting up/strengthening the One Health risk analysis of zoonoses. The infographics are made available via https://onehealthejp.eu/outcome-inventory
Results and impact of COHESIVE in general and particularly of WP4.2 (FCL Web) and WP4.3 (rrisk) were presented at the German OH EJP Mirror Group meeting for stakeholders like e.g. the German Federal Ministry of Food and Agriculture.

The work on FCL Web done within COHESIVE is also highlighted in the final report of the EFSA-BfR Framework Partnership Agreement which will be published soon.

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

In WP2.1 web-based guidelines are drafted to support countries in setting up/strengthening the One Health risk analysis of zoonoses. It is important to promote the existence of the guidelines and why it is important to have a One Health collaboration on risk analysis of zoonoses. Therefore, 3 infographics were made that can be used to promote the guidelines and create awareness. The infographics are made available via https://onehealthejp.eu/outcome-inventory and are free to be used by everyone. It can be useful for both national and international stakeholders, policy makers and professionals in risk analysis of (emerging) zoonoses & AMR.

In WP2.2 an online decision support tool to help users decide on the most appropriate method to use when tasked with conducting a risk assessment for a specific situation was developed and is available via http://cohesive.onehealthejp.eu/. The decision support tool also became part of the Surveillance & Information Sharing Operational Tool (SISOT) initiated by OIE, WHO and FAO in 2020.

FCL Web as a joint outcome of OHEJP COHESIVE WP4.2 and the EFSA-BfR Framework Partnership

Agreement will be interlinked with several tracing tools which serve various tracing purposes on the local, national and European level. It will be closely connected to future EFSA Rapid Outbreak Assessment production procedures. FCL Web also became part of the Surveillance & Information Sharing Operational Tool (SISOT) initiated by OIE, WHO and FAO in 2020.

6.1.2.1.2.6 Data Management Plan

The DMP of COHESIVE can be found on the OHEJP website in the DMP group. At the end of the project all data are fulfilling the FAIR principle. These include models of which also documentation and codes fulfill the FAIR principle. Currently, the DMP will be checked for missing information and action is taken to make sure the DMP is complete by the end of the project.

6.1.2.1.2.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

Regular video meetings take place between EFSA, ECDC and the coordinator of COHESIVE.

In WP2.1 there are regular meetings with FAO and WHO in relation to their TZG and our guideline to help one another with tools and experiences. Also, to align the initiatives and prevent overlap. Several COHESIVE tools are (to be) included in the SISOT toolbox from the Tripartite. The Tripartite will perform some pilots to assess the SISOT toolbox. We will be involved in the pilot in Europe, probably Romania.

Within WP2.1 an online glossary was developed in collaboration with ORION and NOVA, but it remains an ongoing activity to provide new terms. An article has been published. For WP2.1 there is contact with MATRIX to determine whether and how they can proceed with the guidelines on risk analysis of zoonoses within their project. Within WP3.4, together with IDEMBRU a successful workshop on Brucella canis was organised. Together with IDEMBRU, a scientific paper on this topic is being planned. Within task 3.1 there are links to a task within NOVA on early detection and notification of transmissible animal diseases. Within WP4.1.6 there is a complementary collaboration with ORION. In WP4.2 there is a close collaboration with EFSA with focus on tracing solutions in the framework of an EFSA-BfR Framework Partnership Agreement. There were also links to the meanwhile finalised EJP NOVA project in which a module for analysis of sales data was developed to be integrated into FCL Web. On the national level, FCL is involved in a project with the German federal state North-Rhine Westphalia on developing a data entry mask for supply chain data. Several modules of the tracing portal were developed in the framework of these projects and the aim of COHESIVE is to unify all of them under one umbrella – the FCL tracing portal. FCL Web is involved in the Heads of Food Safety Agencies Network on “Food chain analysis tools” and it is used by the U.S. Food and Drug Administration to visualise and analyse tracing data.

In WP4.1 there has been a close collaboration with ORION to integrate ontologies on the prototype COHESIVE Information system (CIS). A prototype has been developed. The project EJP BeOne is interested to work on top of this result. Activities are delayed due to COVID. Additional contacts are made with Matrix and Televir. The two EJP projects are evaluating to build their activities upon results from the Cohesive information system. Activities delayed due to COVID.

6.1.2.1.3 JIP03-R2-IA2.3-CARE

6.1.2.1.3.1 Summary of the work carried out in the Project

In the reporting period of year 4 (9M), CARE updated and adjusted the activities to meet the deliverables and milestones on several occasions. The adjustments were caused by more elaboration being needed to fully complete a task, tasks needed alignment to external activities and Covid-19 related delays. Thus, the task content, the agreed work plan and Gantt charts have been adjusted to allow a more detailed and realistic planning during the Interim meeting (D.0.5). In realization of the delays, CARE submitted the request for a half year with no cost extension. This will grant more time for preparing outputs and dissemination of the outputs, such as writing up scientific manuscripts and publishing the work completed.
The coordination still runs smoothly with the implemented quality control system of finalized deliverables. There are scheduled virtual meetings between the two lead organizations as well as among the WP leads every 14 days and all partners every quarter. Additional virtual meetings have been conducted within each of the WPs to ensure compliance to the work plan and discussion of risks and contingency planning. Some reporting fatigue has been observed in bi-monthly reporting of the work progress, but awareness and the importance of the compliance been highlighted. The two lead organizations have participated in all activities organized by the OHEJP WP4, thereby promoting and disseminating information about the OHEJP and related activities. Including the engagement in recruiting new partners as well as organizing and conducting the One Health EJP Thematic Integrative Meeting led by EJP CARE and EJP OH-HARMONY-CAP entitled «EU surveillance frameworks and infrastructures in a harmonized One Health perspective ».
All reports, documents, deliverables etc. are shared among the partners in the designated DTU share site. In addition, all deliverables are deposited in the OHEJP group site and public deliverables in Zenodo.

The overall aim of WP1 is to develop External Quality Assessment/ Proficiency Testing (EQA/PT) schemes that can be used to assess the combined performance of the OH sector, i.e., the human, food, and veterinary sectors. The work in WP1 has been focusing on planning and execution of the three pilot EQAs which eventually will provide basic knowledge for giving recommendations on future cross sectorial EQAs within the One Health sector. The first cross sectorial culture-based pilot EQA, W1-T2-ST1, is currently being executed. The aim of this pilot PT is to assess the ability of the OH-laboratories to detect and characterise *Campylobacter*, *Salmonella* and *Yersinia* in samples simulated to resemble the real samples analysed at food, veterinary and public health laboratories. The EQA materials were dispatched from Sweden the 12th of April 2021. The participants received five vials each containing a sample mimicking an environmental sample from an abattoir, a stool sample or a composite environmental sample from wild boars. In addition, another five vials containing freeze-dried bacteria of one to three of the target organisms were supplied. The laboratories prepared the samples by mixing the vials with the pathogens and the matrices and analysed the samples using their routine methods. The deadline for submitting results was May 31, 2021. The second pilot EQA, W1-T2-ST2, on typing/characterization based on WGS has been re-scheduled (consult the OHEJP Annual Work Plan for Y5) and are progressing in accordance with the new time plan. Closed genomes of reference strains have been established, the QC data have been accessed, and the reference material is being developed for dissemination to the participating laboratories. The third EQA on outbreak surveillance based on WGS data, W1-T2-ST3, has also been re-scheduled, but also progresses in accordance with the current time plan (previously provided). The data from outbreak and background strains have been selected and include outbreak clusters of *Listeria monocytogenes*, *Salmonella* and *Campylobacter* and work have been done to develop the reporting platform. For both the typing/ characterization PTs an appropriate number of laboratories have confirmed their interest in participating in both exercises.

Progress has also been observed in WP2 and WP3 strengthening the collaboration between the two

WPs. The inventory of available reference material was finalized based on the need assessment document (D.2.3) including more than 2500 reference material strains. In the reporting period, a number of ad hoc calls have been scheduled to discuss the process of filling in the gaps associated to metadata using Whole Genome Sequence providing the characteristics of AMR determinants among others as well as WGS QC metric data.

Currently, the inventory of identified reference materials is being converted into a database for which a web interphase is being developed to ensure easy accessibility and visibility of the reference material. In relation to accessibility and visibility of the reference material, a Massive Open Online Courses (MOOCs) is being developed to teach users how to operate the web interphase to query reference material. Agreements are among the EJP CARE partners to share the data and ensure the sustainability of the resources is still pending but believed to be in reach due to the efforts to clarify ownership etc. thought the several virtual talks about the Nagoya protocol e.g., webinars.

More than 50 individuals from various institutions provided feedback to the risk assessment survey on relevant (meta-)data in relation to quantitative microbial risk assessments conducted by WP4. Thus, feeding into the quantitative microbial risk assessment analysis on data originating from the last five years and provided by 19 EJP members or stakeholders including those from ORION and RADAR (D.4.1.2). The identified (meta-)data in relation to quantitative microbial risk assessments was shared with WP2 and WP3 and stakeholders to assist by increasing the accessibility of data but also to avoid duplication (D.4.1.3). Currently, efforts are made towards developing a User guide and R software suite package “rStain Select” to ease the process of selecting suitable reference strains based on exposure and risks. Thus, feed into the developed strategy by EU authorities to raise the awareness of relevant reference material microbial risk assessment analysis (D.4.2.2).

In summary, the CARE project progresses more or less as planned reaching milestones and deliverables in time and addresses additional assignments from the OHEJP WP4 in a timely manner.

6.1.2.1.3.2 Progress of the project: description of activities

WP0 Coordination

WP0-T1 Over-all project co*ordination*

DTU and SSI constitute the management team and have established a comprehensive management system to ensure that all deliverables and milestones for the vast of the majority are reached in the timeframe envisioned for the project and submitted using the designated OHEJP format. DTU are hosting the secretariat, which is essential for a successful completion of the proposed work programme. Furthermore, DTU has set up and is hosting a CARE share-site for storage of all reports, deliverables, milestones, minutes and other documentation produced as part of CARE.

In February, ANSES, DTU and SSI organized the CARE interim meeting (M.0.2). The meeting was held as a virtual meeting due to Covid-19 and this was facilitated by DTU. The interim meeting allowed all partners to be updated from the CARE management team, WP-lead and on the DMP plan. External stakeholders were invited, there were presentations of OHEJP integrative projects and the OHEJP management team gave an overview of OHEJP projects and presented links between them. There was allocated time for break-out sessions where the content, progress and adjustment to the work plans were discussed. The minutes of the kick-off meeting were reported and shared in the share site (D.0.5).

CARE status meetings for the management team (DTU and SSI) have been conducted every second week. Furthermore, DTU and SSI organized and conducted work package (WP) lead virtual meetings every 14 days to inform, discuss and delegate news and tasks from the OHEJP management as part of Task: JIP IA 2.1-WP0-T1. In addition, WP-leads reported on the work undertaken in each WP as to

progress, deliverables, delays etc. Minutes of all meetings have been reported and shared on the CARE share site. In continuation of the progress management, bi-monthly reports (internal progress reporting templates) (D.0.2) are requested from the WP-leads in which they report WP-progress, deliverables etc. These reports are also stored on the share site. The management team has enforced the WP-leads to provide high quality work and a review system has been implemented for this purpose, where WP1 review the work of WP3 and WP2 the work of WP4 and vice versa.

The management team has organized and conducted joint partner virtual meetings every quarter, primarily to allow the 16 partners to provide feedback to progress and accomplishments. The meetings also allowed for dissemination of information not shared already by email. Minutes of these meeting are shared and saved on the share site.

WP1 Develop of cross-sectional PT´s (proficiency testing)

The overall aim of WP1 is to develop EQA/ PT schemes that can be used to assess the combined performance of the OH sector, i.e. the human, food/feed, and veterinary sectors. The WP are divided into three tasks, a mapping exercise, which is finalized (WP1-T1 Mapping of existing and proposals for new PT schemes), a part where three pilot PTs a organized and documented (WP1-T2 Pilot trials and documentation of outcome), and a third part, WP1-T3 Development of guidance document with suggestions for design of future cross-sectorial PT schemes. This part of the WP has not started yet. The work conducted in the first 9 month of Y4 has been focusing on the planning and execution of pilot PTs:

- *WP1-T2-ST1 Pilot PT on isolation/detection and characterization of pathogens*
- *WP1-T2-ST2 Pilot PT on typing/characterization including WGS*
- *WP1-T2-ST3 Pilot PT on outbreak surveillance based on WGS data*

WP1-T2-ST1 Pilot PT on isolation/detection and characterization of pathogens

The aim of this pilot PT is to increase the understanding of the cross-sectorial capabilities to detect and characterize food-borne pathogens in a matrix relevant to the sectors of food safety, veterinary medicine and public health. The PT is currently being executed. The EQA materials were dispatched from Sweden the 12th of April 2021. The participating laboratories received five vials each containing a sample mimicking an environmental sample from an abattoir, a stool sample or a composite environmental sample from wild boars. In addition, another five vials containing freeze-dried bacteria of one to three of the target organisms, Salmonella, Campylobacter and Yersinia were supplied. The laboratories prepared the samples by mixing the vials with the pathogens and the matrices and analysed the samples. The laboratories were requested to detect and characterize the target pathogens to species level (Campylobacter), to serovar level (Salmonella) and to species and biotype level (Yersinia) according to the methods routinely used by each laboratory. The deadline for submitting results and was May 31, 2021, and the results of the PT is currently being analyses. The PT participants were primarily CARE partners supplemented with a few Swedish laboratories, and both the food and veterinary side and the public health side were well represents.

WP1-T2-ST2 Pilot PT on typing/characterization including WG

The second PT in WG1 aims at assessing the quality and comparability of WGS based on a set of WGS quality metrics and look into the laboratories ability to detect and assign ubtypes/attributes from WGS data from bacterial isolates e.g. sero- and sequence types, antimicrobial resistance genes, toxin subtypes etc. The aim is to access both the individual and the collective performance of the partner participating laboratories.

Work have been done on developing and documenting the reference material that shall be used in the PT, i.e. pure strains and FASTA sequence files from Salmonella, Campylobacter and Escherichia coli.

Participants will be requested to submit reads in FASTQ format to a FTP site and submit the identified sequence types, virulence and resistance genes, etc. present in the strains to an informatic IT module. The submitted reads will be evaluated and individual feedback will be provided to each of the participants. The PT will be launched in the second half of Year 4. The partners that provides EQA services will also be granted access to the informatic IT module that can be used to evaluate an compare assigned WGS quality metrics and typing characteristic, including nomenclature on different species. This will enable the EQA providers to evaluate and compare the performance of their own work streams, pipelines, and nomenclature with other partners working within the field of organizing genomic PTs.

An appropriate number of CARE partner laboratories have been recruited to the PT and these partners were summoned at a TC on April 8, where the ideas for the PT were discussed. At this meeting, the participants were also introduced to the IT module.

WP1-T2-ST3 Pilot PT on outbreak surveillance based on WGS data

The aim of this pilot is to develop proficiency testing schemes for the analysis of whole genome sequence (WGS) data to detect clusters as might be required during an outbreak response.

The pilot distribution will consist of WGS data files, in FASTQ format, from approximately 80 different isolates have been consolidated and consist of outbreak and background stains of Salmonella, Listeria monocytogenes, and Campylobacter. There have been establish and FTP site at SSI for file sharing and work have been conducted to develop a reporting platform for the PT. As the participants will be invited to analyse the data using their routine operational pipelines the reporting also includes information on the methodology used in the laboratories. An appropriate number of CARE partner laboratories have been recruited to the PT. Some of these will participate in the outbreak cluster analysis for a single, two, or all three species depending upon their laboratory specialism. The outbreak exercise is planned for September-October in Y4.

WP2 Creation of EUROpanelOH, a reference database of strains and genomes for effective quality control analysis in food safety and public health protection across sectors

WP2-T1 Inventory of current use and existence of reference materials across CARE / OHEJP partner institutes, for selected, prioritized pathogens and antimicrobial resistances

The first time period has developed a minimum set of descriptive standard information to be associated with individual biological resources among CARE partners. During the kick-off meeting, what bacterial pathogens to focus on in CARE was discussed to ensure feasibility. It was agreed to focus on foodborne bacterial pathogens to align with the scope of the partners official reference laboratory designations, thus, a list of priority pathogens was created. These attributes include qualitative characteristics and physical location of the biological resources as well as contact information of the supplier. An inquiry was made for feedback on the attributes selected by the task leaders. Some additional attributes have been suggested and included in the final excel template of the inventory of resources. Some attributes were considered mandatory information to consider the inclusion of the bacterial strains in the final database, such as the sector of isolation (human, animal, food, feed, and environment), year and country of isolation, while some were instead considered as informative. The template was sent to CARE partners on April 27th. The duly filled templates were expected before June 30th.

A total of 12 partner institutes contributed to the inventory of putative reference materials. Most of them registered more than single pathogens, at least 2 to 3 different pathogens (IP, DTU, SVA,

PIWET, IZSAM, IZSLER) to more than 4 to 8 different pathogens (ANSES, UCN, ISS). Finally, there were 2960 strains that have been considered as candidates for inclusion (D.2.1). Some reference materials were provided from OHEJP JRP projects, especially in the frame of Listadapt (for *Listeria monocytogenes*) and Tox detect (for *Bacillus cereus* and *Staphylococcus aureus*).

The database of resources (D.2.1) was analyzed for the content of information given by the partners. There were only eight strains from which no metadata are available due to confidentiality agreement. Such strains could be used as EQAS materials for dedicated purposes. The compliance with Nagoya protocol regulations has been fully addressed during the inventory timeframe. Countries of origin were unknown or not mentioned for 91 strains. That corresponds to less than 0.03% of the total number of inventoried strains. If the unknown geographical origin of some genetic resources may hamper the future distribution of the strains, it cannot prohibit the handling of the stateless material by CARE partners. Of course, the use and full characterization of such resources will not be favoured in the next phases. It is worth noting that access to the Spanish and French resources will necessitate authorizations from the two National Focal point services.

WGS data are already available for some strains and species. This is not enough however to create the sub-database for antimicrobial resistances as initially planned. Indeed, WGS data are available for 66% of *Listeria* strains, 43% of *Campylobacter* strains, 83% of *Bacillus* strains, 25% of *Salmonella* strains, 24% of *E. coli* strains and 15% of *Yersinia* strains. Only four strains were fully WGS characterized for *Staphylococcus* and none for *Vibrio* and *Streptococcus*. The AMR sub-database (D.2.2) is therefore postponed to December 2021. It will be set up as genome sequencing will progress by using a unique bioinformatics tool (ResFinder) for resistance gene detection. DTU has worked on updating ResFinder to enable and address the need for having a single standardized database that contains all validated AMR genes classified by mechanisms. That will help for full achievement.

WP2-T2 Gap analysis with respect to accessibility, quality and usefulness of existing and potential new reference materials from a One-Health perspective

The task 2 has begun by sending a letter to all CARE partners for calling experts of the target species who will be in charge to select the reference materials in the database of resources. CARE partners reacted swiftly enabling the completion of the need assessment document (D.2.3) intended for conducting the gap analysis. An updated list of strains with information of relevant gap of information identified was circulated to reference contacts for each partner institute, to inform about the strains suggested by the experts for inclusion in the final database and the characterization steps to be performed to fill relevant gaps. It is indeed one of the major purposes of CARE to establish a trustable link between the phenotypes and the genotypes of the strains at the final round of integration in EUROpanelOH. It is expected soon to reach the milestone stating a consensual list of identified gaps that will need to be filled in.

WP2-T3 Production of additional RM to fill in gaps and/or improve characterization if needed

This task will follow in due time when task being a prerequisite has been completed.

WP2-T3-ST1 WGS characterization

This task will follow in due time when task being a prerequisite has been completed.

WP2-T3-ST2 MALDI-TOF characterization

This task will follow in due time when task being a prerequisite has been completed.

WP2-T3-ST3 Completing metadata and phenotypic data

This task will follow in due time when task being a prerequisite has been completed.

WP3 Access and sustainability of well-defined microbial reference materials (RM)

WP3-T1 Development of an information system for making RM more widely accessible and visible

The past year has allowed the construction of the minimum dataset that must be associated with the living Reference Materials (RMs) of the CARE catalogue in order for them to be useful as biological standards and compliant with regulations (D.3.1.1). The work carried out between the partners has also allowed the definition of the principles for the construction of the Information System (IS) that will host the CARE's catalogue of RMs and allow their visibility and distribution (D.3.1.2). A prototype of the definition of the principles for the construction of the Information System (IS) (including database and website) that will host the EUROpanel-OH-CARE's catalogue of RMs and allow their visibility and distribution (D.3.1.2). The choice was made to build a relational database using the commercial Biolomics system (Bioaware), which is the standard chosen by the pan-European MIRRI consortium and appeared to be a realistic solution for having a functional system within the CARE project timeframe. The system will be built by INRAE teams, and a first operational version should be delivered in October 2021 (D.3.1.3). One person in charge of the development of the CARE's catalogue database and of the associated web portal has been recruited allowing to move the process forward and the structure of the catalogue database is yet well defined.

WP3-T2 Ensure the long-term sustainability of RM collections

The objectives of Task 2 to make the living collections of reference material sustainable have been the subject of extensive discussions among CARE partners. Several key points were raised, notably on the important role that official MicroBiological Resource Centers (mBRCs INRAE-CIRM, Institut Pasteur-CRBIP joined by the BVR biobank of IZSLER) operated by CARE partners will play to achieve this objective, ensuring the long-term conservation and distribution of RMs following best practices. The discussions among partners and the organisation by the coordination of a webinar (7th of Dec 2020) dedicated to the Nagoya protocol stressed the importance that RMs must be compliant with the ABS rules. The definition of realistic solutions will still have to be discussed next year with the partners, involving their stakeholders (ministries) who are most often the official owners of the biological resources. On the other hand, the choices that will be made on the physical sites for the conservation of the collections will have important consequences on the architecture of the information system, particularly with regard to functionalities related to data curation, data updating and ordering/distribution of reference materials. This is therefore an important issue to be discussed at the level of project coordination. Partners have been encouraged to enrol in a MOOC on biobanking (June-July 2021) deployed by Institut Pasteur to ensure a common knowledge about operation of culture collections among us.

WP3-T3 Ensuring the long-term accessibility of the RM collection and its existence

A number of agreements will have to be settled between partners in the months to come to ensure the long-term sustainability and accessibility of the EUROpanelOH catalogue:

- Finalizing and signing RMs deposit contract between RM providers and BRCs
- General agreement on RMs exchange (cost of strains for CARE partners and external user of the collection)
- Financial agreement to sustain the maintenance of the EURO-panelOH collection (cost of conservation, Biolomics licences fees).

At the European level, discussions have been initiated by INRAE and IP with the MIRRI (Microbial Research Infrastructure) consortium for exposition of the CARE catalogue through the MIRRI web portal. This option should promote the OHEJP CARE project and give a good visibility to the RM catalogue.

WP4 Investigate, benchmark and improve the availability and the quality of the existing data relevant for risk assessment

WP4-T1 Data and metadata available for risk assessment

WP4 CARE members first established the list of targeted institutes to whom the survey was addressed. This list comprised OHEJP members as well as members from EFSA Microbiological Risk Assessment Network. A small literature review was done to establish criteria to assess the data quality and accessibility in the field of risk assessment.

A survey (D.4.1.1) was intended for the OHEJP members and broader from September 2020 to March 2021 to collect information about existing QMRAs from the last 5 years. The questionnaire was set up on Sphinx online software and was organized into 31 main questions divided into 3 sections.
(https://survey.anses.fr/SurveyServer/s/formation7/questionnaire_RA/questionnaire.htm)

The survey has been fully analysed (D.4.1.3). It revealed that the main hazards assessed were pathogenic *E. coli, Listeria monocytogenes* and *Salmonella spp*. While the starting point of the QMRAs study is diverse, the endpoint is mostly at the consumer stage. Three types of data sources have been described: data produced by the risk assessor organisation, data from literature and data from a scientific network. Part of this data is available but not recorded on a dedicated platform.

In the perspective to establish the roadmap for sharing information for pathogens and AMR useful for exposure/risk assessment, a contact was established with other initiatives. CARE's objective has been presented to the JIP ORION, JRP RADAR, Pathogens-in-foods database and RAKIP projects. Information was collected from these projects related to best practices to improve accessibility and quality assessment of data relevant for QMRA.
A meeting is planned with stakeholders (EFSA and ECDC) to present WP4 CARE project and more particularly the results of the survey and the views shared with other projects on data sharing.

WP4-T2 Metadata web platform

The resources allocated by DTU partner could not be committed in 2021. The other partners of this task propose to carry out the work related to the user guide describing how to access the data available (D.4.2.1) through the extension of the deadlines.

WP4-T3 Dissemination plan

No specific tasks have been carried out related to task 3 during Y4.

6.1.2.1.3.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
CARE	D.0.5	Minutes of the interim meeting - 2021	M38	M38	-	Public	1 (Adm)
CARE	D.2.3	A need assessment document stating what RM we should have	M40	M40	-	Public	2
	D.4.1.2	The establishment of connection of this CARE activity with the databases and initiatives already in place	M45		M47	Confidential The deliverable will be the minutes of the meeting with stakeholders	8
	D.4.1.3	Report on analyzed survey data regarding the quality and accessibility	M42	M43	-	Public The report is fully terminated at the end of M42.	5

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
	D.4.2.1	User Guide for accessing relevant data for risk-assessment	M54			Public The resources allocated by DTU partner could not be committed in Y4 and Y5. The development of D.4.2.3 (web-platform) is cancelled for that reason. The other partners of this task propose to carry out the Y4 work related to the user guide describing accessing relevant data for risk-assessment (D.4.2.1) in Y5.	3
	D.4.2.2	Implementation of developed strategy to raise awareness by EU authorities	M54			Public The resources allocated by DTU partner could not be committed in Y4 and Y5. The development of D.4.2.3 (web-platform) is cancelled for that reason. The other partners of this task propose to carry out the Y4 work related to the user guide describing accessing relevant data for risk-assessment (D.4.2.1) in Y5.	2

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
CARE	M.0.2	Interim meeting 2021	M37	Yes		Held in M38 (3-4 February 2021)
CARE	M.2.2	Consensual list of identified gaps	M45	Yes		Each partner has received info about his list of proposed strains, some contacts still ongoing
CARE	M.3.2.1	Training session on microbial collection management	M52			MOOC (https://www.fun-mooc.fr/fr/cours/biobanking/)
CARE	M.3.2.1	Training session done	M52			
CARE	M.4.1.5	Letter to take contact with JIP/EJP project	M37	Yes		Partially reached (some project still to be contacted)
CARE	M.4.1.6	TC of the different partners of this project to check for synergy and to avoid duplication of the work	M45	Yes		All the identified project leaders have been contacted to present the CARE WP4's objectives and to get their outputs on data sharing
CARE	M.4.2	Data typology and description methodology	M38	No		This milestone is no longer relevant as WP 4.2 objectives have been revised
CARE	M.4.2	Data gaps and accessibility analysis	M40	No		This milestone is no longer relevant as WP 4.2 objectives have been revised

CARE	M.4.2	Inventory of existing data visualization tools	M45	No		This milestone is no longer relevant as WP 4.2 objectives have been revised

6.1.2.1.3.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethics Advisors already accepted your comments. Therefore, this part of the report can be closed.

6.1.2.1.3.5 Publications and additional outputs

N/A

Additional output

- Presentation on the One Health EJP- Online welcome meeting March 3rd, 2021. Cross-sectoral framework for quality Assurance Resources for countries in the European Union (CARE) Proficiency testing and Reference Material. M. Torpdahl.
- Presentation on the One Health EJP CPD Module 2021 Feb. 2021 "rStrainSelect: an R-tool for sampling strains based on metadata in a One Health context Laurent Guillier, French Agency for Food, Environmental and Occupational Health & Safety (ANSES)"
- Posters prepared and accepted for presentation at the OH_EJP ASM 2021:
  - "EJP-OH-CARE: Pilot studies to support guidance and suggestions for cross-sectoral One Health proficiency testing schemes". Authors: Jeppe Boel, Nick Coldham, Sally Hallam, Paula Johnson, Pernille Gymoese, Mia Torpdahl, Elina Lahti, Cecilia Jernberg, Rene Hendriksen, Kees Weldman, Mike Brouwer. Poster at the OHEJP ASM meeting in Copenhagen, Denmark, June 9-11, 2021
  - "Cross sectoral quality assurance systems, an inventory". Authors: Lucas Wijnands and Kirsten Mooijman. Poster at the OHEJP ASM meeting in Copenhagen, Denmark, June 9-11, 2021
  - "Identification of data currently used in quantitative microbial risk assessment". Authors: Juliana De Oliveira Mota, Henk Aarts, Laurent Guillier, Virginie Desvignes. Poster at the OHEJP ASM meeting in Copenhagen, Denmark, June 9-11, 2021
- The presentations of the CARE annual meeting have not been made publically available - only the minutes from the meetings are available via the OHEJP website: https://onehealthejp.eu/jip-care/

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

No papers published yet.

6.1.2.1.3.6 Data Management Plan

A Data Management Plan (DMP) is a key element and the list of data covered in CARE has been further elaborated during the first half of the second year of the project. It covers data from collection to availability and long-term storage. DMP is a living document during the lifetime of the project. Also, the management of the data is discussed proactively and the DMP is regularly discussed with the WP-leaders and the CARE consortium during the project.
The CARE DMP was compiled using the specific tool provided by the OHEJP: Collaborative Data Platform (CDP). The CARE Deputy Leader from SSI is responsible for the CARE DMP and thereby for adding contributions from all participants. Feedback from the OHEJP management team has been received in the end of 2020 and based on this further input from participants has been collected.

The DMP template was filled in and name and email addresses of those representing the datasets included. All datasets generated and foreseen have been collected and will be further updated during the project period if more datasets are identified. The current datasets have been added to the CARE DMP under unique identifiers using the OHEJP developed CDP-tool, and all fulfil the FAIR principles.

6.1.2.1.3.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

Yves Pascal D. VAN DER STEDE (EFSA), Marius Linkevicius (ECDC) and Daniel Palm (ECDC) are all kept in the loop in relation to communication that goes out from EJP CARE, i.e., are informed of activities and also invited for any relevant meetings.

EJP CARE has good communication with the EJP Matrix and the EJP OH-HARMONY-CAP projects and intends to plan and execute the closing meeting in collaboration with these projects.

6.1.2.1.4 JIP04-R2-IA2.2-OH-HARMONY CAP

6.1.2.1.4.1 Summary of the work carried out in the Project

OH-Harmony-CAP (One Health Harmonisation of Protocols for the Detection of Foodborne Pathogens and AMR Determinants) is a 2.5-year project that aims to: 1. Collect information on current capabilities, capacities, interoperability, and adaptability at both the National Reference Laboratory (NRL) and the primary diagnostic level, across Europe by developing an in-depth OHLabCap survey. 2. The quantitative description of current and best practices and the development of harmonised protocols will identify and possibly close the gaps and suggest future studies on how to best detect and characterise food borne pathogens across the One Health sectors

The second year (Y4) of the OH-Harmony-Cap project began with an online consortium meeting January 13th2021 and this summary will include an updated work plan, an updated Annual Work Plan, an overview of the various outcomes and agreements going forward, such as what was decided on the Y4 kick-off-meeting (KOM):

- We have applied for a no-cost project extension so the project will terminate M60. The OH-Harmony-Cap participants all agreed on this particularly, because it will be a challenge to arrange practical workshops, as planned in WP5, here in Y4
- We have welcomed three new partners so that the OH-Harmony-Cap consortium presently comprises 18 partner institutes across 14 countries
- Several recommendations were put forward after the WP2 pilot survey; 1) parasitologists addressed the need to reduce the targeted parasites from 10 to five 2) the adjusted survey need to include an indicator and associated targets on adaptability i.e. the capacity and ability to adjust preparedness, methodology and organisation of each laboratory. As such, we included adaptability in the adjusted survey 3) the survey itself was routed in several ways
- The 59 question OHLabCap was launched in May 2021 by distributing it via several networks and we asked the participants to activate their network
- In Y3, we identified a gap regarding sampling and analyses that are performed on behalf of our food business operators HACCP-based self-control programmes. Therefore, we have included an additional deliverable, which will survey this sector. This new task, JIP4-WP2-T5 is led by 39-SLV. We have developed and launched an anonymous nine question survey using the EUSurvey tool. Participants will distribute this survey in their own country. The deliverable (will be a report in M50).
- In Y3, surveys of relevant laboratories were designed to collate key information on current characterisation practices. The relevant data is currently being analysed to inform the second and third summary report on characterisation and data management
- The evaluation of the collected laboratory protocols is concluded, and the selection of harmonised protocols is ongoing. Experts will decide on the criteria, which will be used for ranking of the protocols. The ranking activity is based on the evaluation tables deployed in JIP4-WP4-T2 and on the original protocols collected.
- The ranked protocols will be sent to the ERLs for comments and suggestions.

Note: We have made necessary changes to the work plan due to difficulties holding practical workshops, as described in WP5. Furthermore, we have postponed our physical full consortium meeting in Dublin, Ireland to November 2021.

6.1.2.1.4.2 Progress of the project: description of activities

WP1 Project coordination

JIP04-WP1-T1: Internal coordination

Progress: As part of the internal engagement, it has been important to effectively communicate the progress and status of the project to the OH-Harmony-Cap participants. At the online Y4 KOM, we communicated a common understanding of the roles and responsibilities, output specifics and expectation.

- Several emails to all participants with project overview, status, and decisions
- *Frequent conference meetings between the project leaders of WP2, WP3, WP4, and WP5*
- *Three conference calls with OH-Harmony-Cap project group (WP leads and deputy leads). Including a welcome online meeting with new partners on May 27th*
- *Y4 KOM with the OH-Harmony-Cap consortium on January 13th*

The Three new partners; The Institute of Health Carlos III (ISCIII), Spain, The Institute of Food Safety, Animal Health and Environment (BIOR), Latvia, and The Finnish Food Authority (RUOKA), Finland, joined the OH-Harmony-Cap in May 2021. At the OH-Harmony-Cap welcoming meeting (May 27th), the WP leaders presented their work packages and identified tasks where BIOR, ISCIII, and RUOKA could contribute. Following the meeting BIOR, ISCIII, and RUOKA were asked to fill in a document listing all the tasks. Consequently, all OH-Harmony-Cap email lists and budgets have been updated to include the new partners.

Note: We have moved our planned consortium meeting in Dublin, Ireland, from September 2021 to November 9-11th to increase the chance of travelling.

JIP04-WP1-T2 : External coordination, outreach and engagement

Progress: As part of the external engagement and to, effectively, find synergies with other projects, disseminate our project, progress and results, OH-Harmony-Cap has participated and presented

- Arranged and participated in a meeting on possible synergies between the FWD AMR-RefLabCap

  (Food- and Waterborne Diseases Antimicrobial Resistance - Reference Laboratory Capacity) project and OH-Harmony-CAP. January 10th, 2021
- Participated in 1st DiSCoVer Stakeholder Web-meeting. January 22nd, 2021
- Presented at OHEJP Welcome meeting to integrate new members / preparatory TC with the OHEJP Coordination team, WP4 and JIPs leaders. February 8th, 2021
- Update meeting between CARE and OH-Harmony-Cap. February 11th, 2021
- Participated in DiSCover STEC meeting. March 2nd, 2021
- Participated in DiSCoVer WP2-T4 meeting. March 16th, 2021
- Update and overlap meeting between MATRIX (Viviane Henaux) and OH-Harmony-Cap. April 21st, 2021
- Presented at the One Health EJP internal meeting at Statens Serum Institut (SSI) (Joint meeting between all the EJP projects that SSI is involved in). May 4th
- Hosted and presented, together with CARE, at the One Health EJP Thematic Integrative Meeting (TIM) "EU surveillance frameworks and infrastructures in a harmonized One Health perspective". May 21st. Note, The report is published in D4.21"
- Oral presentation (Flemming Scheutz) at One Health EJP Annual Scientific Meeting 2021. June 10th

- Poster presentation (WP4, Rosangela Tozzoli, and Melanie Gay) at One Health EJP Annual Scientific Meeting 2021
- Presented and participated in the panel discussion at the One Health Session for EPIET and EUPHEM, August 25th
- Stayed connected with key stakeholders; by inviting them to the TIM panel discussion and to comment on surveys and questionnaires
- Reached out to Rachel Chalmers, Head, Cryptosporidium Reference Unit, Public Health Wales for distribution, and input, of the OHLabCap
- Collaborated with other OHEJP projects, including TOXOSOURCES, MATRIX, DisCoVer, and CARE
- Participation in other OHEJP teleconferences on February 8th, March 3rd, June 8th

JIP04-WP1-T3 : Data management

Progress: The project leader participated in the Data Management Platform (CDP) training on July 29th 2020. The list of data covered in OH-Harmony-Cap DMP was specified during the first year of the project. DMP is a living document during the lifetime of the project. The first DMP version (D-IA.2.2.OH-Harmony-Cap.1.1) included four research datasets. The DMP will be updated again before the end of June.

The final DMP will be published at the end of the project lifetime.

JIP04-WP1-T4: Sustainability

Progress: At this stage, sustainability activities are mainly entailed to external outreach and detailed in JIP4-WP1-T2. However, considering the proximity of the three Integrative Projects CARE (DTU Food, Denmark), OH-HARMONY-CAP (SSI, Denmark) and MATRIX (SSI, Denmark) we have initiated a collaboration to ensure a joint sustainability strategy.

Overall, these activities will be on the agenda at our second Y4 meeting, which is hosted by Teagasc, in Dublin, Ireland.

WP2 Development of the OHLabCap

WP months: M25-M54. WP Leader: 13-SSI. Deputy WP Leader: 30-RIVM
WP2 participants: All OH-Harmony-Cap participants
The aim of WP2 is to develop a benchmarking instrument "OHLabCap", by surveying OneHealth laboratory interoperability, capacity and capability across EU. The focus will be on the detection and typing of selected priority foodborne pathogens, AMR determinants, and priority parasites across each sector (AH, PH, and FS). The main deliverable will result in the establishment of an OHLabCap instrument that is generic, adjustable and sustainable at both an EU and National level.

JIP04-WP2-T1: Development and testing of the pilot survey

Task month: M25-M30

Task Leader: 13-SSI. Deputy Task Leader: 30-RIVM

Task Participants: All OH-Harmony-Cap participants

Description of task: The development of a pilot survey, targeting NRLs, was expanded to include the primary diagnostic services (primary sector). This pilot survey, which will later become the OHLabCap, was drafted covering; [1] capability, [2] capacity, and [3] interoperability (JIP04-WP2-T2-S1, S3). The pilot survey covered six priority bacteria, ten priority parasites, and antimicrobial resistance (AMR)

testing of *Salmonella* and *Campylobacter*. The pilot survey was circulated among the OH-Harmony-Cap consortium.

Progress: Concluded. Deliverable, D-IA.2.2.OH-Harmony-Cap.2.1.

JIP04-WP2-T2: Scoring of Collected Data and Chosen Indicators

Task month: M31-M38
Task Leader: 32-FHI. Deputy Task Leader: 30-RIVM
Task Participants: All OH-Harmony-Cap participants

Description of task: The result of the pilot survey was analysed and recommendations for the adjusted survey (which will become the OHLabCap) was derived and described in D-IA.2.2.OH-Harmony-Cap.2.2.
Progress: Concluded. Deliverable, D-IA.2.2.OH-Harmony-Cap.2.2.

JIP04-WP2-T3: Development and testing of an adjusted survey

Task month: M37-M42
Task Leader: 13-SSI. Deputy Task Leader: 30-RIVM
Task Participants: All OH-Harmony-Cap participants
Task description: The outcome of JIP04-WP2-T2 will generate several recommendations, which will be incorporated into the pilot survey in order to develop the OHLabCap.
Progress: Several modifications to the pilot survey was undertaken and incorporated into the questionnaire which will become the OHLabCap:

- Continue with the EUSurvey tool
- The number of target parasites was reduced from 10 to five: *Cryptosporidium* spp., *Echinococcus granulosus (sensu lato)*, *Echinococcus multilocularis*, *Toxoplasma gondii* and *Trichinella* spp.
- Questions targeting laboratory adaptability have been included
- The survey was routed on several levels *i.e.,* organism, laboratory functionalityand methods
- The classification and scoring of indicators, measuring capacity, capability, interoperability, adaptability, and communication has been clarified
- The "NA" option has been added to several questions
- The final revised tool is now able to target score distribution by discipline (human, animals, food/feed) and dimension scores (human, animal and food/feed) by country (maps)

After multiple reviews and pilot tests, the OHLabCap was distributed on May 7th with a July 31st deadline. The survey link was accompanied by a letter of invitation and a "how to save the survey document". To ensure an optimal response, the OHLabCap was distributed via the OH-Harmony-Cap consortium, Cryptosporidium Reference Unit, FWD-network, OHEJP JIPs/JRPs and the Scientific Steering Board, EURL-VTEC, EURL-Listeria, EURL-Campylobacter, and EURL-Salmonella. Concurrently, OH-Harmony-Cap recipients were also asked to help with the distributions and circulation of the OHLabCap, particular outreach to in-country primary laboratories. Identifying laboratory contact points have been a challenge and, therefore, the distribution is ongoing. As of September 3rd, there have been 122 responses.

JIP04-WP2-T4: Scoring of collected data and chosen indicators

Task month: M43-M54 (ongoing)
Task Leader: 13-SSI. Deputy Task Leader: 30-RIVM
Task Participants: All OH-Harmony-Cap participants
Description of the task: JIP04-WP2-T4 will evaluate, assess, and score the collected data from the OHLabCap top repare the One Health map of the levels of system

capability/capacity/interoperability/adaptability for the National reference and primary diagnostic laboratories in each of the EU/EEA countries and dissemination.
The overall outcome will be an integrated One Health map of the levels of system capability/capacity/interoperability/adaptability for each of the chosen organisms for the NRLs and primary diagnostic laboratories in the EU/EEA countries. This tool will not include an evaluation of surveillance capacities and capabilities (EUEpiCap), which is included in MATRIX. However, collaboration is planned, including a joint workshop between OH-Harmony-Cap and MATRIX if both projects are funded (WP5-T3).
Progress: 32-FHI and 13-SSI have started the analysis and we expect a draft report M48. If needed, and for any clarifications, we will follow up with the participating laboratories.

JIP04-WP2-T5: Development of a survey targeting the food sectors self-control

Task month: M37-M50 (ongoing)
WP Leader: 39-SLV. Deputy WP Leader: 13-SSI
Task participants: 1-ANSES, 9-BfR, 13-SSI, 33-NVI, 35-INIAV, 30-RIVM, 44-BIOR, 45-Ruoka, 43-ISCIII
Task description: The primary aim of this task is to provide an overview of the sampling and analyses that are performed on behalf of our food business operators' HACCP-based self-control programmes. Due to the lack of knowledge and overview of how many samples that are taken and processed by the food business operators, the project is undertaking a survey targeting food business operators' HACCP-based self-control programmes. This mini survey is – to our knowledge – the first of its kind. It is expected to give a first glimpse of which food categories that are examined, the number of positive samples for each of the pathogens, and will provide information on accreditation, participation in EQA programmes and storage and/or sharing of positive samples. This is an important data collection exercise to help describe the One Health microbiology system by surveying food laboratories across all EU/EEA countries. We will use this information to identify gaps and needs necessary to develop and implement harmonised and compatible protocols for the detection and typing of foodborne pathogens across One Health. It is our hope that this initiative will encourage further communication between private food business operators and One Health authorities. In the letter of invitation, it was clearly stated that participation in the survey is anonymous. Additionally, the gathered information will be; 1) included in a technical report, and 2) the results of the survey will be open source data and will be published on https://onehealthejp.eu/jip-oh-harmony-cap/, and 3) data will not be used for commercial purposes.

Note: This is an additional task not described in the original work plan. However, it is described in the DMP. As part of WP2, participants from the Swedish Food Agency (39-SLV) suggested that we perform an anonymous survey in Y4 specifically targeting the primary laboratories in the food sector. SLV informed the OH-Harmony-Cap consortium by email on December 17th, 2020 and this task was finalised on the January 13th 2021 consortium meeting.
Progress: The survey was adapted for the EU online survey tool, drafted covering sampling and analysis performed in food business operators HACCP-based self-control programs and focuses on; six priority bacteria, five priority parasites, and antimicrobial resistance (AMR) testing of *Salmonella* and *Campylobacter*.

They include:

- Shiga Toxin-producing *E. coli* (STEC)**,** *Salmonella***,** *Campylobacter***,** *Shigella***,** *Yersinia*, and *Listeria.*
- *Cryptosporidium* spp*., Echinococcus granulosus (sensu lato), Echinococcus multilocularis, Toxoplasma gondii, Trichinella spp.*

The nine-question routed survey was shared with the OH-Harmony-Cap consortium. Partners interested in participating tested the survey. The survey was finalised on May 19th, 2021, and an email

sent out to the OH-Harmony-Cap consortium inviting all to participate. Interested partners received a letter of invitation, and link to the survey, for the distribution to relevant food laboratories in their own countries. The initial deadline to fill out the survey was June 25, 2021. However, this deadline has now been set to July 31st to accommodate the new partners. We have received 44 responses and has started the analysis, which will be included in a technical report (D-IA2.2.OH-Harmony-Cap 2.5)

WP3 One Health Laboratory Interoperability Guidance

WP months: M25-M54. WP Leader: 26-Teagasc. WP Deputy Leader: 36-INSA

WP3 Participants: 1-ANSES, 9-BfR, 13-SSI, 21-APHA, 26-Teagasc, 27-ISS, 30-RIVM, 33-NVI, 34-PIWet, 35-INIAV, 36-INSA, 39-SLV, 40-FOHM, 41-SVA

The overall aim of this WP is to map the existing knowledge gaps and propose new studies and/or methods that are needed to fill them. The main deliverable is quantitative and will be a guidance, of the chosen model organism for systematic methodological improvement with respect to One Health laboratory interoperability. Four pathogens will be selected as an example for the project's purposes:

Target Pathogens:

1. Shiga Toxin producing *E. coli* (STEC)
2. Enterotoxigenic *E. coli* (ETEC)
3. *Cryptosporidium*
4. AMR for *Salmonella* and *Campylobacter*

Target areas:

1. Sampling & testing
2. Characterisations
3. Data management & harmonised reporting

JIP04-WP3-T1: Sampling and Testing

Task description: Harmonised testing, including sampling algorithms and laboratory methods for the detection of foodborne pathogens and AMR determinants in food, feed, animal and human samples in Europe. The outputs will include recommendations on a harmonised OH approach, based on the most effective methods currently available, including opportunities offered by new technologies such as WGS.

Progress: Concluded. Deliverable, D-IA.2.2.OH-Harmony-Cap.3.1

JIP4-WP3-T2: Characterisation

Task month M37-48 – Ongoing

Task Leader: 21-APHA. Deputy Task Leader: 36-INSA

Task Participants: 1-ANSES, 9-BfR, 13-SSI, 21-APHA, 26-Teagasc, 27-ISS, 30-RIVM, 33-NVI, 34-PIWet, 35-INIAV, 36-INSA, 39-SLV, 40-FOHM, 41-SVA

Task description: The primary aim of this task is to establish what characterisation of the target pathogens is ongoing in public health, veterinary and food/feed/environmental testing laboratories throughout the EU and to harmonise this work to ensure the data required for food safety policy development and more efficient risk assessment/management is supported in the future.

Progress: A survey of relevant laboratories throughout the EU was undertaken in the previous period using a questionnaire, which was designed to collate key information on current characterisation practices in these laboratories. The relevant data from this questionnaire is currently being analysed for the summary report, which forms the deliverable for this task. Teams of participants are currently collating this information (JIP4-WP3-T2S1). This output is being combined with information from a literature review, focusing on recommendations contained in relevant EFSA Opinions, FAO reports, etc and the expertise within the project team to draft the summary report, covering; 1) current routine strain characterisation (typing, AMR & virulence gene testing) in Europe; 2) gaps in strain characterisation that are inhibiting food safety regulation/policy in the EU; 3) typing methods, AMR & virulence gene testing as part of a future harmonised approach to strain characterisation and 4) how a harmonised strategy should be implemented.

JIP4-WP3-T3: Data Management

Task start month: M46

Task end month: M54

Task Leader: 26-Teagasc. Deputy Task Leader: 36-INSA

Task Participants: 1-ANSES, 9-BfR, 13-SSI, 21-APHA, 26-Teagasc, 27-ISS, 30-RIVM, 33-NVI, 34-PIWet, 35-INIAV, 36-INSA, 40-FOHM, 41-SVA

Description of the task: This task aims at describe harmonised reporting including foodborne pathogen data management within individual MSs and data transfer to EFSA and ECDC.

Sub-task WP3-T3-ST1 (M46-M50): The responses to the questionnaire (sub-task WP3.T1.S1) will be used to establish current data management practices with individual MSs and how this data is communicated to EFSA and ECDC.

Sub-task WP3-T3-ST2 (M49-54): Experts from ICT companies will be invited to present their data management systems to a panel of personnel (with relevant experience) from within the project and external experts (eg. personnel in EFSA and ECDC who collate and analyse this data). Recommendations on best practice used to form the basis for a harmonised system for recording, storing and transferring data with the EU.

The deliverable will be a summary report (drafted by the WG but reviewed by all project participants) describing; [1] currently used data management systems in Europe; [2] a harmonised data management system for the future and [3] recommendations on how this system should be implemented (hard ware, software, motivating MSs, etc.).

WP4 Harmonisation of Protocols

WP months: M25-M48. WP Leader: 27-ISS. WP Deputy Leader: I-ANSES

WP participants: 1-ANSES, 9-BfR, 13-SSI, 21-APHA, 27-ISS, 32-NIPH, 30-RIVM, 33-NVI, 34-PIWet, 35-INIAV, 36-INSA, 39-SLV, 40-FOHM, 41-SVA, 44-Bior

The aim of WP4 is to produce recommendations on how to harmonise the methodology for the detection and typing of the model pathogens. These pathogens will be selected as examples for the project's purposes, and identified candidates include Shiga toxin producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC) and *Cryptosporidium* and AMR for *Salmonella* and *Campylobacter*. The strategy and the approaches adopted in the framework of WP4 activities may be transferred to other pathogens, enabling harmonisation of methodologies in use in the EU/EEA laboratories

JIP4-WP4-T1: Collection of the Laboratory Protocols for Model Organisms

Progress: Concluded.

JIP4-WP4-T2: Evaluation of the Collected Laboratory Protocols

Task month: M31-M39

Task Leader: 41-SVA. Deputy Task leader: 1-ANSES

Task description: Evaluation of the laboratory protocols for the detection and typing of selected foodborne pathogens, including STEC, ETEC, *Cryptosporidium* and AMR for *Salmonella* and *Campylobacter* in use in the laboratories operating in public health and veterinary sectors. The information about the methodologies applied in the EU/EEA laboratories will be circulated among the WP4 participants and will be assessed with the purpose of identifying differences and similarities in the methodological approaches applied in the different laboratories. This will be the basis for the definition of harmonised protocols.

Progress: The evaluation of the protocols collected was initiated as scheduled and finished on M39. In particular, the activities of this task concerned the evaluation of the laboratory protocols for the detection and typing of selected foodborne pathogens, including STEC, ETEC, *Cryptosporidium* and AMR for *Salmonella* and *Campylobacter* in use in the laboratories operating in public health and veterinary sectors. This primary evaluation would be the basis for identifying similarities among the protocols applied and for the definition of harmonised procedures.

The task has been subdivided in three subtasks:

WP4-T2-S1: Evaluation of the laboratory protocols for the detection and typing of pathogenic *E. coli* STEC/ETEC

WP4-T2-S2: Evaluation of the laboratory protocols for the detection and typing for AMR *Salmonella* and *Campylobacter*

WP4-T2-S3: Evaluation of the laboratory protocols for the detection and typing of *Cryptosporidium*

Three groups consisting each of about four to six experts in pathogenic *E. coli*, *Cryptosporidium* and AMR, participating in OH-Harmony-CAP project, have been formed, with the aim of facilitating the comparison of the procedures collected in the framework of task JIP4-WP4-T1. To do so, evaluation tables have been deployed by task JIP4-WP4-T2 leaders and agreed within the groups of experts, to aid the primary assessment of the protocols. The following protocols have been evaluated:

WP4-T2-S1: 53 protocols STEC/ETEC

WP4-T2-S2: 10 protocols for *Campylobacter* AMR and 9 protocols for *Salmonella*

WP4-T2-S3: 18 protocols for *Cryptosporidium*

Three evaluation tables corresponding to each subtask have been produced and stored in the same folder with the original protocols received, which will be an important output to be used in the JIP4-WP4-T3.

JIP4-WP4-T3: Ranking and choice of laboratory protocols

Task months: M39-M48 (ongoing)

Task Leader: 34-PIWet. Deputy Task leader: 1-ANSES

Task description: WP4 participants will be enrolled in the preparation of the protocols according to

their area of expertise. They will rank and evaluate the harmonised laboratory protocols for the detection and typing of "model organisms". The protocols will be distributed to the Medical and Veterinary Laboratories participating in the WP4 and those who participated in the collection of the protocols (JIP4-WP4-T1).

Progress JIP4-WP4-T3 takes place in Y4 as scheduled, and particularly in M37-M48. The aim of this task consists in choosing and proposing harmonised protocols. To do so, experts participating in WP4 will be enrolled for the ranking of the procedures collected and preliminarily evaluated in JIP4-WP4-T2. Since the beginning of Y4, three meetings have been held among WP4-T3 leader, co-leader, WP4 coordinator and OH-Harmony-Cap coordinator, to discuss the roadmap of this task. Moreover, a specific meeting with WP4 participants was held in M40, to discuss the progress of the WP activities and to present the outcomes of JIP4-WP4-T1 and JIP4-WP4-T2 and, further, decide on the criteria, which will be used for ranking of the protocols. We have established a preliminary list of requirements, such as PCR targets, and criteria, *i.e.* specificity, sensitivity, man hours, training requirements, which will be in support for ranking of the protocols. The groups of experts have been identified and discussed (July 8th) on a proposal of a ranking table, which will be fine-tuned according to the specific model microorganisms included in this WP. Furthermore, the ranking activity has been initiated based on the evaluation tables deployed in JIP4-WP4-T2 and on the original protocols collected, which will be made available.

The WP4 leads, JIP4-WP4-T3 lead, and the project leader discussed (June 8th) the possibility of prolonging this task to M50. This has been updated in the AW4. The outcome of this task will be used directly in JIP4-WP5-T3, which is dedicated to practical workshops and E-learning on the harmonised protocols.

WP5 Training sessions on harmonised protocols

WP5 leader: 27-ISS. Co-Leader: 13-SSI.

WP months: M40-M60 (ongoing)

WP5-T1 participants: All OH-Harmony-Cap participants. WP5-T2 participants: All CARE, OH-Harmony-Cap, and MATRIX participants. WP5-T3 participants: 13-SSI and 35-INIAV

The aim of this WP consists in increasing EU capacity to deal with foodborne zoonoses, antimicrobial resistance and emerging threats across the One Health interface through partner-to-partner delivery of training. The training will provide opportunities for better communication, knowledge exchange, and knowledge integration within the One Health interface, and not just for the OH-Harmony-Cap participants. As such, two workshops will be held during the final meeting in Copenhagen.

JIP4-WP5-T1 Facilitation and communication of the OHLabCap and T2 Joint workshop between CARE, OH-Harmony-Cap, and MATRIX

Task months: M40-M57 (ongoing)

Task Leader: 13-SSI. Co-leader: 27-ISS

Task participants: All OH-Harmony-Cap participants

Task descriptions: A workshop for communicating and discussing the outcome of the OHLabCAP survey and the joint meeting between the three joint integrative projects, CARE (IA2.1), OH-Harmony-Cap (IA2.2), and MATRIX (IA2.3) and the joint research project DisCoVer

Progress: the workshop for communicating and discussing the outcome of the OHLabCAP survey conducted in the framework of WP2 (T1) and the joint meeting between the three integrative projects, CARE (IA2.1), OH-Harmony-Cap (IA2.2), and MATRIX (IA2.3) (T2), will be delayed according to the extension of OH-Harmony-Cap until M60. The project leaders of all three projects have agreed to postpone the exit meeting until M57. The planning of the two workshops will start in M49 after the

conclusion of WP2, WP3, and WP4.

JIP4-WP5-T3: Practical workshops (S1) and e-learning activities (S2)

Task months: M40-M60 (ongoing)

Task Leader: 27-ISS. Co-leader: 13-SSI

Task descriptions (*JIP4-WP5-T3S1)*: Three practical workshops, focusing on the model organisms (STEC, ETEC, *Cryptosporidium*, and AMR for *Salmonella* and *Campylobacter*) considered in this project, will be held at ISS and SSI. The trainings will consist in three days of laboratory activities on the application of the harmonised procedures developed in WP4. At ISS, training on harmonised procedures for the detection and typing of pathogenic *E. coli* and parasites (*Cryptosporidium*), will be provided to eight scientists for each pathogen in two rounds (for a total of 16 scientists). The training on harmonised procedures on AMR determinants for *Salmonella* and *Campylobacter* will be provided at SSI.

Progress: The planning of this subtask has not started and will be postponed. As such, the practical workshops will be held M54-M60.

Task descriptions (JIP4-WP5-T3S2): Development of *e-learning* activities dedicated to the application of the harmonised protocols developed in WP4 on a set of foodborne pathogens.

Progress: A meeting (February 23rd) was organised among WP5 leader, co-leader, WP4 coordinator and OH-Harmony-Cap coordinator, to start planning the WP5 tasks. The following was agreed:

- Webinars based on presentations and videos, with emphasis on the critical control points (CCP) of the harmonised method/s. It was suggested to develop a common template (ex: ISO standards, SOPs) to be applied to all harmonized protocols developed in WP4. The webinars will be delivered before the practical training, with the aim of eliciting the participant interest
- Presentations and videos on the harmonised protocols to be published on a dedicated web-page on the OHEJP web site (with the help of the communication team), in order to disseminate the project achievements.

All the training delivered will have a final step of assessment of the achievement of the learning objectives.

Furthermore, the WP5 leader participated to the web-meetings on the WP update and the welcome and update to the three new participating institutes.

Note: On September 2021 Tosini Fabio, 27-ISS will be the new WP5 leader.

6.1.2.1.4.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
OH-HARMONY-CAP	D-IA.2.2.OH-Harmony-Cap.2.2	Technical report of the survey results	M38	M38		Public https://zenodo.org/record/4588483#.YJt4wIdxc2w	2, 4, 6
OH-HARMONY-CAP	D-IA.2.2.OH-Harmony-Cap.2.3	Adjusted survey	M42	M41		10.5281/zenodo.5464722 (public)	2, 4, 6
OH-HARMONY-CAP	D-IA.2.2.OH-Harmony-Cap.2.3	Food sector survey	M42	M42		10.5281/zenodo.5465061 (public)	2, 4, 6

** Categories of Integrative activities: 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
OH-HARMONY-CAP	M-IA2.2. OH-Harmony-Cap.6	Assessing protocols for detection and typing of the selected foodborne pathogens	M39	Yes, M39		

6.1.2.1.4.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethic Advisors accepted the comments you provided last January. Therefore, this part of the report can be closed.

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Recommendations Ethics Advisors, Apr 2021
On the ethics checklist you have ticked a safety issue, therefore the beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	None received, none implemented	Assume this means there is no response from the researchers, please reminded the team a response is needed (Reply to be clarified, what does 'none received' mean?)	Apologies for the unclear answer. We meant that no specific issues had been identified. The laboratories are used to working with the pathogens included in the work. Appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in the project.	Satisfactory reply

6.1.2.1.4.5 Publications and additional outputs

Publications

N/A

Additional output

At the OHEJP ASM 2021 two OH-Harmony-Cap WPs were disseminated; 1) Oral presentation "OH-Harmony-Cap: Development of an OHLabCap tool" (Flemming Scheutz, 13-SSI), and 2) poster presentation "Collection of protocols for the detection and characterisation of Shiga toxin-and enterotoxin producing *Escherichia coli* (STEC/ETEC), *Cryptosporidium* spp. and antimicrobial resistance in *Salmonella* and *Campylobacter*" (Rosangela Tozzoli, 27-ISS and Melanie Gay. 1-ANSES)

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

The development of the first OHLabCap tool using the EU online survey tool is in itself a major achievement, which is open for adaptation by other key stakeholders in the OH field such as FAO and WHO, who are conducting similar surveys. The inclusion of private food operators and laboratories in the mini

survey of this project could potentially represent an improvement in the communication between the private sector and One Health authorities. Both parties could benefit from the results. The involvement of these stakeholders could in return serve as inspiration for the further development of tools intended to measure the dimensions and targets in the OHLabCap tool (D-IA2.2.OH-Harmony-Cap 2.4).

6.1.2.1.4.6 Data Management Plan

The deliverable describing the DMP is available at Zenodo. The final DMP will be published at the end of the project lifetime. The DMP was updated on September 3rd.
The FAIR principles are fulfilled.

6.1.2.1.4.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

On May 21st, OH-Harmony-Cap co-hosted a Thematic Integrative Meeting (TIM) "EU surveillance frameworks and infrastructures in a harmonized One Health perspective" in collaboration with JIP CARE. More than 80 people participated and the meeting concluded with a panel discussion with representatives from ECDC (Johanna Takkinen) and EFSA (Valentina Rizzi and Mirko Rossi). A set of questions, as agreed by CARE and OH-Harmony-Cap, where sent to the panel in advance and they included:

1. Do ECDC, EFSA and the Commission see the outcome of the Joint Integrative Projects (JIPs) as a part of an EU strategy going forward?

2. What are the plans for OH networks on the organisms that are targeted in OH-HARMONY-CAP and CARE?

3. In general, how do we further coordinate with WHO and FAO?

4. How will ECDC, EFSA and the Commission use the output from the JIP's? Are there any plans for sustainability?

5. Does the EU have plans to develop a bacterial/parasitical analytic manual for diagnostics?

If so, will it be done in a OH-health environment?

There was a wide agreement, that there is a need for a continuation of the activities performed in the JIPs. Further, there is support from Johanna Takkinen, ECDC, that activities needed to implement the results (JIP outcome), should go beyond dissemination. Specific actions could be considered such as, implementation projects, including developing electronic support systems, training sessions and on site guidance. Importantly, The OH-Harmony-Cap WP5 is dedicated to the dissemination of results and we are very aware of the need for sustainability measures. Notably, the EU commission did not participate in the discussion panel.

Collaboration with EURLs and the PH-NRLs includes ongoing sharing of outputs, reports, meeting minutes allowing the oppotunity for these stakeholders to suggest and comment on activities related to the OH-HARMONY-CAP project.

6.1.2.1.5 JIP05-R2-IA2.1-MATRIX

6.1.2.1.5.1 Summary of the work carried out in the Project

MATRIX is an EU-co-funded project within the framework of the One Health European Joint Programme (OHEJP). It aims to advance the implementation of One Health (OH) surveillance in European countries, by identifying and extending existing cross-sectorial programmes including animal health (AH), public health (PH) and food safety (FS). Currently, nineteen partners from twelve European countries constitute the MATRIX Consortium[6].

During the reporting period of this document – month-37 (M37) to M45 – the major achievements of the project included:

1. Updating a review of commonalities and differences of various surveillance frameworks in AH, PH, and FS; identifying recommendations to adapt existing frameworks into a common sectorial framework for AH, PH and FS;
2. Mapping surveillance chains for the MATRIX hazard tracks i.e. *Listeria, Salmonella*, *Campylobacter,* and emerging threats including Hepatitis E;
3. Initiating a review of the available evidence about output based surveillance within the AH, PH and FS sectors; completing internal inventories of relevant surveillance methodologies among MATRIX partners;
4. Finalizing a report on the necessary criteria to evaluate OH capacities and capabilities for integrated surveillance;
5. Initiating the design of a roadmap for integrated OH surveillance; consolidating the OH Knowledge-Integration Platform and training/dissemination activities;
6. Initiating the design of user-driven dashboards for collaboration and decision making in OH surveillance;
7. Continuing to ensure that MATRIX work encompasses relevant pathogens by pairing the activities of Work Packages with that of Hazard Tracks.

In 2020, MATRIX demonstrated an excellent degree of adaptability to the SARS-CoV-2 pandemic and could adequately respond to the challenges that it posed to the project work plan. However, the pandemic is still affecting the work of many MATRIX partner institutes across Europe and it is hard to imagine a swift return to normality in the coming months. Therefore, in May 2021, the MATRIX Consortium applied for a 6-month no-cost extension of the project to the end of 2022. In this way, MATRIX will i) benefit from the results produced by other EJP projects, which were also extended; ii) expand the synergies among the different sectors, which is at the core of MATRIX's aim, and therefore achieve the project's objectives under the best possible conditions.

MATRIX i) reviews available national and multi-national One Health surveillance frameworks, linking across animal health, human health and food safety sectors: ii) spreads cross-sectorial best-practices for data collection and analysis, and dissemination of surveillance results; iii) develops guidelines for the design, implementation and evaluation of official controls within the food sector using output-based standards; iv) develops a benchmarking tool for characterizing, monitoring and evaluating One Health surveillance capacities and capabilities (EUEpiCap); v) designs a roadmap for the development and implementation of national One Health surveillance, considering differences across countries; iv) creates dashboards of One Health surveillance inputs and outputs with a focus on possible pitfalls and biases in multi-sectorial data analysis. Finally, MATRIX encompasses four hazard-specific tracks that cross all activities: Campylobacter, Salmonella, Listeria and emerging threats (including antimicrobial resistance).

In 2021, MATRIX faced an additional challenge: as per January 2021, the National Food Institute, Technical University of Denmark (DTU Food) left the MATRIX Consortium. Despite the scientific loss related to the specific DTU Food expertise in food safety, the Consortium managed to take over the related activities. This was the second withdrawal of a partner institute from MATRIX since the project started.

On the other hand, a new partner, the Finnish Food Authority, Ruokavirasto (45-RUOKA), joined the OHEJP and specifically the MATRIX project Consortium in June 2021.

In conclusion, MATRIX activities are currently aligning to the project's initial plans and, given the continuous availability of the expected resources including a 6-month extension, it is reasonable to imagine that its objectives will be achieved.

6.1.2.1.5.2 Progress of the project: description of activities

WP1 Existing frameworks and OH capacity

JIP-MATRIX-WP1-T1 Generate an inventory of existing frameworks for surveillance of inter-sectorial hazards, by sector (AH, PH, and FS) – The task does not span the reporting period of this document. The task is completed. However, during the whole time of MATRIX, the inventory will be updated to capture new surveillance systems. The related deliverable D-WP1.1 Report on the commonalities and differences of the different operational frameworks in Animal Health (AH), Public Health (PH), and Food Safety (FS), which was submitted as per deadline in M36, has been updated during Year 4 (Y4, 2021) with new available evidence from MATRIX partner institutes before being made public via the Zenodo platform.

JIP-MATRIX-WP1-T2 Generate suggestions for adaptation of existing frameworks into a common sectorial framework (AH, PH, FS) which can support surveillance of inter-sectorial hazards (OHS) – The task is ongoing. This task provides recommendations to adapt existing sectorial frameworks into a common one for AH, PH and FS. MATRIX partner institutes are sharing information on existing OH surveillance systems, with a focus on relevant hazards i.e. Listeria, Salmonella, Campylobacter, emerging threats, including antimicrobial resistance. This work is done in collaboration with other MATRIX Work Packages (WP). This work informs the content of deliverable D-WP1.2 Description of a common framework for OH surveillance. The deliverable was originally expected by M48. Following the 6-month extension of the project, the new deadline in the Year 5 Work Plan is M54.

JIP-MATRIX-WP1-T3 Evaluation of the sectorial frameworks, for specific inter-sectorial hazards – The task does not span the reporting period of this document.

WP2 Best-practices and multi-sectorial collaboration

JIP-MATRIX-WP2-T1 Mapping of the surveillance chain across all sectors for each hazard-track – The task originally did not span the reporting period of this document. The mapping started in Year 3 and is still ongoing, since additional countries have been involved at a later stage. Per each hazard-track, including Hepatitis E (chosen as the emerging pathogen of interest), a specific food chain was selected to explore in detail the different realities. Twelve online questionnaires were implemented based on the "farm to fork" approach for the three sectors AH, PH and FS, and each hazard-food chain combination. Surveillance activities in place were investigated in at least two countries for each combination. Salmonella was investigated in humans and pork meat; Listeria in humans and dairy products; Campylobacter in humans and poultry meat; Hepatitis E in humans and wild boar meat. Answers were categorized in order to be graphically displayed i.e. events, actors, data, metadata, event producing data, identified data sources, and sharing potential. Categorized answers represent the starting point for the identification of cross-sectorial linkages across surveillance chains. At the moment, the following countries have been investigated: i) Salmonella in humans and pork meat, in France, Spain, Germany, Norway and England; ii) Listeria in humans and dairy products, in Norway and

Italy; iii) Campylobacter in humans and poultry meat, in France, Norway, Germany and Denmark (pending); iv) Hepatitis E, in humans and wild boar meat, in Portugal, The Netherlands, Italy and Norway.

JIP-MATRIX-WP2-T2 Identify cross-sectorial surveillance chain linkages, particularly, which outputs should be shared and how they should be shared for OH orientated decision making – The task is ongoing. Building on the work done and the information collected about maps of integrated hazard-food surveillance chains in JIP-MATRIX-WP2-T1 during Y3 (2020), this Task identifies cross-sectorial linkages for integrated OH surveillance systems. The deliverable *D-WP2.1 Mapping of the surveillance chain for all hazard tracks, and cross-sectorial linkages* was originally expected in M36 and it was then postponed to M42 during Y3 (2020). In May 2021, MATRIX requested to postpone it by 6 months to December 2021 (M48), which was granted.

JIP-MATRIX-WP2-T3 Propose best-practice guidelines and effective strategies for data collection, analysis, and dissemination aimed at multi-sectorial OH collaboration, for each specific hazard-track – The task is ongoing. This task develops best-practices for multi-sectorial collaboration and puts OH surveillance into practice, also addressing the limitations related to data sharing, data analysis, interpretation and dissemination of outputs. The work in this task is hazard specific i.e. Listeria, Salmonella, Campylobacter and Hepatitis E. The deliverable D-WP2.2 Suggested best-practices for multi-sectorial collaboration in order to achieve OHS, hazard-specific (hazard tracks) was originally expected in M48. Following the 6-month extension of the project, the new deadline in the Year 5 Work Plan is M54.

JIP-MATRIX-WP2-T4 Identify commonalities across the tracks, to propose a common OHS framework when applicable – The task does not span the reporting period of this document.

WP3 Output-based surveillance evaluation

JIP-MATRIX-WP3-T1 Inventory of previous work and current practice – The task does not span the reporting period of this document. However, WP3 has been heavily impacted by the turnover of key personnel during 2020, including the WP leader. Therefore, activities related to this task continue being implemented during the reporting period of this document. No deliverable is attached to this task. A review of evidence (both peer-reviewed and grey literature) about output-based surveillance within the three sectors AH, PH and FS has begun.

JIP-MATRIX-WP3-T2 Identification of operational partners and stakeholders – The task is ongoing. The originally planned stakeholder mapping exercise was delayed due to the pandemic and Avian Influenza, as this WP is primarily implemented by partners working in the AH sector. However, this task spans the entire duration of MATRIX and the delay should not affect its outcome. This task uses input from the RISKSUR project (about risk-based surveillance systems) and from the SIGMA project (about stakeholder mapping). The WP is setting up a case study in Y4 (2021), focusing on a specific pathogen. No deliverable is attached to this task.

JIP-MATRIX-WP3-T3 Selection of appropriate output-based surveillance systems – The task is ongoing. Participating partners have identified appropriate output-based methodologies according to the specific objective of the surveillance system they have selected. Deliverable D-WP3.1 Report on the output-based surveillance system selection methodology for each country and as many of the approaches as possible was originally expected in M48. Following the 6-month extension of the project, the new deadline in the Year 5 Work Plan is M54.

JIP-MATRIX-WP3-T4 Development of evaluation strategies for surveillance systems based on output-based metrics

The task is ongoing. This task initiates amidst the reporting period of this document and is in

consequence affected by the aforementioned delays. This task is looking at strategies to evaluate the effectiveness of surveillance systems within the three sectors: AH, PH and FS. Participating partners are reviewing the appropriate output metrics that are to be used to develop a country-specific standard for evaluation. Deliverable D-WP3.2 Combined report for all subtasks within the development of output-based metrics was originally expected in M54. Following the 6-month extension of the project, the new deadline in the Year 5 Work Plan is M58.

WP4 Benchmarking tool for characterising, monitoring and evaluating OH capacities and capabilities (EUEpiCap)

JIP-MATRIX-WP4-T1 Review of existing methods for surveillance systems evaluation – The task is completed. This task was delayed during Y3 (2020) and eventually produced the expected deliverable as per plans in M39: D-WP4.1 Report on the selected set of criteria for evaluation of epidemiological capacities for One Health surveillance. The task reviewed existing OH evaluation tools, identifying indicators that are common across the different tools. Inspired by the EU-LabCap approach, the EU-EpiCap tool is structured with three dimensions and four targets per dimension.

JIP-MATRIX-WP4-T2 Development of the surveillance capacity benchmarking tool – The task is ongoing. Building on JIP-MATRIX-WP4-T1, this task works to define a scoring system. The EU-EpiCap tool will be tested on some combinations of hazards and countries (e.g. antimicrobial resistance and/or Salmonella in France) in 2021. An R Shiny-based interface is being developed to record data for each indicator included in the assessment. The interface will automatically generate synthetic country profile reports to facilitate the dissemination of results. The interface will allow the exploration of the outcomes of the assessment(s) by way of multiple interactive visualization options. A tutorial will be created to explain how to use the tool. Deliverable D-WP4.2 EUEpiCap tool and tutorial was originally expected in M48. Following the 6-month extension of the project, the new deadline in the Year 5 Work Plan is M54.

JIP-MATRIX-WP4-T3 Application and evaluation of the tool
The task does not span the reporting period of this document.

WP5 Outreach and roadmap

JIP-MATRIX-WP5-T1 Perform a requirement analysis for national OH surveillance roadmaps – The task is ongoing. Due to the mutual benefits for both parties and high-level synergies between the two tasks, Statens Serum Institut (SSI) and the National Veterinary Institute (SVA) agreed to form a unique collaboration of shared leadership for JIP-MATRIX-WP5-T1 and JIP-MATRIX-WP5-2, inviting all partners from both tasks to contribute. The requirement analysis started to investigate barriers and facilitators to OHS implementation at the national level and will aid in the development of a national OHS roadmap. A review of other OHEJP project outputs will serve as a jumping off point for the requirement analysis, which will also comprise of a systematic literature review of both peer-reviewed and grey literature, and a series of interviews/user stories/group discussions with other task participants and member states, with particular focus on the MATRIX hazard tracks. An initial workshop was conducted in April of 2021, which included WP 1, 2, and 4 leaders, hazard track leaders, and an EFSA representative to discuss various inputs and ideas for the requirement analysis and the roadmap. Deliverable D-WP5.1 Report on requirement analysis for “OHS roadmap template” was originally expected in M45. In May 2021, MATRIX requested to postpone it by 3 months to December 2021 (M48), which was granted.

JIP-MATRIX-WP5-T2 Develop a national OH surveillance roadmap – The task is ongoing. Due to the mutual benefits for both parties and high-level synergies between the two tasks, Statens Serum Institut (SSI) and the National Veterinary Institute (SVA) agreed to form a unique collaboration of shared leadership for JIP-MATRIX-WP5-T1 and JIP-MATRIX-WP5-2, inviting all partners from both tasks to

contribute. This task is developing a roadmap for country level. The work will build on deliveries from previous OHEJP projects (i.e. JIP COHESIVE, NOVA and ORION), and will consider OH collaboration at different levels such as work on strategic questions (long term planning and prioritization), regular issues (routine surveillance) and emergency situations (outbreaks). Deliverable D-WP5.2 OHS roadmap template document was originally expected in M54. Following the 6-month extension of the project, the new deadline in the Year 5 Work Plan is M58.

JIP-MATRIX-WP5-T3 Knowledge-Integration Platform – The task is ongoing. The development of the Knowledge-Integration Platform (KIP) is progressing in frequent task-specific meetings. In the past, it was decided to extend the OHS Codex that was developed in the JIP ORION project (https://oh-surveillance-codex.readthedocs.io/en/latest/). Consequently, the KIP is available as the same readthedocs-web page. To reflect the extension of the OHS Codex through the MATRIX project, we decided to rename the webpage to "OHS Codex: The Knowledge integration platform"; in the following we will refer to the KIP as OHS Codex. In general, it is planned to add relevant tool, sections, and, if needed, extend the OHS Codex framework to extend the OHS Codex. The following extensions/changes were decided: (1) extend the scope and purpose, (2) add a description of the MATRIX project in the introduction of the OHS Codex, (3) update the existing principles, (4) create new sections for training materials and examples for OHS reports, (5) add MATRIX as funding, (6) a new principle that focuses on surveillance planning and management (the exact definition is an ongoing process). Agreed changes 1 to 5 are already in the draft mode that is evaluated and added by the OHS Codex team (see for the draft mode https://docs.google.com/document/d/1W69Lcc0-5fudoex7-Gjl_BxTpQyVjxHoJkUmELu1-8o/edit). The collection of suggestions for resources that might be interesting to add to the OHS Codex and information, e.g., whether these suggestions were approved by the MATRIX team are listed in a living document (https://seafile.bfr.berlin/f/0ad237213cb844588190/); currently, it contains 35 entries (effective 7th June 2021). In the past, it was decided that the OHS Codex should support the aspect of exchanging of mathematical models from different OH sectors. To accomplish that, it will be necessary that the dedicated OHS Codex section supports harmonized information exchange formats, e.g., the Food Safety Knowledge Exchange (FSKX) Format (former FSK-ML, see https://foodrisklabs.bfr.bund.de/fsk-ml-food-safety-knowledge-markup-language/). FSKX format is the basis for various tools that support in re-using and exchanging of knowledge. A dose-response model was implemented and published in FSKX format. This model is available in the executable article journal (https://fmj.pensoft.net/article/63309/) and can be made available in model repositories as well. The work on re-implementing a QMRA model of Trichinella in FSKX format has started. The extension of the FSKX format towards PH/OH has started. Re-implementation of existing OH models as FSKX compliant files has started with the purpose of validating the proposed PH/OH FSKX extension. The process goes along with the re-implementation of a source-attribution model that is in the draft model, which will be submitted soon. The collection of potential use cases of the FSKX-format within MATRIX has started. NVI will explore the option to apply the FSKX format to annotate/share IRIDIA sequencing pipelines. The Milestone M-WP5.1 "Knowledge-platform operational" was successfully accomplished in M42.

JIP-MATRIX-WP5-T4 Training and dissemination – The task is ongoing. Please see the following enumeration for MATRIX-internal and public achievements. MATRIX partners are contributing to enrich the opportunities for training and dissemination, which are planned in frequent task-specific meetings. Among others, there is the plan to explore how MATRIX outputs can contribute to some of the ECDC public health trainings initiatives. No deliverable is attached to this task.
MATRIX-internal resources: video to inform the MATRIX community on demand about the FSKX format.

Dissemination activities

OHS Briefings - inform about OHS initiatives and projects on EU level - will actively involve stakeholders, such as EFSA and ECDC - e.g. webinars/meetings	**Training materials** e.g., tutorials, videos, and guidelines	**External trainings** - external trainings to share the generated knowledge and train external experts - internal and external partners
Participation on EJP JRP DiSCoVeR stakeholder meeting, Jan 2021		Presentation by Kyrre Kausrud on the OHEJP ASM 2021 Satellite workshop Software Fair, June 2021
SafeConsume-ORION-MATRIX meeting that deals with OHS Codex/KIP, Jan 2021 & communication in April 2021		Presentation by Esther Sundermann on the OHEJP ASM 2021 Satellite workshop Software Fair, June 2021
Presentation about WP5 related work entitled "Food Safety Knowledge Exchange (FSKX) Format: a Flexible Exchange Format for (Joined) Models in the Area of Food Safety and One Health" by Esther Sundermann on the OHEJP ASM 2021, 9-11 June 2021		MATRIX team members supported the organization of the OHEJP ASM 2021 Satellite workshop Software Fair, June 2021
Poster entitled "GIS AS A TOOL FOR MAPPING SALMONELLA SEROVARS IN PIGS IN POLAND BETWEEN 2014-2018" by Anna Ziętek-Barszcz on the OHEJP ASM 2021, 9-11 June 2021		Co-chairing by Guido Benedetti in the "One Health Session" of the ECDC EPIET/EUPHEM Fellowship Project Review Module on August 25th, 2021. Participation in the related round table and brief presentation about the MATRIX project and integrated One Health surveillance.
Poster entitled "BUILDING POLITICAL WILL FOR ONE HEALTH RISK ANALYSIS SYSTEM" by Zuzana Norden on the OHEJP ASM 2021, 9-11 June 2021		
Poster entitled "EVOLVING AT THE SPEED OF RISK" by Kyrre Kausrud on the OHEJP ASM 2021, 9-11 June 2021		
Poster entitled "MAPPING THE SURVEILLANCE ACTIVITIES FOR FOOD-HAZARDS ACROSS EUROPE" by Francesca Cito on the OHEJP ASM 2021, 9-11 June 2021		

WP6 Decision and collaboration dashboards

JIP-MATRIX-WP6-T1 Country-based Identification of existing cross-sectorial OHs activities – This task does not span the reporting period of this document. However, this WP has been heavily impacted by the pandemic in year 2020, and its activities fully took off in 2021. This task gathers information from the first round of EJP projects i.e. the lessons learned in JIP ORION regarding multi-agency collaboration and data interoperability in OH; the documentation from JIP COHESIVE on dissemination of surveillance results; and the achievements of JRP NOVA recommending novel methods for analysis of surveillance data. No deliverable is attached to this task.

JIP-MATRIX-WP6-T2 Defining the content needs for cross-sectorial decision and collaboration dashboards – The task is ongoing. Following the work in JIP-MATRIX-WP6-T1, and considering the hazards of relevance to MATRIX, this task invites partners to have inter-agency discussions on the possibilities and problems that can be addressed with joint analyses and/or visualization of data. The design of cross-sectorial decision and collaboration dashboards is driven from the end user perspective, including information gaps and methodological limitations, technical and legal barriers associated with data sharing. Deliverable D-WP6.1 User manual for construction and implementation of OH dashboards using open source tools (source codes) was originally expected in M54. Following the 6-month extension of the project, the new deadline in the Year 5 Work Plan is M58.

JIP-MATRIX-WP6-T3 Technical implementation and testing of dashboards
The task is ongoing. This task implements country-specific, cross-sectorial decision and collaboration dashboards. The deliverable of this task will be a dashboard inventory, to be used within MATRIX and hopefully beyond. D-WP6.2 A practical manual to the use of dashboards in OHS practice, including recommendations for sustainability was originally expected in M54. Following the 6-month extension of the project, the new deadline in the Year 5 Work Plan is M58.

MATRIX Hazard Tracks

The hazard tracks (HT), which are a signature feature of MATRIX, ensure that the work in MATRIX packages is directly relevant to specific pathogens/hazards. The HTs were chosen based on the operational priorities of MATRIX partner institutes and their OH relevance. They are *Listeria, Salmonella, Campylobacter* and emerging threats, including antimicrobial resistance. During the 2020 kick-off meeting, it was remarked that not all WPs specifically work on these hazards.

Hazard Track – Listeria – The HT contributed to i) the development of a questionnaire about sampling and analytical methods and capacities of *Listeria monocytogenes* in the animal, food and human sectors and collection of responses from three countries; ii) based on operational observations about OHS during Y3 (2020), the development of checklists for best practices e.g. in outbreak management and active surveillance; the work is ongoing (WP2); iii) planning the inclusion of two new pillars to the OHS Codex i.e. sampling and management pillars (WP5); iv) planning a Listeria pilot as a part of "Sykdomspulsen" in Norway, focusing on official surveillance data and selected metadata, for food and human illness cases; this activity further builds on the JRP ListAdapt protocols and experience from JIP ORION (WP6).

Hazard Track – Salmonella – The HT continued the collaboration with other WPs e.g. mapping surveillance chains for Salmonella in the United Kingdom (WP2).

Hazard Track – Campylobacter – The HT initiated related activities during 2021. The HT identified opportunities in collaborating with WP1, WP2, WP4, and WP6. The work is ongoing.

Hazard Track – Emerging threats (including antimicrobial resistance) – The HT is exploring opportunities for collaboration within the different WPs; possible topics include Hepatitis E (WP2 and WP4), and antimicrobial resistance; COVID-19 is also an OH emerging threat, which is cross-cutting with other OHEJP JIP (namely COVRIN), with ongoing close interaction.

WP0 Coordination

JIP-MATRIX-WP0-T1 Internal coordination – activities included: i) managing the handover of the MATRIX leadership and vice-leadership; ii) managing the withdrawal of the partner 12-DTU Food from the MATRIX Consortium (starting on January 1st, 2021), including the redistribution of the partner activities and budget; iii) welcoming and orientating new colleagues in the Consortium e.g. after hand over at partner institute level; iv) managing routine meetings with the MATRIX Consortium and the

WP/HT leaders; v) supporting the routine management of WPs; vi) defining a Consortium internal procedure for the validation of the project deliverables; vii) continuous documentation/reporting of the project progresses; drafting the 9-month report (Y4, 2021) and revising the Y5 (2022) work plan; viii) routine budgetary management; ix) ongoing housekeeping of the project webpage; x) initiating an internal discussion about peer-reviewed publications.

JIP-MATRIX-WP0-T2 External coordination and communication – activities included: i) routine communication with OHEJP WP4 and OHEJP Coordination Team; ii) inclusion of new partners i.e. Ruokavirasto (Finland) expressed their interest to participate in MATRIX; therefore, an activity plan and an estimated budget were drafted and submitted for approval ; Ruokavirasto (45-RUOKA) is now a partner of the MATRIX Consortium; iii) communicating with other OHEJP projects (from the first and second call), and with external relevant projects and stakeholders; iv) applying for the extension of 2 project tasks and related deliverables (see section 3 of this report); v) applying for a 6-month no-cost extension of the project (agreed at Consortium level).

JIP-MATRIX-WP0-T3 Data management – see paragraph 6 of this report.

JIP-MATRIX-WP0-T4 Sustainability – activities included: i) considering the proximity of the 3 Integrative Projects CARE (DTU Food, Denmark), OH-HARMONY-CAP (SSI, Denmark) and MATRIX (SSI, Denmark) a collaboration was initiated to ensure a joint sustainability strategy; ii) the inventory created during OHEJP JIP ORION project and being updated in MATRIX WP1, has been made available as a shiny-app on the FLI-website; this will be available even after the conclusion of the MATRIX project; iii) continuous identification and documentation of sustainability challenges and opportunities to address them; iv) contributing to the expansion of the project Consortium and network.

6.1.2.1.5.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
JIP05-MATRIX	D-WP2.1	Mapping of the surveillance chain for all hazard tracks, and cross-sectorial linkages	Originally M36; then M42; postponed to M48 (see comments)		M48	The deliverable was originally expected in M36. It was then postponed to M42. In May 2021, a request was made for its postponement until M48. MATRIX staff from the SVA, leader of Task 2.2 that includes this deliverable, have experienced a full turnover in the early months of 2021. This has required an adjustment of the workflow and the creation of new working methods. This happened in the general pandemic scenario, which is still impacting routine operations. The extension was accepted by OHEJP WP4 and it will allow for the completion of this deliverable under better conditions to ensure its quality. The deliverable will be public.	1, 6
JIP05-MATRIX	D-WP4.1	Report on the selected set of criteria for evaluation of epidemiological capacities.	M39	M39		Public. Zenodo link: https://zenodo.org/record/4750976#.YJuOxrVKguU	1, 6

JIP05-MATRIX	D-WP5.1	Report on requirement analysis for "OHS roadmap template"	Originally M45; postponed to M48 (see comments)		M48	The deliverable was expected in M45. A request was made in May 2021 for its postponement until M48. MATRIX staff from SSI, leaders of Task 5.1, which includes this deliverable, have experienced a full turnover in the early months of 2021. This has required an adjustment of the workflow and the creation of new working methods. This happened in the general pandemic scenario, which is still impacting routine operations. The extension was accepted by OHEJP WP4 and it will allow for the completion of this deliverable under better conditions to ensure its quality. The deliverable will be public.	1, 6

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2021 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
MATRIX	M-WP3.1	End of the first round of OHEJP projects: this will coincide with the end of the inventory and requirement analysis phase in most WPs	M36	No	M48	The end of the first round of OHEJP Projects was extended due to the SARS-CoV-2 pandemic. Therefore, MATRIX inventories and requirement analysis obtained the relevant resources directly from the projects instead of relying on the publicly available expected outcomes. A full achievement of this milestone is expected during 2021.

MATRIX	M-WP5.1	Knowledge-platform operational	M42	Yes		The milestone is delivered on time. The platform is operational at: https://oh-surveillance-codex.readthedocs.io/en/latest/index.html

6.1.2.1.5.4 Follow-up of the recommendations and comments by the Ethics Advisors

The Ethics Advisors accepted the comments you provided last January. Therefore, this part of the report can be closed.

6.1.2.1.5.5 Publications and additional outputs

Publications

Publication title, DOI reference and Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? If yes please provide the embargo length and the manuscript release date	Is it a Gold Open Access? If yes please provide the processing charges (in €)
Sundermann EM, Nauta M, Swart A (2021) A ready-to-use dose-response model of *Campylobacter jejuni* implemented in the FSKX-standard. Food Modelling Journal 2: e63309. Doi reference: https://doi.org/10.3897/fmj.2.63309 Zenodo link: https://zenodo.org/record/4923018	Yes		Yes, 0 Euro

Additional output

See the list of activities covered by the JIP-MATRIX-WP5-T4 Training and dissemination. See section 2 of this report.

Relevant MATRIX outputs from WP2 will be included in the Ph.D. project of an expert from 28-IZSAM, member of the MATRIX Consortium and specifically working on WP2, as a case study on the application of the One Health Approach. The project focuses on case studies about the application of One Health in different contexts i.e. vector-borne, food-borne and emerging diseases. The project belongs to the Ph.D. course in "Infectious Diseases, Microbiology and Public Health" at the Sapienza University of Rome (Italy).

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

n.a.

6.1.2.1.5.6 Data Management Plan

Within the MATRIX Consortium, the German Federal Institute for Risk Assessment (BfR) has the responsibility of the MATRIX Data Management Plan since spring 2021. BfR is sending quarterly reminders for updates. A continuous update of the data management plan in the CDP-tool is ongoing.

6.1.2.1.5.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

Several MATRIX members were also members of the JIP ORION project, and thus they are able to transfer results and add value to them within MATRIX. WP1 leaders in MATRIX were also leaders of the work package on epidemiological knowledge sharing in OH in JIP ORION. Leaders of MATRIX WP5 and WP6 were part of an initiative in JIP ORION – called "supra-national One-health pilot" that was working closely with EFSA and ECDC to determine how the OH tools developed in JIP ORION can be applied to the European Union level. We expect that work to benefit directly the roadmap and outreach work that we will develop in MATRIX's WP5.

WP1 has been working closely together with WP2 of JIP ORION, where 10-FLI was actively involved. At the national level, collaborations were continued with 9-BfR as well. Furthermore, 10-FLI is engaged in different working groups and projects with EFSA, working on surveillance and different animal diseases, and EFSA, regarding their terminology and inventory variables.

WP2 leaders have also been leaders of the JIP COHESIVE project WP4 regarding a data platform to facilitate risk-analysis and outbreak control, and deputy leaders of the JRP NOVA project WP2, task 2.1 about the availability of food purchase data and barriers. WP2 is also collaborating with the JRP BeONE in the evaluation and implementation of national level data sharing as lead and deputy lead on several tasks. In addition, national-level collaborations were developed in France with surveillance actors: French Agency for Food, Environmental and Occupational Health & Safety, national reference laboratories on Salmonella and Campylobacter; and Santé Publique France, the National Public Health Agency.

In addition, the WP2 leader is the coordinator of the SIGMA Consortium, a project funded by EFSA, which aims at finding solutions for facilitating the exchange of data between Member States and EFSA. Although the SIGMA project is limited to animal health issues, the data mapping solutions and the tools under development in this project can be of interest for MATRIX, as possible approaches applicable also in food safety. IZSAM is also the coordinator of the EFSA Tender on One Health Zoonoses. The tender aims to provide support to EFSA and ECDC in the production of the EU One Health Zoonoses yearly report, in the related zoonoses online interactive data visualisation dashboards and story maps.

WP3 connected and received support from EFSA, which provided information on current output based surveillance within the EU and links to the EFSA catalogue browser.

In the framework of the WP4, national-level collaborations were developed in France with the Food chain surveillance platform (website, in French: https://www.plateforme-sca.fr/ - description of platform objectives and organisation), especially the research group on Salmonella surveillance. International collaborations were identified with ECDC regarding the EU-LabCap tool and with FAO regarding the SISOT tool.

WP5 is collaborating extensively with other projects. A WP5 member participated in the first JRP DiSCoVer Stakeholder Web-meeting and got the chance to determine potential synergies and collaboration. The extension of the FSKX format planned in WP5 is also relevant for the EJP RADAR project. In the RADAR project, a model repository was developed that is already based on the FSKX format. We are in close communication with the responsible partners. In Task JIP5-WP5-T3, models compliant to the FSKX format are implemented. Mathematical models and their exchange are relevant in multiple other EJP projects e.g. JRP DiSCoVer. The development of FSKX format and the including

metadata schema to annotate knowledge started within the research project “Risk Assessment Modelling and Knowledge Integration Platforms (RAKIP)”. The RAKIP community, with partners from 1-ANSES, 12-DTU Food, and 9-BfR, continues the collaboration to improve and extent the community-driven metadata schema. The work done in WP5 shows high potential for synergies with the work of the WHO/OIE/FAO SISOT-team (Surveillance and Information Sharing Operational Toolkit). We are in contact with the team to align the work and potentially identify reciprocally beneficial tasks. Based on the presentation held at the Cogwheel workshop in November 2020, the SafeConsume community is interested in supporting the OHS Codex. It will be invited to joining upcoming meetings. The close collaboration between MATRIX and JIP ORION was continued i.e. members of MATRIX participated regularly in relevant JIP ORION calls. As the JIP ORION project finished, JIP ORION OHS Codex team members were invited to join the MATRIX OHS Codex call.

WP6 deals with the creation of “data for decision dashboards”. The WP is collaborating with a similar WP in the OHEJP project BeONE.

6.1.2.1.6 JIP06- SARS-CoV2 Research Integration and Preparedness-COVRIN

6.1.2.1.6.1 Summary of the work carried out in the Project

The research work within the COVRIN project will focus on two main overall operational objectives: A) to identify drivers for the emergence and spread of SARS-CoV2. B) to generate data and build models for risk assessment of SARS-CoV2. To achieve this, the COVRIN integrative research activities encompass 4 main areas: 1) Detection of SARS-CoV2 in animal reservoirs and hosts, and the environment: Integration of studies on SARS-CoV2 spread in livestock, wildlife and pets. Optimization and harmonization of RNA detection methods for SARS-CoV-2 detection in wildlife and environmental samples. Definition of bioavailability of virus in fomites, water, and the environment. 2) SARS-CoV2 characterization: Genome analyses; Next generation sequencing of detected isolates and metagenomic sequencing of different samples. In vitro and ex vivo biological characterization of circulating SARS-CoV2 strains. Development, optimization and harmonization of animal models for SARS-CoV2 characterization. Analyses of virus traits related to zoonotic and/or reverse zoonotic transmission. 3) SARS-CoV2 risk assessment and surveillance: Integration of surveillance activities in wildlife, food producing animals, pets and the environment (incl. sewage). Mapping of surveillance data obtained from wildlife, food producing animals, pets and the environment (incl. sewage). Identifying risk factors for virus transmission in wildlife reservoirs, food producing animals and the environment. Establishing models for transmission routes and risk assessment in a One health perspective. 4) Coronavirus preparedness: Study of virus – host interactions, looking into virus evolution and host adaptation. Identifying drivers of virus emergence through evaluation of phylodynamics and cross-species interactions with focus on zoonotic and reverse zoonotic aspects and adaptations. Use of generated data and research outcomes for virus emergence risk modelling to improve surveillance and control strategies, intervention strategies and prevention.

6.1.2.1.6.2 Progress of the project: description of activities

WP0

JIP06-WP0-T0.1

A share-point for data exchange of the different work packages is under construction. This sharepoint will be included in the COVRIN website. Format and procedures are discussed. Management reports are drafted.

JIP06-WP0-T0.2

The COVRIN project page and group have been set up on the OH EJP website. A comprehensive list of contact details for each workpackage, task and subtask has been compiled and uploaded to the secure area of the OH EJP website and is in use for organising meetings and compiling reports.

JIP06-WP0-T0.3

A very successful kick-off meeting was held in March 2021. Work package presentations have been made available through the COVRIN website. A series of regular work package meetings was started by June 2021 and all work packages have had update meetings. Several presentations of the COVRIN project were held for stakeholders, REA, SSB and PMT. The scoping exercise to avoid overlaps with other EU funded projects on Covid-19 has been completed and a report submitted on time.

WP1

JIP06-WP1-T1.1

Data from SARS-CoV-2 genome testing in the different partner institutes are being generated for sharing.

JIP06-WP1-T1.2

An inventory of methods for virus antigen detection in clinical samples from animals, in the different partner institutes is being made with regular updates at task leader meetings.

JIP06-WP1-T1.3

The contributors to this important task assessing the bioavailability of the virus in various matrices have met and agreed an approach. These activities require experiments in high containment with associated detailed planning which is underway.

WP2

JIP06-WP2-T2.1

Sequencing methodologies for SARS-CoV-2 in the different partner institutes are exchanged. SARS-CoV-2 variants are compared and reference samples exchanged.

JIP06-WP2-T2.2

Existing data on in-vitro and in-vivo characterisation of Sars-CoV-2 has been shared at two progress meetings. Antigenicity studies have progressed with initial antigenic maps including variants of concern produced for internal review.

JIP06-WP2-T2.3

Optimisation of animal models is a very specialised activity and the selected partners contributing to this have already shared techniques and ongoing work. The candidate animal models have been selected and experiments are underway and being planned.

JIP06-WP2-T2.4

These experiments on virus traits related to zoonotic transmission are dependent on T2.2 and 2.3 and are currently being planned.

WP3

JIP06-WP3-T3.1

The format/procedure for sampling/surveillance data on wildlife, food producing animals, pets, and the environment, from partner institutes, suitable to inform risk assessment, has been evaluated.

JIP06-WP3-T3.2

Current surveillance activities being carried out in partner institutes are being evaluated.

JIP06-WP3-T3.3

This task, risk factors for virus transmission has been initiated through meetings of task leader and contributors and will be dependent on the outcomes from T3.1, 3.2 and Workpackage 1. This will be facilitated through regular workpackage leader meetings.

JIP06-WP3-T3.4

An overview is made of the different dynamics models and required parameters to test hypotheses regarding the role of animals as potential reservoir hosts and parameters needs.

An overview is made of the different dynamics models and required parameters to test hypotheses regarding the role of animals as potential reservoir hosts and parameters needs.

WP4

JIP06-WP4-T4.1

Plans are underway for this task: Animal coronaviruses will be passaged in vitro and in vivo and ex-vivo in their homologous host or in alternate animal hosts, cells or tissues. Experimental co-infections will be performed in vitro or ex-vivo and the genetic make-up of viral progeny will be investigated by deep sequencing. Isolation of hedgehog coronavirus will be attempted and zoonotic potential of possible isolates will be assessed experimentally using models of Covid19.

JIP06-WP4-T4.2

An inventory of sample types and hot spots for SARS-CoV2 infections is being made. Virus sequencing protocols are being evaluated and discussed.

JIP06-WP4-T4.3

This task is underway following successful task leader meetings: Specific animal communities will be followed over a predefined period of time (RT-PCR, NGS) to monitor intra-community CoV genetic

variation. Data from other WPs and other WP4 tasks will be used to assess the potential of CoV genetic variation on future emergences (for animal species and humans).

6.1.2.1.6.3 Progress of the research project: deliverables and milestones

Deliverables

JRP/JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2021 (month)	Date delivered on Project Group (month)	If deliverable not submitted on time: Forecast delivery date (month)	Comments *Please mention: public or confidential, the Zenodo reference, reason and justification of delay (for instance COVID), other comments*	Proposed category* *(1 to 8) (several categories may be applicable)*
COVRIN	D0.1.1	Share-point for data exchange of the different work packages	M42		51	Deliverable delayed due to delayed project start and staffing issues due to COVID-19. Data currently being exchanged via closed COVRIN members group on OH EJP website	3
COVRIN	D0.3.1	Report scoping exercise	M45	M45		https://zenodo.org/record/5537781#.YVSqy	2,5
COVRIN	D2.1.1	Report on the established website protocol repository.	M45	M45		D-COVRIN.2.1.1 Report on the established website protocol repository | Zenodo	2
COVRIN	D2.3.1	Catalogue of animal models in use, pros and cons, and designed protocol sharing and harmonisation.	M45	M45		COVRIN WP2 D2.3.1 Catalogue of Animal Models | Zenodo	2
COVRIN	D3.1.1	Report on the format and procedure for data integration.	M45	M45		D3.1.1: Report on the format and procedure for integration of SARS-CoV-2 surveillance data | Zenodo	4

COVRIN	D3.4.1	List of existing models and required parameters that need quantification	M45	M45		Public - OHEJP COVRIN D3.4.1: Overview of models and parameters to assess transmission in animals | Zenodo	3,4,5
COVRIN	D4.2.1	Report on relevant sample types and hot spots for Coronavirus sampling.	M42		M48	Deliverable delayed due to delayed start to the project.	2

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020 (month)	Achieved (Yes/No)	If not achieved: Forecast achievement date (month)	Comments
COVRIN	JIP06-WP4-M4.1	In vitro and in vivo experiments performed	M54			On target
COVRIN	JIP06-WP4-M4.2	Samples collected under farming and natural conditions	M57			On target
COVRIN	JIP06-WP4-M4.3	Coronavirus evolution under experimental and natural conditions analyzed	M63			On target
COVRIN	JIP06-WP4-M4.4	impact on the capacity to cross the species barrier evaluated and risk modelized	M63			On target

6.1.2.1.6.4 Follow-up of the recommendations and comments by the Ethics Advisors

Project number	Project name	Requirements Ethical Reviewers, August 2021	Comments Project Leaders, Sep 2021	Recommendations Ethics Advisors, January 2022	Comments Project Leaders, 2022	Recommendations Ethics Advisors, 2022
JIP06	COVRIN	**Human Sample**: In case Human samples will be manipulated, their source must be revealed and the relevant authorisations to obtain them must be presented;	Importance of this cannot be understated and is acknowledged. All partners handling human samples have the necessary national authority and licenses in place. Sources of samples and relevant approval will be included in the deliverable reports			
JIP06	COVRIN	**Animal:** in Tasks 2.3 animal models of SARS-CoV2 infection will be developed, the beneficiaries must demonstrate the implementation of the 3Rs and must present the ethical authorization to conduct the experiments in these animals;	Experiments involving animals will only be undertaken following independent ethical review and under relevant national licences. These will be explicitly stated in the relevant deliverable reports.			

JIP06	COVRIN	**Safety:** the beneficiaries must demonstrate that appropriate safety procedures have been put in place to protect the staff involved in the inoculation studies using different coronaviruses. Also, in case of the manipulation of contaminated Human samples, appropriate safety procedures must be established.	Work on Sars-CoV-2 requires very specialist containment facilities and partners contributing to tasks involving virus propagation and culture are only those with facilities and procedures that meeting or exceed relevant national and international standards.			

6.1.2.1.6.5 Publications and additional outputs

N/A

Additional output

Med-Vet-Net-Association Workshop: Sars-CoV-2 at the Human Animal Interface, Wednesday 1st September

Multiple presentations from COVRIN partners, with over 150 attendees: https://www.mvnassociation.org/Event?id=22

Outcomes (deliverable, publication, folder, tool, etc.) of the project that might be suited for communication purposes to various audiences, for instance food safety or AMR scientists, national and international stakeholders, specific professionals, the general public, etc.

N/A

6.1.2.1.6.6 Data Management Plan

Data management plan training has been undertaken, and the data management plan is currently being drafted

This proposal is explicitly aiming at developing and implementing FAIR data use and management practices. This is key to underpin all future COVRIN initiatives. Therefore, we aim at “FAIRyfication” by design that will keep data practices as simple as possible, using for example the Yoda/iRods portal, to safeguard compliance and sustainability.

6.1.2.1.6.7 Contacts and cooperation with national or international projects, organizations (e.g. ECDC, EFSA, EMA, EEA, FAO, OIE, WHO), networks, or national ministries

A communication channel through REA will be established in order to inform the European Commission and EU Agencies (EFSA, ECDC and EMA) on (interim) knowledge/data.
Knowledge/data interim and final results that could serve for potential Covid19-related risk assessment and/or risk management activities, will be communicated to REA and to relevant EU stakeholders (European Commission and EU Agencies EFSA, ECDC and EMA).

6.1.3 Task 4.3: Integrative support

6.1.3.1 *Subtask 4.3.1: Alignment with strategic initiatives at EU level:*

The cogwheel workshops (CWs) are also being organised by WP4 to allow key actors and relevant partners within the OHEJP, typically Project leaders or WP leaders within Joint Research Projects (JRPs) or Joint Integrative Projects (JIPs), to identify synergies, joint priorities, and opportunities for collaboration within the OHEJP or with other EU initiatives. Strategic interactions with OHEJP are identified in collaboration with WP2 and WP5, to avoid redundancy and to further leverage the alignment at EU level.

The target for the seventh CW was the Versatile Emerging infectious disease Observatory (VEO) project (https://www.veo-europe.eu) and MOnitoring Outbreak events for Disease surveillance in a data science context (MOOD) project (https://mood-h2020.eu). This CW was held as a recorded online meeting with 54 participants; 18 OHEJP projects were represented in total, including one PhD project. Key elements in VEO and MOOD relevant for each One Health EJP project were listed in the meeting summary. For more information see deliverable 4.23.

An *ad hoc* report based on a questionnaire regarding cogwheel workshops was concluded in Y4. The result from this survey showed that the cogwheel workshop concept seems to fulfill its intention and is considered satisfying. As there was still potential in reaching out to more participants in the OHEJP, both regarding information on which external projects to invite and the invitation to the cogwheel workshops, WP4 used these inputs when organizing the seventh CW.

The eighth cogwheel workshop will be arranged in the end of September as an online meeting targeting the project ELIXIR.

The planning process for an OHEJP simulation exercise has started. The exercise will build on the ECDC handbook on simulation exercises and be a Command Post Exercise. This will be a possibility for the OHEJP partners to test cross-sectorial collaboration. A project organisation has been set, an exercise leader recruited and a Steering Board as well as an Advisory Board formed. In the project organisation there is expertise representing Public Health, Animal Health and Food Safety. In the Advisory Board there are representatives from the OHEJP organisation and Stakeholders ECDC and EFSA. The exercise will be performed at the national level with the aim to test the One Health capability, capacity, and interoperability of authorities in public health, animal health and food safety to work together. Skills to be tested are the sharing of outbreak data, intra- and interorganizational communication and the role and functionality of available systems. Participating partners can also add national aims. The exercise will be executed for 3-5 consecutive days per country during the period May to July, 2022. In September the Project Exercise team and the Steering Board had their first meetings.

6.1.3.2 *Subtask 4.3.2: Support function for integration of additional partners in ongoing JIP:*

This subtask is responsible for so called integrative missions, aimed at helping OHEJP partners that are not originally partners of the JIPs to join. Since six new partner institutes joined the OHEJP during Y4, all JIPs were asked to provide options for the new partners to join the projects. The WP4 support team have facilitated this process and guided the new partners in the OHEJP procedures. Three of the new partners have joined CARE (3), OH-HARMONY CAP (1), MATRIX (1) and COVRIN (1). The new partner institutes have also shown a great interest in the Simulation exercise and in various no-cost OHEJP activities.

Because of the COVID-19 crisis, the budget for Short Term Integrative Missions (STIMs) and Integrative Mentoring (IM) Y4 was reallocated for other needs and no such activities have been performed during Y4. However, the close collaboration between the JIPs and the Stakeholders have been further developed. ORION and COHESIVE have provided input to EFSA and the global SISOT tool,

being developed in collaboration between FAO, WHO and OIE. As an example, the OHEJP Glossary and One Health Surveillance (OHS) Codex have been included as dedicated resources into the SISOT toolkit. All JIPs have contact persons at EFSA and ECDC and keep them updated about the project progress.

6.1.3.3 *Subtask 4.3.3: Scientific meetings to enhance and leverage integration:*

Thematic integrative meetings (TIMs) between Joint Research Projects (JRPs) and/or Joint Integrative Projects (JIPs) are also arranged by WP4. The TIMs aim to facilitate integration across domains, but within themes, addressing specific thematic integrative needs and opportunities. This year, the third TIM was held as an online workshop on the subject "*EU surveillance frameworks and infrastructures in a harmonized One Health perspective*", following the invitation from CARE and OH-HARMONY-CAP. The theme was about reference materials, proficiency testing and laboratory protocols. The target groups were Joint Research Projects, Joint Integrative Projects, Stakeholders and EURLs. Encouraging discussion between specialists in different fields was a key objective. The interest for this format seems to increase every year and this TIM attracted 94 participants. For more information see deliverable 4.21. The topic for the next TIM is under discussion with WP3.

6.1.4 Task 4.3: Organisation of call for additional JIPs for the period Y3-Y5

N/A.

6.1.5 Task 4.5: Open data management

All projects have appointed DMP Leaders that manage the project specific Data Management Plans (DMPs). For the first call projects three different templates have been used. The final versions are or should be uploaded to the DMP Group on the OHEJP website and to Zenodo. The second call projects use the online software tool CDP (Lisam®). All second call DMP Leaders have got training in the CDP-tool. In collaboration with WP5 a link from the CDP-tool to the Outcome Inventory has been set up and the public content of the DMPs can be reached by the stakeholders via a Reader´s view. In this way data can be accessed before the projects are finished. Finalized DMPs were reviewed by the DMP Committee and suggestions for improvements communicated to the DMP and Project Leaders. The final DMPs are uploaded to the OHEJP website and when approved to Zenodo. The DMPs of ongoing JRP/JIPs are annually reviewed by the DMP Committee. The overarching OHEJP DMP is also under revision. All OHEJP WPs and Comms Team are asked to provide data and other relevant outcomes that are not listed elsewhere. The work to put this into the CDP-tool is made by WP4. The original OHEJP DMP, found in D4.4 Data Management Plan will be completed with an D4.4 annex.

6.2 Deliverables and Milestones

6.2.1 Deliverables

Del. Rel. No	Deliverable title	Expected Submission	Notification
D4.21	Report from thematic meeting III	M42	Delivered M42
D4.22	3rd periodic report on JIPs	M39	Delivered M39
D4.23	Report from 7th cogwheel workshop	M42	Delivered M40
D4.24	Report on evaluation of finalised JIPs, 1st round	M48	WP4 wishes to postpone this deliverable since only ORION has finished. A combined report on

			the first call projects ORION and COHESIVE will be submitted in M54.
D4.25	Report from 8th cogwheel workshop	M48	

6.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS61	Thirty-five STIMs	M38	Budget Y4 reallocated because of the Covid-19 pandemic.
MS62	Fifteen IM visits	M40	Budget Y4 reallocated because of the Covid-19 pandemic.
MS60	External evaluators for JIPs	M45	External evaluators recruited.

7 WP5 - Science to Policy translation to stakeholders

7.1 Work carried out to date

7.1.1 Task 5.1: Identification of the stakeholders and establishment of communication links

The fourth reporting year (M37-M45) was focused on consolidating the relation with the Key EU stakeholders ECDC and EFSA, and with other European and global stakeholders, as well as with national stakeholders (representatives of ministries of authority, represented in the POC).

The Stakeholders Committee

Exchange with contact officers from the stakeholders' organisations continued to be very good. A formal contact was established with OIE, the organisation representing the last piece in a committee, the Stakeholders Committee, now covering the whole One Health spectrum (human-animal-environment), at the European as well as international level. The Stakeholders Committee is in fact now composed of the key EU Stakeholders, ECDC and EFSA, the European Environment Agency (EEA), the European Medicines Agency (EMA), as well as the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE), and the World Health Organization regional office for Europe (WHO-Euro). Contact representatives of such organisations were appointed to join the Stakeholders Committee of the One Health EJP and to follow the progress of the consortium.

Communication with stakeholders was active by email, on the One Health EJP website, and by web-meetings. One of the major instrument is the Stakeholders Committee Meeting (SCM).

The Stakeholders Committee Meetings

The 6th SCM took place on the 12th of October 2020 as an online event (due to the COVID-19 restrictions). It was attended by EFSA, ECDC (thus overcoming the momentary loosening of the interaction during the third year due to the COVID-19 crisis and response, in which the representative of ECDC was, and still is, deeply involved), EEA, FAO, WHO-Euro, and for the first time OIE.

Unfortunately due to pressing commitments, our contact representative at EMA could not take part in the meeting. She was kept up to date and received all the informative material (presentations, meeting's minutes etc.). The EU funded projects JPIAMR and EU-JAMRAI also participated.

Like the previous SCMs, the 6th SCM aimed at giving an update on current activities of the OHEJP, discussing the needs of the stakeholders and how they are complemented and addressed by the OHEJP, and at discussing the dissemination strategy and impact of the OHEJP outcomes. In addition part of the meeting was themed around AMR, as AMR was identified during the 5th SC meeting as a topic of particular interest to several of the stakeholders. 4 JRP projects with focus on, or dealing with AMR, presented their work including the ongoing collaborations with the represented stakeholders, as well as collaboration with other EU institutions, like the European Union Reference Laboratories (EURL). 2 JIPs of particular interest for the key EU stakeholders also presented their progress. WP5 offered support to strengthen bilateral collaborations between stakeholders and projects. The aims of the meeting were reached well.

In preparation to the meeting, WP5 distributed to stakeholders the 4th Targeted Report to Key EU Stakeholders, and the One Health EJP Thematic Report on AMR (Task 5.4).

The 6th SCM took place in conjunction with the PMC-POC meeting, where representatives of ECDC, EFSA, OIE and WHO-Euro gave their testimonies on their interaction and collaboration with the One Health EJP.

The 7th SCM took place on the 11th of June as a hybrid event allowing participation in person (at the SSI, Copenhagen) and online. It was attended by ECDC, EFSA, FAO, OIE, and WHO-Euro. The aims were to update on current activities of the One Health EJP, to present outcomes and ongoing activities of research projects with a particular focus on environmental aspects, to inform on future dissemination approaches and approaches to increase impact, and to present the sustainability strategy of the One Health EJP. This latter point was of particular importance, and the SCM provided the ideal platform to gather the feedback and advices of EU and international stakeholders to assure optimal sustainability of the lesson learnt during the One Health EJP.

The need of dedicating a section of the meeting to discuss environmental issues was a need expressed by the stakeholders during the previous SCM, as well as during private communications, and often identified during the activity "scanning of stakeholders' documents" (Task 5.2). 4 JRPs presented their work with particular focus on how they address environmental aspects of One Health. The newly started JIP COVRIN, which deals with SARS-CoV2, also presented its progress. The aims of the meeting were fully reached.

In preparation to the meeting, WP5 distributed to stakeholders the 5th Targeted Report to Key EU Stakeholders, and the Thematic report on environmental aspects addressed in One Health EJP activities (Task 5.4).

The 7th SCM took place in conjunction with the ASM2021, in which representatives of the stakeholders agencies took part, and gave welcome speeches.

In the various SCMs, it was stakeholders' opinion that to maximise impact of the One Health EJP outcomes, targeted activities (workshops, seminars etc.) are a suitable tool. To cover this need, WP5 identified specific projects and outcomes of interest by the stakeholders, supported this interaction and improved the links between the projects and stakeholders' nominated contacts (Task 5.3).

In regards to dissemination, reports which are targeted to specific stakeholders or deal with a specific topic were considered optimal by the stakeholders (Task 5.4) and praised for being clear, brief and on the spot. In addition, the stakeholders were always kept up to date with the tools developed to make outcomes available to a broader audience of stakeholders: the One Health EJP Outcome Inventory (OHOI, Task 5.3) and the Data Management Plan (DMP) Reader (Task 5.4).

The 8th SCM is planned for November 2021, in association with the POC/PMC meeting.

Involvement in the enlargement campaign

WP5 was involved in the enlargement campaign of the OHEJP, which aims ideally at the representation of all EU countries in the consortium. In the reporting year 3 a detailed list of organisations suitable for membership was drafted for all the EU countries not yet participating in the OHEJP, as well as for the countries which lack the representation of either an animal health or of a human health organisation. During Y4, WP5 continued to support the Coordination Team with the enlargement campaign, which resulted in six new institution joining the One Health EJP: BIOR (Latvia), ISCIII (Spain), Nébih/NFCSO (Hungary), NMVRVI (Lithuania), Ruokavirasto (Finland), and THL (Finland).

The One Health EJP as a forum to create contacts between the stakeholders

Beside establishing communication links between the One Health EJP and the stakeholders, initiatives of WP5 were also aimed at creating links between the stakeholders. The most recent example is the support given to the Tripartite. WP5 was contacted by the Tripartite team at FAO responsible for the Surveillance and Information Sharing Operational Tool (SISOT), an operational tool of the Tripartite Zoonoses Guide. The Tripartite team aimed at setting a SISOT pilot in the European region and the identified country was Romania, a consortium member state of the One Health EJP. With support of the Coordination Team, informal links were established between the Romanian colleagues and ministries of authority, and the Tripartite team. This initial contact initiated by the One Health EJP is leading to the preparation of the SISOT pilot in an EU Member State. This is an example of how the One Health EJP is seen as a high level forum for exchanges between stakeholders.

7.1.2 Task 5.2: Identification of the research needs of EU stakeholders

WP5 has a number of tools in place to gather the needs of EU and international stakeholders.

Scanning of stakeholders' documents

Regular scanning of stakeholders' documents (publications, reports, regulations, press releases, speeches etc.) is performed, and identified needs are summarised in monthly documents stored in thematic groups of the OHEJP website, where they are available to consortium members and stakeholders. To ease access to such documents, they are also accessible through a dedicated page of the One Health EJP website. The document consist of initial pages with highlights of the month, followed by news summarised in order to highlight information useful for the One Health EJP. This activity is performed in order to keep the consortium up to date with stakeholders' needs, knowledge gaps, policy trends, new regulations, future risks etc.

The websites scanned are updated as needed. At the moment the following websites are scanned: ECDC, EFSA, EMA, EEA, EC Press Corner, European External Action Service, DG-SANTE, European Health Union, JRC, Scientific Committee on Health, Environmental and Emerging Risks (SCHEER), European Council and Council of the EU, European Parliament, EU Action on AMR, European Health Data Space, EC public health, European Parliament Think Tank, FAO (including regional offices), WHO (including regional offices), OIE (including regional offices), EMM Health and Food Safety, ProMED-AMR, Horizon Magazine, Avian Influenza and the Pandemic Threat News Pouch, One Health Initiative, One Health Commission, Eco Health Alliance, Food Navigator, CIDRAP, Health Policy Watch.

WP5 dissemination activities are complementary to the general dissemination activities of the One Health EJP (WP1, Communications Team) and the results of the scanning of stakeholders' documents are available to support other means of dissemination (e.g. social media). In addition the identified needs also help shaping the Strategic Research and Innovation Agenda (SRIA, WP7 Task 7.2).

Direct communication of needs

While the activity of scanning of stakeholders' documents helps keeping the consortium up to date with general needs of the stakeholders, it is an indirect way to identify needs. Direct, active dialogue with stakeholders is important and focuses on research and integrative needs in the area of foodborne zoonoses, antimicrobial resistance and emerging threats. The two approaches supplement each other.

Following earlier discussions, a virtual helpdesk was established to allow easy communication about ad hoc needs. The helpdesk is mainly run by communication through email and can be complemented through a thematic group of the One Health EJP website, allowing documentation of the steps taken to respond to the communicated needs if needed. Most importantly, personal communication via email – formal as well as informal – is well established, and a number of web-meetings were organised to discuss the identified needs, expectations of the stakeholders, and the support that the One Health EJP can offer.

Involvement in sustainability

WP5 also contributes to the sustainability aspects of the OHEJP. Because not all of the stakeholders' needs can be addressed within the lifespan of the consortium, WP5 is involved in drafting the Strategic Research and Innovation Agenda (SRIA), a task led by WP7 (Task 7.2). Furthermore, WP5 is contributing to the shaping of the upcoming European Partnerships to be included in the Horizon Europe Strategic Plan.

7.1.3 Task 5.3: Linking of the scientific capacity available in the EJP with the stakeholders' identified needs: closure of knowledge gaps

The One Health EJP Outcome Inventory

The One Health EJP Outcome Inventory (OHOI, previously referred to as "capacity map") is a tool linking the stakeholders' needs with the scientific and integrative results of the consortium. It highlights outcomes of the OHEJP, supports dissemination of results of the various activities within the consortium (e.g. research projects, integrative activities), and depicts to some extent complementarity with activities outside the One Health EJP. The OHOI is a public online database accessible to all. As such it targets not just ECDC and EFSA and international stakeholders, but also national stakeholders, and supports internal and external collaboration and dissemination. The OHOI was seen as a valuable tool for dissemination of results of the OHEJP to national and international stakeholders, to other One Health initiatives, as well as within the OHEJP. As an internal dissemination tool, the OHOI helps identifying potentially overlapping activities as they emerge, thus minimizing the risk of duplication of work.

The OHOI is accessible though the dedicated page in the One Health EJP website. It is split between an Outcome section and an Updates section.

The Outcome section lists the outcome of the consortium (databases, biobanks, computational methods, pieces of hardware, etc.), gives general information on the specific outcome, highlights the added value by depicting to a certain extent similar activities in place outside of the consortium, and links to specific resources if available. Most importantly it gives the contact information of the persons in charge, facilitating contacts in case more insights are desired.

The Updates section illustrates the progress of the different areas covered in the OHOI in a timely manner. It gives updates on the activities of JIPs and JRPs which are completed, in progress or planned, with reference to the source material where the information was acquired. If an update deals with a specific outcome, it links to the Outcome section of the OHOI. Contacts of the persons responsible for the update are also given.

Both the Outcome and the Updates section open with a short text introducing the tool, and linking to other resources where outcomes are available, like the publication page, the projects' page and, importantly, the Data Management Plan Reader (Task 5.4). Both the section have a search function to

ease navigation and browse the inventory, as well a "new update" column that highlights newest updates. In addition to browsing the database, data of the OHOI can also be downloaded.

The backbone of the capacity map was implemented in the form of a relational database, using the commercial software Caspio.

The OHOI was shaped following thorough discussion with the PMT and the key EU stakeholders. A beta version was deployed in Y3 and improved in Y4 based on users' inputs. The OHOI is now fully available and functional, however suggestions and comments are still being taken into consideration and based on them the OHOI is being constantly improved. One major improvement following feedback was, for example, the addition of outcomes and updates of PhD projects.

Besides the design, initial population of the database, deployment, and technical curation, WP5 is also in charge of the continuous update of the OHOI. WP5 performs two major updates of the OHOI per year, mostly taking information from the annual reports, deliverables and publications, but also from personal communications. WP5 gathers information, which, after being validated by the project leaders, is uploaded on the OHOI. Alternatively, project representatives can require an immediate editing/addition by contacting WP5.

As of May 2021, the Outcome section consists of 84 entries, and the Updates section of 868 entries.

All projects within the One Health EJP are encouraged to use this platform to describe their approaches, skills, tools etc. and to include links to their specific activities and outcomes.

The OHOI is advertised on newsletters, at meetings, at international conferences, and on the One Health EJP website.

Overall, the OHOI increases transparency of the consortium depicting complementarity of ongoing and completed activities as well as improving understanding where future approaches can build on work already performed, increasing thus the sustainability of the consortium. It supports closing of identified gaps and strengthens understanding on different levels (EU, national and institutional) as well as across domains and areas. It provides scientific support to enhance exploitation of results, as well as to follow up on the development of the activities (capacity building, better preparedness).

After the lifespan of the One Health EJP, the OHOI will still be available on the One Health EJP website as downloadable file.

To avoid duplication of efforts within the OHEJP and to support dissemination, WP5 is also involved in the DMP Committee (Task 5.4), supervised by WP4, to coordinate and link the DMP with the OHOI.

WP5 is also in charge of more targeted means of dissemination: the targeted reports and the thematic reports, in which the identified needs of the stakeholders are linked with the OHEJP activities (Task 5.4).

<u>Other ad hoc support</u>

Another way in which knowledge gaps are being closed is through the allocation of resources to support specific stakeholders' needs with specific actions, as well as through the development of specific strategies addressing interests of national, EU and international stakeholders, as agreed following consultation with stakeholders. Members of the Stakeholders Committee (Task 5.1) supported the idea of dissemination workshops, organised in collaboration with WP3, WP4 and WP6 (Task 5.4).

To close knowledge gaps as they emerge, additional funding can be allocated for targeted activities on a case by case base. WP5 coordinates, supervises and facilitates these activities. One example of timely support was the response to the request for assistance of the WHO-Global Outbreak Alert and Response Network (GOARN). In Y3 the One Health EJP was invited to join the WHO-GOARN, and the vast network of the One Health EJP was put at disposal of WHO-GOARN to disseminate its Request for

Assistance aimed to the One Health community. This collaboration continued during Y4 and expanded to include the One Health Commission. Together with WHO-GOARN and the One Health Commission, OHEJP ran a global survey to examine the value of One Health workforce and of One Health networks in response to the COVID-19 pandemic.

Contribution to stakeholders' consultations and other EU and international One Health initiatives

The One Health EJP was involved as stakeholder in a number of consultations, either following invitation or by its own initiative, thus employing the knowledge created by the One Health EJP consortium to shape the international One Health environment.

The Coordination Team, together with WP5, gave input to the Tripartite's "Call for Public notice and comments on the Terms of Reference of a High-Level Expert group on One Health (FAO, OIE, WHO)", the EC's "EU4Health stakeholders consultation", EFSA's "consultation on the draft EFSA Strategy 2027", and is currently involved in the preparation of PREZODE. The One Health EJP offered additional support to all of the aforementioned stakeholders' activities

Thanks to informal networking activity and to the activity of scanning of stakeholders' documents (Task 5.2), the One Health EJP consortium is kept up to date with opportunities to give its input to One Health initiatives at the EU and international level.

Support of bilateral collaborations

In addition to the vast array of WP5 mediated activities, bilateral collaborations between stakeholders and projects are in place. WP5 encourages stakeholders' representatives to participate in meetings and activities of JIPs and JRPs. One of such instances is the appointment of point of contacts at EFSA and EMA for the new JIP COVRIN, an initiative mediated by WP5.

Survey of interactions with national stakeholders

Given the reference mandate of One Health EJP members, a number of solutions produced by the projects are already in place at the national level. A number of methods optimised thanks to One Health EJP research are, for example, already in use at national Reference Laboratories. Because these interactions are managed by the single projects, they sometimes fall under the radar. To close this gap WP5, in collaboration with the Support Team, has been working on strategies to survey such interactions. WP5 aims to collect instances of interactions with national stakeholders in order to understand in which fields the work of the One Health EJP is having the most impact at the national level.

This mapping exercise is already in place in the form of a German Pilot, with the support of the German Mirror Group.

Efforts of the One Health EJP in closing stakeholders' knowledge gaps were acknowledged by the stakeholders at One Health EJP meeting (e.g. by giving testimonies at the POC-PMC meeting or at the Annual Scientific Meetings), and in stakeholders' publications (e.g. in this EFSA editorial).

7.1.4 Task 5.4: Dissemination of new knowledge, tools and materials

WP5 implemented a variety of general (e.g. the OHOI, Task 5.3) and tailored dissemination strategies in order to meet the needs of national, European and international stakeholders. This maximises the impact of the consortium's outputs and ensures taht the tools and results of the OHEJP are used in a timely manner.

The overall dissemination strategy is regularly discussed with stakeholders, particularly during the SCMs, and subjected to revision.

The various activities of WP5 were presented at the Annual Scientific Meeting 2021 in the form of 5 poster presentations and 1 oral presentation.

Targeted and Thematic Reports

ECDC and EFSA are kept up to date about the scientific outputs of the consortium through Targeted Reports. These reports consist of a concise description of results and ongoing work of JIPs and JRPs, focusing on projects of highest interest for the stakeholders, as well as general announcements from the OHEJP consortium, and scientific publications of all the projects. The documents also supports linkage with external resources, as well as with contact persons in case more insights on specific topics are needed.

Two targeted reports to Key EU stakeholders per year are produced and distributed. Even though these reports are targeted mainly to ECDC and EFSA, the 4th and 5th targeted report were distributed also to EEA, EMA, FAO, OIE and WHO-EURO. The feedback received was very positive, and the brief and clear nature of the Targeted Reports was particularly appreciated.

In addition to these regular reports, to highlight the responsiveness and timeliness of the OHEJP, WP5 produces ad hoc Thematic Reports. These reports are written in response to stakeholders' needs and depict how the work of the One Health EJP closes knowledge gaps of stakeholders' organisations. The report "One Health EJP Thematic Report on environmental aspects addressed in One Health EJP activities" was published in May 2021, and provides an overview of the key outcomes produced by the One Health EJP projects on environmental aspects of One Health, by end of April 2021. Projects, which outcomes are described in the report, were selected in an unbiased way based on frequency of keywords reported on projects' annual and final reports. The report highlights the importance of considering environmental issues in the light of recent EU policies.

All the ad hoc reports are published on the dedicated page of the One Health EJP website, as well as on the Zenodo platform. To maximise its visibility, the "One Health EJP Thematic Report on environmental aspects addressed in One Health EJP activities" was disseminated by a number of means, including to the stakeholders' agencies (ECDC, EFSA, EEA, EMA, FAO, OIE, WHO-Euro) with the request of internal distribution, as well as through the general dissemination channels of the One Health EJP (Communications Team). The feedback from the stakeholders was very positive.

As of June 2021, 6 ad hoc reports were released and are available on Zenodo and on the dedicated page of the One Health EJP website. Due to the particular importance of one of such reports, "Links between COVID-19 related needs of stakeholders and One Health EJP activities", the document was further made into an extra deliverable, D5.12, and submitted in M38.

Involvement in the Data Management Plan committee and the DMP Reader

WP5 collaborates with WP4 in the Data Management Plan (DMP) committee. In particular, this collaboration between WPs ensures the complementarity between Outcome Inventory (OHOI) and the projects' DMPs.

WP5 supported the adaptation of the DMP Collaborative Data Platform (CDP) for the use of the One Health EJP projects (led by WP4, Task 4.5), and was involved in the assessment of a number of DMPs of first round projects.

Final DMPs are publicly available at the end of each project. While this provides FAIRness to the data, it does not ensure the timely availability of outcomes. In order to bridge this gap, WP5 in collaboration with the Communications Team, with WP4 and with the company providing the software (Lisam), set up a DMP Reader, where entries of projects' DMPs could be publicly accessed before the end of the projects. The DMP leader of each project can chose whether to give public accessibility to each entry immediately, or to keep the entry "as draft" until he/she deems appropriate. The entries set as publicly accessible are immediately visible to all users through the ad hoc set up DMP Reader. The DMP Reader is accessible through the DMP page of the One Health EJP website. In this way DMP data are made available for the use of a large group of stakeholders, notably national and EU risk assessors, and other One Health initiatives.

The DMP Reader was presented to stakeholders during the 7th SCM and in more than one occasion to project representatives (for example during the DMP leaders meeting, and during the Project Leaders Forum).

Dissemination of One Health EJP events to the stakeholders

The broad spectrum of initiatives of the One Health EJP, not limited to WP5 initiatives, are often of interest of the stakeholders. WP5 played a role not just in disseminating information to the stakeholders' agencies, but also, in collaboration with WP6, in distributing VIP places. These VIP places consist in facilitated access for stakeholder agencies representatives (e.g. free of charge entries). Sometimes the high interest in VIP places from the stakeholders' side led to the establishment more VIP places than was initially planned. This was the case, for example of the Summer School 2021 on Environmental Issues in One Health, and of the Annual Scientific Meeting 2021.

All the stakeholders' agencies (EFSA, ECDC, EEA, EMA, FAO, OIE, WHO-Euro) joined One Health EJP organised initiatives. They played an active role in such events not merely by participating, but by giving seminars, talks and lectures.

Calls for One Health EJP initiatives were further disseminated by stakeholders (e.g. through EFSA newsletter, social media, and through internal dissemination), and by other One Health initiatives (e.g. One Health Commission newsletter).

Dissemination of stakeholders' calls/material to the One Health EJP

Dissemination efforts go both from the consortium to the stakeholders, and from the stakeholders to the consortium. One example of the latter is the hosting on the One Health EJP website of policy documents and recommendations produced by EU-JAMRAI, which ended in February 2021.

Other examples are the support given to the International Student One Health Alliance and JPIAMR in disseminating their call to the One Health EJP network of institutions.

Dissemination to national stakeholders: WP5 presence at national stakeholders' meetings

Beside EU and international stakeholders, the One Health EJP addresses the needs of national stakeholders. Member institutions of the consortium have in fact mandate from line ministries, which also accepted the One Health EJP scientific agenda. National stakeholders are represented in the Programme Owners Committee (POC), in the Programme Managers Committee (PMC), and in the Scientific Steering Board (SSB). These committees are composed of representatives of ministries, CEOs/Directors of the One Health EJP institutions, and scientific directors respectively. To maximize the visibility of the One Health EJP and WP5 to national stakeholders, presentations were given at the SSB meetings (the latest one being in March 2021) and will be given at the POC-PMC meeting (November 2021). In such meetings thematic reports (Task 5.3) were/will be presented and disseminated to national stakeholders.

Dissemination to national, EU and international stakeholders: the dissemination workshops

Dissemination workshops are a form of scientific support to stakeholders which addresses specific needs of national, EU and international stakeholders.

Target audience is mostly national stakeholders: experts at the level of ministries (e.g. risk managers with a scientific background) and decision/policy makers (upper level of the hierarchy, POC members with limited scientific background). Although addressed primarily at national stakeholders, EU (ECDC, EFSA, EEA, EMA) and international (FAO, OIE, WHO-Euro) stakeholders are welcome to participate.

Each dissemination workshop is centred around one topic, with a number of project presenting different aspects of the topic. Topics were pre-selected based on the WP3 survey on common activities, on analyses of reports by WP5, and on further discussion between WP3, WP4, and WP5. Pre-selected topics were presented at the SSB meeting the 18th of March 2021, and participants to the

meeting were given the possibility to vote for the topics, and to suggest further topics. The topic of the first dissemination workshop will be metagenomics. Project dealing with aspects of metagenomics (e.g. detection, standardisation of methods, source attribution) were identified, and a preparatory meeting is planned. This first workshop is planned for the second half of 2021.

Focus of the dissemination workshops is on the application of the solutions presented, e.g. case studies, how were the solutions used in a country, how could they potentially be used in specific situations.

The format of the dissemination workshops should be appealing to the target audience. Because the audience is composed of experts with some scientific background, as well as representative of national ministries with limited scientific background, the workshops are divided in an initial part slightly more technical, and a second part more concise for non-technical audience.

In order to maximise the usefulness of the dissemination workshops, and to ensure its sustainability, the workshops are complemented with a dissemination package that includes: a briefing with information on the scope of the workshop distributed before the workshop itself; the recording of the workshop; and a concise report of outcomes (highlights, key messages, incl. recommendations discussed and future needs) distributed after the workshop. This dissemination package could be used after the workshop itself, thus maximising its impact.

The dissemination workshops were presented to project leaders at the Project Leader Forum of the 8th of June 2021, and at the 7th SCM.

7.2 Deliverables and Milestones

7.2.1 Deliverables

Del. Ref.	Deliverable title	Expected ssion	Notification
D5.12	Report on "Links between COVID-19 related needs of stakeholders and One Health EJP activities"	M38	Timely submitted
D5.9	Fourth annual report on dissemination activities to international stakeholders	M48	Timely submitted

7.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS71	Identification of new knowledge gaps and research needs n°3	M48	Milestone duly delivered thanks to regular scanning and communications mechanisms in place
MS75	Scientific support provided n°3	M48	Beside regular support, ad hoc support provided when needed (e.g. WHO-GOARN, SISOT), and targeted to specific stakeholders' needs and groups (e.g. dissemination

			workshops primarily for national stakeholders)

8 WP6 - Education and training

8.1 Work carried out to date

In M37, the calls for the Education and Training activities planned for 2022 were all launched on 18th January 2021 (M37) and were initially given a deadline of 14th June 2021 (M42). To try to increase the number of applications to all our calls, a dedicated Education and Training "open calls" page was created on the OHEJP website where a short video was created with the WP6 Project Manager promoting the key points of each call, complemented with a dedicated flyer which were made available on the website in collaboration with the Communications Team, with links to the relevant guidelines and application form on the private space of the OHEJP website.

The validated procedures and protocols were followed for the calls that received applications. Six STM applications were received through the STM 2022 call. One application was received to organise the ASM Satelite Workshop 2022.

There were no applications received to organise the third or fourth CPD modules or the fourth summer school. These calls were extended until M47.

8.1.1 Task 6.1: Short-Term Missions

The four STMs selected through the STM 2021 call have been affected by the ongoing travel restrictions. These missions have now been postponed until 2022. Similarly, the two STMs funded through the STM 2020 call which were already postponed, have been further postponed until 2022.

In M38, D6.10, report no.2 of the annual STMs completed was submitted on time as planned and uploaded to the website. The two reports of the successful STMs that took place in early 2020 before the lockdown were included in the deliverable, case studies were consequently created and uploaded to the STM 2020 page of the website, and these were disseminated via several internal and external channels- e.g. social media, the monthly Education and Training bulletin and OHEJP newsletters.

Six STM applications were received through the STM 2022 call launched in M37. Each application was sent to three independent reviewers. Two of six applications were amended based on feedback provided by the reviewers. The final versions of all applications were validated by the PMT, communicated to the SSB and were uploaded to the new STM 2022 page on the website.

8.1.2 Task 6.2: Workshop programme (satellite to Annual Scientific Meetings)

The ASM Satellite Workshop 2021 was organised by the German Federal Institute for Risk Assessment (with support from the WP6 and communications teams based at the University of Surrey) and took place successfully as an online event in M42. The workshop was open to internal and external audiences and was targeted towards anyone with an interest in One Health related digital applications, and in particular, PhD candidates and junior researchers and professionals were encouraged to apply and participate.

As with all WP6 previous events, a dedicated page on our website for this event was available where the event flyer, programme, bios and photos of the presenters and an information about the workshop and associated deadlines was found. The link to apply to attend could also be found on this webapage. The ASM Satellite workshop 2021 page can be found here. This page was used as a base for the promotional campaign on social media and other routes such as the monthly Education and Training bulletin, the EFSA newsletter, and the OHEJP consortium and external newsletters. The promotional campaign took place between February and June 2021, and more information about the campaign will be detailed in D6.11. The event website page and promotional branded tools for the event were produced by the OHEJP communications team.

The promotional campaign was a success, and 70 delegates from 29 countries worldwide registered to attend the workshop.

The workshop was titled 'Online Software Fair' and took placed on 7th June 2021. Researchers and One Health professionals who deal with data generation, data integration and data analysis tasks, for example during disease surveillance or outbreak investigations, need to be continuously informed about new digital solutions that support these activities and promote the One Health compliant exchange of information across sectors. The Online Software Fair allowed participants to get informed on 9 selected innovative digital software tools, and provided members of all interested OHEJP projects, a platform to present their new tools/software in greater detail compared to a classical conference presentation setup.

The online event started with pitch presentations from the nine presenters to provide a brief overview on each tool to all workshop participants followed by nine individual breakout rooms which allowed participants to get detailed information and presentations from the presenters.

The BfR team created an e-learning course to access pitch recordings and slides from the workshop, along with slides from the breakout rooms. On the same platform, delegates completed the event evaluation survey and request a certificate of participation.

After the workshop, WP6 wrote a blog post in collaboration which was validated by the local organisers describing the key contents of the programme, successes and highlights from the workshop. This was posted on the OHEJP blog page and was used to promote the success of our education and training activities. This was promoted on social media, the monthly education and training bulletins, OHEJP newsletters, and meetings with the SSB, PMC-POC and PMT.

The ASM 2021 deliverable report D6.11 will be submitted in M48 as planned and will have the full details of the organisation and achievements of the event.

Finally, the call to organise the ASM satellite workshop in 2022 was launched in M37. One application was received from the University of Surrey and NUIGalway. This application was reviewed by PMT who provided their validation and feedback to the applicants who adapted the programme to ensure these points were addressed. The WP6 team have organised monthly meetings with the local organisers and communications team between September 2022 and the event to monitor and support the organisation of this event. Like with previous training events, WP6 have also provided the local organisers with a branded procedure which describes the input and expectations of the WP6 team, communications team and the local organisers, and also the optional services offered by the communications team. In addition, the WP6 team manage a central spreadsheet to mintor the event organisation milestones from the start to delivery.

8.1.3 Task 6.3: 'One health' Summer School for medical and veterinary science

The Summer School 2021 organised by the Italian National Institute of Health - Istituto Superiore di Sanità, ISS (with support from the WP6 and communications teams based at the University of Surrey) and took place successfully as a two-week online event from 26th July to 6th August 2021. The event was targeted towards early career researchers (post docs, junior researchers, and staff) and PhD candidates involved in all aspects of One Health. This was a global event open to both internal and external audiences.

As with all WP6 previous events, a dedicated page on our website for this event was available as described above. The Summer School 2021 page can be found here. This page was used as a base for the promotional campaign on social media and other routes such as the monthly Education and Training bulletin, the EFSA newsletter, and the OHEJP consortium and external newsletters. The promotional campaign took place between October 2020 and July 2021, and more information about

the campaign will be detailed in D6.12. The event website page and promotional branded tools for the event were produced by the OHEJP communications team.

The Summer School was titled 'Environmental Issues in One Health- from risk assessment to surveillance'. The environment is one pillar of One Health, and environmental issues need to be illustrated and discussed in their multi-faceted aspects: risk assessment, role of ecosystem related factors, role of manmade factors, the farm as environmental modifier, and the issue of sustainability. Therefore, this Summer School was split into four modules and provided interdisciplinary training on a range of One Health topics. The first module included One Health topics such as planetary health, environmental risk assessment, climate change, zoonoses and antimicrobial resistance in the natural environment, the use of genomics in surveillance, wildlife, feeds, health sustainability and epidemiology. The second module included a workshop on One Health, sustainability and food security delivered by the FAO. The third module included two workshops from our partners in Italy (IZS and ISS). These workshops worked on two different One Health case studies: One Health surveillance of AMR and a multidisciplinary One Health approach to environmental pollution. The final module included topics such as integrated risk analysis, One Health governance and science to society understanding. The programme also included a mini workshop on communication in One Health led by the One Health EJP communications team with input from the WP5 Science to Policy team, and communication experts in our consortium.

Invited speakers provided up-to-date insights on environmental issues in One Health from multiple viewpoints, including interventions of experts from EFSA, EEA, FAO, OIE and WHO. Working groups were organised at the start of the Summer School and were met daily to foster the interactive potential of the training.

The deadline to apply closed in M41, and 280 applications were received. This was the most successful round of applications of our summer schools to date. In addition, six VIP places were taken from EFSA, EEA, OIE and FAO. Five VIP places were initially offered, however due to the popularity, an additional place was offered.

After the summer school, WP6 are collaborating on a blog post with the local organisers to describe the key contents of the programme, successes and highlights from the event. In addition, the WP6 team are working with the local organisers and an external company to create a post-success video for the summer school using the recordings and other content captured (with permission) from the event. One finalised, this will be posted on OHEJP website and will promote the success of our education and training activities through the internal and external communication channels including social media.

The full details of the Summer School will be available in D6.12, which will be submitted in M48.

Finally, the call to organise the Summer School in 2022 was launched in M37. There were no applications received for this call, and the call was extended to M47 with the expectation that this event will take place in Y5. In addition to promoting the call via the education and training monthly bulletin and newsletters, WP6 made specific requests to PMT, SSB and new consortium partners to forward to contacts in their respective institutes.

8.1.4 Task 6.4: Doctoral Training Programme

In M37-M45, WP6 continued to support and monitor the progress and dissemination activities of the PhD projects, and provide guidance to the PhD main supervisors and their respective PhD candidates to ensure WP6 can monitor the progress of the projects effectively through submission of their project deliverables and publications. WP6 ensured that the PhD deliverables submission, dissemination, and reporting procedures were updated and adhered to, along with the scientific publication policy.

In total, in M37-M45, 15 deliverables were submitted (5 confidential deliverables, 10 public deliverables) and one more publication was produced in addition to two previous publications in M35

and M36. Further details of the progress of each PhD project are discussed in more detail in the individual PhD reports in this deliverable.

All public deliverables and publications were uploaded to Zenodo by the PhD supervisors, and subsequently, to the PhD project pages on the website by the OHEJP communications team, who wherever possible, promoted these dissemination activities through our social media channels.

All dissemination activities were also reported to the coordination team via the Internal Events Survey. All confidential deliverables have been uploaded to the private space of the OHEJP website in individual project groups to which the supervisor and student have access and control. A confidentiality deliverable statement form has been created by WP6, which will be used going forwards (and retrospectively) to have in place until the deliverable can be made public, as evidence that this deliverable exists, and details of when it will be made public.

In M37-38, all PhD supervisors and their students submitted their 12-month reports reporting on the progress, outcomes and collaborative activities over M25-36. WP6 worked closely with the supervisors and advised them as required to complete the template reports. WP6 then worked with the individual 12-month reports to produce the deliverable report – D6.21 – third periodic report on the PhDs, which was submitted on time in M39.

The PhD candidates participated to a great extent at the Annual Scientific Meeting in M42. All 17 PhD candidates submitted an abstract. Sixteen PhD project abstracts were accepted for poster presentations, and one PhD project (Sarah Humboldt-Dachroeden, SUSTAIN project) was accepted for an oral presentation. Of the sixteen poster presentations, one poster (Olivia Turner, WILBR project) was awarded the Poster Prize selected from 160 posters. As this was virtual, there was an opportunity for students to submit a short oral presentation of the posters.

WP6 also hosted and organised the second virtual Three Minute Thesis (3MT) PhD competition at the ASM through Zoom, which was broadcasted live on 10th June through the ASM livestream platform. Unlike the first 3MT competition, the competition this year was made to be live to make the experience as close to a physical event as possible, and to train the students to prepare for such presentations. All PhD candidates participated and showcased their projects in this competition to a very high standard. This was evident from the high scores and positive comments received from the evaluation panel who were three independent members of the ASM Scientific Committee. Finally, the winner of the 3MT 2021 was selected and announced at the close of the ASM - Marieke de Cock, DESIRE project.

Two PhD candidates (Sarah Humboldt-Dachroeden, SUSTAIN and Mahbod Entezami, MACE) also volunteered to organise and host the first ASM Social Quiz following guidelines prepared by the WP6 team in collaboration with the ASM organisers. The ASM social quiz was managed and setup on Kahoot and was a very successful social interaction and strategy to engage the audience, especially those who attended virtually. The quiz was held at the start of each coffee break (5 mins) and the winner from each session was an awarded a certificate after the conference. One of the students hosted the quiz and attended the conference on-site, which helped make the quiz work seamlessly. Both students were awarded certificates for 'ASM 2021 Quiz Master'.

Some PhD candidates also participated in the other training activities by WP6 to learn and expand their networks with a new generation of 'One Health' researchers e.g., participated in the second CPD module and the third Summer School. One of the STMs funded to take place in 2021 was awarded to an OHEJP PhD student (Marieke de Cock, DESIRE), but has been postponed due to travel restrictions implemented because of COVID-19. Two PhD students applied to the STM 2022 call, and their applications were selected to be co-funded – Laura Gonzalez Viletta (EnvDis) and Ingrid Cardenas Rey (VIMOGUT).

PhD candidates also not only attended, but also presented at the 6th cogwheel workshop organised by Work Package 4 in collaboration with Versatile Emerging infectious disease Observatory. These are

meetings between OHEJP partners and strategically interesting external projects or initiatives. The objective of these workshops is to avoid duplication of research activities and to find synergies and opportunities for knowledge transfer and/or collaboration. For more details, please read D4.20 – report from the 6th cogwheel workshop. PhD candidates continue to attend cogwheel workshops to take advantage of the unique learning and collaborative opportunities available.

To report the progress of the project and to evaluate the COVID-19 impact, PhD supervisors and students submitted a 9-month summary progress report to WP6 through the templates provided, to inform the content this Summary Progress Report Y4. The 9M PhD reports can be found here below.

8.1.4.1 *Detail of the PhDs activity reported in 9M reports*

8.1.4.1.1 PhD01-R1-AMR2-ECO-HEN

8.1.4.1.1.1 Summary

During these 9 months, from January 2021 to September 2021, the student has carried out the following activities.

- She has joined in June the annual scientific meeting that took place online. During the congress, the student had the opportunity both to share part of her work and to listen to the work of other colleges. In addition, she presented a poster.
- During the same congress, the student also participated in the "3 minutes thesis" competition, in which she was able to summarise her project and present it to her colleagues. This contributed to the development of her oral and written dissemination skills.
- Her writing abilities were further developed due to the preparation of a manuscript as well as the preparation of an abstract for the oral presentation of an upcoming congress.
- The student participated during these months in the university activity called: doctoral seminars. Through the preparation and presentation of the seminar, the following competencies were developed: summarizing, processing and critical analysis of the data produced and, the public presentation of her activity. By attending other seminars, the student acquired competencies and skills in critical analysis and public questioning, and came to be more familiar with the methodologies and objectives of other topics of research.
- In February, the student participated in Digital innovations for One Health practitioners workshop, training on innovative digital software solutions supporting risk assessment, zoonotic outbreak investigations and data interoperability. She had the opportunity to familiarise herself with other One Health EJP projects as ORION, COHESIVE and RADAR and she was introduced to open-source software tools focused on different disciplines.
- The student also participated as an assistant in the laboratory activities of the Microbiology and Immunology subject belonging to the Veterinary degree, acquiring teaching skills.
- The student participated as a delegate in the summer school 2021, which took place online from 26 July to 6 August. She learned about the role of the environment within One Health, as well as how to set up surveillance systems in different countries for different diseases.

8.1.4.1.1.2 Overview of Project Progress

Tasks related to the characterization of *E. coli* isolates from animals (WP1 -WP4 and WP6) are almost finished, and two manuscripts are in preparation.
Tasks related to the characterization of *E. coli* from eggs (WP5, WP7 and WP8) will be addressed along July to October.

8.1.4.1.1.3 Progress of the research performed in the PhD project and key scientific results

During the reporting period (January 2021 to September 2021) the PhD work was focused on the in deep analysis of cefotaxime- resistant *E. coli* strains isolated from the studied farm.

WP5. Reconstruction of plasmids spreading AMR genes from animals' to egg shell's isolates

By studying cefotaxime-resistant isolates, we identified different plasmids harboring genes responsible for cephalosporin resistance:

- IncI1/ST3 plasmids were found harboring $bla_{CTX\text{-}M\text{-}1}$ gene in *E. coli* isolates from day-old chicks, pullets and laying hens. These IncI1/ST3 plasmids also harbored the sulfonamides resistance gene *sul2* and tetracycline resistance gene *tet(A)*. $Bla_{CTX\text{-}M\text{-}1}$ has also been found in the chromosomal DNA in some isolates, together with an IncX1 plasmid harboring beta lactam resistance gene $bla_{TEM\text{-}1B}$ and tetracycline resistance one *tet(A).* both $bla_{CTX\text{-}M\text{-}1}$ genes, the one

identified in plasmid and the one identified in chromosome, have been identified close to an insertion sequence *ISECp1*.

- IncI1/ST26 plasmids have been identified harboring the ESBL bla_{SHV-12} gene. These plasmids have been identified harboring also the *tet(A)* gene and a class 1 integron with streptomycin/spectinomycin resistance genes *aadA1* and *aadA2*, chloramphenicol resistance gene *cmlA1* and sulfonamides resistance gene *sul3*. Isolates with this plasmids have been identified in the farm in isolates from pullets and laying hens. Moreover, this isolates also had IncX1 plasmids with bla_{TEM-1B} and quinolones resistance gene *qnrS1*.
- The ampC resistance gene bla_{CMY-2} has been identified in two different plasmids in the farm. IncI1/ST plasmids have been identified in laying hens harboring this gene, presenting these isolates also a class 1 integron in a non-typeable plasmid. In addition, bla_{CMY-2} gene has been main identified in a IncK2 plasmids in isolates from all the growing phases in the farm (from day-old chicks to laying hens). Gene in both plasmids has been identified downstream of an insertion sequence *ISECp1*, which can facilitate gene insertion in mobile genetic elements.

Plasmid stability experiments. We studied the stability of a large plasmid harboring the gene of interest *dfrA36*. This experiment was conducted up to 100 generations in the absence of antibiotic selective pressure. After 100 generations, more than 80% of the cells lost the plasmid. The stability of this plasmid in the presence of antibiotic was also studied up to generation 20, with 20% of the cells lost the plasmid up to this generation, compared to 50% lost after 20 generations in the absence of selective pressure. This means that this large plasmid is lost over generations even with antibiotic pressure, although less abruptly in the presence of antibiotic in the medium.
In addition, a conjugation experiment was performed with the same plasmid. A rifampicin-resistant strain of *E. coli* was used as receptor, and the isolates with the plasmid of interest as a donor. Conjugation efficiency was calculated by counting donor colonies (ampicillin-resistant), receptor colonies (rifampicin-resistant) and transconjugants (ampicillin and rifampicin resistant) with the formula:

$$Conjugacion\ efficiency\ (CE) = \log\left(\frac{Transconjugants\ (T)}{\sqrt{Donor\ (D)\ x\ Receptor\ (R)}}\right)$$

A result of 6.4% conjugation efficiency was obtained.

WP6. Flow of AMR isolates between animals. The objective of this WP is to follow the dynamics of AMR, both, isolates and associated platforms, from day-old chicks to pullets and laying hens. M28-M33 (april / sept – 2020).

All the sequenced isolates obtained along the study (including isolates from eggs) will be used for a phylogenetic analysis to address this WP.
Deviation: this task will be taken together with WP7 for improving the analysis, and, although a preliminary analysis has been performed, we have to wait until WP.7.1 (Checking on the isolates database looking for non-sequenced egg isolates) will be finished.

WP7. Flow of AMR isolates from animals to egg shells

As explained above, WP6 and WP7 will be merged for improving our knowledge about AMR flow in the farm including both animals and eggs.

WP8. Writing of PhD thesis.

This activity will be delayed until October 2021.

8.1.4.1.1.4 Progress of the research project: milestones and deliverables

8.1.4.1.1.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD1-AMR2-ECO-HEN	D-PhD01-5.1.	A list of plasmids putatively present on *E. coli* isolates	M27 (March 2020)	M31 (July 2020)		Delay due to the coronavirus pandemic.
	D- PhD01-6.1	A list of *E. coli* from animals isolates to be sequenced	M28 (April 2020)	M33 (July 2020)		Delay due to the coronavirus pandemic
	D- PhD01-6.2.	Manuscript	M33 (September 2020)		M47	Delayed due to laboratory work.
	D- PhD01-7.1.	A list of *E. coli* from eggs to be sequenced	M37 (January 2021)	M36 (December 2020)		
	D- PhD01-8.1.	Manuscript	M42 (June 2021)	M43		Sent to a journal July 2021
	D- PhD01-9.1.	Doctoral thesis draft	M48 (December 2021)		M53	Delayed

8.1.4.1.1.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD1-AMR2-ECO-HEN	M-E14-1	Updated list of phenotypic features of the *E. coli* isolates collection	M15 (March 2019)	Yes		
	M-E14-2	Training of the PhD-student on bioinformatic analysis of raw sequences	M17 (May 2019)	Yes		Achieved September 2019
	M-E14-3	New sequenced *E. coli* isolates from animals	M33 (September 2020)	Yes		Achieved April 2021
	M-E14-4	New sequenced *E. coli* isolates from eggs	M37 (January 2021)	No	M47	Delayed

8.1.4.1.1.5 Soft Skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
OHEJP Summer School 2021	Environmental issues in One Health	26th July to 6th August 2021	ISS (Italian Ministry of Health)

8.1.4.1.1.6 Publications and Patents

Two communications were presented at two different congresses:

1. Dynamics of extended spectrum β-lactamase resistance gene *bla*$_{SHV-12}$ in a laying hen commercial farm (Poster presentation in Annual Scientific Meeting 2021 of One Health EJP, Hybrid event, 06/09/2019-06/11/2021)

2. *Bla$_{CMY-2}$* gene dynamics in a commercial egg production farm (Oral presentation in XXVII Microbiology Spanish Society National Congress of Microbiology, Online event, 06/28/2021-07/02/2021)

8.1.4.1.1.7 Remarkable outcomes

N/A

8.1.4.1.1.8 Impact and Relevance

As mentioned in previous reports, during this nine-month period (January-September 2021) the PhD project has not been in direct collaboration with external institutes; nevertheless, the PhD student is in close contact with VISAVET researchers working in other ongoing OneHealthEJP projects like ADONIS, DISCOVER and MATRIX for improving their bioinformatics skills.

8.1.4.1.1.9 Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers comments have been accepted and this is closed.

8.1.4.1.1.10 Impact of COVID-19 crisis on the project

As it was mentioned in the previous yearly report, a delay of all the milestones and deliverables scheduled from March 2020 for approximately four/five months must be applied.

8.1.4.1.1.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	Yes[1]
Loss of technical training staff delaying progress of the work	Yes[2]
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No[3]
Other risks (please describe)	

Additional information:

[1]The DTU supervisor, Dr. Valeria Bortolaia was not working at DTU. A new DTU supervisor, Dr. Pimlapas Leekitcharoenphon, is now in charge.

[2] The UCM PhD co-supervisor Prof. Dr. Alicia Gibello passed away last May, so some rearrangements are in motion.

[3] Not included those related to coronavirus pandemic.

8.1.4.1.1.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

N/A

8.1.4.1.1.13 List of dissemination and communication activities

Name of the activity:	*Poster presentation and 3MT Thesis Competition*
Date:	*9th – 11th June 2021*
Place:	*Online Event*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	**Yes**
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition	**Yes**	Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		Number
Scientific Community (Higher Education, Research)	**550+**	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			
Name of the activity:	Digital Innovations for One Health Practitioners		
Date:	15^{th}-19^{th} February 2021		
Place:	Online Event		

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	Yes
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		Number
Scientific Community (Higher Education, Research)	**55**	Media	

Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*PhD seminar*
Date:	*12th May 2021*
Place:	*Veterinary School, Complutense University of Madrid*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition	***Yes***	***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS

	Number		*Number*
Scientific Community (Higher Education, Research)	***30***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.2 PhD02-R1-AMR2/3/6-LIN-RES

8.1.4.1.2.1 Summary

Occurrences among faecal samples of poultry, pigs and veal calves and nasal swab samples from pigs (results viewable in previous PhD reports) were reported in a paper submitted to the Journal of Antimicrobial Chemotherapy (under reviewing). In this paper, we also reported the presence of mutations or genes conferring resistance to linezolid but also the genetic organisation surrounding these genes and the relatedness between(MLST and cgMLST) the animal isolates isolated during the selective monitoring, the human isolates sent by the collaborators from the National Reference Centre-*Staphylococcus aureus* located in Erasme hospital in Brussels and from public databases. The LIN-RES project results were included in the Ph.D. thesis manuscript of Michaël Timmermans entitled « Etude de la résistance aux antibiotiques : développement d'une technique multiplexée de détection de gènes de résistance chez les bactéries Gram-négatives (AMR-ARRAY) et étude de la résistance au linézolide chez les bactéries gram-positives (LIN-RES) », submitted to the university (Université Libre de Bruxelles) in the beginning of August. Finally, conjugation experiments are in progress.

8.1.4.1.2.2 Overview of the PhD project progress

The task 3.1 is completed (genetic organization of the contigs carrying LIN-RES genes as well as incompatibility groups were investigated) and the task 3.2 was already started: establishment and test of the protocol and a first conjugation experiment was performed to assess the transferability of linezolid resistance genes. Conjugation experiments are ongoing (task 3.2) but results have to be analysed and transconjugants isolates obtained have to be analysed by PCR and WGS if PCR results are satisfactory.
A paper has been written and submitted (under reviewing). The epidemiological analysis including investigation of putative risk factors related to antibiotic consumptions is ongoing.

8.1.4.1.2.3 Progress of the research performed in the PhD project and key scientific results

All the linezolid resistant isolates collected during the selective monitoring were sequenced by whole genome sequencing (WGS), assembled and analysed. A cgMLST analysis was conducted to study the relatedness of these isolates and compare with published sequenced of linezolid-resistant isolates.
A paper was submitted and is currently under reviewing.
Conjugation experiments are in progress and results have to be analysed.
A study about antibiotic consumption at farm level was started to look after a potential correlation between linezolid resistant gene occurrences and phenicol consumption (as linezolid resistant genes confer also resistance to phenicols)

The task 3.2 (Laboratory experiments to demonstrate transferability of linezolid resistance genes and estimate transfer rates) is ongoing: establishment and test of the protocol and first conjugation experiments were performed to assess the transferability of linezolid resistance genes.

8.1.4.1.2.4 Progress of the research project: milestones and deliverables

8.1.4.1.2.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD2-AMR2/3/6-	D-PhD02-2.4	Results of in silico analysis of genetic scars of	M36		M48	request of a delay (until M48) was approved to submit it at the same time as

PhD LIN-RES		horizontal transfer or recombination events				D3-3, to deliver together in silico and in vitro transferability study results.
PhD2-AMR2/3/6-PhD LIN-RES	D-PhD02-3.3	Results of laboratory experiments to demonstrate transferability of linezolid resistance genes	M48		M48	Conjugations are in progress
PhD2-AMR2/3/6-PhD LIN-RES	D- PhD0-4.1	Summarize of the risk factors analysis (epidemiological and NGS data)	M48		M48	
PhD2-AMR2/3/6-PhD LIN-RES	D- PhD0-5.1	Final synthesis and reporting	M48		M48	

8.1.4.1.2.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD2-AMR2/3/6-PhD LIN-RES	M-E27-5	Final synthesis and reporting	M48		M48	

8.1.4.1.2.5 Soft skills and Continuing Professional Development training

No training courses have been undertaken during this reporting period.

8.1.4.1.2.6 Publications and patents

A paper was submitted and is currently under reviewing.

8.1.4.1.2.7 Remarkable outcomes

N/A

8.1.4.1.2.8 Impact and Relevance

This project allowed us to strengthen the links with the National Reference Center of *Staphylococcus aureus* in Belgium. It also, through the different presentations in international meetings, raised the awareness of the other countries and inside Belgium about the resistance to linezolid and gave the opportunity to share the scientific expertise between labs. The different conferences organized by the EJP gave the possibility to have a better visibility among the scientific community. The links with the collaborators (ANSES, Veterinair Microbiologisch Diagnostisch Centrum (VMDC) Utrecht University, Bundesinstitut für Risikobewertung (BfR) have been reinforced through this project and thanks to the Annual Scientific Meeting's.

8.1.4.1.2.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers comments have been accepted and this is closed.

8.1.4.1.2.10 Impact of COVID-19 crisis on the project

N/A

8.1.4.1.2.11 List of critical risks

Description of risk	Yes/No

Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

8.1.4.1.2.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

We work with the FASFC (Federal Agency for the Safety of the Food Chain) and we work with samples of the nationwide antimicrobial resistance surveillance program.

8.1.4.1.2.13 List of dissemination and communication activities

Name of the activity:	*One Health EJP ASM 2021 (presentation of a poster and participation in 3MT competition)*		
Date:	*9-11 June 2021*		
Place:	*Digital conference*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*Yes*
Exhibition	***No***	***Brokerage Event***	*No*
Flyer	***No***	***Pitch Event***	*No*
Training	***No***	***Trade Fair***	*No*
Social Media	***Yes***	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	***No***	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	***No***		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	*0*
Industry	***0***	***Investors***	*0*
Civil Society	***0***	***Customers***	*0*
General Public	***0***	***Other***	*0*
Policy Makers	***0***		

8.1.4.1.3 PhD03-R2-AMR2.1-HME-AMR

8.1.4.1.3.1 Summary

This project, which focuses on the impact of the presence of heavy metal (in this case zinc) in soil on the resistome both in the soil and foods produced from the land, commenced in February 2021. As reported previously there were significant delays in student recruitment due to COVID-19 related travel restrictions. The student started in February and work has been progressing well since then. Initial work focused on the preparation of a scoping literature review on the impact of heavy metals on antimicrobial resistance in primary production. All literature searches have been completed on a number of databases and relevant literature has been selected for review and analysis and this process is ongoing. In addition to this the student has completed a number of training modules in relation to health and safety, lab practices, manual handling, statistics, presentation skills and database usage.

The first experimental trial of the project has commenced. Field plots were set up in two geographically distinct regions, with zinc added to some plots and not to others in each site. Soil samples were taken at both sites (22 zinc added, 22 no zinc added) and returned to the lab. Each sample was analysed for the presence of Enterobacteriaceae, ESBL Enterobacteriaceae, carbapenamase producing Enterobacteriaceae and ciprofloxacin resistant Enterobacteriaceae using culture based SOPs in place in the lab. Presumptive isolates obtained from this sampling will be identified by MALDI-TOF prior to further characterisation. Soil samples from each plot were also stored for later DNA extraction for metagenomics analysis.

8.1.4.1.3.2 Overview of the PhD project progress

There was a substantial delay to the commencement of this project due to recruitment issues and the COVID-19 crisis. A suitably qualified candidate commenced the project in February 2021. Initial work focused on the preparation of a scoping literature review on the impact of heavy metals on antimicrobial resistance in primary production. The student has also completed a number of training modules in relation to health and safety, lab practices, manual handling, statistics, presentation skills and database usage. Work has commenced on the first experimental trial. Field plots were set up in two geographically distinct regions, with zinc added to some plots and not to others at each site. Soil samples were taken at both sites (22 zinc added, 22 no zinc added) and returned to the lab for analysis. Each sample was analysed for the presence of Enterobacteriaceae, ESBL Enterobacteriaceae, carbapenamase producing Enterobacteriaceae and ciprofloxacin resistant Enterobacteriaceae using culture-based SOPs in place in the lab. Presumptive isolates obtained from this sampling will be identified by MALDI-TOF prior to further characterisation and inclusion in study 4. Soil samples from each plot were also stored for later DNA extraction for metagenomics analysis.

Due to the late start of the project and to align with the seasonality of field trials, the first experimental work to be undertaken is in relation to study 3 (D-PhD03-1.2), focused on the impact of heavy metal amendments on AMR in soil. In the initial project description this deliverable was associated with the application of zinc containing pig slurry. However, this trial is now being undertaken in plots where ready to eat food crops will be grown and therefor slurry was not a suitable amendment. Therefore, the zinc was added directly to the soil. This was also deemed more applicable because high level zinc oxide supplementation of pig diets will no longer be permitted in the EU from 2022 and therefore it is expected that the zinc levels present in pig slurry will decrease.

Sampling for achievement of D-PhD03-1.1 associated with study 1 will commence in the second half of 2021. Initial meetings have taken place with Geological Survey Ireland to identify suitable locations, and these will be further defined in the coming months. The analysis to be undertaken will be analogous to that used for study 3 and therefore all SOPS will be in place.

8.1.4.1.3.3 Progress of the research performed in the PhD project and key scientific results

The task titles below are as outlined in the original project description.

Task 1 (Study 1) Comparison of the resistome in low and high metal containing soils

- Initial discussions on site selection have taken place with Geological Survey Ireland. Sampling will commence in late 2021. Analytical SOPs are in place.

Task 2 (Study 2) Comparison of the resistome in bovine milk filters from cattle grazing on grass in low and high metal areas

- Sampling for this study will follow from site selection for Study 1 and will take place in the summer of 2022. Analytical SOPs are in place. Suitable farms will be selected through consultation with the Teagasc advisory (extension) service.

Task 3 (Study 3) Analysis of the resistome on sites following manure application from pigs with heavy metal included in feed and from pigs with no/minimal heavy metal in feed

- As noted above high level zinc oxide supplementation of pig diets will no longer be permitted in the EU from 2022 and therefore it is expected that the zinc levels present in pig slurry will decrease. Due to this and the desire to reduce the variables it was deemed appropriate to focus on the impact of direct zinc amendment and compare soils in paired plots with and without amendment to monitor and changes in the resistome. This also provides the opportunity to monitor changes in AMR organisms present in crops produced in these soils. The deliverable associated with this study is unchanged.
- Work on this task has commenced and is progressing well. All necessary SOPs are in place and sampling has been completed on two sites (44 samples in total). Presumptive isolates of interest are undergoing analysis currently.

Task 4 (Study 4) Genotypic comparison with antimicrobial resistant Enterobacteriaceae with human, environmental and animal isolates from Irish and EU reference laboratories

- Dr Burgess and Prof Morris have suitable banks of isolates from animals and the environment for comparison with isolates obtained as part of this project and will utilise public repositories of sequenced isolates and collaborations with reference laboratories to increase the diversity of isolates for comparison. The selection of strains for comparison will be dependent on the identity of isolates obtained in studies 1, 2 and 3 of this project. This work will commence in late 2022.

8.1.4.1.3.4 Progress of the research project: milestones and deliverables

8.1.4.1.3.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from	Actual Delivery Date	If not achieved: Forecast	Comments

			Annual Work Plan		achievement date	
PhD03-AMR2.1-HME-AMR	D-PhD03-1.1	Report on AMR bacteria present in soils of differing heavy metal content	M48		M60	Delay in student starting project and rearrangement of study commencement order will delay the first deliverable being achieved.
PhD03-AMR2.1-HME-AMR	D-PhD03-1.2	Report on the impact of the application of heavy metal containing amendments to AMR bacteria in soil	M54		M54	The study linked with this has commenced first and therefore it is currently envisaged that this deliverable will be achieved on time.
PhD03-AMR2.1-HME-AMR	D-PhD03-1.3	Report on AMR bacteria present in milk filters from animals grazing in areas of differing heavy metal content	M59		M66	Delay in student starting project will delay the achievement of this deliverable.

8.1.4.1.3.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD03-AMR2.1-HME-AMR	1	SOP in place for sampling culture based analysis	27	Yes	M40	Delayed due to the late recruitment of student but the SOPs for culture based analysis are now in place and have been utilised in the first sampling study undertaken.
PhD03-AMR2.1-HME-AMR	2	Sequences of sufficient quality obtained from metagenomic analysis	35	No	M48	Delayed due to late recruitment of PhD student

8.1.4.1.3.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Biosafety Awareness	Health and Safety	23/04/21	Teagasc
Lab risk assessment	Health and Safety	29/04/21	Teagasc
Lab chemical safety awareness	Health and Safety	19/04/21	Teagasc
Lab safety basics	Health and Safety	17/03/21	Teagasc
Manual handling	Health and Safety	08/02/21	Teagasc

Antimicrobial resistance – theory and methods	Relevant to PhD topic	04/02/21	Coursera
Metagenomics applied to surveillance of pathogens and antimicrobial resistance	Relevant to PhD topic	19/02/21	Coursera
Scopus workshop	Database interrogation	24/03/21	NUIG
Present your research with confidence	Presentation skills	24/05/21	Environ 2021
Statistical analysis with R	Statistics	14-17-18/06/21	Teagasc

8.1.4.1.3.6 Publications and patents

Not applicable yet.

8.1.4.1.3.7 Remarkable outcomes

Not applicable yet.

8.1.4.1.3.8 Impact and Relevance

Dr Burgess and Prof Morris currently collaborate on the Irish EPA funded project AREST which examines antimicrobial resistance in the environment and contribute to One Health orientated AMR research and policy making at a national and international level. This project is highly complementary to those activities and will facilitate an increasing collaborative relationship between the research groups. Dr Burgess has worked previously with Dr Johannessen of the Norwegian Veterinary Institute as part of the HUPLANT Control COST action focusing on microbial food safety issues and this project will further develop this relationship. This project has also led to Dr Burgess forging collaborative linkages with Geological Survey Ireland in relation to heavy metal mapping and with the SFI funded VISTA MILK research centre.

8.1.4.1.3.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers comments have been accepted and this is closed.

8.1.4.1.3.10 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
All		M69	All		M66	Recruitment delay	0	133,900

Comments: COVID-19 has had a severe impact on recruitment for this project. Prior to the pandemic we reported issues with recruiting a suitable PhD candidate. A suitable candidate was identified in March 2020 and the position offered but failed to secure a visa by October 2020. Another advertising round commenced and another suitable candidate was selected and started in February 2021. Tighter sampling schedules and rearrangement of study order will be employed to address project delays to date.

Description of risk	Yes/No
Loss of PhD supervisor(s)	Not foreseen

Loss of technical training staff delaying progress of the work	Not foreseen
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	Not foreseen
Lack of commitment between the collaborative partners that support the PhD	Not foreseen
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	Not foreseen
Change in PhD student circumstances requiring temporary leave	Not foreseen
Other risks (please describe)	

8.1.4.1.3.11 List of critical risks

Additional information:
As outlined there has been a significant delay to the project start and associated delay in work plan execution and reporting. Nonetheless, Dr Burgess and Prof Morris believe the project remains achievable with the right candidate and slightly tighter timelines. This has been made possible through collaboration with other relevant projects as outlined in Section 12.

8.1.4.1.3.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

- An initial meeting has taken place with Geological Survey Ireland and through collaboration with the Tellus survey (https://www.gsi.ie/en-ie/programmes-and-projects/tellus/Pages/default.aspx) this will enable suitable high and low metal content soils across Ireland for resistome analysis as part of study 1 and farms for selection as part of study
- As part of Study 3 we will collaborate with a nationally funded project which is focused on the impact of different soil amendments on cadmium uptake in food crops. We will focus on plots where zinc has been applied and use the corresponding control (no amendment) plots for comparison.
- This project is complementary to the EPA funded AREST project (https://www.nuigalway.ie/medicine-nursing-and-health-sciences/medicine/disciplines/bacteriology/research/arest/) which is examining antimicrobial resistance in the environment. Prof Morris is the coordinator of this project and Dr Burgess is a participant. The culture based methodologies being employed in AREST will be particularly relevant for HME-AMR. The student will have the opportunity to collaborate with these colleagues for methodologies and isolate characterisation.
- Dr Burgess and Prof Morris both contribute to Ireland's National Action Plan for Antimicrobial Resistance which is currently being updated and the results of this project will contribute to achieving the objectives of that plan.
- HME-AMR is complementary to an ongoing PhD project in Dr Burgess' group focusing on the impact of zinc oxide supplementation on the porcine resistome which will facilitate a smooth transition for the HME-AMR student in relation to methodologies and analysis.
- HME-AMR is complementary to a project led by Dr Orla O'Sullivan as part of the SFI funded VistaMilk project (https://vistamilk.ie/) which is examining the soil resistome in different sites across Ireland. Dr Burgess and Dr O'Sullivan will collaborate to ensure synergy of the projects and avoid duplication.

8.1.4.1.3.13 List of dissemination and communication activities

Name of the activity:	*OHEJP ASM*

Date:	*June 9-11 2021*
Place:	*Copenhagen/online*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		**Participation to a Conference**	*Yes (poster)*
Organisation of a Workshop		**Participation to a Workshop**	
Press release		**Participation to an Event other than a Conference or a Workshop**	
Non-scientific and non-peer-reviewed publication (popularised publication)		**Video/Film**	
Exhibition		**Brokerage Event**	
Flyer		**Pitch Event**	
Training		**Trade Fair**	
Social Media		**Participation in activities organized jointly with other H2020 projects**	
Website		**Other**	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	**550+**	**Media**	
Industry		**Investors**	
Civil Society		**Customers**	
General Public		**Other**	
Policy Makers			

Name of the activity:	*OHEJP ASM (Thesis in 3 minutes)*
Date:	*June 9-11 2021*
Place:	*Copenhagen/online*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		**Participation to a Conference**	*Yes*
Organisation of a Workshop		**Participation to a Workshop**	
Press release		**Participation to an Event other than a Conference or a Workshop**	
Non-scientific and non-peer-reviewed publication (popularised publication)		**Video/Film**	
Exhibition		**Brokerage Event**	
Flyer		**Pitch Event**	*Yes*
Training		**Trade Fair**	
Social Media		**Participation in activities organized jointly with other H2020 projects**	
Website		**Other**	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.4 PhD04-R2-AMR2.1-KENTUCKY

8.1.4.1.4.1 Summary

Salmonella enterica serovar Kentucky (*S.* Kentucky) is a common causative agent of gastroenteritis in humans. It is one of most notorious *Salmonella* serotypes, as it is strongly associated with antimicrobial resistance (AMR). Ciprofloxacin-resistant S. Kentucky is able to gain additional antibiotic resistance determinants through the acquisition of locally circulating plasmid-borne ESBL, AmpC and/or carbapenemase genes. Most recently, the situation has worsened, as ECDC launched an Urgent Inquiry (UI-464) on a CIP^R S. Kentucky ST198 strain carrying a chromosomally integrated $bla_{CTX\text{-}M\text{-}14b}$ gene encoding for cephalosporin resistance. The insertion event was traced back to Malta, but the strain has already spread to Belgium, UK and five other EU countries. In this PhD project, we will investigate (i) what explains the evolutionary success of the multidrug resistant S. Kentucky ST198 clone, and (ii) what is the mechanisms of the integration (and potential further transfer) of the ESBL gene in its chromosome.

In the second year of the PhD research, we continued the investigation of the IncHI-type, mega-plasmid carrying the ISECP1-$bla_{CTX\text{-}M\text{-}14B}$ transposition unit. Due to its clinical significance, the plasmid was chosen as a focal point for our fluorescent tagging strategy. Yet, wet-lab experiments backed with *in silico* analysis indicated that the plasmid is non-conjugable, most probably due to truncation in the transfer region. Thus, we reasoned to shift to the prototype IncHI1 plasmid (R27), a self-transmissible plasmid with fully characterized conjugation machinery (Craig et al.,2000) and is capable of transfer between members of the *Enterobacteriaceae*.

The transposition mechanism of ISECP1 is obscure so we decided to develop fluorescent reporters based on the partitioning system parS/ParB to differentially label the pR27 backbone and the ISEcp1-$bla_{CTX\text{-}M\text{-}14b}$ unit. parS is a cis-acting centromere-like sequence to which a partitioning protein ParB selectively binds. Two alternative partitioning systems were instigated namely, P1 Phage-derived and *Lactococcus lactis*-derived parS/ParB. The later system has minimal interference with DNA replication. Yet, the system is derived from gram-positive bacteria and needs to be optimized to a gram-negative host.

First, we labeled the pR27 backbone with a P1-parS sequence. Next, we tagged ISECP1-$bla_{CTX\text{-}M\text{-}14B}$ transposition with lactis-parS sequence *in vitro*, followed by cloning the assembled unit into pR27. The construct was confirmed by sequencing. Then we codon-optimized lactis-parB protein according to *Salmonella* codon usage and fused the optimized-parB to msf-GFP under an IPTG inducible promoter. We tagged optimized parB at the N- terminus and C- terminus with both flexible and rigid linkers. Yet we failed to obtain localized foci in vivo.
We decided to move to alternative fluorescent reports based on P1/ pMT partitioning system (Nielsen et al., 2006). In parallel, the in vitro- mating assays of pR27 indicated a conjugation efficiency of 10^{-5}-10^{-6} at 25C which is challenging to study under time-lapse fluorescent microscopy. Thus we replaced the conjugation repressor *htdA* with pMT parS sequence to obtain a 2 log increase in the conjugation efficiency (Whelan et al., 1994). Next we -in vitro-labeled the ISECP1-blaCTX-M-14B transposition with P1-parS sequence and cloned it into pR27. Currently, we are optimizing the cognate parB proteins.

8.1.4.1.4.2 Overview of the PhD project progress

The PhD is going according to schedule, although the project start suffered from the Covid-19 crisis. Due to direct consequences of the pandemic, the original candidate (Jasper Van der Peet) had to return permanently to The Netherlands in M28. He was replaced by Alaa Albasiony, who will pick up activities in M32. The transition between both candidates was smooth and Jasper is still involved in the project as advisor, with two of the five deliverables (D-PhD04-1.1 and D-PhD04-1.2) completed. However, a necessary visit to INRAE (MedVet travel grant obtained) is still postponed to 2022. This academic year,

Alaa is taking courses in omics and bioinformatics, and supervises a master thesis in bioinformatics (Biancamaria Florenzi, KULeuven), who is perform the database mining described above.

8.1.4.1.4.3 Progress of the research performed in the PhD project and key scientific results

Insertion of P1-parS and lactis-parS labeled transposition unit into pR27

pKD46, a plasmid with temperature-sensitive replicon, encoding lambda Red recombinases was electroporated into MA8508ΔpSLT strain. Next, pR27 was conjugated by filter mating to MA8508ΔpSLT containing pKD46. In short, cultures of the donor (E.coli, pR27)and recipient strains (MA8508ΔpSLT containing pKD46) were grown in LB supplemented with the required antibiotics at 25°C in static conditions for 16 h. Cells were washed with 0.1M MgSO4 to eliminate the antibiotics. The recipient strain suspension (0.4 ml) and donor strain suspension (0.1 ml) were mixed on a 0.22 µm membrane and incubated at 25°C for 2 h. Serial dilutions were plated inappropriate media to select for trans-conjugants (MA8508ΔpSLT containing pKD46 and pR27).

P1-parS was then PCR amplified as P1-parS- frt-cat-frt fragment and electroporated into MA8508ΔpSLT containing pKD46 and pR27 to be inserted in an intergenic region on pR27 backbone via lambda red recombineering (Datsenko et al; 2000). Next, we flipped the chloramphenicol cassette using pCP20, a plasmid encoding Flp recombinase.

To label the ISEcp1-bla $_{CTX\text{-}M\text{-}14b}$ unit with *Lactococcus lactis*-derived parS, we PCR amplified the unit as two fragments with the 18bp parS sequence as an overhang (GGGGCTAAATTTAGCCCC). The two fragments were then assembled in vitro by gibsson assembly and inserted into pR27 backbone by homologous recombination (Datsenko et al; 2000). The 18bp parS sequence was inserted downstream the terminator region of the ISEcp1 gene and upstream the promoter region of bla $_{CTX\text{-}M\text{-}14b}$ gene. We then confirmed the insertion of P1 and *Lactococcus lactis*-derived parS together with the conservation of the ISEcp1,bla $_{CTX\text{-}M\text{-}14b}$ genes by sequencing.

Optimizing P1-ParB and Lactis-ParB Proteins

First, we separately inserted by recombineering the two parS sequences into the chromosomes of MA8508ΔpSLT to serve as a positive control for fluorescent microscopy. Next, we transformed the MA8508ΔpSLT labeled with P1-parS and lactis-parS with pALA2705 and pMK17-01 respectively. pALA2705 plasmid encodes P1-parB N-terminally fused to mCherry whereas pMK17-01 encodes lactis-parB C-terminally fused to msfGFP. Time-lapse microscopy experiments showed only localized foci with P1-parB-mCherry, indicating that lactis-parB failed to bind its cognate parS sequence.

Thus we reasoned to codon optimize the lactis-parB protein to a gram-negative host using the genescript™ platform. Then the codon-optimized lactis-parB protein was then cloned into a variant of pALA2705 encoding GFP to obtain pALA2706 (lactis-parB N-terminally fused to GFP). pALA2706 was then transformed into MA8508ΔpSLT labeled with lactis-parS. Microscopy experiment showed again diffused fluorescence. We thought to change the fusion type into C- terminal fusion and try different linkers. In short, pALA2706 was PCR linearized to remove GFP- lactis-ParB. Then msfGFP and lactis-ParB were PCR amplified, assembled in vitro to produce lactis-parB C-terminally fused to msfGFP with two different linkers (SSSRGSGGEAAAKAGS, $(SGGGG)_4$), and ligated back to PCR-linearized pALA2706 by gibsson assembly. The two variants of the plasmid were then separately transformed to MA8508ΔpSLT labeled with lactis-parS. yet we failed to obtain localized foci.

The inability of lactis ParB to bind its cognate parS sequence in vivo may be attributed to improper folding in gram-negative host despite being codon-optimized. It is also possible that parB fail to oligomerize upon binding so we don't observe the localized focus over the background diffused fluorescence. Moreover, in *E.coli*, the integration host factors (IHF) bind specific sequence on P1-parS and facilitate the binding of P1-parB to P1-parS (Barbara Funnel., 1991) and since lactis-parS does not carry an IHF-binding sequence it might have failed to attract the partitioning machinery.

Construction of a reporter plasmid based on P1, pMT parS/ ParB partition system

pMT parS was PCR amplified as pMT-parS- frt-cat-frt fragment and the fragment was electroporated into MA8508ΔpSLT containing pKD46 and pR27 to replace *htdA* gene. HtdA is involved in the repression of four tra operons and has a pivotal role in the growth phase dependency of R27 conjugation. In vitro conjugation assay has shown a 2 log increase in the conjugation efficiency of pR27, *htdA* mutant as compared to the wild-type. We then labeled the transposition unit with P1-parS as previously described followed by electroporation into MA8508ΔpSLT containing pKD46 and pR27(pMT-labelled, Δ*htdA*). We are currently optimizing the cognate parB proteins and if the system were to be orthogonal as per the literature (Nielsen et al., 2006), we will assemble the P1-parB and pMT-ParB with the fluorescent markers into a chromosomal operon under the control of Ptet inducible promoter to avoid ectopic expression from the plasmid.

8.1.4.1.4.4 Progress of the research project: milestones and deliverables

8.1.4.1.4.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD4-AMR2.1-KENTUCKY	D-PhD04-1.1	Genomic Characterization of clinical Salmonella Kentucky strains	M32	M35		
PhD4-AMR2.1-KENTUCKY	D-PhD04-1.2	Construction of reporter strain, able to show transfer of ESBL gene between plasmid and chromosome	M42		M44	Reporter strain is constructed, but still ongoing final checks for functionality.
PhD4-AMR2.1-KENTUCKY	D-PhD04-1.3	Peer-reviewed paper on the database search on the abundance of transferable AMR genes in public databases	M48			
PhD4-AMR2.1-KENTUCKY	D-PhD04-1.4	Peer-reviewed paper on the molecular mechanisms behind the hopping of AMR elements	M54			
PhD4-AMR2.1-KENTUCKY	D-PhD04-1.5	PhD dissertation finalized	M60			

8.1.4.1.4.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD4-AMR2.1-KENTUCKY	M1	Quality training completed at Sciensano	M23	Y		
PhD4-AMR2	M2	S. Kentucky strain and genome collection completed	M30	Y		

.1-KENTUCKY						
PhD4-AMR2.1-KENTUCKY	M3	Completion of hybrid assemblies of four S. Kentucky strain	M36	Y		
PhD4-AMR2.1-KENTUCKY	M4	Finalization of characterization of MGEs and selection of elements for further study at KULeuven	46	N	46	On time.

8.1.4.1.4.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
OMICS techniques and data analysis	Lab skills / Bioinformatics	01/02/2021 – 30/06/2021 (ongoing)	KULeuven
Exploitation of research - knowledge & technology transfer	IP skills	Planned for 2021 (Q4)	KULeuven
Academic English: presentation and seminar skills	Language course	Planned for 2022	KULeuven

8.1.4.1.4.6 Publications and patents

Albasiony, A., Ceyssens, PJ., Aertsen, A. (2021) Exploring the ISECP-1 mediated chromosomal integration of blaCTX-M-14 in Salmonella Kentucky. Annual Scientific Meeting OHEJP, June 9-11th, 2021. Poster presentation.

8.1.4.1.4.7 Remarkable outcomes

None to report so far.

8.1.4.1.4.8 Impact and Relevance

The Kentucky project units **Sciensano, INRAE and** Laboratory of Food Microbiology of **KULEUVEN** (BEL), which clearly have complementary expertise. Sciensano holds the National Reference laboratory of Samonella, and has experience in short-and long read sequencing. **Prof. Abram Aertsen**'s group uses analytical genetics and live (single-)cell biology approaches to study the spread, establishment and adaptive phenotypic impact of mobile genetic elements. **Benoît Doublet** and his team have long-term expertise in plasmid biology, and will study the transfer dynamics of these elements. It is clear these groups, which have never collaborated before, will greatly learn from each other and will exchange knowledge, strains and experiences along the way. The final goal is to improve our methodologies and understanding of transfer dynamics of these mobile elements, and its impact on antimicrobial resistance.

8.1.4.1.4.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers comments have been accepted and this is closed.

8.1.4.1.4.10 Impact of COVID-19 crisis on the project

1. Due to consequences of the Covid-19 crisis, the original candidate (Jasper Van der Peet) had to return permanently to The Netherlands in M28. He was replaced by Alaa Albasiony who

picked up activities in M32. This caused an inevitable delay in lab progress, although all objectives of the Kentucky project remain in reach.

2. The Kentucky project only foresees budget for personnel cost, so the budgetary impact is limited. If the program will be extended with two months to compensate for the lab closures (during Y3), we would need a budgetary injection of €6.000 to pay the salary of the PhD candidate.

8.1.4.1.4.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	NO
Loss of technical training staff delaying progress of the work	NO
Delay in work plan execution	YES
Conflicts between the collaborative partners that support the PhD	NO
Lack of commitment between the collaborative partners that support the PhD	NO
Delay in duties, tasks or reporting	NO
Poor working relationships within the PhD project team	NO
Change in PhD student circumstances requiring temporary leave	YES
Other risks (please describe)	NO

Additional information: The planned research visit to INRAE (M32) is still uncertain at this time, although crucial for buildup of expertise in conjugation and plasmid transfer methodology. Hopefully this visit can be planned in late Y4.

8.1.4.1.4.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Long-read sequencing was performed according to methodology derived from the Full Force project (JRP-14). No interactions with OHEJP stakeholders were made in this stage of the project.

8.1.4.1.4.13 List of dissemination and communication activities

Name of the activity:	*OHEJP Annual Scientific Meeting 2021 – poster presentation and three minute thesis competition*		
Date:	*09-11 June 2021*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*NO*	***Participation to a Conference***	*YES*
Organisation of a Workshop	*NO*	***Participation to a Workshop***	*NO*
Press release	*NO*	***Participation to an Event other than a Conference or a Workshop***	*NO*
Non-scientific and non-peer-reviewed publication (popularised publication)	*NO*	***Video/Film***	*NO*
Exhibition	*NO*	***Brokerage Event***	*NO*
Flyer	*NO*	***Pitch Event***	*YES*
Training	*NO*	***Trade Fair***	*NO*
Social Media	*NO*	***Participation in activities organized jointly with other H2020 projects***	*NO*
Website	*NO*	***Other***	*NO*
Communication Campaign (e.g. Radio, TV)	*NO*		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	***0***
Industry	***0***	***Investors***	***0***
Civil Society	***0***	***Customers***	***0***
General Public	***0***	***Other***	***0***
Policy Makers	***0***		

8.1.4.1.5 PhD05-R2-AMR2/6.1/ET5-METAPRO

8.1.4.1.5.1 Summary

2021 has been a productive year so far. We have been able to advance in the first sampling proposed for the project, but we are still working to be able to finish it at the end of the year. Metagenomic analysis has been the main focus during the first month of the years and we have optimised our pipeline to obtain interesting results from the metagenomic data from our database. We have been able not only to detect 16 rRNA methyltransferases but also to assemble them and know more about their genetic environment. This has led us to make some changes on some milestones and in our workflow. We expect to be able to isolate the bacteria that act as reservoirs from plazomicin resistance determinants. However, we decided to move the second sampling to the next year to first complete the one that is currently ongoing.

8.1.4.1.5.2 Overview of the PhD project progress

– Spanish sampling design: since at the beginning we had some difficulties with the pandemic situation we had difficulties accessing sampling points, a metagenomic analysis of the data of previous projects from the lab was performed. In addition, samples from different ecological niches are already collected and we have established contact with other potential sampling points.

– Spanish sampling execution: samples from pig and poultry farms have been obtained and sampling for the rest of the ecological niches that we aim to analyse is intended to be done during 2021.

– Metagenome sequencing of the Spanish sampling: sequencing of the samples taken is currently being performed.

– Enterobacteria isolation and WGS from the first sampling: we are making a slight change in this task. We are no longer looking for just Enterobacteria, but we are aiming to isolate and culture the bacteria that act as a reservoir of the acquired 16S rRNA methyltransferases.

– Analysis of the genomic and metagenomic data from the Spanish sampling: we started some of the analysis using metagenomic data obtained in previous projects from the lab. We tested part of our pipeline and we were able to detect already acquired 16S rRNA methyltransferases with our read-based approach. Assembly-based approach is also giving us hints of where are these resistance mechanisms maintained in the different niches.

– Sampling design in the United Kingdom: contact with our UK partners has been established to discuss timeline and sampling possibilities.

– UK Sampling execution: this task is not due to take place until next year.

– Metagenome sequencing of the UK sampling: this task is not due to take place until next year.

– Enterobacteria isolation and WGS from the UK sampling: this task is not due to take place until next year.

– Analysis of the genomic and metagenomic data from the UK sampling: this task is not due to take place until next year.

– Writing of a guideline for early detection of plazomicin resistance determinants to preserve plazomicin for human clinical use: this task is not due to take place until June 2022.

– Writing of the Thesis manuscript: this task is not due to take place until June 2022.

– Thesis defence: this task is not due to take place until February 2023.

8.1.4.1.5.3 Progress of the research performed in the PhD project and key scientific results

During this year we continued with the metagenomic analysis of the data of pig and poultry farms from Spain belonging to previous projects. With a full metagenomic analysis we were able to detect 16S rRNA methyltransferases in a low prevalence in both types of farms using a read based approach. The 16S rRNA methyltransferase-positive samples were analysed as well with an assembly approach to study the potential genetic environment of the methyltransferases. It suggested that the Order *Eubacterium* may play a role in the maintenance of these resistance determinants. At present, we are extending this analysis to samples from pig and poultry farms across Europe to enlarge our dataset. We are also including human metagenomic data accessible via SRA to compare if we can detect 16S rRNA methyltransferases.

Since our metagenomic analysis suggest that the prevalence of 16S rRNA methyltransferases is low and associated to the Order *Eubacterium*, it is possible that in many samples we will not obtain enterobacteria via culturing. Therefore, we changed the milestone 4 ("Enterobacteria isolation and WGS from the first sampling") to look for all the bacteria that may act as reservoirs of the plazomicin resistance. Currently, we are testing different culture media to isolate potential plazomicin resistant bacteria and proceed with their Whole Genome Sequencing to identify more accurately the genetic environment of the methyltransferases.

In addition, we were able to collect samples from a few different ecological niches. We are still processing them now, and the metagenome will be sequenced via Illumina and Nanopore technologies soon.

8.1.4.1.5.4 Progress of the research project: milestones and deliverables

8.1.4.1.5.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD05-AMR2/6.1/ETS-METAPRO	D-PhD05-4.1	Sampling questionnaire	JAN 2021	APR 2021		https://zenodo.org/record/4662681#.YMxl7JMzb0p

8.1.4.1.5.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD5-AMR2/6.1/ET5-METAPRO	M3	Metagenome sequencing of the first sampling	MAR 2021	No	DEC 2021	We are currently sequencing the first samples collected, as we collect the rest we will be sequencing them

	M4	Enterobacteria isolation and WGS from the first sampling	MAR 2021	No	DEC 2021	We made an adjustment to this milestone, so we are not longer for enterobacteria but we are looking for the bacteria that act as reservoir of plazomicin resistance determinants
	M5	Design of the second sampling	JUN 2021	No		Ongoing
	M6	Second sampling execution	SEP 2021	No		We expect to start at the end of 2021/beginning 2022 with this task

8.1.4.1.5.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
MicroMundo Symposium	AMR	27-28th/04/2021	Universidad Complutense de Madrid
Webinar Series 'Jornadas sobre la Carrera Investigadora'	Scientific Research	14th/01/2021 – 29th/04/2021	Universidad Complutense de Madrid
Course 'Hojas de Cálculo con Excel I'	Excel	15th/03/2021 – 30th/06/2021	Universidad Complutense de Madrid
Course 'Hojas de Cálculo con Excel II'	Excel	14th/06/2021 – 29th/10/2021	Universidad Complutense de Madrid

8.1.4.1.5.6 Publications and patents

Proceedings of the 3rd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Copenhagen (Denmark)/Online meeting, June 9th - 11th, 2021, page 132

Bosco R. Matamoros, Roberto M. La Ragione, & Bruno Gonzalez-Zorn. (2021). Questionnaire on farm management, biosecurity, health and antibiotic use in livestock farming (METAPRO_D-PhD05-4.1). Zenodo.
DOI: 10.5281/zenodo.4662680

Proceedings of the 31st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Online Congress, 9th – 12th July, 2021, page 200

Proceedings of the XXVIII Congreso de la Sociedad Española de Microbiología (SEM). Online Congress, 28th June – 2nd July, 2021, page 214

8.1.4.1.5.7 Remarkable outcomes

N/A

8.1.4.1.5.8 Impact and Relevance

This PhD project connects three major laboratories focused on the fight of Antimicrobial Resistance, having all of them participated in other European Projects in the past. This offers a possibility to maintain the great relationship that has been always present between institutes. This fact is

highlighted by the various exchanges of students that have had part and how successful they have been. In addition, since the PhD project supervisors study AMR from different perspectives, this new collaboration brings the opportunity to combine them all together to get the most out of it. But it is not only limited to that. Being part of the EJP networks allows to connect the partners and the student with different backgrounds and perspectives that are often needed.

8.1.4.1.5.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers comments have been accepted and this is closed.

8.1.4.1.5.10 Impact of COVID-19 crisis on the project

N/A

8.1.4.1.5.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	NO
Loss of technical training staff delaying progress of the work	NO
Delay in work plan execution	YES
Conflicts between the collaborative partners that support the PhD	NO
Lack of commitment between the collaborative partners that support the PhD	NO
Delay in duties, tasks or reporting	YES
Poor working relationships within the PhD project team	NO
Change in PhD student circumstances requiring temporary leave	NO
Other risks (please describe)	NO

8.1.4.1.5.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

- FARMED: Fast Antimicrobial Resistance and Mobile-Element Detection using metagenomics for animal and human on-site tests (https://onehealthejp.eu/jrp-farmed/). Since the objectives and the techniques of both projects are similar, the candidate is taking part actively in the tasks performed at UCM associated to this project.

- EFFORT: Ecology from Farm to Fork Of microbial drug Resistance and Transmission (http://www.effort-against-amr.eu/). The group participated actively in the EFFORT project and its activities and resources are the foundation of the present research project.

- JPIAMR: Joint Programming Initiative on Antimicrobial Resistance. The lab is part of the NEAR-AMR (https://www.jpiamr.eu/near-amr/) and of the GAP-ONE (https://www.jpiamr.eu/gap-one/) projects. The candidate can benefit of these networks to extend the search of plazomicin resistance determinants in other parts of Europe and Africa or to estimate the economic burden of plazomicin resistance.

- AVANT: Alternatives to Veterinary Antimicrobials (https://avant-project.eu/). The student will also be involved in the development and test of alternatives to antimicrobials.

- DAMR-Una Europa: “Disseminate antimicrobial resistance knowledge and the use of whole genome sequencing on relevant bacterial pathogens during COVID-19 world emergency”

(https://www.una-europa.eu/). The student is taking part in this project teaching a webinar on metagenomics and collaborating on the sequencing of bacterial pathogens.

8.1.4.1.5.13 List of dissemination and communication activities

Name of the activity:	*OHEJP Three Minute Competition 2021 and Poster Presentation*		
Date:	*10th June 2021*		
Place:	*Copenhagen/ONLINE*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	*Yes*
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	*Yes*
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*Seminar presentation 'Identificación y caracterización molecular de mecanismos de resistencia a antibióticos emergentes'*		
Date:	*12th May 2021*		
Place:	*Faculty of Veterinary Medicine, Universidad Complutense de Madrid*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	*Yes*
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	

Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***50***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*31st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) + Poster Presentation*
Date:	*9th- 12th July 2021*
Place:	*ONLINE*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)		***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*XXVIII Congreso de la Sociedad Española de Microbiología (SEM) + Poster Presentation*
Date:	*28thJune – 2nd July 2021*

Place:	*ONLINE*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***900+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.6 PhD06-R1-ET5-PEMbo

8.1.4.1.6.1 Summary

WP1: Establishment of reference sequences from the main French clonal groups
Task-1.2: Sequencing and de novo assembly (M26-M41)

Since the last report, Ciriac has modified the bacterial genomic DNA extraction protocol which has enabled him it to obtain DNA of sufficient quantity and quality to perform 3rd generation sequencing of 10 *Mycobacterium bovis* (*M. bovis*) strains with the MinION (Nanopore) technology available in his laboratory. Two of them have also been sequenced with PacBio technology in Genoscreen platform and will used to compare the quality of the genome sequences obtained with these two 3rd generation sequencing techniques. The 10 strains were also sequenced with the Illumina technology in Genoscreen that provide high fidelity DNA sequencing data but on short reads. Using long read -MinION's data- *de novo* assembly and short reads -Illumina's data- for genome correction, Ciriac obtained new reference genomes. The assembly strategy was discussed with bioinformaticians during a mission in Tours and the strategy has been validated on reference genomes AF2122/97 and Mb3601 with short read and long read sequencing simulation (with ART and Badread bioinformatic tools).

WP2: Genome sequence analysis
Task-2.2: Genomic markers analysis (M32-M43)

IS*6110* is an insertion sequence of the *Mycobacterium tuberculosis* complex –to which *M. bovis* belongs-, with an important role in genome plasticity and in the bacterium evolution caused by IS*6110* transposition.
In the previous report we showed that IS*6110* is present in multiple copies in endemic French *M. bovis* groups and could lead to phenotypic changes that explain their epidemiologic success. Since the last report, we have found out that some IS*6110* interrupt or could regulate (with their strong promoter) important genes implied in virulence, host persistence or environmental stress resistance. The insertion positions were confirmed by analysis of *M. bovis* Mb3601 reference sequence because this strain belongs to one of the studied groups. The new reference genomes of this thesis project will refine the IS*6110* position in other clonal groups.

A pangenomic study has been perform on the 10 genomes and showed a high similarity between them. Whole genome SNP (single nucleotide polymorphism) was performed on short read sequencing of the 10 strains in comparison to one of the current *M. bovis* reference genomes. The results of these comparative studies may allow Ciriac to define genetic targets explaining differential phenotypical characteristics of the different *M. bovis* groups. This work will be done in the WP3.

Once the complete genomes will be thoroughly annotated, we will be able to compare them to discern genetic events (deletions, insertions, SNPs,…) to better define the French clonal group. These first analyses have been discussed and improved during the mission in Tours. This task is still in progress.

WP4: Valorisation and dissemination of results (M37-M56)
Task-4.2: Communication
Ciriac presented his project at two conferences. He presented his work on IS*6110* during the scientific day of his doctoral school (ABIES doctoral days 2021) with oral communication and during the ASMOHEJP2021 (Annual Scientific Meeting One Health European Joint Program 2021) with a poster presentation. During the ASMOHEJP2021, he presented also his strategy to obtain the new reference genomes with another poster presentation and took part in the Three Minute Thesis Competition. Ciriac participated in similar challenge during "ma thèse en 180 secondes" competition.

8.1.4.1.6.2 Overview of the PhD project progress

WP1: Establishment of reference sequences from the main French clonal groups

Task-1.2: Sequencing and *de novo* assembly (M27-M41)

Ten genotypes were sequenced in MinION and Illumina technology. Two strains were also sequenced at Genoscreen, Lille, with the PacBio technology.

We performed a comparison of the results obtained by using different assemblers (with MinION and Illumina sequencing results) for a same genotype and selected of the best one.

Assembly strategy was validated on reference genomes.*De novo* assemblies were performed with the best tools.

This task has been delayed because of the COVID-19 crisis (initially programmed in M37) but could be carried out during M43.

WP2: Genome sequence analysis

Task-2.1: Sequencing of supplementary strains (M38-M41)

This task, which depends on the genome sequencing of strains belonging to additional clonal complexes, which is a non-essential task of the project, could start on month M45 if possible.

Task-2.2: Genomic markers analysis (M33-M44)

IS*6110* position found in BCG-21 strains can be confirmed and precisely determined with the use of Mb3601; interruption and possible up or down regulation of important genes will also be analyses.

IS*6110* position in the other strain endemic groups will be confirmed when the new reference genomes closer to French field strains will be available.

Pangenomic studies was carried out on the 10 genotypes and show a list of shell genes which must be validated.

Whole genome SNP was performed with the short read sequencing of the 10 strains in comparison to Mb3601 reference genome.

The search of genetic events is going on.

WP4: Valorisation and dissemination of results (M37-M56)

Task-4.1: Publication of results (M44 and M53)

The first manuscript is underway.

Publication of the 10 new complete genomes will done soon on genbank database.

Writing of the second manuscript will start soon.

Task-4.2: Communication

Oral communication at ABIES doctoral days 2021.

Posters presentation at ASMOEHP2021.

Participation in the 6th edition of “ma thèse en 180 secondes ”.

8.1.4.1.6.3 Progress of the research performed in the PhD project and key scientific results

WP1: Establishment of reference sequences from the main French clonal groups

Task-1.1: Culture, cloning and extraction of M. bovis strains (M21-M36)

→ This task has been delayed because of COVID-19 crisis, lockdown and technical problem in DNA extraction protocol but was finally accomplished in M36 (programmed for M26).

Task-1.2: Sequencing and de novo assembly (M27-M41)

M. bovis strains MinION sequencing at ANSES.
M. bovis strains PacBio and Illumina sequencing at Genoscreen (Lille France).
Ciriac used MinION and Illumina sequencing results and compared several assemblers (Raven, Flye, Unicycler and Spades).
Finally, Ciriac used bioinformatic tool Trycycler to obtain 10 consensus assemblies which take into account Raven, Flye and Unicycler assemblies.
Assembly strategy was validated on reference genomes.
Ten strains assembly with *de novo* assembly was performed.
→ This task was delayed because of COVID-19 crisis, lockdown and technical problem in WP1 task 1-1 (programmed for M37 but being finished in M41).

WP2: Genome sequence analysis

Task-2.1: Sequencing of supplementary strains (M38-M41)

To be done according to time disponibility.

Task-2.2: Genomic markers analysis (M33-M44)

Pangenomic studies on the 10 new reference genomes is ongoing.
Whole genome SNP on the new reference genomes and ancient reference genomes is being made.
Identification of genomic events (insertion / deletion or broad sequence polymorphism (LSP)) by comparison study of genetic variation between the new reference genomes sequenced and comparison between the new reference genomes and other genome already sequence with Illumina technology must be done.
In silico result will show the possible mutation, deletion or overexpression of a gene which will be good molecular targets to be explored in WP3.

WP3: Analysis of the antigenic variability: biochemical and lipidomic studies

Task-3.1: Protein profiles determination (M40-M49)

A confirmation of first *in silico* result will be reinforced with an appropriate biochemical or microbiological approach.

Task-3.2: Lipidomic analyses (M40-M49)

A confirmation of first *in silico* result will be reinforced with an appropriate biochemical or microbiological approach.

WP4: Valorisation and dissemination of results

Task-4.1: Publication of results (M36-M56)

Two publications in peer review journals are planned. One of them will present the number and localization of IS*6110* in the diversity of *Mycobacterium bovis* French strains. This article is currently being written and should be finished the next September.
Ten complete genomes have been obtained and should be publish in genbank soon.

The second publication will present the new reference genomes with their analysis, the genetic differences between them and the possible consequences in their phenotype.

Task-4.2: Communication (M36-M56)

Ciriac presented his thesis subject at the speech competition in ASMOHEJP2021. Ciriac also presented two posters in the same event (one in the IS*6110* work and one about obtaining new reference genomes).

The *M. bovis* congress scheduled on June 2020 has been postponed to June 2022 because of the COVID-19 crisis. We had been selected for a poster presentation on the 2020 planned session. According to our new results, we hope to be able to present a poster or oral communication on the 2022 session.

In September 2021, the student will participate to SFM Microbes 2021 (organized by French Society of Microbiology).

In March 2021, Ciriac presented on live his thesis subject during "ma thèse en 180 secondes challenge". The video is available on Youtube plateform: https://www.youtube.com/watch?v=NDdasDgzt2Y. The goal of this challenge is to present his thesis subject in 3 minutes.

Task-4.3: PhD manuscript writing (M36-M56)

The student must write thesis manuscript.

8.1.4.1.6.4 Progress of the research project: milestones and deliverables

8.1.4.1.6.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD06-ET5-PEMbo	D-PhD06-6.2	Second steering committee report	M42	M41		
PhD06-ET5-PEMbo	D-PhD06-6.4	first publication in microorganisms MDPI	M40		M46	

8.1.4.1.6.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD06-ET5-PEMbo	M-E5-1	PacBio + Illumina sequencing for obtaining reference genomes	M30	Yes	M41	Some strains are not sequence with PacBio technology but with MinION.
PhD06-ET5-PEMbo	M-E5-2	Assembly and annotation of reference genome	M38	No	M43	The genome are assembled and the assembly strategy was tested on reference genomes.
PhD06-ET5-PEMbo	M-E5-3	Illumina sequencing on supplemental strains	M32	Yes		
PhD06-ET5-PEMbo	M-E5-4	Mapped genome	M36	No	M44	ongoing
PhD06-ET5-PEMbo	M-E5-5	Strain selection for WP3	M40	No	M43	ongoing

PhD06-ET5-PEMbo	M-E5-6	SNP matrix	M44	No		
PhD06-ET5-PEMbo	M-E5-7	List of SNP in virulence genes	M44	No		

8.1.4.1.6.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Ma thèse en 180 secondes	To learn how to explain thesis subject quickly and clearly to a non-scientific people.	11/02/2021	Paris Est
MinION sequencing training	Presentation of MinION sequencing and how to run it	22-26/02/2021	ANSES
Bionumerics training	Presentation of Bionumerics	19/04/2021	ANSES
ABIES doctoral days 2021	Scientific meeting on "transition" organised by Ciriac's doctoral school.	5-6/05/2021	Doctorale school ABIES - AgroParisTech

8.1.4.1.6.6 Publications and patents

C. Charles' second thesis project steering committee report
19 May 2021
doi: 10.5281/zenodo.4772557
https://zenodo.org/record/4772558

8.1.4.1.6.7 Remarkable outcomes

Not Applicable

8.1.4.1.6.8 Impact and Relevance

The aim of this thesis project, a collaborative study between ANSES, Animal Health Laboratory (Maisons-Alfort) and INRAE, Infectiology and Public Health laboratory (Nouzilly), two French EJP Partners, is to better understand the complex biology of *M. bovis* through the study of the complete genomes of a large panel of isolates of interest. The first two parts aimed at obtaining reference sequence (WP1) and identifying large genomic events (WP2). This part of the project will be carried out at ANSES and with the support of Genoscreen, Lille, for the strains' Illumina and PacBio sequencing. The third part of the project will consist on studying phenotypic traits that the genetic events disclosed by the genomic studies through analyses of antigenic variability, lipidomics and proteomics studies. This part of the project will be carried out at INRAE with the support of IPBS laboratory at Toulouse for molecular target selection.

8.1.4.1.6.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers comments have been accepted and this is closed.

8.1.4.1.6.10 Impact of COVID-19 crisis on the project

The delay observed in the last report has been almost completely made up. The task 2.2, 3.1 and 3.2 should not be affected.

8.1.4.1.6.11 List of critical risks

Description of risk	Yes/No

Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

8.1.4.1.6.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Not Applicable

8.1.4.1.6.13 List of dissemination and communication activities

Name of the activity:	*Ma thèse en 180 secondes*		
Date:	*09/03/2021*		
Place:	*Youtube https://www.youtube.com/watch?v=NDdasDgzt2Y*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	*Yes*
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media	***Yes***	***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)		***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public	*160 during the live and 143 in youtube plateform*	***Other***	

Policy Makers			

Name of the activity:	*C. Charles' second thesis project steering committee report*
Date:	*19/05/2021*
Place:	*Zenodo: https://zenodo.org/record/4772558*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)	*Yes*	***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***15***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*ABIES doctoral days 2021. Oral presentation.*
Date:	*06/05/2021*
Place:	*Zoom*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	

Social Media		*Participation in activities organized jointly with other H2020 projects*	
Website		*Other*	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	*170*	*Media*	
Industry		*Investors*	
Civil Society		*Customers*	
General Public		*Other*	
Policy Makers			

Name of the activity:	*ASMOHEJP2021. Two posters presentation.*
Date:	*10/06/2021*
Place:	*https://onehealthejp.eu/asm-satellite-workshop-2021/*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		*Participation to a Conference*	*Yes*
Organisation of a Workshop		*Participation to a Workshop*	
Press release		*Participation to an Event other than a Conference or a Workshop*	
Non-scientific and non-peer-reviewed publication (popularised publication)		*Video/Film*	
Exhibition		*Brokerage Event*	
Flyer		*Pitch Event*	
Training		*Trade Fair*	
Social Media		*Participation in activities organized jointly with other H2020 projects*	
Website		*Other*	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	*More than 800*	*Media*	
Industry		*Investors*	
Civil Society		*Customers*	
General Public		*Other*	
Policy Makers			

Name of the activity:	*ASMOHEJP2021. 3MT challenge.*
Date:	*9-11/06/2021*
Place:	*https://onehealthejp.eu/asm-satellite-workshop-2021/*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***More than 800***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.1 PhD07-R2-ET2.1-MACE

8.1.4.1.1.1 Summary

A review of cystic echinococcosis surveillance and control methods, as well as historical mathematical modelling approaches used, was drafted for an internal report due 15 months after the start of the PhD (Confirmation report). This report is available in Zenodo (relates to D-PhD07-6.1).

Methodology for a new surveillance programme for cystic echinococcosis in dogs for Uruguay has been developed and will be implemented by our colleagues in the National Commission for zoonoses (NCZ). This framework is being extended and adapted to support current planning of a screening programme across the country, in collaboration with the Ministry of Agriculture (relates to D-PhD07-6.2).

The elicitation questionnaire is currently being planned to be conducted in a representative sample audience of stakeholders in South America. We are currently in communications with partners and are planning to share the questionnaire following a short presentation of the objectives and outcomes of the survey (relates to D-PhD07-6.3).

A stochastic, individual-based transmission model of the dynamics of cystic echinococcosis between dogs and sheep is currently being developed, with significant progress made (relates to D-PhD07-6.4).

8.1.4.1.1.2 Overview of the PhD project progress

The PhD project has been progressing mostly as originally planned. A draft of review of surveillance and control tools for CE (D-PhD07-6.1) was written for the confirmation process and uploaded to Zenodo. An inital spatio-temporal model of CE was fitted to data in Uruguay (D-PhD07-6.2). The student's current focus country in South America is still Uruguay, with very good engagement from local stakeholders and new initiatives being planned where new data will become available. The elicitation questionnaire (D-PhD07-6.3) that was originally moved forward was developed, but it was not possible to deploy yet due to COVID19, we are currently engaging stakeholders to deploy it in South America. Significant progress has been made coding the mathematical model to simulate different control scenarios (D-PhD07-6.4).

8.1.4.1.1.3 Progress of the research performed in the PhD project and key scientific results

The relevant literature has been flagged (MACE.Y2.A), with a draft finalized for the student's confirmation report (due in M39), this output relates to deliverable (D-PhD07-6.1), which is available in Zenodo.

An initial pilot version of the spatial model was developed based off data from Uruguay between the years of 2008 – 2015 (D-PhD07-6.2), however due to concerns regarding the spatial coverage of the data used, it was decided not to make the analysis public yet. Instead we are adapting and extending the framework to support a screening programme that Uruguay is currently planning, so that the model can be revisited with better data. We have designed the methodology for the new campaign, and we are currently waiting for the sharing of locations of possible sample locations. The spatial model will be further developed once the surveillance campaign is finished and data is shared.

The main focus of the student during the last year has been to conduct an elicitation with surveillance and One Health experts at an international conference. This exercise will allow to capture in the mathematical model developed in this project more realistic considerations regarding "willingness to pay" and "willingness to accept" of different control and surveillance strategies. This task relates to MACE.Y5.3; and was originally scheduled for later in the project (milestone MACE.Y5.A; M53; updated as deliverable D-PhD07-6.3), however a unique opportunity presented itself to conduct this task with the support of the organizers of the 4th International Conference on Animal Health Surveillance (ICAHS). This exercise has never been done for surveillance in a One Health context, and will have unique policy relevant impact, which will be shared with key stakeholders. Due to Covid-19, the ICAHS conference was delayed and eventually cancelled, the framework developed here was then adapted

(upon a request by a WHO colleague) to support the COVID-19 response, by conducting the questionnaire within the WHO COVID-19 Stakeholder meeting in the context of investment in improving sensitivity for contact-tracing of cases in the WHO South-East Asian Region (SEAR). However, due to the surge of cases in India in early 2021, the activity could not be carried out. The current aim is to conduct the elicitation, as originally planned, with One Health experts in South America, during the summer 2021, and finalize it by M44.

A multi-host, individual based transmission model is currently being developed to, initially, simulate the dynamics of the disease between sheep and dogs (D-PhD07-6.4). This will be extended to include different control options. Coding of the model has started, but not finished (MACE.Y3.A). Simulations still need to be finished (MACE.Y3.B).

Posters have been submitted and presented in the OHEJP ASM 2021, BSP annual conference 2021, and the Surrey Vet School Symposium. A poster presentation, participation in a 3MT competition, and organisation of a quiz for a hybrid audience for the OHEJP ASM 2021.

8.1.4.1.1.4 Progress of the research project: milestones and deliverables

8.1.4.1.1.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD07-R2-ET2.1-MACE	D-PhD07-6.1	Draft of review of surveillance and control tools for CE	M37	M37		Report completed and uploaded on Zenodo.
PhD07-R2-ET2.1-MACE	D-PhD07-6.2	Spatial Temporal Model of CE validated in Uruguay	M37		M44	Spatio-Temporal model completed and fitted to Historical data from Uruguay (by M37). However, concerns were raised by our local partners in Uruguay regarding the data. We are supporting planning for more data collection and want to revisit this later in the project.
PhD07-R2-ET2.1-MACE	D-PhD07-6.3	Questionnaire to Elicit WTP/WTA of One Health Surveillance Activities	M36		M44	This deliverable was moved up during the first year of the PhD due to an Opportunity presented at the time. Due to Covid-19 the venue was cancelled and an alternate venue is being organised.
PhD07-R2-ET2.1-MACE	D-PhD07-6.4	Final draft of publication with the model and control scenarios	M44			Progress has been made with the model.

8.1.4.1.1.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD07-R2-ET2.1-MACE	MACE.Y2.A	Relevant literature on surveillance and control of CE identified	M23	YES		Relevant literature identified. Draft document included in student's Confirmation report (due M39)
PhD07-R2-ET2.1-MACE	MACE.Y2.B	Training in Mathematical modelling	M36	YES		Some training activities delayed due to COVID
PhD07-R2-ET2.1-MACE	MACE.Y3.A	Fitted model of CE to data from Uruguay	M37	YES		First iteration of statistical model fitted to Uruguay historical data.

PhD07-R2-ET2.1-MACE	MACE.Y3.B	Simulations of different control scenarios	M44			Simulations to be run once transmission model is fitted
PhD07-R2-ET2.1-MACE	MACE.Y4.B	Eastern European data	M40	NO	M48	Due to COVID, engagement with stakeholders has been challenging. We plan to conduct some data analysis later in the year.
PhD07-R2-ET2.1-MACE	MACE.Y5.A	Online polls with stakeholders completed	M36	Partial	M44	Poll finalised. Deployment pending.

8.1.4.1.1.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Writing A Confirmation Report	Advice on writing the confirmation report	12/Jan/2021	University of Surrey - Doctoral College
GGA Spanish Stage 3	Spanish Language	29/Sept/2020 – 5/May/2021	Universtiy of Surrey - Global graduate award
OHEJP Summer School 2021	One Health	26th July – 6th August 2021	OHEJP

8.1.4.1.1.6 Publications and patents

Review of surveillance and control tools for CE
DOI : 10.5281/zenodo.4672250
Link : https://doi.org/10.5281/zenodo.4672250
Public publication

This was published as part of the deliverable D-PhD07-6.1.

8.1.4.1.1.7 Remarkable outcomes

We are engaging with colleagues in Uruguay (which includes the national commission of zoonosis and the Ministry of Agriculture). We are directly impacting work that is being done on the surveillance programme on CE in dogs.

8.1.4.1.1.8 Impact and Relevance

This project has allowed collaboration with partners within the consortium (namely between UoS and ISS), as well as supporting stakeholders in South America. The PhD student has engaged in activities in Brazil and Uruguay, supporting ministry of health officials through data analysis of current surveillance and control programmes.

We have been collaborating with the National Commission for Zoonoses in Uruguay and assisting them in planning a survey for 2021, sampling dogs for cystic echinococcosis. We are extending our network of collaborations and partnerships to maximize the impact of the work developed, currently engaging with partners in the Eastern European region.

8.1.4.1.1.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers comments have been accepted and this is closed.

8.1.4.1.1.10 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables			Associated budget		
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			D-PhD07-6.3 MACE.Y4.B	M53 M40	M46 M48	The elicitation was originally moved forward, however the original venue where the elicitation was going to be conducted in was cancelled due to COVID This was delayed as collaboration with stakeholders has been challenging		

8.1.4.1.1.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	No
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	

8.1.4.1.1.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

We are collaborating with Comision Zoonosis in Uruguay and their Programa de Equinococosis quística (Programme of Cystic Echinococcosis). They are planning on initiating a national survey for cystic echinococcosis in dogs. We are preparing to assist with the planning of said programme.

In terms of OHEJP stakeholders, this project is a close collaboration between UoS and ISS, Rome. We are working closely with Dr. Adriano Casulli from the Istituto Superiore di Sanità in Italy, who leads the MEmE JRP. We are also planning on collaborating with Professor Majid Fasihi Harandi from the Kerman University of Medical Sciences in Iran, with the studies they are conducting on cystic echinococcosis within the region's dog population.

8.1.4.1.1.13 List of dissemination and communication activities

Name of the activity:	*OHEJP ASM 2021 – three minute thesis competition, poster presentation, and quiz organisation*		
Date:	*9th – 11th June 2021*		
Place:	*Virtual*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	***Yes***	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	***Yes***
Communication Campaign (e.g. Radio, TV)	*No*	***Organisation of Social Activities***	
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*BSP annual meeting 2021*
Date:	*21st – 25th June 2021*
Place:	*Virtual*
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories	

	Yes / No		Yes / No
Organisation of a Conference	*No*	***Participation to a Conference***	*No*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*Yes*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		Number
Scientific Community (Higher Education, Research)	***N/A***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*Surrey Vet School Symposium*
Date:	*1st and 2nd July 2021*
Place:	
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories	

	Yes / No		Yes / No
Organisation of a Conference	*No*	***Participation to a Conference***	*No*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*Yes*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		Number
Scientific Community (Higher Education, Research)	***N/A***	***Media***	

Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.2 PhD08-R2-ET5.1/4.1/FBZSH3-DESIRE

8.1.4.1.2.1 Summary

In the first 7 months of 2021 the PhD candidate has focused on the NGS analysis, literature review and field work. Furthermore, the PhD student has participated in multiple educational activities, both on specific topics to be used in this PhD (e.g. a Microbiome Data Analysis Workshop) and on general scientific topics, such as Scientific publishing and Scientific writing.

8.1.4.1.2.2 Overview of the PhD project progress

The main activities this far in 2021 have been the NGS analysis, literature review and field work. Furthermore, the PhD student has participated in multiple educational activities, both on specific topics to be used in this PhD (e.g. a Microbiome Data Analysis Workshop) and on general scientific topics, such as Scientific publishing and Scientific writing.
In 2021 a visit was initially planned to a collaborating partner (FLI) in July. This visit has been rescheduled to the end of the year, due to the extended field work. It depends on the corona measures and lab activities whether this visit can take place at that moment. This is currently not clear.

8.1.4.1.2.3 Progress of the research performed in the PhD project and key scientific results

In 2021, it was planned to continue the field study, which indeed is progressing as anticipated. It was decided in 2020 to collect more samples from a third city rather than initiate a new type of field study. It is expected that the field study will result in sufficient data to use for hypothesis 2 and 4.

Also the collection of monitoring data of rat populations by app continued in 2021. However, the number of reports is currently too low and too geographically clustered to be used for analyses. The monitoring app is running and will still continue, but the PhD will focus on a different study : a genetic study, assessing the genetic relatedness of rat populations accros a city, and studying the influence of green and blue areas on the dispersal of wild rats.

The PhD student has familiarised herself with the analyses of the NGS data. This resulted in preliminary results, which we then wanted to confirm with more traditional techniques. This is currently ongoing in a collaboration with the partner institute WBVR. This therefore is taking up some more time than anticipated, but we think this will improve the final result. Also with the WBVR, the PhD student has studied the virome of a selection of rats. However, preliminary analyses do not show any results yet that require further follow-up.

Additional to the initial work plan, a literature review on zoonoses of wildlife has started, for which several wildlife species that are commonly found in the urban environment have been selected. This should aid in prioritising research and funds, and should give more insight in ecological mechanisms influencing the wildlife populations and/or the zoonotic pathogens.

8.1.4.1.2.4 Progress of the research project: milestones and deliverables

8.1.4.1.2.4.1 *Deliverables*

No deliverables planned for this period.

8.1.4.1.2.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achiev	Comments

					ement date	
PhD8-ET5.1/4.1/FBZ SH3-DESIRE	M3	Visit laboratories of partner institutes WBVR and FLI	June (M42)	partly	Nov (M47)	The partner institute WBVR has been visited several times for various collaborations, of which the study of the virome is most notable. The FLI will be visited later this year, if the corona situation allows this.

8.1.4.1.2.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Introduction to R	How to work with R	12-01-21 t/m 02-02-21	Wageningen University
Scientific publishing	Scientific publishing	09-03-21	Wageningen University
Scientific writing	Scientific writing skills	29-03-21 t/m 10-05-21	Wageningen In'to languages
Microbiome Data Analysis Workshop (MDAW)	Data analysis of microbiome data	20-04-21 t/m 23-04-21	Hasselt University
Supervising students	How to supervise BSc and MSc students	17-06-21 & 18-06-21	Wageningen University

8.1.4.1.2.6 Publications and patents

Not Applicable

8.1.4.1.2.7 Remarkable outcomes

One Health EJP Annual Scientific Meeting Three Minute Thesis competition winner 2021

8.1.4.1.2.8 Impact and Relevance

The PhD student has regular contact with scientists from the WBVR about different aspects of the study: virome analysis, analysis of 16S NGS results and specific pathogen testing. The supervisors involved in this PhD meet once every 3 months to discuss the progress of the research.
Also, we are currently discussing potential collaborations for the genetic study with the Wageningen University and the University of Amsterdam.

8.1.4.1.2.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers' comments have been accepted and this is closed.

8.1.4.1.2.10 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to	Expected end date	Associated Milestone or	Deadline according to	New proposed	Reason for delay	Budget that will	Budget that will

	AWP 2020	due to crisis	Deliverable	AWP 2020	deadline		not be spent	be spent with delay
NGS analyses	M57	M59	D-PhD08-3			Delay due to inefficient supervision possibilities	NA	NA

8.1.4.1.2.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes, due to COVID-19
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

8.1.4.1.2.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

The PhD student and PI's are involved in a grant proposal about "Ticks and the city", which has been submitted again and has made it to the second selection round. If this is granted, this would result in extensive collaboration between the projects. In the selection of the field sites this has already been taken into account.
Also, collaboration is present with our RIVM colleagues working on AMR: samples that contain MRSA or antibiotic resistant Enterobacteriaceae are further typed together with this group.
Furthermore, we share samples with a PhD student who is based at the Erasmus MC and who is part of the *One Health PACT – Predicting Arboviruses Climate Tipping points* project from the Netherlands Centre for One Health. This PhD student will test the samples for arboviruses that we initially had not included in our study plan, of which our PhD can also use the results. This collaboration therefore is beneficial to both PhD's.

8.1.4.1.2.13 List of dissemination and communication activities

Name of the activity:	*Poster presentation at the WIAS Annual conference*		
Date:	*28 & 29 April 2021*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*

Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***30***	***Media***	*0*
Industry	***0***	***Investors***	*0*
Civil Society	***0***	***Customers***	*0*
General Public	***0***	***Other***	*0*
Policy Makers	***0***		

Name of the activity:	*ASM OHEJP 2021 – 3 minute thesis oral presentation and poster presentation*		
Date:	*9-11 June 2021*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*Yes*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	*0*
Industry	***5***	***Investors***	*0*
Civil Society	***0***	***Customers***	*0*
General Public	***0***	***Other***	*0*
Policy Makers	***5***		

8.1.4.1.3 PhD09-R2-FBZSH3/AMR2.1-UDOFRIC

8.1.4.1.3.1 Summary

The student has been making progress in the project's current objectives. The nine-month Y2 report has been submitted as the first deliverable. The literature review focussing on the background and previous research conducted on fluoroquinolone (FQ) resistance (FQR) in *Campylobacter* has been completed. The project has access to six distinct datasets over a 24-year period. *Campylobacter* samples were obtained either by sampling caecal contents of broilers at slaughter or neck skin from broiler carcasses after processing. Data analysis has been carried out on a subset of the dataset available to this project, obtained by previous UK national research and surveillance studies in poultry, analysing the trends in FQR over time and production factors associated with FQR (e.g. bird age, farming methods, bird weight). Work has also been conducted identifying *Campylobacter* lineages and aims to determine if there is a link between MLST or whole genome sequence information and FQR. Historic isolates have been recovered from the APHA *Campylobacter* National Reference Laboratory archives, their species classification has been confirmed and DNA from these isolates has been extracted and whole genome sequencing (WGS) has been carried out on isolates from 1995, 2008 and 2020. Work is currently being carried out to determine genotypic and phenotypic resistance profiles and multi-locus sequence types (MLST).
In vivo experiments have begun with the student relocating to ANSES, France. The student is currently undergoing relevant training for the analysis of samples produced via the outcome of these experiments.

The student has also been taking part in journal clubs, attending local presentations/seminars at the APHA and meeting with staff members who have extensive experience and knowledge on the historic use of antibiotics (ABs). The student also presented a poster at the 2021 annual EJP scientific meeting, he also entered the 3MT competition and presented a poster the medical school post graduate conference with the University of Warwick.

8.1.4.1.3.2 Overview of the PhD project progress

- **Literature review**

The first section of the literature review focused on *Campylobacter* within broiler production systems, its impact on industry and on human health. Also, publications on *Campylobacter* genetics, including virulence mechanisms, mutations, and gene transfer have been reviewed.

The second section of the literature review has also been completed, with a distinct focus on FQs. The first subsection focusses on discovery of FQs, their chemical structure and the differences between different types of FQs, their uses, importance and mechanisms of action. Then, the review describes the historic trends in the use of FQs and how this usage has developed over time. The text mainly focusses on when literature first discovered a link between the use of antibiotics both in medicine and in agriculture and how use in these sectors selected for antibiotic resistance, including the environmental impact. This link was then recognised by government bodies and the use of antibiotics as growth promoters was legally prohibited in the EU by 2006.

The third section focuses on the specific relationship between FQs and *Campylobacter,* and how this has developed over time. It summarised the research in the mechanisms of FQR in *Campylobacter*, the spread of this resistance and previously identified factors associated with FQR in *Campylobacter.*

- **Data collection and identification of data gaps**

Information has been gathered and reviewed of six datasets taken from research and surveillance of broiler production systems from 1994 to 2020. These datasets include information on phenotypic resistance to antimicrobials, genomic data (including some whole genome sequence data) and epidemiological meta-data.

Key work has been undertaken in the collation of the datasets included in this project. A sample size of isolates has been collected for years (1995, 2008 and 2020) where WGS information was missing

and work is ongoing to process this. Isolates sampled from 1995 have been profiled using MLST and we are currently working on building a fluoroquinolone resistant snps database for *Campylobacter* to determine genotypic resistance. From 1995, 2008 and 2020 we have also determined a sample list and outlined methods for MICs to determine phenotypic resistance profile to compare with our genotypic resistance findings.

Further work has been carried out in the analysis of previously characterised datasets to identify trends in resistance in several associated production variables (such as farming method, abattoir of slaughter etc). Initial observations follow that of previous studies, indicating that FQR in *Campylobacter* has increased over time and that certain clonal complexes and sequence types may be specifically associated with FQR. Similar analysis is needed on one final dataset the student has yet to acquire.

- **Training in WGS and bioinformatics**

Training in Whole Genome Sequencing (WGS) and bioinformatics has been undertaken earlier than planned, due to COVID 19 safety precautions preventing access to lab-based training. Bioinformatics training has involved the building of a database of known antimicrobial resistance (AMR) genes and mutations for *Campylobacter*, and initial training to assemble genomes, to identify multi locus sequence types using $SRST_2$ and detecting AMR genes and mutations using the APHA SeqFinder WGS analysis pipeline. The student has now categorised the first set of WGS information (1995 dataset) into MLST profiles, completed training in the use of APHA SeqFinder and is finalising the *Campylobacter* AMR database to determine a genotypic resistance profile. The student has also attended a week-long course with EURL and an online training course of "Bioinformatics for Biologists: An Introduction to Linux, Bash Scripting, and R" with Wellcome Genome Campus Courses and Conferences to train in different detection methods of AMR and utilising WGS information.

- **In vivo trials**

In vivo FQ resistance generation has begun, with the birds being inoculated on the 16 of Sep 2021.Tests began on the 22 of Sep 2021 to determine colonisation of the chicks by the intended strains. Confirmation of the chicks were *Campylobacter*-free status was also carried out on the first day of experimentation. The student has been trained on plate replication methods, PCR and specific cell culturing techniques whilst at ANSES to prepare for future work.

8.1.4.1.3.3 Progress of the research performed in the PhD project and key scientific results

- **Literature review of FQ in *Campylobacter***

The student has completed the literature review on the topic as planned. It consists of three major sections: *Campylobacter*; Fluoroquinolones and Fluoroquinolone resistant *Campylobacter*.

- **Description of the diversity of FQR and acquisition of resistance variants over time**

Unplanned supervisor leave has affected the procurement of some datasets delaying the milestone's progress. Work has been carried out on previously characterised datasets, from 2007-2009 (including caeca and carcass samples) and 2012-2015 (carcass samples only). Of previously characterised datasets we are currently attempting to obtain 2013-2016 caeca dataset, while also carrying out our own analysis on WGS information obtained from 1995, 2008, and 2020 caeca samples.

- **Report on the relationship between WGS and phenotype**

Work has been completed on WGS from historic isolates from 1995, 2008, and 2020. Work is currently ongoing to determine genotypic FQ resistance of these isolates and a sample list has been compiled of the same years to determine phenotypic resistance by MIC. The determination of phenotypic resistance has been delayed due to COVID-19.

- **Identification of strains for fitness trials**

Strains have been selected for use in *in vivo* experimentation with the first set of trials already begun. Strains selected for use have been labelled as wt-01, wt-02, wt-04 and wt-05 and 2 backup strains have

also been transferred to ANSES as a backup in case of any issues in the process. Generation of counterpart strains for comparison has started with *in vitro* alternatives also created.

- **Selection and characterization of isogenic resistant strains**

This work has not begun, and it is not due until Jan 2022

- **In vitro fitness study: competition growth assays and growth kinetics**

This work has not yet started, and it is not due until Mar 2022

- **In vitro fitness study: comparison of survival on abiotic surfaces and on food matrices (e.g. Chicken skin model)**

This work has not yet started, and it is not due until May 2022

- **In vivo study: comparison of colonisation using chicken models**

This work has not yet started, and it is not due until Sep 2022

8.1.4.1.3.4 Progress of the research project: milestones and deliverables

8.1.4.1.3.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD09-FBZSH3/AMR2.1-UDOFRIC	D-PhD09-1.2	Literature review of FQ in Campylobacter	M37	M38		
PhD09-FBZSH3/AMR2.1-UDOFRIC	D-PhD09-1.3	PhD Annual review	M38	M38		
PhD09-FBZSH3/AMR2.1-UDOFRIC	D-PhD09-2.1	Description of the diversity of FQ resistance and acquisition of resistance variants over time.	M41	M46		
PhD09-FBZSH3/AMR2.1-UDOFRIC	D-PhD09-2.2	Report on the relationship between WGS and phenotype.	M42	M46		
PhD09-FBZSH3/AMR2.1-UDOFRIC	D-PhD09-2.3	Identification of strains for fitness trials	M48			

8.1.4.1.3.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments

PhD09-FBZSH3/AMR2.1-UDOFRIC	1	Completion of literature review	M37	Yes		
PhD09-FBZSH3/AMR2.1-UDOFRIC	6	Annual review	M38	Yes		
PhD09-FBZSH3/AMR2.1-UDOFRIC	7	Completion of phenotyping and WGS	M39	No	M46	Deadline delayed due to national pipette shortage
PhD09-FBZSH3/AMR2.1-UDOFRIC	8	Descriptions of genomic diversity and temporal trends in resistance	M41	No	M46	Deadline delayed due to complications in the acquisition of datasets
PhD09-FBZSH3/AMR2.1-UDOFRIC	9	Relationship between WGS and phenotype	M42	No	M46	Deadline delayed due to national pipette shortage
PhD09-FBZSH3/AMR2.1-UDOFRIC	10	Identification of strains for fitness study	M45	Yes		
PhD09-FBZSH3/AMR2.1-UDOFRIC	11	Completion of 9-month review	M45	Yes		

8.1.4.1.3.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
OHEJP Annual Scientific Meeting	Virtual scientific conference	09/06/21 - 11/06/21	One Health European Joint Project
Warwick University Medical school Post-graduate symposium	Virtual scientific conference	26/05/21	Warwick University
EURL-AR online training course	Training course	26/04/21 - 29/04/21	EURL

8.1.4.1.3.6 Publications and patents

N/A

8.1.4.1.3.7 Remarkable outcomes

N/A

8.1.4.1.3.8 Impact and Relevance

Fluoroquinolone resistance in *Campylobacter* is a threat, with the World Health Organisation (WHO) naming it on their list of 12 priority pathogen/AMR combinations. The information gathered during this project on drivers of resistance will help policy makers, the scientific community and the agricultural industry make decisions to prevent or reduce the continuous spread of FQR in *Campylobacter*. The study will interrogate archives of *Campylobacter* from UK research and surveillance activities in broilers from 1995 to 2020 (APHA isolates, phenotypes, MLST, WGS, production metadata), to determine the development and diversity of resistance to FQ over time (temporal trends). In addition, data from French broilers and from other potential sources of *Campylobacter* exposure to people (livestock/environment) will be interrogated wherever possible (ANSES and Public access archives and databases).

The UDoFRiC project combines the collaborative experience of the APHA, ANSES, and Warwick University. It combines the expertise of microbiologists, epidemiologists, and bioinformatics throughout various institutions in the UK and France. The project is supervised by Dr John Rodgers who leads the National reference lab for *Campylobacter* in animals, Professor Muna Anjum who leads bacterial characterisation workgroup and is the AMR research lead and Dr Manal Abu Oun who is the AMR and virulence characterisation team lead at the Animal and Plant Health agency (APHA). Professor

Noel McCarthy is the lead of Evidence in Communicable Disease Epidemiology and Control at Warwick University. The project also includes a year at the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) under the supervision of Dr Isabelle Kempf who is a researcher microbiologist specialized in AMR; Leading the Mycoplasmology Bacteriology Antimicrobial Resistance Unit in ANSES, Ploufragan and Dr Katell Rivoal, who works in the Hygiene and Quality of Poultry and Pork Products research unit and is leading scientific projects on zoonotic pathogens (*Salmonella, Listeria*, and *Campylobacter*) in poultry productions.

8.1.4.1.3.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers' comments have been accepted and this is closed. *In vivo* protocols have been prepared and accepted by the ethics committee and Ministry of education and research

8.1.4.1.3.10 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Completion of training in bacteriology and MIC	Sept 2020	December 2020	D-PhD09-2.2	Sept 2020	Apr 2021	COVID-19 isolation guidelines have prevented lab-based training. The impact of COVID testing has also impacted the supply of pipette tips needed to carry out MICs	n/a	n/a
WGS and bioinformatics training	Dec 2020	March 2021	D-PhD09-2.1	Dec 2020	March 2021	COVID-19 has prevented face-to-face interaction and therefore negatively affected teaching time. The limitations on lab-based training also prevented the acquisition of some WGS data for teaching exercises	n/a	n/a

8.1.4.1.3.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	Yes
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No

Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

Additional information:
The main supervisor was on extended from Mar-Aug2021, however other supervisors intervened to provide guidance and support.

8.1.4.1.3.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Not Applicable

8.1.4.1.3.13 List of dissemination and communication activities

Name of the activity:	One Health European Joint Project Annual Scientific Meeting – 3 minute thesis and poster presentation		
Date:	09-11 Jun 2021		
Place:	Online		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	Warwick Medical School Post-Graduate Symposium poster presentation
Date:	26 May 2021
Place:	Online

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		*Participation to a Conference*	*Yes*
Organisation of a Workshop		*Participation to a Workshop*	
Press release		*Participation to an Event other than a Conference or a Workshop*	
Non-scientific and non-peer-reviewed publication (popularised publication)		*Video/Film*	
Exhibition		*Brokerage Event*	
Flyer		*Pitch Event*	
Training		*Trade Fair*	
Social Media		*Participation in activities organized jointly with other H2020 projects*	
Website		*Other*	
Communication Campaign (e.g. Radio, TV)			

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	*100+*	*Media*	
Industry		*Investors*	
Civil Society		*Customers*	
General Public		*Other*	
Policy Makers			

8.1.4.1.4 PhD10-R2-FBZSH3/AMR2.1-WILBR

8.1.4.1.4.1 Summary

A literature review has been written on the role of wild birds in dissemination and persistence of antimicrobial resistance (AMR) in the farm environment. The review includes sections on identifying the current situation regarding AMR in different environments; drivers for AMR; the role of vectors and the environment in persistence and dissemination of AMR; the role of AMR surveillance; and evaluation of different methodologies for identifying AMR, both by phenotype and genotype, in bacteria.

Pig and presumptive gull faecal samples were collected over three time points in October 2017, 2018, and 2019 from a low antimicrobial usage pig farm as part of the APHA's work on the ARDIG project within the One Health European Joint Programme (https://onehealthejp.eu/jrp-ardig/). These were grown on antibiotic free and antibiotic selective agar, and 632 *E. coli* were purified from pig and gull faeces (n=342 and n=290 respectively) (see Annex 2, Table 1). 176 gull isolates (110 from T3, 66 from T5) that had not been subject to previous analyses were identified and underwent whole genome sequencing. WGS data for all isolates was run through APHA Seqfinder pipeline and Abricate to identify AMR determinants and plasmids. Seven allele MLST was carried out using SRST2 to identify the *E. coli* Sequence Type (ST) of isolates, which was used as an indicator of *E. coli* diversity in pig and gull populations. A total of 91 different sequence types were identified from the isolates included in this study, with 63 and 57 STs identified in gull and pig isolates, respectively. Considering the SNP differences is the next step to understand if the same "clone" or strain has been transferred rather than an AMR plasmid A core genome SNP alignment was generated from WGS data of all isolates using Snippy version 4.6.0 to compare the core genes that are present in all members of the same species, and a SNP distance matrix was generated using snp-dists version 0.7.0 to estimate the SNP differences between isolates to determine the levels of genetic variation in the ST744s, allowing any transmission and persistence of clones across host species and time points, on this farm, to be identified. Preliminary analysis allowed AMR profiles to be linked to ST types that are circulating in both wild bird and pig populations.

Another outdoor pig farm, known to have wild birds persistently present on farm, had been recruited for a longitudinal study, but due to Covid-19 restrictions we were unable to undertake any farm visit to collect environmental samples, faecal samples from pigs, and caecal samples from corvids present on farm. Visits to this farm have now been pushed back, with the current plan being to start sampling in August 2021. An opportunity to examine the dissemination of AMR in wild bird populations across Great Britain for a 12 month period through the APHA's wild bird scanning surveillance programme is currently being pursued.

A poster was submitted to the OHEJP ASM 2021 meeting, and an oral presentation on the PhD was presented virtually in the 3MT competition for the OHEJP ASM 2021, as part of communicating their research. The student won the prize for the best poster presentation at the OHEJP ASM 2021. The student has also attended two virtual plasmid workshops hosted by International Society for Plasmid Biology and Other Mobile Genetic Elements in order to further develop their skill set for tasks later on in the project.

8.1.4.1.4.2 Overview of the PhD project progress

Due to the Covid-19 pandemic in UK only desk and lab-based tasks have been recommended; currently farm visits are not permitted for research projects. A literature review has been completed on the role of wild birds in dissemination and persistence of antimicrobial resistance (AMR) in the farm environment. The review includes sections on identifying the current situation regarding AMR in different environments; drivers for AMR; the role of vectors and the environment in persistence and

dissemination of AMR; the role of AMR surveillance; and evaluation of different methodologies for identifying AMR, both by phenotype and genotype, in bacteria.

Over two hundred archived E. coli isolates from gull faeces that had not been subject to previous analysis have undergone whole genome sequencing, and downstream bioinformatics is now currently taking place. These isolates were collected as part of the OH-EJP ARDIG project. Work is still being undertaken so results are not complete.

An opportunity to examine the dissemination of AMR in wild bird populations across Great Britain for a 12 month period through the APHA's wild bird scanning surveillance programme is currently being pursued. It has been agreed that the wild bird scanning surveillance will provide 0.5-1.0g of caecal/intestinal contents from every wild bird submitted as part of the APHA's wild bird scanning surveillance for a 12 month period. This study would allow a unique opportunity to establish the burden of AMR in the wild bird population of Great Britain. Sampling over 12 months will allow me to observe any changes throughout the different seasons, which may be impacted by wild bird migratory patterns. The study will also provide context to our on farm studies by allowing wider comparisons to be made, highlighting any correlation between the AMR profiles identified in the farm environment and those found in wild birds across Great Britain.

8.1.4.1.4.3 Progress of the research performed in the PhD project and key scientific results

Deliverable 1 in AWP for Y4 is being undertaken.

Milestone 1 in AWP has been delayed as due to further Covid-19 restrictions in the UK, all fieldwork at APHA has been stopped. The original plan was that the farms successfully recruited for the longitudinal study would be visited once in Y1 and pig faecal samples will be collected. The new plan will involve visiting the farm at four timepoints over a 12 month period where environmental, pig faecal samples, and corvid caecal samples will be collected. The APHA epidemiology department has been consulted and a farm questionnaire and an initial sampling plan designed. The final sampling plan will be dependent on the farmer's questionnaire responses and we'll use a statistical test to calculate appropriate numbers to match wild birds. Due to further Covid-19 restrictions in November the fieldwork for this study was delayed again, after last summer, by several months.

Some historical E. coli isolated from gull faeces, collected from an outdoor pig farm, during a longitudinal study in the OH-EJP project ARDIG are being included in this PhD project as a result of the delays caused by COVID-19. Over two hundred isolates archived from this farm have undergone whole genome sequencing, and downstream bioinformatic analysis is now being carried out in line with Milestone 2. This will result in a dataset including over 300 gull isolates and over 400 pig isolates from three time points that can be compared. AMR profiles and phylogeny are being generated from whole genome sequencing data to assess the diversity of *E. coli* strains and associated ARGs in gulls and pigs on the same farm, and assess the potential for transmission of AMR bacteria between wild birds and livestock. This work is still being carried out so the results are not yet complete.

An opportunity to examine the dissemination of AMR in wild bird populations across Great Britain for a 12 month period through the APHA's wild bird scanning surveillance programme is also being pursued. The wild bird scanning surveillance programme examines wild bird species for all disease and mortality investigations, including infectious and non-infectious disease. They receive submissions from all over Great Britain, and from many different species. It has been agreed that the wild bird scanning surveillance will provide 0.5-1.0g of caecal/intestinal contents from every wild bird submitted as part of the APHA's wild bird scanning surveillance for a 12 month period. Final logistics are being confirmed, taking in to account the risk from human and livestock pathogens that wild birds could be carrying.

8.1.4.1.4.4 Progress of the research project: milestones and deliverables

8.1.4.1.4.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD10-FBZSH3/AMR2 .1-WILBR	D-PhD10-1.3	Completion of 9 month review	M45			
PhD10-FBZSH3/AMR2 .1-WILBR	D-PhD10-1.4	Completion of 12 month review	M48			

8.1.4.1.4.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD10-FBZSH3/AMR2 .1-WILBR	1	Collection of microbiological samples for longitudinal study	M48			
PhD10-FBZSH3/AMR2 .1-WILBR	2	Molecular analysis of bacterial isolates and antimicrobial resistance	M48			
PhD10-FBZSH3/AMR2 .1-WILBR	3	Characterisation of mobile elements transmitting/proliferating AMR	M48			
PhD10-FBZSH3/AMR2 .1-WILBR	4	Completion of 9 month review	M45			
PhD10-FBZSH3/AMR2 .1-WILBR	5	Completion of 12 month review	M48			

8.1.4.1.4.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
EURL-AR Training Course	Working with bacterial sequence data in relation to the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria	26-29/04/2021	Technical University of Denmark

8.1.4.1.4.6 Publications and patents

No publications or patents.

8.1.4.1.4.7 Remarkable outcomes

The student won the prize for the best poster presentation at the OHEJP ASM 2021.

8.1.4.1.4.8 Impact and Relevance

The supervisory team brings together leading experts in veterinary, wildlife and environmental AMR, with expertise spanning veterinary and molecular microbiology, bioinformatics, microbial ecology and evolution, as well as wildlife disease.

William Gaze is working with the United Nations Environment Project on AMR in the environment, having recently authored the UNEP Frontiers report on AMR and the environment. He is currently located within two interdisciplinary units, Exeter's Centre for Environment and Human health, and the Environmental and Sustainability Institute, which is also part of the University of Exeter

Beside his work as a researcher within SVA, Stefan Börjesson is involved in the Swedish AMR monitoring program and is also a senior lecturer in clinical microbiology with an emphasis on AMR in a One-health perspective at Linköping University at the Department of Clinical and Experimental Medicine.

Muna Anjum leads the Bacterial Characterisation Workgroup and is the AMR Research Lead at the APHA working at the interface of molecular and veterinary microbiology, within the One Health remit. As lead for the AMR research, she is also involved in supporting national AMR surveillance activities and APHA's response to national outbreaks, and identifying new and emerging threats. She is a member of the DEFRA Antimicrobial Resistance Coordination Group, which advises and reviews the DEFRA activities on antimicrobial usage in animals and AMR in microorganisms from feedstuffs, animals and food, the APHA lead for the Defra AMR in the Environment group, and works closely with colleagues in Public Health England in various research projects and national activities.

8.1.4.1.4.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers' comments have been accepted and this is closed.

8.1.4.1.4.10 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			2	M33	?	COVID-19	not yet known	not yet known

Comments:

The project has been significantly affected by COVID-19 as the student by this point should have been visiting the farm recruited for longitudinal sampling. The sampling has been delayed and if these restrictions persist it may have a major impact on the proposed PhD plans. The original plan was to sample across four time points in one year, but this is unlikely to happen as a result of COVID-19. A new estimated completion date is hard to predict, as it is reliant on external factors, such as restrictions being lifted to allow access to the farms. To help mitigate risks posed by Covid-19 on visiting and sampling on farms, we have already included characterization of archived isolates and are also planning to explore bird caecal samples collected from national surveillance of wild birds.

8.1.4.1.4.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	None

8.1.4.1.4.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

This PhD overlaps with the ARDIG project, where wild birds on farm have already been sampled.

8.1.4.1.4.13 List of dissemination and communication activities

Name of the activity:	*ISPB Plasmids Around the Globe*		
Date:	*06/05/2021*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	
Organisation of a Workshop		***Participation to a Workshop***	*Yes*
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***60***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*ISPB Plasmids Around the Globe*		
Date:	*25/05/2021*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	
Organisation of a Workshop		***Participation to a Workshop***	*Yes*
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***60***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*OHEJP ASM 2021 (poster and 3MT competition)*		
Date:	*09-11/06/2021*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	*Yes*
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	

Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.5 PhD11-R1-FBZ4/5- EnvDis

8.1.4.1.5.1 Summary

A major deliverable of the PhD (D-PhD11-1.3) was accomplished on March with the successful submission and defence of the Confirmation Report, which supported the feasibility of the project as well as the adequate progression towards completion. The examiners provided a useful viewpoint (e.g. define more precisely the research question, consider changing the model used as method) that will be taken into account to further improve the project. It also meant a good learning process towards drafting a document similar to the final thesis and the best strategy to approach it (i.e. constant reading and drafting along the process).

The updated daily human salmonellosis data was received from PHE in April, completing the disease data required for performing a thorough analysis for England and Wales. Together with other gathered data (such as altitude, detailed demographics with the number of residents by age and sex, year to year), an exploratory analysis of the data was performed confirming the notably higher prevalence of salmonellosis in the younger age groups (i.e. 0-4 years old). *S.* Enteritidis was also confirmed as the predominant serotype among the rest.

A spatial analysis is ongoing to determine the influence of the location over the cases of salmonellosis. To this geographic analysis we will link the corresponding weather variables to identify the influence of the particular climate condition of each region.

The exploration of further climate variables to include in the model on top of temperature lead to include vapour pressure and cloud cover to our analysis, as identified with potentially relevant influence. A conditional incidence of the combined effect of these variables as well as the effect of extreme climate conditions is on progress. This work will potentially lead to writing of 1-2 papers.

Since animals are the source of disease for humans, we are gathering as much information as it is available to us to investigate relevant relationships, such as the proximity of animal premises to the disease events or the linkage of the peak of animal salmonellosis with human incidence. So far, we have collected farm animal (cattle, pig, and poultry) numbers per county from APHA, being animal salmonellosis a sensitive and difficult data to be shared. In this regard, we have proposed APHA to set a short research collaboration so that the data could be analysed internally instead. This is still under revision within APHA but does not seem to be happening in the near future.

8.1.4.1.5.2 Overview of the PhD project progress

Under the WP1, with the objective of *developing a general tool to assess the risk of infectious diseases (in particular zoonosis) when we have information of relevant environmental factors*:

- *Task 1 « Test and discuss findings of the model for salmonellosis; validate the model with data from another European country; include animal role in the model from available data; write up thesis »* has been completed for preliminary data, but it is a transversal task that will progress until the end of the PhD in December 2022 (60M). Preliminary results suggest that the three parameters considered so far (eggs, chicken and temperature) play a big role on the seasonality of salmonellosis. Humidity and cloudiness are now to be evaluated in isolation and in combination with the other in the relevance of salmonellosis incidence.
- *Subtask 1 « Test and discuss findings of the model for Salmonellosis »* more specifically, where temperature has been seen to be a determinant seasonality factor, but more variables (e.g. humidity) will be taken into consideration applying a conditional incidence analysis to the model. This task is due in December 2021 (48M).
- *Subtask 2* «* Validate the model with data from another European country; include animal role in the model from (if) available data*»* due in June 2022 (54M). We have contacted RIVM as

collaborator who is willing to work together in providing salmonellosis incidence data for the Netherlands and thus complementing our model. A Short-Term Mission (STM) travel grant was awarded by the OHEJP to this purpose.

- *Subtask 3 « Write up thesis »* due in December 2022 (60M).

8.1.4.1.5.3 Progress of the research performed in the PhD project and key scientific results

WP1, TASK 1 (sT-1-1.1*).*

Subtask 1.1 (sT-1-1.1): *« Test and discuss findings of the model for salmonellosis »:*

- **Literature review**:
  - Importance of *Salmonella* as a foodborne agent of disease both in developed and developing countries. The follow-up of constant outbreaks linked to *Salmonella* in different countries state the relevance of the topic nowadays.
  - Empirical/experimental studies of the impact of environment on *Salmonella* identified cloudiness and vapour pressure as two variables determinant in the seasonality of salmonellosis. These will be further explored.
  - Water activity will be explored as a food parameter in close relation to the humidity of the environment and the growth of *Salmonella*.
- **Improving R coding skills.**
  - Exploratory epidemiologic analysis was performed over the definitive PHE salmonellosis cases, including relative frequency of salmonellosis incidence per age group (normalized) and total of subspecies identified every year.
    - Higher prevalence of salmonellosis in the younger age groups (i.e. 0-4 years old) is evident, followed by 20-24 and 25-29 years old. The average age of infected individual is 30 years old. For the rest of the categories, there is a smooth bell curve.
    - *S.* Enteritidis is the clear predominant serotype (94%). There is a non-negligible number of unknown serotypes, meaning that there could be a very wide diversity of serotypes responsible for a considerable amount of cases. *S.* Thyphimurium and S. Virchow are the following most common serotypes.
  - The seasonality of all the years combined (1989-2020) shows an increase in the summer with a peak in August and September. The peak may be delayed as much as one month as compared to the analysis of weekly cases evidenced in previous outcomes for the 90's.
  - Spatial analysis will follow. Using both QGIS and R, I am looking for a spatial link between the amount of cases registered across the country and the geo-climatic characteristics of the location, as well as the distribution of rural/urban areas and presence of livestock.
- **Data management and manipulation:** the format of data provided by PHE was explored:
  - Some delay in the data coming from PHE was experienced given the ongoing COVID circumstances. The complete dataset was received (i.e. 32 complete years of daily salmonellosis diagnosis in humans in England and Wales from 1989 to 2020).
  - The data comprises 560,842 salmonellosis records, with information on the *Salmonella* subspecies and phagetype, the age of the patient, laboratory of identification and type of sample analysed.
  - A surprising number of 83 records corresponded to patients of 100 years or older, with one record aged 127. This finding is compatible with the UK Office for National

Statistics, according to whom, there were 202,490 people censed to be ≥100 for the years 2007-2019. Yet, we will consider removing these outliners from our analysis.

- **Constant tailor the research question(s) and approaches to use next:**
  - The Wavelet analysis is a way for demonstrating seasonality of the weather parameters selected over time. It will be used to compare different models and choose the most adequate. The preliminary analysis performed over weekly cases confirms the seasonality identified year after year. It does not identify other less obvious trends separated greater spans of time.
  - Perform different length duration aggregation averages (7, 14, 30 days) of weather parameters to reduce the effect of incubation delay from the weather variation to its perception in human cases detection.
  - Perform a conditional incidence analysis combining more than one weather factors (e.g. temperature + humidity/vapour pressure).
  - Adjust the model to fit historic prevalence data by incorporating more variables (weather, and other relevant food sources, if found).
  - Explore the effect of environment on animal salmonellosis.
  - Re-adapting the method from Lo Iacono et al., 2020. for campylobacteriosis to salmonellosis with additional weather variables will follow.

Subtask 1.2* (sT-1-1.2): *« ~~Complete the same tasks for Leptospirosis~~ »*. Following our last periodic review with the internal and external supervisory team (PHE and Zoetis), this subtask has been changed in the Annual Work Plan to: *« Validate the model with data from another European country; include animal role in the model from (if) available data »* to strengthen the relevance of the project:

- Yearly census of farm animals (cattle, pigs, chicken, duck, geese, other poultry) from 1999 to 2016 per county were provided by APHA. This will be used to find a link with the presence/proximity of animals to the cases.
- Salmonellosis data in chicken farms have been requested to APHA. This information will be very interesting to find similarities in the seasonality in animals/humans and the moment of the highest peak of incidence.
- Collaboration with a similar project at the Dutch National Institute for Health and Environment (RIVM) has been established. We will perform a data and methods exchange to improve our model and validate it with the Netherlands.

8.1.4.1.5.4 Progress of the research project: milestones and deliverables

8.1.4.1.5.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD11-FBZ4/5-EnvDis	D- PhD11-2.1	Presentation of findings (e.g. conferences, internal school seminars) Y2	M42	M42		• Poster presentation at the OHEJP ASM, • OHEJP ASM 3-minute thesis competition

						• Participation on a 3-minute thesis competition of the Doctoral College of the University of Surrey, • Organization committee member and participation on the Vet School Research Symposium .
	D- PhD11-2.2	End of Year Progress Review Y2	M48	TBC	M48	Will be finalised at the end of the year

8.1.4.1.5.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD11-FBZ4/5-EnvDis	M-PhD11-1	Test and discuss findings of the model for Salmonellosis	M48	No	M48	Ongoing

8.1.4.1.5.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
French language classes (weekly)	Languages/ Interdisciplinary skills	07/11/20 - 28/04/21	Global Graduate Award - UoS
Writing retreat	Writing skills	08/01/2021	Research and Development skills
International Student One Health Association (ISOHA) mentorship program	Networking	11/12/20 - 31/05/21	One Health Commission
Managing your supervisor	Interpersonal skills/ Managerial skills	15/12/20	Doctoral college - UoS
Genomics & Bioinformatics workshop	Interdisciplinary skills	14/12/20-24/12/20	Arnoud van Vliet - UoS
Research Data Management and Open Data	Research skills	19/01/21	Library and Learning Support Services - UoS
OHEJP CPD Workshop – Digital innovation for One Health practitioners	Research skills	15/02/21-19/02/21	OHEJP – German Federal Institute for Risk Assessment (BfR)
Demonstration GIS	Teaching skills	23/02/21 25/02/21 01/03/21	University of Surrey
Demonstration ELISA	Teaching skills	08/03/21	University of Surrey

OHEJP Cogwheel workshop – Versatile Emerging Infectious disease Observatory (VEO)	Research skills	25/02/21	OHEJP - Swedish National Veterinary Institute (SVA) & Norwegian Veterinary Institute
Building your professional network workshop	Networking	19/03/21	Research and Development skills - UoS
1 to 1 Career support	Research skills	24/03/21	Doctoral college - UoS
International Women in STEM workshop	Interdisciplinary skills/ Networking	25/03/21	Queen Mary University - QMUL
Performing Data analysis in R studio	Interdisciplinary skills	26/03/21	Academic Skills and Development - UoS
3MT competition practice	Presentation skills	29/03/21	Doctoral college - UoS
The Conversation media training	Presentation skills/ Research skills	20/04/21	Doctoral college - UoS
Demonstration heart dissection	Teaching skills	22/04/21	University of Surrey
Demonstration Herp practical	Teaching skills	05/05/21 06/05/21	University of Surrey
Storytelling for researchers	Presentation skills/ Research skills	10/05/21	University of Surrey - ESRC Impact Acceleration Account
Communication with impact	Presentation skills/ Research skills	12/05/21	University of Surrey - ESRC Impact Acceleration Account
Vet School Research symposium participation and member of the organising committee	Research skills/ Presentation skills/ Networking/ Managerial skills	18/03/21-02/07/21	School of Veterinary Medicine - University of Surrey
23 Things International participant & Pod chair	Networking/ Managerial skills	08/03/21-07/06/21	Research and Development skills - UoS
Summer School	Research skills	26/07/21 – 06/08/21	OHEJP – Italian Higher Institute of Health (ISS) & CERSAG
Organisation & participation on News and updates meeting with the NTD group	Research skills/ Managerial skills	Biweekly	University of Surrey
Member of a programme promoting physical activity at the university	Managerial skills/ Transversal skills	Annual	SurreyMoves - University of Surrey
Co-author of papers (2)	Writing skills/ Research skills	Ongoing	University of Surrey
Progress analysis reviews	Writing skills/ Project Management skills	Quarterly	OHEJP - UoS

8.1.4.1.5.6 Publications and patents

No publications.

8.1.4.1.5.7 Remarkable outcomes

None yet.

8.1.4.1.5.8 Impact and Relevance

The project involves the University of Surrey, Public Health England (PHE) and Zoetis. EnvDis project builds on current work being done at PHE by Dr Gillingham and Prof Nichols and Dr Lo Iacono. Essentially PHE will focus on a phenomenology of *Salmonella* in England and Wales, and Laura's work will complement this by using mechanistic models. The project fits also very well with research interests of Dr Kanellos in Zoetis in understanding better the disease and how to prevent it; and the activities led by Prof Cook in vHive (https://vhive.buzz/). By collaborating with the Dutch National Institute for Health and Environment (RIVM), we are strengthening the versatility of the model in understanding and measuring the impact of the environment on infectious diseases in different geographic scenarios.

8.1.4.1.5.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers' comments have been accepted and this is closed.

8.1.4.1.5.10 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables			Associated budget		
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
sT-1-1.1	NA	NA	M-PhD11-1	April 2021	December 2021	Delayed data on origin	NA	NA
sT-1-1.2	NA	NA	M-PhD11-2	August 2021	July 2022	Changed subtask2. New collaborator found	NA	NA

Comments:

M- PhD11-1 The data were received in April 2021, which was the deadline foreseen for the result of the analysis.
M- PhD11-2 As a result of the 2021 OHEJP Annual Scientific Meeting, Laura contacted a PhD student working on a similar OHEJP project (ADONIS) and convened with the supervisory team to apply for a short visit (STM). This will take place early next year, following OHEJP's bases, and will serve as a validation approach for when the model is ready next year.

8.1.4.1.5.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes*
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

Additional information:

- Due to COVID we have received the salmonellosis data 16 months after the start of the PhD. In this meantime, I focused on learning how to code maps in R and explore spatial distribution as well as proceeding with further literature review. Even though parallel work has been done in the meantime, it has irremediably delayed the progression on analysis of the outcomes.

8.1.4.1.5.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

A short-term mission was applied to work together with the OHEJP ADONIS project (https://onehealthejp.eu/jrp-adonis/) at the RIVM (https://www.rivm.nl/en).

ADONIS and EnvDis are two OHEJP projects with a similar research scope, yet with different approaches, where human salmonellosis is studied with the aim of better understanding the epidemiology of the disease:

ADONIS focuses on explaining the trends in the time-series of salmonellosis' incidence. Their approach is based on the variations of explanatory factors, such as national surveillance systems in humans and poultry, following a longitudinal approach. EnvDis aims to estimate the incidence of salmonellosis conditional to certain environmental exposures. It assumes that this conditional incidence is, in general, not depending on time. In addition, it aims to incorporate mechanistic processes, such as bacterial growth in the food source of human transmission. A key benefit of working closely together is to bring the temporal dimension, typical of timeseries analysis, into the conditional incidence approach. Vice-versa, by applying the conditional incidence approach to different settings we would be able to assess whether or not the relationship between salmonellosis and exposure is universal. This collaboration will enhance the robustness of our findings and it will help in overcoming the intrinsic limitations of the different approaches.

8.1.4.1.5.13 List of dissemination and communication activities

Name of the activity:	*OHEJP Annual Scientific Meeting 2021 – poster presentation and three minute thesis competition*		
Date:	*09-11/06/2021*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*Yes*
Training	*No*	***Trade Fair***	*No*
Social Media	***Yes***	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			

	Number		Number
Scientific Community (Higher Education, Research)	***550+***	***Media***	*1,935*
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*International Day of Women in Science post*		
Date:	*11/02/2021*		
Place:	*LinkedIn*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*No*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	***Yes***	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)		***Media***	*1,935*
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*OHEJP Cogwheel workshop – Versatile Emerging Infectious Disease Observatory (VEO)*		
Date:	*25/02/21*		
Place:	*Virtual/ Swedish National Veterinary Institute (SVA) & Norwegian Veterinary Institute*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*No*
Organisation of a Workshop	*No*	***Participation to a Workshop***	***Yes***

Press release	*No*	*Participation to an Event other than a Conference or a Workshop*	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	*Video/Film*	*No*
Exhibition	*No*	*Brokerage Event*	*No*
Flyer	*No*	*Pitch Event*	*No*
Training	*No*	*Trade Fair*	*No*
Social Media	*No*	*Participation in activities organized jointly with other H2020 projects*	*Yes*
Website	*No*	*Other*	
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***55***	*Media*	
Industry		*Investors*	
Civil Society		*Customers*	
General Public		*Other*	
Policy Makers			

Name of the activity:	*Vet School Research Symposium*		
Date:	*01/07/2021 - 02/07/2021*		
Place:	*Virtual/ Vet School University of Surrey*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	***Yes***	*Participation to a Conference*	***Yes***
Organisation of a Workshop	*No*	*Participation to a Workshop*	*No*
Press release	*No*	*Participation to an Event other than a Conference or a Workshop*	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	*Video/Film*	*No*
Exhibition	*No*	*Brokerage Event*	*No*
Flyer	*No*	*Pitch Event*	*Yes*
Training	*No*	*Trade Fair*	*No*
Social Media	*Yes*	*Participation in activities organized jointly with other H2020 projects*	*No*
Website	*No*	*Other*	
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***75***	*Media*	
Industry		*Investors*	
Civil Society		*Customers*	
General Public		*Other*	

Policy Makers			

Name of the activity:	*Modelling in Animal Health conference (ModAH²)*		
Date:	*16/09/2021*		
Place:	*Virtual/ INRAe*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*Yes*
Training	*No*	***Trade Fair***	*No*
Social Media	***Yes***	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	*--*	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.6 PhD12-R2-FBZSH9-AptaTrich

8.1.4.1.6.1 Summary

1) Whole Larvae SELEX

The PhD student has been trained at McGill institute (extra EJP-partner) for the SELEX (Systematic Evolution of Ligands by Enrichment) method (cell-SELEX, protein-SELEX,…). The protocols to adapt the SELEX method to *Trichinella spiralis* whole Muscle Larvae (ML) have been established and optimized at Anses.

T. spiralis ML has been recovered from infected mice and fixed in ethanol. They have been used and are currently in use for the whole larvae-SELEX method defined.

Selection of single stranded DNA-based aptamers specific for *T. spiralis* whole muscle larvae (ML) has begun using a highly variable and randomized ssDNA library by SELEX method (Systematic Evolution of Ligands by Enrichment). Intact *T. spiralis* whole ML are incubated with the DNA library for multiple rounds (up until now, 7 successful cycles of selection have been performed) with a progressive decrease in ML number and interaction time at each round to increase sequence selection stringency.

In the past few months, much work has been accomplished in producing single-stranded DNA (ssDNA) sequences from double-stranded DNA (dsDNA) PCR products, an absolutely vital step in the successful isolation of target specific aptamers.

2) Monitoring SELEX

Following each cycle of selection, the quantity and quality of selected aptamer pools is evaluated by concentration readings (Qubit) and gel electrophoresis. Furthermore, qPCR and amplification/melting curve analysis was performed on cycles 2, 3, 4, 5, 6, and 7 of selection to qualitatively evaluate the success of aptamer selection. Following 7 cycles of selection, a reduction in sequence pool diversity and an increase is sequence homogeneity has been observed. This is made clear by the gradual movement of melting peaks from a temperature of 65C to 80C. While these finding need to be strengthened with confocal microscopy binding studies, the results are encouraging.

3) Excretory/Secretory Protein SELEX

A previous study (Bolas-Fernandez et al. 2009) has demonstrated the importance of micro-environmental conditions, specifically aerobic and anaerobic conditions, in modulating *T. spiralis* protein secretion and infectivity of L1 larvae. In light of this information, *Trichinella spiralis* muscle larvae (L1) were recovered from infected mice by means of artificial digestion. Following isolation and counting, larvae were maintained in nutrient medium under aerobic (non-infective) and anaerobic (infective) conditions. Following 18 hours of incubation, the culture medium supernatant (containing *T. spiralis* excretory/secretory (ES) antigents) was collected, concentrated, filtered, and dyalized against PBS in preparation for Top-down Mass Spectrometry proteomic analysis at the McGill Institute (RI-MUHC). Proteins upregulated in the aerobic condition, thought to be vital for infection to occur, will be indentified and eventually used as targets to generate potential diagnostic aptamers.

8.1.4.1.6.2 Overview of the PhD project progress

- The project has begun and the student has been well trained in the whole-larvae SELEX methodologies along with other key laboratory protocols. Furthermore, many *Trichinella spiralis* muscle larvae have been produced and fixed in ethanol for use as targets in aptamer selection.

- As shown in the gel image below (Figure 1), following 6 cycles of whole-larvae SELEX, DNA sequences have been successfully isolated from the surface of the muscle larvae.

- Recent qPCR (amplification curve and melting curve) analysis of *Trichinella spiralis* L1 muscle larvae specific sequences indicate a successful selection protocol. When comparing the amplification and melting curves of rounds 2 to 7 of aptamer selection, there is indication of sequence convergence and increase in population homogeneity.

- Recently, *T. spiralis* excretory and secretory (ES) antigens have been produced. These proteins are being kept
at -80C for further identification by Liquid Chromatography Mass Spectrometry (LC-MS/MS). This work is to be
conducted at the Canadian partner institution. Upon identification of potential biomarkers, work on producing
recombinant proteins will begin to initiate protein SELEX.

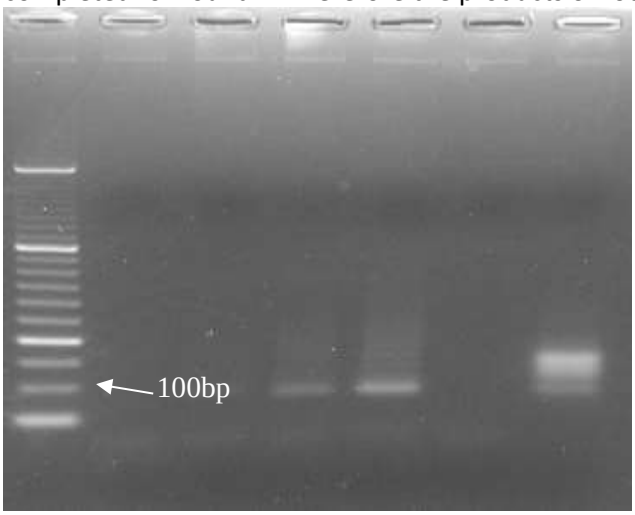
8.1.4.1.6.3 Progress of the research performed in the PhD project and key scientific results

Key Scientific Findings

1) Aptamer selection against *T. spiralis* L1 muscle larvae

- 7 rounds of aptamer selection against whole *T. spiralis* L1 muscle larvae have been successfully completed
- Following incubation with ssDNA sequences amplified from round 5 of selection, muscle larvae were washed and heated to release surface-specific sequences
- These sequences were subjected to a step or preparative PCR, where a number of amplification cycles is performed and imaged in order to determine the optimal settings prior to large-scale amplification.
- In **Figure 1**, products of 100bp, corresponding to double-stranded DNA (dsDNA) aptamers, can be seen. This implies that, following 6 rounds of aptamer selection, some sequences are still binding to the target (*T. spiralis* L1 muscle larvae) even under increasingly stringent selection.

Note: While 7 cycles of selection have been performed, the step of preparative PCR has not yet been completed for round 7. Therefore the products of round 6 are shown below.



Gel Loading Sequence

1 – 50bp ladder (5ul)
2 – 5 cycle PCR product
3 – 10 cycle PCR product
4 – 12 cycle PCR product
5 – 14 cycle PCR product
7 – NTC negative control
8 – 1uM library positive control

Fig 1: Electrophoretic profile of aptamers amplified for 5, 10, 12, and 14 cycles of PCR (lanes 2 – 5).

2) qPCR Amplification Curve (AC) and Melting Curve Analysis (MCA)

- Rounds 2 to 7 of aptamer selection were monitored by qPCR and a combined strategy of amplification curve and melting curve analysis were applied in parallel to monitor the progression of aptamer selection.

Note : R2(4), R3(4), R4(4), R5(4), R6(4), and R7(4) represent rounds 2 to 7 of aptamer selection, respectively.

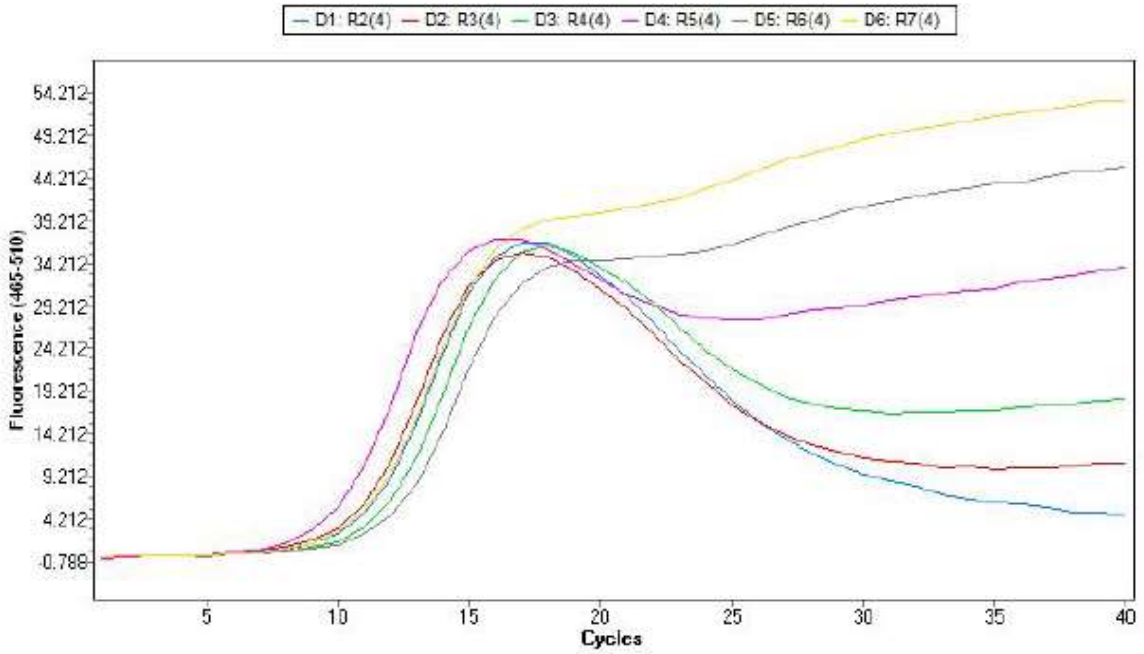


Fig 2 : Amplification curves of rounds 2 – 7 of aptamer selection against *T. spiralis* L1 muscle larvae.

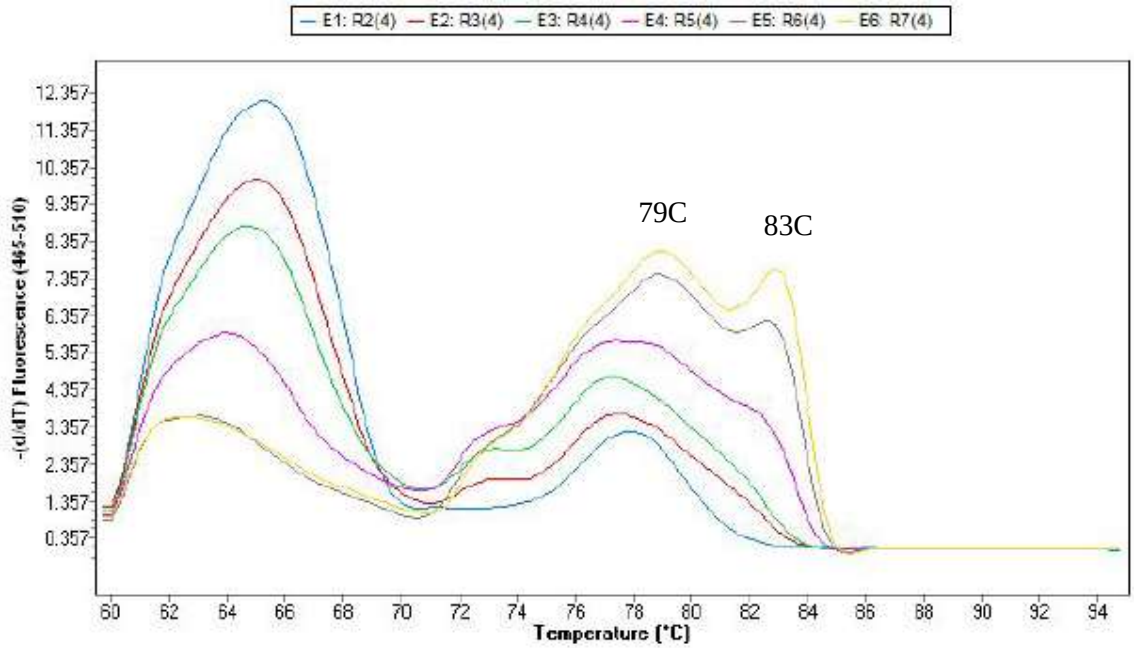


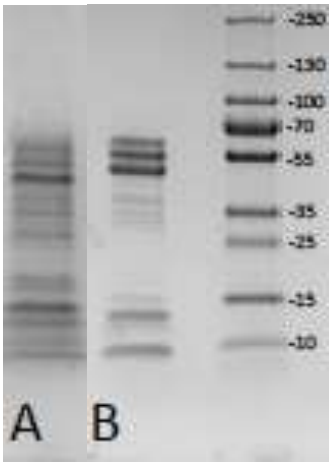
Fig 3 : Melting curves of rounds 2 – 7 of aptamer selection against *T. spiralis* L1 muscle larvae.

- Both **Figures 2 and 3** suggest the convergence of aptamer species towards a population of lower diversity and higher homogeneity. In **Figure 2**, fluorescence drops after 20 cycles of amplification in

rounds 2 to 5 of selection. As selection progresses, the sequences of rounds 6 and 7 display a maintained fluorescence, indicating higher population homogeneity.
- The melting curves of **Figure 3** strengthen the results of the amplification curve. A gradual increase in higher melting temperature peaks, from rounds 2 to 7, means that a larger proportion of the sequence population is composed of homoduplex species which require higher temperatures for their denaturation.
- Importantly, the apparition of a subpopulation with a high melting temperature of approximately 83C can be seen in the melting curves of rounds 6 and 7.

3) Production of *T. spiralis* Excretory/Secretory (ES) Antigens

- Following maintenance of *T. spiralis* L1 muscle larvae in nutrient medium for 18 hours under Aerobic and Anaerobic conditions, the ES antigens were extracted, concentrated, fltered, and dialyzed.
- Concentrated proteins were imaged on a 4 – 20% precast polyacrylamide gel and subjected to 100V for 40 minutes.
- Figure 4 illustrates the ES antigen profiles produced under both Aerobic (A) and Anaerobic (B) culture conditions.



Gel Loading Sequence

1 – Aerobic ES Antigens
2 – Anaerobic ES Antigens
3 – 10kDa ladder

Fig 4 : Protein profile of *T. spiralis* ES antigens produced under **Aerobic (A)** and **Anaerobic (B)** conditions

- A marked difference in the electrophoretic profiles of ES proteins produced under aerobic and anaerobic conditions implies up and/or down regulation of specific proteins.

8.1.4.1.6.4 Progress of the research project: milestones and deliverables

8.1.4.1.6.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD12- FBZSH9-AptaTrich	D-PhD12-3	Aptamers set on stage specific proteins is selected	36	NA	50	

8.1.4.1.6.4.2 *Milestones*

No milestones during this reporting period.

8.1.4.1.6.5 Soft skills and Continuing Professional Development training

No training during this reporting period

8.1.4.1.6.6 Publications and patents

Not Applicable

8.1.4.1.6.7 Remarkable outcomes

Not Applicable

8.1.4.1.6.8 Impact and Relevance

The partners involved in the project offer various elements of expertise to optimally support a PhD student in the development of a new method. The partners come from institutes with a wide interdisciplinary background.
ANSES laboratories conduct their activities in three major areas: animal health and well-being, food safety (chemical and biological) and plant health. The French NRL is also an OIE Collaborating Centre, it is part of the Animal Health Laboratory of Anses, which is internationally renowned and carries out critical missions for France, Europe and World in the field of animal health and public health, food safety, epidemiology. Researchers of the NRL work in close collaboration with medical doctors, veterinarians and give their expertise when outbreaks occur.
The Federal Institute for Risk Assessment (BfR) is the German national scientific institution in the area of consumer health protection, food safety, authenticity and risk assessment/ risk communication. The NRL for Trichinella mainly operates in the field of diagnostics, food safety and risk assessment, but also provide consultant support in human diagnostics.
The Canadian National Reference Centre for Parasitology (NRCP) is located within the Research Institute of the McGill University Health Centre (RI-MUHC) and provides reference serological and molecular diagnostics for parasitic diseases. Investigators at the Centre are active in several areas, including clinical parasitology, parasite diagnostics, parasite epidemiology, vaccine immunology, as well as cold-climate parasitoses and circumpolar health.
The existing close collaboration between the partners, which operate between the medical, veterinary and food science fields, will facilitate the success of the proposed PhD project under a One Health approach.

8.1.4.1.6.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers' comments have been accepted and this is closed.

8.1.4.1.6.10 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Aptamers selection on *T. spiralis* whole larvae	M29	M32	D-2 & M-3	M36	M39	Lab closed during the crisis	11250	11250
Production of stage specific proteins and apply protein-SELEX	M36	M42	D-3 & M-3	M36	M42	Lab closed during the crisis		

8.1.4.1.6.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

8.1.4.1.6.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Not applicable

8.1.4.1.6.13 List of dissemination and communication activities

Name of the activity:	*Poster presentation at OHEJP ASM 2021 (Presentation of poster and participation in 3MT competition)*		
Date:	*(9-11)-05-2021*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	*Yes*
Training	***Yes***	***Trade Fair***	
Social Media		***Participation in activities organised jointly with other H2020 projects***	
Website		***Other : Poster presentation***	*Yes*

Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.7 PhD13-R2-FBZ8/AMR2-VIMOGUT-AMR

8.1.4.1.7.1 Summary

The VIMOGUT project consists of *in vivo* analysis of the microbiome in the caecum of healthy broilers and the set-up of an *in vitro* model to study the transmission dynamics of antimicrobial resistance in caecal microbial communities . In the first period of 2021, the PhD student has worked on both aspects of the project.
For the *in vivo* component, further data analysis was performed to complete the first manuscript titled "Succession in the caecal microbiota of developing broilers colonised by Extended-spectrum β-lactamase-producing Escherichia coli" which will be submitted in September 2021. The collection of additional broiler caecal samples at farms was postponed due to COVID-19, but preparations are currently underway with the hope to perform the sampling in the second half of 2021.
The *in vitro* chicken caecum model was first tested in 2020 after which further optimisation was necessary. Currently, some challenges with the oxygen sensors are still under investigation but the model is ready for further testing. The initial tests in the semi-automated in vitro chicken have been run for ~8 days. Analyses of data collected (process real time monitoring and 16S rRNA genesanalysis) have shown reproducibility and reliability of the system between experiments.

8.1.4.1.7.2 Overview of the PhD project progress

In WP 1, the analysis of the data from the first experiment has been finalised. The student has written the results into a manuscript which is currently ready to be submitted to a peer-reviewed journal.
For WP2, the *in vitro* chicken caecum model was further adjusted and optimised to minimise the presence of oxygen in the culture media, underlined by the high presence of anaerobic bacteria in the microbiome. Currently, we still attempt to monitor the presence of oxygen in the system by testing a different set of sensors. The first experiments using two feed additive phytochemicals as intervention strategy were used to test the system, 16S rRNA genes sequencing and microbiome analyses were performed. In preparation of the planned experiments, an approach of fluorescent labelling of plasmids has been selected which was previously carried out at university of Copenhagen. A short term mission proposal was submitted and selected to co-fund the student to visit this research group in order to create her own fluorescently labelled plasmids efficiently.

8.1.4.1.7.3 Progress of the research performed in the PhD project and key scientific results

Before the start of VIMOGUT, a number of broiler caecal samples was collected on which 16s sequencing was performed. For WP1, additional samples of this collection were sequenced for VIMOGUT to allow proper statistical analysis of the data set. Challenges in the analysis arose due to a difference in 16S rRNA genes amplification between these datasets. It was impractical to analyse both datsets together due the nature of the research question and the possible bias influencing the results of the microbiome downstream analysis. Precautions have been made to prevent this problem from occurring in any further datasets that are generated during VIMOGUT.
Analysis of the data generated in VIMOGUT indicates that the broiler microbiome matures over three successional stages and increases in diversity over the first 21 days of the boiler's life, (see figure 1). Animals with a greater diversity in the microbiome were colonised later than animals with a less complex microbiome suggesting a possible window of opportunities for intervention strategies (see figure 1). A manuscript which describes the relationship of the chicken gut microbiota and the colonisation of the gut by ESBL *E. coli*, has been prepared and is currently circulating amongst co-authors. It is expected that the manuscript will be submitted for peer-review shortly, thus completing deliverable.
Preparations for sampling at additional farms are currently on-going.

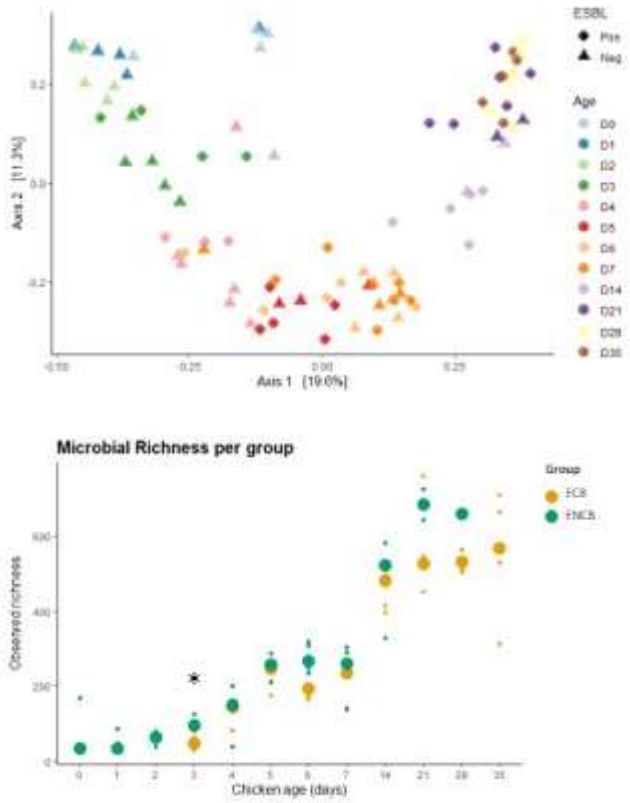


Figure 1. Principal coordinate analyses based on dissimilarity metrics. Triangles depict the microbiome of animals colonised by ESBL *E. coli*, and circles non-colonised by ESBL *E. coli*, colours show the age of the animal. Right panel; Comparisonof observed microbial richness between broilers colonised and not colonised by ESBL *E. coli.*

For WP2, an *in vitro* chicken caecum model was set up. The model is still challenged by difficulties using the oxygen sensors for which the calibration to a completely anaerobic environment appears to be inaccurate. Replacement with new sensors has not helped to overcome this problem and tests with a different type of sensor are currently ongoing. Additional optimisation of the system included the addition of a second gas inlet that creates an overlay of nitrogen on top of the culture media in the bioreactor to prevent any oxygen from dissolving into the culture. Two experiments were performed in which phytochemical feed additives were added to the culture at 3 days after the start of the experiment. After DNA isolation, 16S rRNA genes amplicon sequencing was performed and the data was analysed. In both experiments, the relative abundance of phyla of the *in vitro* microbiome was relatively stable and consisted primarily of anaerobic bacteria, confirming the lack of oxygen in the bioreactors, see also Figure 2. The control bioreactors in which no intervention was added showed similar stabilisation of the microbiome after 3 days, suggesting that any interventions can be started from the timepoint.

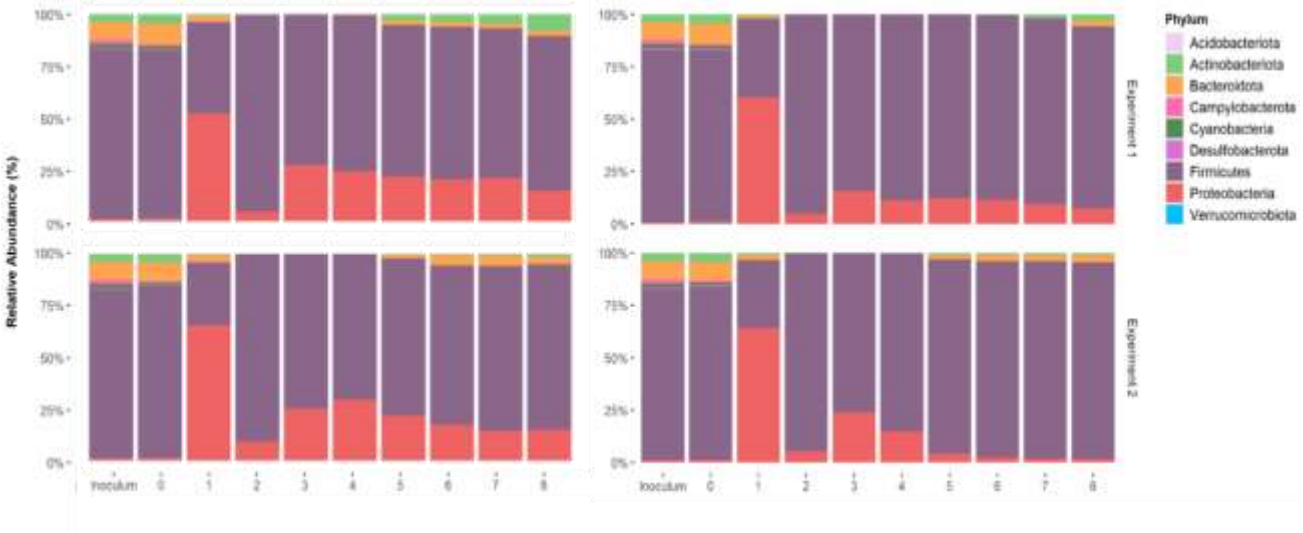


Figure 2. Relative abundance of different phyla measured in the *in vitro* caecal microbiome. The numbers below the bar indicate the experiment timeline (when the sample was acquired). The two upper plots represent the experiment 1, the lower plots are for experiment 2. The left panels represent the control reactors, while the right panels show the effects of two phytochemicals which are added to the bioreactors from day 3.

8.1.4.1.7.4 Progress of the research project: milestones and deliverables

8.1.4.1.7.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD13-R2-FBZ8/AMR 2	PhD13-1.1	Manuscript on preliminary findings for the relationship between chicken gut microbiome maturation and ESBL colonisation.	36		45	Challenges in data analysis delayed the preparation of the manuscript.
PhD13-R2-FBZ8/AMR 2	PhD13-1.2	Manuscript on relationship between chicken gut microbiome maturation and ESBL colonisation over several farms.	48		56	Farm visits could not be planned in the first half of 2021 due to COVID-19.

8.1.4.1.7.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD13-R2-FBZ8/AMR 2	M6	Perform initial test runs on in vitro gut model to determine CFU for reliable ESBL colonisation.	36	No	48	Delayed due to closure of lab facilities in 2020.
PhD13-R2-FBZ8/AMR 2	M7	Write manuscript on initial results relationship between chicken gut	36	No	45	See deliverable 1.1

		microbiome maturation and ESBL gut colonisation.				
PhD13-R2-FBZ8/AMR 2	M8	Perform 16S sequencing and analysis of caecal samples from OH-EJP VIMOGUT.	42	No	52	Samples were not collected yet due to the inability to visit broiler farms.
PhD13-R2-FBZ8/AMR 2	M9	Write manuscript on relationship between chicken gut microbiome maturation and ESBL colonisation over several farms.	48	No	56	See deliverable 1.2

8.1.4.1.7.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Peer review discussion meeting	Communication	12/01/21	GSS-WUR
Metagenomics webinar (DADA2)	Data analysis	21/01/21	Loop Genomics
Scicom IG meeting	Communication	16/02/21	WUR
Imposter syndrome	Personal development	24/02/21	
7th OHEJP cogwheel workshop	Science	25/02/21	CACTUS
Global Tricycle Surveillance ESBL *E.coli*	Science	03/03/21	WHO
Applications of -omics technologies in poultry health and productivity: where are we now?	Science	22/04/21	*IHSIG*
Designing and attractive and effective poster	Communication	17/05/21	PE&RC - WSG
Effective and efficient verbal communication in academia and beyond	Communication	26/05/21	PE&RC - WSG
Mindful productivity for PhD Candidates	Personal development	28/05/21	PE&RC - WSG
Multivariate analysis course	Statistical analysis	23 – 25, 28 – 29 /06/21	PE&RC – WSG
RMarkdown	Data analysis	5-6 / 7/21	VLAG - WSG

8.1.4.1.7.6 Publications and patents

Currently not applicable.

8.1.4.1.7.7 Remarkable outcomes

Currently not applicable.

8.1.4.1.7.8 Impact and Relevance

The analysis of the initial *in vivo* 16s data set has been performed with Dr. Stephanie Jurburg at iDiv Germany. The positive collaboration in the VIMOGUT project has led to preparation of a new collaborative project which started in 2021.

The *in vitro* gut model experiment to compare the effects of phytochemicals described above has been conducted in collaboration with Dr. Adam Roberts at Liverpool School of Tropical Medicine. The PhD student of Dr. Roberts, Mr. William Hutton visited WBVR at the end of 2020 for one month to perform this experiment. The collaboration has boosted the implementation of the *in vitro* model. Furthermore, a consortium led by Dr. Roberts has submitted a pre-proposal and is now

preparing a full proposal for the JPI-AMR call in which the *in vitro* gut model at WBVR is an important component.

As part of the work for VIMOGUT, the PhD has engaged with the research group of Prof. Luca Guardabassi at University of Copenhagen in order to receive technical assistance in constructing fluorescently labelled plasmids, as the group previously described it in the literature. As such, she has written a proposal for a short-term mission in the 2021 call and would like to visit Prof. Guardabassi's group for 2 months in 2022. The fluorescently labelled plasmids will be utilised in the *in vitro* model to study the host range of ESBL-encoding plasmids.

8.1.4.1.7.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers' comments have been accepted and this is closed.

8.1.4.1.7.10 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			D2	48	56	Farm visits could not be planned in the first half of 2021 due to COVID-19.	-	-
			D3	60	68	Lab facilities were closed for several months in 2020.	-	-
			M6	36	48	Lab facilities were closed for several months in 2020.	-	-
			M8	42	50	Farm visits could not be planned in the first half of 2021 due to COVID-19.	-	-
			M9	48	56	Farm visits could not be planned in the first half of 2021 due to COVID-19.	-	-

Comments:

The COVID-19 crisIs has had some effects on the feasibility of meeting deadlines of the milestones. The work on the *in vitro* model has had significant delays in Y3 due to the temporary closure of the laboratory facilities and the postponement of an essential course to properly commence this part of the work, but progress has since been made and most of the work will be delivered by the end of the project.

For the *in vivo* work, sampling at farms has been delayed until the PhD student has been vaccinated against COVID-19. Preparations are made to start this work in Q3/Q4 of 2021.

8.1.4.1.7.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No

Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

Additional information:
'Delay in work plan execution', see 'Impact of COVID-19 crisis on the project'

8.1.4.1.7.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Currently not applicable.

8.1.4.1.7.13 List of dissemination and communication activities

Name of the activity:	*OH-EJP 3-minute thesis competition at OH-EJP ASM 2021*		
Date:	*10-06-21*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	*Yes*
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*Poster presentation at OHEJP ASM 2021*
Date:	*09-06-21 / 11-06-21*
Place:	*Online*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.8 PhD14-R2-FBZ4-ToxSauQMRA

8.1.4.1.8.1 Summary

The present research project aims to answer to the scientific question: "What is the attribution of the traditional raw pork products in the human Toxoplasma infection? " based on three main areas of study consisting of (i) a thorough investigation of *T.gondii* predilection sites in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst) (WP1); (ii) evaluate the impact of the manufacturing process (including different incorporation rates of nitrites and NaCl) and the conservation of dry sausage on the viability of *T. gondii* (WP2); (iii) a quantitative microbiological risk assessment analysis to be conducted for the various raw pork products (dry sausage, dry ham, etc)

(WP3). The 3-year project spans over four Annual Periods (Y2-Y5). The key activities, results and achievements for the reporting period of January to September of this year are the following:

Key scientific results:

1. Complete artificial digestion of meat samples from "tissue cysts" pig group
2. Complete MC-PCR analysis of tissue samples and dry sausages
3. Complete data analysis of *T. gondii* tissue distribution and resistance in dry sausages

Challenges:

1. Delay in PCR reagents (Taq polymerase, primers, etc) delivery (manufacture, synthesis, etc) due to internationally high demand of PCR Covid19 testing
2. Optimisation of MC-PCR protocol with a new UV-light lamp according to the latest published protocol (Algaba et al., 2017)

Adaptations:

1. Involvement of under-graduate students for helping us with the digestions and PCR
2. Selecting the MC-PCR protocol that gave us the best results in terms of reproducibility (Opsteegh, et al., 2010)

8.1.4.1.8.2 Overview of the PhD project progress

WP1 - Investigation of *T.gondii* predilection sites in pigs experimentally infected with two different stages (tissue-cyst versus oocyst)

WP1-T1: Experimental infection of pigs **– successfully completed last year**
WP1-T2: Predilection sites of *T.gondii* in pigs **– successfully completed this year**

WP2 - Assessment of the persistence of *T. gondii* during the production and storage of dry sausages and dry ham

WP2-T1: Manufacture of dry sausages and dry ham **– successfully completed last year**

WP2-T2: Assessment of the persistence of viable *T. gondii* in pork delicatessen **– underway/ to be completed by the end of this year**

WP3 - Quantitative microbiological risk assessment

WP3-T1 Review of prevalence of *T. gondii* in various dry pork delicatessen**– underway/ to be completed by the end of this year**

WP3-T2 QMRA modelling for human *T. gondii* infections**– started/ to be completed by the end of next year**

8.1.4.1.8.3 Progress of the research performed in the PhD project and key scientific results

The present research project aims to answer to the scientific question: "What is the attribution of the traditional raw pork products in the human Toxoplasma infection? " based on three main areas of study consisting of (i) a thorough investigation of *T.gondii* predilection sites in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst) (WP1); (ii) evaluate the impact of the manufacturing process (including different incorporation rates of nitrites and NaCl) and the conservation of dry sausage on the viability of *T. gondii* (WP2); (iii) a quantitative microbiological risk assessment analysis to be conducted for the various raw pork products (dry sausage, dry ham, etc) (WP3). The 3-year project spans over four Annual Periods (Y2-Y5). The present 9-month report focuses on the progress and activities from WP1 (delayed due to covid 1st and 2nd confinement), WP2 and WP3. Within the WP1 (Investigation of *T.gondii* predilection sites in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst)), we have finished the work of WP1-T2: Predilection sites of *T.gondii* in pigs which has been delayed due to covid 1st and 2nd confinement.

Within the WP2 - Assessment of the persistence of *T. gondii* during the production and storage of dry sausages and dry ham we have worked mainly on Task 2 : WP2-T2: Assessment of the persistence of *T. gondii* since Task 1: WP2-T1: Manufacture of dry sausages and dry ham has been completed last year.

However, a short summary of WP1-T1 and WP2-T1 is mentioned for a better understanding of the workflow over the last 2 years. All the work has been completed with the help of external partners : Institut du Porc (IFIP) and Université de Champagne-Ardennes (URCA).

For WP1-T1, the main focus was to be able to collect meat massively contaminated with *Toxoplasma gondii*. Therefore an infection with a high dose (1000) of parasites was carried out (strain ME49) in pigs (WP1-T1). These tests were carried out with 2 parasitic forms that may be at the origin of contamination in pigs: the oocyst (ingestion from the environment) and the tissue cyst (ingestion from infested meat). Three pigs were inoculated with oocysts and 3 others with tissue cysts. Serological monitoring of the serum of these pigs was carried out by Modified Agglutination Test (MAT) on a weekly basis until D30, then every 15 days until the end of the protocol (3-4 months/80-90 kg).After euthanasia of the MAT positive pigs, 4 tissues /pig were collected for analysis: heart, breast, shoulder and ham as tissues used in the manufacture of dry sausage. For each of the anatomical regions studied, the different muscles (4 for breast, 7 for shoulder and 13 for ham) were pooled. Some 40 supplementary muscles per carcass were individually collected, representing the most important anatomical regions. One hind leg was collected for each pig for dry ham production.

Concerning the WP1-T2, the main focus was to identify the predilection sites of *T.gondii* presence in pig tissues. Therefore, mouse bioassays and quantitative PCR was performed on a part (200g) of the pooled sample per region. The remaining parts of the pools were used for industrial (salting, smoking, etc) processing (WP2).

The analysis of tissue samples ([breast, shoulder, ham, heart + 40 supplementary muscles] x 6 pigs) by MC-PCR, delayed by the 1st and the 2nd confinement due to COVID19 sanitary crisis in France, has been completed this year, for both parasitic stages (oocysts and tissue cysts). This involved the digestion, DNA extraction and PCR for each individual tissue sample.

Within the WP2-T1 the manufacture of dry sausages was carried out on a pilot scale by IFIP, according to a protocol representative of those used by a commercial factory. Briefly, after mincing, the muscle pool was divided into seven portions (corresponding to the 7 combinations of nitrites (as sodium NaNO2 nitrites) and NaCl concentrations that was to be compared: 120 (maximum dose of nitrites mentioned in the Code of Practice), 60 and 0 ppm of nitrites combined with the usual dose of 26 g/kg NaCl or a reduced dose of 20 g/kg NaCl or 0 g/kg NaCl. For each of the tested formulations, 3 dry sausages was collected at different dates (D0, D2, D10, D20, D30 and D50). On each analysis date, IFIP has carried out a physico-chemical monitoring (pH, Aw, weight loss) and a count of the lactic flora from a dry sausage per formulation, in particular to check that the process is running properly. A total of 168 dry sausages was required for this study (3 dry sausages × 7 recipes × 6 analysis dates for *T. gondii* monitoring as well as 1 dry sausage × 7 recipes × 6 analysis dates for physico-chemical and bacteriological analyses).

The dry hams were meant to be sent and manufactured by INRAe Corte (Corsica), using two traditional salting techniques (a long one : 2.5 days/kg and a short one: 1 day/kg). However due to a local strike in the harbour of Marseille, the hams arrived with 10 days of delay, causing the sanitary quality of meat to be questionable in terms of manufacturing. Therefore, a long salting technique has been applied only, with 300g of product that was taken at D30 and D90 due to Covid19 shut-down.

Concerning the WP2-T2 the main focus was the analysis of the dry sausages and ham for the presence of viable *T. gondii* by bioassay in mice that has been performed in the animal facility of URCA. In total 90 mouse bioassays were performed (1 dry sausage x 7 recipes × 6 analysis dates x 2 mice + 2 dry hams x 3 analysis dates) with the last data being collected 6 weeks after the last bioassay was performed. In the same time, the presence of *T. gondii* DNA in the inocula, resulting from the dry sausage digestion, was quantified by URCA using a (conventional) qPCR and by ANSES by MC-qPCR developed by Opsteegh et al., (2010).

Within the WP3 - Quantitative microbiological risk assessment we focused on the WP3-T1 Review of prevalence of *T. gondii* in pigs and pork products and WP3-T2 QMRA modelling for human *T. gondii* infections. During Y4, the main focus was the review of the prevalence data of *T.gondii* in pigs and pork products. Therefore data from various studies involving long-last processing pork delicatessen (Jambon de Parme; Jambon Serrano, etc) are about to be collated, building on the systematic reviews performed for EFSA and EJP Toxosources. In the same time the we initiate the first steps in WP3-T2 QMRA modelling for human *T. gondii* infections by providing the animal *T.gondii* prevalence for the QMRA model that needs to be build.

Key scientific results:

4. Complete artificial digestion of meat samples from "tissue cysts" pig group
5. Complete MC-PCR analysis of tissue samples and dry sausages
6. Complete data analysis of *T. gondii* tissue distribution and resistance in dry sausages

Challenges:

3. Delay in PCR reagents (Taq polymerase, primers, etc) delivery (manufacture, synthesis, etc) due to internationally high demand of PCR Covid19 testing
4. Optimisation of MC-PCR protocol with a new UV-light lamp according to the latest published protocol (Algaba et al., 2017)

Adaptations:

3. Involvement of under-graduate students for helping us with the digestions and PCR
4. Selecting the MC-PCR protocol that gave us the best results in terms of reproducibility (Opsteegh, et al., 2010)

8.1.4.1.8.4 Progress of the research project: milestones and deliverables

8.1.4.1.8.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD14-R2-FBZ4	D-PhD-ToxSauQMRA-WP2.2	Final report on the *T.gondii* detection in collected tissues after processing	M46		M46	On time
PhD14-R2-FBZ4	D-PhD-ToxSauQMRA-WP3.1	Report on prevalence of *T. gondii* in various dry pork delicatessen	M46		M48	Expecting 2 months delay since there are other partners involved in this task, that are overwhelmed for the moment

8.1.4.1.8.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD14-R2-FBZ4	M-PhD-ToxSauQMRA-01	Data on quantitative PCR, Magnetic-capture PCR and mouse bioassay on tested tissues after processing to be collected	M46	Yes		Being helped by under-gratuated students we advanced quicker than initially planned

PhD14-R2-FBZ4	M-PhD-ToxSauQ MRA-02	Prevalence of *T.gondii* in relevant meat products to be collected	M46		M48	We are expecting 2 months delay since there are other partners involved in this task, that are overwhelmed for the moment
PhD14-R2-FBZ4	M-PhD-ToxSauQ MRA-03	Prevalence of *T.gondii* in relevant meat products is provided as input to QMRA	M51			On time

8.1.4.1.8.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Cours Français Langue Etrangère niveau élémentaire	Language	18/01/21 - 15/04/21	Université Paris-Est Créteil
Les enjeux de la bioéconomie	Bio-economics	19/04/21 -22/04/21	Agreenium, AgroParisTech
Intégrité scientifique et éthique de la recherche	Ethics	06/05/21 – 21/05/21	Université Paris-Est Créteil

8.1.4.1.8.6 Publications and patents

For this period Filip DAMEK, presented his PhD project with the results up to date and the results from Toxosources concerning T.gondii animal prevalence review as a poster, in the 3rd Annual Scientific Meeting of EJPs (09-11.06, Copenhagen, Denmark) (n=2). In the same time, these results were presented in the 28th International Conference of the World Association for the Advancement of Veterinary Parasitology (19-22.07, Dublin, Ireland) as posters (n=2).
Furthermore, his results will be submitted for publication in a Special Issue "**Advances in the Diagnosis, Detection, Epidemiology, and Control of *Toxoplasma gondii***", in collaboration with /Microorganisms/ (ISSN 2076-2607,ImpactFactor4.152)

8.1.4.1.8.7 Remarkable outcomes

Not Applicable

8.1.4.1.8.8 Impact and Relevance

From the beginning, the PhD project was built as an interdisciplinary project involving partners from the Vet (Anses, RIVM), Med (URCA, RIVM) and industrial (IFIP) area. The topic itself touches all three domains, fitting perfectly into the OneHealth approach. Therefore the PhD student is working in an inter-disciplinary environment, gathering together vets, researchers, doctors, pharmacists, engineers, technicians with a broad spectrum of activities such as parasitology, food-product manufacture, risk assessment analysis, statistics, epidemiology. Precisely, the experimental infection of pigs has been performed by JRU BIPAR, the sausages were manufactured by Institut du Porc (IFIP), and tested both by JRU BIPAR and URCA. Later on the results will be interpreted with the help of statisticians (ANSES) and the QMRA model will be run and performed in RIVM. The PhD project and the PhD student are playing perfectly the role of a pivot within this interdisciplinary environment. Without them this part of the research would not have been possible.

8.1.4.1.8.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers' comments have been accepted and this is closed.

8.1.4.1.8.10 Impact of COVID-19 crisis on the project

1. No specific delay for specific activities just a general delay in PCR reagents delivery. Due to an important internationally demand for PCR reagents to be used in the COVID19 testing

(diagnostic), the R&D activities are not on the priority list for the biochemical companies generating delays in work plan execution, duties, tasks or reporting.

8.1.4.1.8.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	NO
Loss of technical training staff delaying progress of the work	NO
Delay in work plan execution	YES
Conflicts between the collaborative partners that support the PhD	NO
Lack of commitment between the collaborative partners that support the PhD	NO
Delay in duties, tasks or reporting	YES
Poor working relationships within the PhD project team	NO
Change in PhD student circumstances requiring temporary leave	NO
Other risks (please describe)	YES

Additional information:

Filip DAMEK is an excellent PhD student, dynamic and pro-active. From the beginning of the PhD project we had to deal with several unexpected problems, but this has not discouraged him, demonstrating he can adapt and is well suited to finalise this project successfully. For the moment the challenges include:

1. Delay in PCR reagents delivery. Due to an important internationally demand for PCR reagents to be used in the COVID19 testing (diagnostic), the R&D activities are not on the priority list for the biochemical companies generating delays in work plan execution, duties, tasks or reporting.
2. The collaboration with RIVM is excellent with monthly meetings and ad-hoc discussions to proceed with the PhD project. However, a 3 months internship of Filip in RIVM was discussed at the beginning of his PhD. This internship has been postponed due to COVID19 safety protocols since. Hopefully the situation will change in the future.

8.1.4.1.8.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Filip DAMEK has been involved in the EJP JRP Toxosources, as part of the Anses team, participating at the kick-off meeting (3-4.02, Copenhague, Denmark) and since then participating at all the videoconferences of the various WPs, where Anses is involved in. He took a very active role in the activities of WP2 (human/animal prevalence review, questionnaires, etc), as well as in activities of WP3 (T.gondii detection in RTE salads).

Similarly, part of the Anses team, Filip DAMEK is involved in the national research project n° 0917003490 financed by the French Ministry of Agriculture through the France Agri Mer agency with the title: Study of the tropism and persistence of *Toxoplasma gondii*: from pork carcass to sausage, gathering the above mentioned partners (Ifip, URCA, Inrae Corte), representing in the same time the fundament/basis of his PhD programme.

8.1.4.1.8.13 List of dissemination and communication activities

Name of the activity:	*On-line poster defence and Three Minute Thesis competition within the 3rd Annual Scientific Meeting of OHEJP (n=2)*		
Date:	*9-11.06.21*		
Place:	*Copenhague, Denmark*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*

Organisation of a Conference	*NO*	***Participation to a Conference***	*YES*
Organisation of a Workshop	*NO*	***Participation to a Workshop***	*NO*
Press release	*NO*	***Participation to an Event other than a Conference or a Workshop***	*NO*
Non-scientific and non-peer-reviewed publication (popularised publication)	*NO*	***Video/Film***	*NO*
Exhibition	*NO*	***Brokerage Event***	*NO*
Flyer	*NO*	***Pitch Event***	*YES*
Training	*NO*	***Trade Fair***	*NO*
Social Media	*NO*	***Participation in activities organized jointly with other H2020 projects***	*NO*
Website	*NO*	***Other***	*NO*
Communication Campaign (e.g. Radio, TV)	*NO*		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*On-line poster defence within the 28th International Conference of the World Association for the Advancement of Veterinary Parasitology*
Date:	*19-22.07*
Place:	*Dublin, Ireland*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*NO*	***Participation to a Conference***	*YES*
Organisation of a Workshop	*NO*	***Participation to a Workshop***	*NO*
Press release	*NO*	***Participation to an Event other than a Conference or a Workshop***	*NO*
Non-scientific and non-peer-reviewed publication (popularised publication)	*NO*	***Video/Film***	*NO*
Exhibition	*NO*	***Brokerage Event***	*NO*
Flyer	*NO*	***Pitch Event***	*NO*
Training	*NO*	***Trade Fair***	*NO*
Social Media	*NO*	***Participation in activities organized jointly with other H2020 projects***	*NO*
Website	*NO*	***Other***	*NO*
Communication Campaign (e.g. Radio, TV)	*NO*		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)		***Media***	
Industry		***Investors***	

Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.9 PhD15-R2-FBZ5-TRACE

8.1.4.1.9.1 Summary

In the beginning of this year there was still some delay due to the COVID19 crisis.
Regarding TRACE, in the previous year, some progress was made on establishing optimisation of sample (pre)processing (enrichment) to make whole genome sequencing possible (WP1). The producer/supplier of the enrichment product made a change in this product. For this reason, we had to start with another method. We didn't succeed in our first attempts. We started setting up a new enrichment method, in June.
Because of the delay of WP 1 we were not able to start in WP2 (phylodynamics) with the analyses of newly produced sequences. However, we started setting up a method for HEV analyses for timed-phylogeny of known sequences from the NCBI. This should enable us to later apply this method for sequences we generated ourselves.

8.1.4.1.9.2 Overview of the PhD project progress

WP1. During the first year of the project work was done to establish a sample (pre)processing (enrichment) to make whole genome sequencing possible. Different methods were used and DNA depletion treatments were explored. The mRNA of 18S (host) and 16S (bacterial) origin was successfully removed. HEV whole genome sequences were successfully generated using above enrichment methods and specific primers.
The producer/supplier of the enrichment product made a change in this product. For this reason, we had to start with another method. We decided to develop a test based on multi primer sequencing method for HEV. Probably the genome structure of HEV is a little bit complicated therefore we didn't succeed with this method. In June, we started setting up a new enrichment method. This method is based on the principle of the first original procedure.

WP2. This work was planned to start in the beginning of 2021, however the delays of WP1 meant that we were not able to start in WP2 analyses of new produced sequences. However, we started setting up a method for HEV analyse for timed-phylogeny of known sequences from the NCBI, with the idea that we can apply the method for our own generated sequences later on.

WP3. Not planned to start yet.

WP4. Not planned to start yet.

8.1.4.1.9.3 Progress of the research performed in the PhD project and key scientific results

- Workpackage 1 Whole genome sequencing.

During the first year of the project, work was done to establish a sample (pre)processing (enrichment) to make whole genome sequencing possible. Different methods were used and DNA depletion treatments were explored. The mRNA of 18S (host) and 16S (bacterial) origin was successfully removed. HEV whole genome sequences were successfully generated using above enrichment methods and specific primers. Currently work is being done to analyse sensitivity.
The producer/supplier of the enrichment product made a change in this product. For this reason, we had to start with another method. We had a successful method for sequencing SARS-CoV-2, based on a multi primer sequencing method. Therefore, we decided to develop a test based on the same principle for HEV. However, we didn't succeed with this method, and this may be because the genome structure of HEV is more complex.
In June, we started setting up a new enrichment method. This method is based on the principle of the first original procedure.
HEV culture preceding sequencing may be considered to increase the amount of HEV virus (work on a culture method is part of the One Health EJP's BIOPIGEE project).

- Workpackage 2 HEV phylodynamics.

Using HEV whole genome sequences data generated in WP1, we will construct a timed-phylogeny of HEV based on core-genome SNP analysis. From this analysis, we can infer time-points of evolutionary divergence of different HEV types. Coupled to geographic location data of the isolates, we will also include a spatial dimension to the phylogeny which will inform us on the geographical origin and spread of HEV types over time. Comparison of sequences over time will also be used to identify sequences that differ between time periods in which we observed a different epidemiology of HEV, giving indications of probable increased colonization success in pigs, increased environmental survival and/or increased virulence. This work was planned to start in the beginning of 2021, however, the delays of WP1 meant that we were not able to start in WP2 -analyses of new produced sequences. However, we started setting up a method for HEV analyses for timed-phylogeny of known sequences from the NCBI, with the idea that we can apply the method for our own generated sequences later on.

- Workpackage 3 Identification of HEV virulence genes and HEV quasispecies.

Work on this work package is planned to start in month 48

- Workpackage 4 Data analyses and data evaluation

Work on this work package is planned to start in month 48

8.1.4.1.9.4 Progress of the research project: milestones and deliverables

8.1.4.1.9.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD15-FBZ5-TRACE	D-PhD15-1.1	Sample processing protocol for HEV RNA positive target samples of different origin and associated deep sequencing procedure for HEV from such samples	48			We are working on getting it ready on time.
PhD15-FBZ5-TRACE	D-PhD15-2.2	Report/publication on HEV dynamics including information about the geographical origin of predominant virulent strains and identification of genetic traits changed over time.	48			WP2 is partially dependent on WP1. A general procedure how to perform a timed-phylogeny analyses for HEV could be finished before moth 48

8.1.4.1.9.4.2 *Milestones*

No milestones for this reporting period.

8.1.4.1.9.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Digital innovations for one health practitioners	Digital innovations for one health practitioners	15th-19th February 2021	BfR

8.1.4.1.9.6 Publications and patents

One Health EJP ASM 2021 poster - https://zenodo.org/record/4973989#.YUtPbeySk2w

8.1.4.1.9.7 Remarkable outcomes

Not applicable

8.1.4.1.9.8 Impact and Relevance

OHEJP SRA 1 Updated List and Descriptions of Priority Research and Integrative topics: "risk factors and infection dynamics".
Development and harmonisation of deep sequencing-based methods for detection and tracing of foodborne zoonotic agents and emerging threats have been identified as a main research item within the updated EJP One Health SRA. Data from this project will lead to improved surveillance and more harmonized data analyses on the foodborne zoonosis HEV. This will contribute to broader and flexible actions to detect actual hazards, main reservoirs, trends and routes of transmission as well as common approach and timely analysis and data sharing which will be needed more and more with ongoing globalisation.

8.1.4.1.9.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers' comments have been accepted and this is closed.

8.1.4.1.9.10 Impact of COVID-19 crisis on the project

In 2020 there was significant impact of COVID-19 on this project and we extended the expected delivery dates of deliverables. Although there is still some impact of COVID-19 overall we do not expect a lot of extra extension is required.

8.1.4.1.9.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	N
Loss of technical training staff delaying progress of the work	N
Delay in work plan execution	Y
Conflicts between the collaborative partners that support the PhD	N
Lack of commitment between the collaborative partners that support the PhD	N
Delay in duties, tasks or reporting	N
Poor working relationships within the PhD project team	N
Change in PhD student circumstances requiring temporary leave	N
Other risks (please describe)	N

8.1.4.1.9.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Not Applicable

8.1.4.1.9.13 List of dissemination and communication activities

Name of the activity:	*OH-EJP 3-minute thesis competition and poster presentation at OH-EJP ASM 2021*
Date:	*09-11-06-21*

Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	*Yes*
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.10 PhD16-R2-FBZ2/AMR6.1-Codes4strains

8.1.4.1.10.1 Summary

Aim

Whole genome sequencing allows the tracking of pathogenic strains and informs infection control, diagnostic and sometimes treatment strategies. To track strains globally, and as they spread between the environment, food, animals and humans, universal strain nomenclatures are necessary. The core genome Multilocus Sequence Typing (cgMLST) approach is an accurate, reproducible and portable strain genotyping method that underlies widely used strain nomenclatures, in which groups are generally determined by single-linkage clustering. However, cgMLST groups are unstable due to the possibility of group fusion upon subsequent sampling. Recently, a new coding approach named LIN (Life Identification Number) was introduced by Marakeby et al. (1). It provides a numerical code for each genome based on its similarity (estimated using the Average Nucleotide Identity, ANI) to the closest genome already encoded. As LIN codes are attributed to genome rather than groups, they are stable. A common feature of both approaches is that single linkage groups and LINcodes can be defined using several similarity cutoffs, in which case they inherently convey phylogenetic proximity information. cgMLST additionally provides, through the number of allelic mismatches among cgMLST profiles, intuitive human-understandable metrics of differences among strains involved in epidemiological events (*e.g.*, '5 cgMLST allele differences').
The aim of the PhD project is to develop a novel genome-based genotyping approach taking the best of the two above classification approaches, *i.e.,* combining the advantages of cgMLST (discrimination, standardization) with those of the LIN code approach (complete stability). That is, we aim to develop and explore the strain classification utility of cgMLST-based LIN code (cgLINcodes) systems, where the pairwise distance is based on the number of allelic mismatches, rather than ANI. We also aim to compare the cgLINcodes approach with other existing classification approaches: the SNP address and multi-level single-linkage classifications (Multilevel Single Linkage, MLSL). We use *Klebsiella pneumoniae* (Kp) and *Escherichia coli* (Ec) as two important pathogens to develop and evaluate our approach.

Report on the period of January to September 2021

Before the reporting period, we had selected our pilot genome dataset for the pathogen Kp (December 2019, **D-PhD16-2.1**).
We developed procedures (bioinformatics, algorithmic) to follow our goal of a cgMLST-based LIN code systems. First, we generated cgMLST profiles from genomes, using defined schemes. To this aim, the choice and the evaluation of the cgMLST schemes was finalized, for Kp and Ec (**D-PhD16-3.1**).
As a second task, the development of the cgMLST-based LIN code algorithm has been defined and a bioinformatics implementation was developed in Python (**D-PhD16-3.2**).

During this reporting period, we have consolidated our analyses of LINcodes and their comparison with MLSL. We have developed a novel way to define the population structure of species, called MSTclust; we have optimized our inheritance algorithm to provide backwards compatibility of MLSL groups with previous nomenclature identifiers from 7-gene MLST; we have defined the optimal input order for LIN encoding; we have compared the ANI metric with the cgMLST metric; we devised a method to identify recombination in cgMLST data; we have incorporated the MLSL identifiers into the BIGSdb platform to make them publicly available; and have started writing a publication on the cgLIN codes concept and its implementation in Kp with comparison with multi-level single-linkage classifications (**D-PhD16-4.1**).

References:

1. Marakeby, H. *et al.* A System to Automatically Classify and Name Any Individual Genome-Sequenced Organism Independently of Current Biological Classification and Nomenclature. *PLOS ONE* **9**, e89142 (2014).

8.1.4.1.10.2 Overview of the PhD project progress

The initial objectives proposed for the PhD project are the following:

- To define a collection of pilot dataset of genomes including published outbreak sets
- To develop the cgLINcode approach and compare with MLSL and SNPaddress approaches on large genomic datasets
- To simulate genomic evolution and use the three approaches to encode resulting evolved genomes; compare resulting codes with expectations
- To write publications on the cgLINcodes concept and implementation with comparison with MLSL and SNPaddress approaches
- To disseminate the cgLINcodes approach towards collaborating partner institutes and to evaluate this approach in one Health and Global Health contexts
- To write-up the PhD dissertation

The first two objectives have been achieved over the period September 2019 – December 2020. The second objective was divided into 4 subtasks: define the cgMLST schemes to be used for the two pathogens; collect a full dataset of public genomic sequences and scan them to define their cgMLST allelic profiles; development of the cgLINcodes algorithm; and create and/or analyze the SNPaddress database and compare cgLINcodes with MLST and SNPaddress approaches.

We have decided to leave the simulation out of the scope of the PhD work, as the data analysis of the real dataset is thorough enough for us to conclude that LIN codes and MLSL provide fully congruent results. Therefore, there is no point in simulating datasets to evaluate the differential performance of one against the other method. This is an unexpected outcome of our evaluation of a large dataset of 7060 Kp genomes.

The scheduled work that is not complete yet is the development and analysis of the SNPaddress approach for Kp. We are first finalizing the manuscript on cgLINcodes for Kp, as this is the most innovative development and the manuscript is already complex and long. We thus reserve the comparison with SNP address for the next period, or possibly for an ulterior project outside of the PhD work.

The planned objectives for the next period are (i) to complete the writing of the publication on the cgLINcodes approach; (ii) to disseminate this approach towards our partners and explore its practical implementation in a One Health context; and (iii) We also intend to apply the LIN code approach and the tools we developed (MSTclust and inheritance to nomenclature identifiers) to other pathogens, even though this was not initially planned.

8.1.4.1.10.3 Progress of the research performed in the PhD project and key scientific results

D4.1. Simulated dataset analysed

We have decided to leave the simulation out of the scope of the PhD work, as the data analysis of the real dataset is thorough enough for us to conclude that LIN codes and MLSL provide fully congruent results. Therefore, there is no point in simulating datasets to evaluate the differential performance of one against the other method. This abandoned DL is largely compensated by our additional DLs outlined below.

D4.2. Publication on the cgLIN codes concept and its implementation in Kp with comparison with multi-level single-linkage classifications

The publication is being written at the moment; The current version of the abstract of the publication is the following:

Sublineages within microbial species can differ widely in their ecology and pathogenicity, and their precise definition is important from basic research to industrial or public health applications. Whereas the classification and naming of prokaryotes is unified at the species level and higher taxonomic ranks, universally accepted definitions of sublineages within species are largely missing, which confuses communication in population biology and epidemiology. Currently existing nomenclatures based on the single linkage algorithm are unstable.

We propose, in the publication, a broadly applicable genomic classification and nomenclature approach for bacterial strains, using as model the prominent public health threat *Klebsiella pneumoniae*. Phylogenetic and clustering analyses of >7000 genomic sequences captured population structure discontinuities, which were used to guide the definition of 10 infra-specific genetic distance thresholds. Based on a 629-gene core genome multilocus sequence typing (cgMLST) scheme, we devised both a multilevel single linkage (MLSL) clustering classification and a stable proximity-based nomenclature based on the life identification number (LIN) concept. Genomic sublineages and clonal groups identifiers were attributed by maximizing their inheritance from the widely used 7-gene multilocus sequence typing (MLST) nomenclature. The taxonomy is provided through a community-curated web-accessible database (https://bigsdb.pasteur.fr/klebsiella) that also enables identification of user's query genomic sequences.

The proposed strain taxonomy system combines stable phylogenetic-rich barcodes (cgLIN codes) with unstable but human-readable MLSL group identifiers. Our species-specific operational approach for creating unified genomic taxonomy of microbial strains is broadly applicable and should enhance the collective understanding of epidemiology, emergence and microevolution of microbial pathogens.

D4.3. (NEW) A novel tool to define the population structure of species, called MSTclust

To profile the population structure, we developed MSTclust (https://gitlab.pasteur.fr/GIPhy/MSTclust) and used this tool to perform single linkage (SL) clustering of allelic profiles into partitions, at all possible thresholds *t* (from 0 to 629 allelic differences). SL clustering was applied on the pairwise distances among allelic profiles, defined as the number of allele mismatches normalized by the number of loci with alleles called in both profiles (therefore accounting for missing data). For each threshold *t*, the degree of clustering (goodness-of-fit of the clustering to the population) of the corresponding allelic profile partitioning was assessed by three different measures: (i) The overall average silhouette width S_t (2), which estimates the overall clustering quality based on maximizing inter-group distances and minimizing intra-group distances; (ii) the clustering robustness index in face of data subsampling, estimated by the Wallace index W_t (3) ; and (iii) The adjusted Rand index R_t (4,5) to assess the global concordance between the SL partitioning and those induced by any external classifications. For the application on Kp, we used as external classifications the 7-gene MLST sequence types, subspecies and species classifications.

D4.4. (NEW) Optimization of our inheritance algorithm to provide backwards compatibility of MLSL groups with 7-gene MLST

In order to attribute to each sublineage (SL) and clonal group (CG), an identifier that would maximally reflect the widely adopted 7-gene ST identifier of the corresponding isolates, we developed a set of naming rules that prioritize the most abundant ST observed among isolates of each group.
We briefly describe the process for the clonal groups (CG) level, and it would also apply in the same way for sublineages (SL). The data (*e.g.,* a list of CG-ST pairs) can be formalized as a bipartite graph, in which each CG or ST are nodes, and each CG-ST pair is an edge. The weight of each edge is equal to the number of isolates sharing the corresponding CG and ST identifiers. Based on this representation, the algorithm will consist in following all edges in the input graph, in the order of decreasing weight. The approach prioritizes the most frequent ST/CG pairs of isolates, *i.e.*, those that are epidemiologically

predominant, and thus naturally transfers to the CG nomenclature, the identifiers of the epidemiologically major STs. Rules were implemented to treat the cases of equality of representation of two or more STs connected to the same CG. Once all edges were removed from the graph, it may happen that some CGs were not yet named (for example, because the identifier of their unique corresponding ST was already attributed to another CG). For these orphan CGs, iteratively, the attributed identifier corresponds to the maximal CG identifier already attributed, plus one. Finally, to highlight identifiers that were attributed with no reference to 7-gene MLST identifiers, their numbering was initiated at 10000.

D4.5. (NEW) Comparison of the ANI metric with the cgMLST metric

We compared ANI-based distances with the number of mismatches between cgMLST profiles (**Figure1).** While the ANI-based distances varied from 92.8% to 100%, the cgMLST distances varied from 0 to 100% (*i.e*., 629 allele mismatches). Although both distributions had four to five modes, the first two modes of the ANI distance distribution, composed of inter-phylogroup strain comparisons, were at 93.5% to 95.5% ANI, whereas in the cgMLST distribution these distance pairs were centered around 2%, corresponding to the first mode of this distribution. In turn, whereas intra-species cgMLST distances varied from 5% to 100%, the range was only 98% to 100% for ANI values.

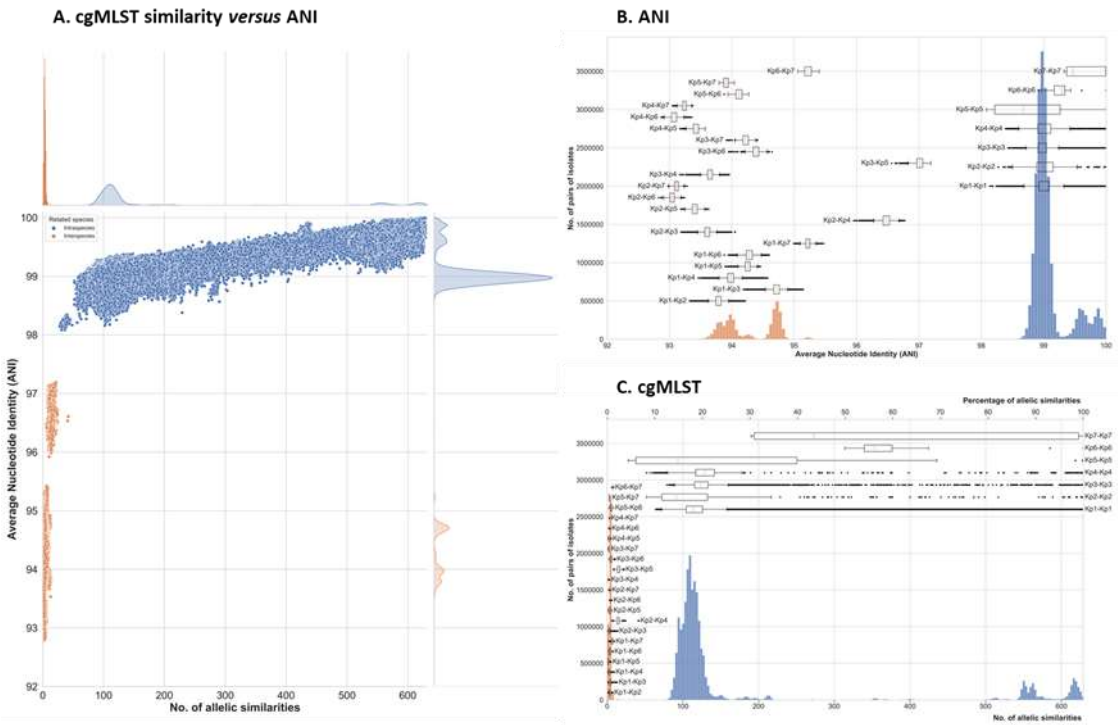


Figure 1. The distribution of pairwise distances of 7060 genomes of Kp based on Average Nucleotide Identity (ANI) and cgMLST. (A) Each point represents a pair of distances between two strains, the X-axis the distance cgMLST and the Y-axis the distance ANI, the distributions are shown on the outside of the graph. We have colored the points corresponding to intergroup and intragroup distance. (B) Distribution of cgMLST pairwise distance. (C) Distribution of ANI pairwise distance.

The ANI metric was not designed to define the similarity of closely related genomes, such as those of strains within species; for example, high similarity values (e.g. 99.9990% and 99.9991%) are not intuitive to compare. In addition, the ANI metric is non-reciprocal, is highly dependent on comparison parameters, and is sensitive to sequencing or assembly artifacts. This shortfalls of the ANI metric lead to imprecision and non-reproducibility that are particularly impactful for comparisons between very similar genomes.

In contrast, the metric based on a cgMLST scheme, comprising 629 highly conserved and syntenic genes, was appropriate to reveal discontinuities that are relevant for strain nomenclature purposes.

D4.6. (NEW) Designed a method to identify recombination in cgMLST profiles

Horizontal transfer of large portions of the genome can occur among isolates belonging to distinct KpSC phylogroups (6). Additionally, MLST or cgMLST alleles may have been transferred horizontally from non-KpSC members, for example *E. coli*. For the purpose of phylogeny-based classification, we excluded such hybrid genomes. To define genomes that result from large inter-phylogroup recombination events, we leveraged our gene-by-gene approach to define an original strategy, outlined briefly here: we first attempted to determine for each allele of each locus, its KpSC phylogroup of origin; we then quantified a phylogroup homogeneity index of each genome by simply summing phylogroup origins of individual loci over the 629 scgMLSTv2 loci. The index was defined as the number of loci in the predominant phylogroup, divided by the number of alleles called in the profile. We then established a distribution of the phylogroup homogeneity indices to define genomes as having a hybrid origin.

D4.7. (NEW) Integration of MLSL identifiers into the BIGSdb platform to make them publicly available

The MLSL nomenclature was incorporated into the Institut Pasteur *K. pneumoniae* MLST and whole genome MLST database (https://bigsdb.pasteur.fr/klebsiella). For this purpose, classification schemes and attached information fields were implemented as a novel BIGSdb functionality in BIGSdb version 1.21.0. The cgMLST profile of all isolates with less than 30 missing cgMLST-629 alleles were assigned to a core genome sequence type (cgST), and these were grouped into single-linkage partitions at the 10 defined mismatch levels. For SL and CG levels, a custom classification group field (named SL or CG within the system) was populated with 7-gene MLST inherited identifiers. All cgMLST profiles and their corresponding classification identifiers were made available for public use.
To allow users to easily identify *K. pneumoniae* isolates, a profile matching functionality was developed, allowing to search for cgMLST profiles related to a query genome sequence. This was implemented and is available from the website sequence query page (https://bigsdb.readthedocs.io/en/latest/administration.html#scheme-profile-clustering-setting-up-classification-schemes). This functionality returns the classification identifiers (including MLST-inherited clonal group and sublineage identifiers) of the cgMLST profile that is most closely related to the query genomic sequence, along with its number of mismatches compared to the closest profile.

References:

1. Marakeby, H. *et al.* A System to Automatically Classify and Name Any Individual Genome-Sequenced Organism Independently of Current Biological Classification and Nomenclature. *PLOS ONE* **9**, e89142 (2014).

2. Rousseeuw, P. J. Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. *J. Comput. Appl. Math.* **20**, 53–65 (1987).

3. Wallace, D. L. A Method for Comparing Two Hierarchical Clusterings: Comment. *J. Am. Stat. Assoc.* **78**, 569–576 (1983).

4. Hubert, L. & Arabie, P. Comparing partitions. *J. Classif.* **2**, 193–218 (1985).

5. Carriço, J. A. *et al.* Illustration of a Common Framework for Relating Multiple Typing Methods by Application to Macrolide-Resistant Streptococcus pyogenes. *J. Clin. Microbiol.* **44**, 2524–2532 (2006).

6. Holt, K. E. *et al.* Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. *Proc Natl Acad Sci U A* **112**, E3574-81 (2015).

8.1.4.1.10.4 Progress of the research project: milestones and deliverables

8.1.4.1.10.4.1 *Deliverables*

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD16-FBZ2/AMR6.1-Codes4strains	D-PhD16-3.4	SNapperDB Kp implemented for full dataset	M36		M48	We did not create SNapperDB for the pathogen Kp yet. We are finalizing the manuscript on cgLINcodes for Kp first.
	D-PhD16-4.1	Simulated dataset analysed	M42		M48	Priority to writing the publication on the cgLINcodes approach
	D-PhD16-4.2	Publication on LINcode approach and comparison with cgMLST and SNP address approaches	M48		M48	
	D-PhD16-4.3	A novel tool to define the population structure of species, called MSTclust		M40		
	D-PhD16-4.4	Optimization of our inheritance algorithm to provide backwards compatibility of MLSL groups with 7-gene MLST		M42		
	D-PhD16-4.5	Comparison of the ANI metric with the cgMLST metric		M42		
	D-PhD16-4.6	Designed a method to identify recombination in cgMLST		M40		
	D-PhD16-4.7	Integration of MLSL identifiers into the BIGSdb platform to make them publicly available		M42		

8.1.4.1.10.4.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
	M-PhD16-3.3	SNapperDB databases set-up for both pathogens	M36	Yes/no		Yes for Ec; not yet for Kp
	M-PhD16-4.1	Simulations completed	M42	No		Priority to writing the publication on the cgLINcodes approach

	M-PhD16-4.2	Publication submitted	M48	No		In the process of being written
	D-PhD16-4.3	A novel tool to define the population structure of species, called MSTclust	M40	Yes		
	D-PhD16-4.4	Optimization of our inheritance algorithm to provide backwards compatibility of MLSL groups with 7-gene MLST	M42	Yes		
	D-PhD16-4.5	Comparison of the ANI metric with the cgMLST metric	M42	Yes		
	D-PhD16-4.6	Designed a method to identify recombination in cgMLST	M40	Yes		
	D-PhD16-4.7	Integration of MLSL identifiers into the BIGSdb platform to make them publicly available	M42	Yes		

8.1.4.1.10.5 Soft skills and Continuing Professional Development training

No training undertaken in this reporting period.

8.1.4.1.10.6 Publications and patents

In addition to the main project, I also contribute to side projects. The PhD host lab comprises the National Reference Center for Corynebacteria of the diphtheriae complex (i.e., C. diphtheriae, C. ulcerans and phylogenetically related species; https://www.pasteur.fr/fr/sante-publique/CNR/les-cnr/corynebacteries-du-complexe-diphteriae). It thereby collects and characterizes all potentially toxigenic strains identified in French metropolitan area and overseas territories, since 11 years. This unique collection of isolates and the associated clinical and epidemiological data has been an essential resource for various related projects.

I have been interested in and contributed to a few of these projects, including the study of the diversity and evolution of Corynebacterium diphtheriae and the computational research of antimicrobial resistance. Below is a list of articles where I have made contributions:

Hennart, M., Guglielmini, J., Maiden, M.C., Jolley, K.A., Criscuolo, A. and Brisse, S., 2021. A dual barcoding approach to bacterial strain nomenclature: Genomic taxonomy of Klebsiella pneumoniae strains. bioRxiv 2021.07.26.453808. https://doi.org/10.1101/2021.07.26.453808

Badell, E., Alharazi, A., Criscuolo, A., Almoayed, K.A.A., Lefrancq, N., Bouchez, V., Guglielmini, J., Hennart, M., Carmi-Leroy, A., Zidane, N., Pascal-Perrigault, M., Lebreton, M., Martini, H., Salje, H., Toubiana, J., Dureab, F., Dhabaan, G., Brisse, S., Rawah, A.A., Aldawla, M.A., Al-Awdi, E.M., Al-Moalmy, N.M., Al-Shami, H.Z., Al-Somainy, A.A., 2021. Ongoing diphtheria outbreak in Yemen: a cross-sectional and genomic epidemiology study. Lancet Microbe 0. https://doi.org/10.1016/S2666-5247(21)00094-X (GOLD, 4300 EUROS)

Badell, E., Hennart, M., Rodrigues, C., Passet, V., Dazas, M., Panunzi, L., Bouchez, V., Carmi-Leroy, A., Toubiana, J., Brisse, S., 2020. Corynebacterium rouxii sp. nov., a novel member of the diphtheriae species complex. Res. Microbiol. https://doi.org/10.1016/j.resmic.2020.02.003; PMID: 32119905 (GREEN, 12 months).

Hennart, M., Panunzi, L.G., Rodrigues, C., Gaday, Q., Baines, S.L., Barros-Pinkelnig, M., Carmi-Leroy, A., Dazas, M., Wehenkel, A.M., Didelot, X., Toubiana, J., Badell, E., Brisse, S., 2020. Population genomics and antimicrobial resistance in Corynebacterium diphtheriae. Genome Med. 12, 107. https://doi.org/10.1186/s13073-020-00805-7; PMID: 33246485; PMCID: PMC7694903. (GOLD OPEN ACCESS, 3000 EUROS)

Others patents:

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 71.

8.1.4.1.10.7 Remarkable outcomes

Our publication on the cgLIN codes concept and its implementation in *Klebsiella pneumoniae* in comparison with multi-level single-linkage classifications should soon be available on bioRxiv.

8.1.4.1.10.8 Impact and Relevance

The project will define, implement and evaluate a novel bioinformatics strategy to classify and name strains within pathogenic bacteria, from the level of deep subspecific lineages down to shallower levels of diversity that differentiate epidemiological related strains from non-related ones. It will be first tested on Ec and Kp, two important ubiquitous 'One Health' pathogens. However, the general applicability of the approach means that in the future, the classification and nomenclature of strains of other pathogens could benefit from the PhD project outcomes.
By facilitating in the future, communication on bacterial strains across sectors and countries, the project is highly relevant to multiple topics and objectives of One Health EJP: antibiotic resistance clonal dissemination, emerging pathogens, cross-sector transmission, public health and basic microbiology integration.
The project will deliver a novel nomenclature system of bacterial pathogens genomes that will be stable by design, unlike existing systems based on SNPs and cgMLST single linkage groupings. This has a far-reaching impact on possibilities to integrate efforts of agencies (e.g., at the international levels, ECDC, EFSA, PulseNet international) to detect, monitor, understand and control the spread of pathogens.

8.1.4.1.10.9 Follow -up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers' comments have been accepted and this is closed.

8.1.4.1.10.10 Impact of COVID-19 crisis on the project

No impact, as our bioinformatics work was not affected.

8.1.4.1.10.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	No

Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	None

8.1.4.1.10.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

The novel method developed in the PhD project will find natural dissemination ways via the existing networks of collaborations in which the main investigators are involved: MedVetKlebs (just finished JRP), KlebNET, SpARK, kleb-GAP; Nor-Kleb-Net for Kp (see MedVetKlebs final report) and KlebNET-GSP (funded by BMGF); and Ec surveillance networks and national and international levels (e.g., French NRC & NRL @Pasteur and ANSES; ECDC; PulseNet international).

The novel nomenclature system will be compared with existing nomenclatures (dictionaries of nomenclature correspondence between LIN codes and current SNP/cgMLST nomenclatures for wider communication and backwards-compatibility) and will need in the future to be integrated in existing platforms that serve nomenclatures of bacterial strains (e.g., SnapperDB, EnteroBase, BIGSdb-Oxford and PulseNet international for E. coli; BIGSdb-Pasteur for K. pneumoniae). Future interactions with 'dev-op' specialists (application developers) will be established with free software such as EnteroBase, BIGSdb and Innuendo; or commercial software such as BioNumerics or SeqSphere.

Following the writing of the first draft of our publication on the cgLIN codes concept and its implementation in Kp with comparison with multi-level single-linkage classifications, Martin M.C. Maiden and Keith A. Jolley became aware of it and were interested in applying our methods on other pathogens of interest to the One Health perspective (Campylobacter) or Clinical/Antimicrobial resistance (N. gonorrhoeae)

8.1.4.1.10.13 List of dissemination and communication activities

Name of the activity:	*"Journées Boris Ephrussi" 2021* Poster : **A new approach for naming bacterial strains, combining cgMLST and LIN codes**		
Date:	*May 27th -28th 2021*		
Place:	*Digital Conference*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	*Yes*
Training		***Trade Fair***	

Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)		***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*Poster presentation at OH-EJP ASM 2021*		
Date:	*09-11-06-21*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

8.1.4.1.11 PhD17_SUSTAIN

8.1.4.1.11.1 Summary

Fieldwork:
No fieldwork was done in the period from January to September 2021. Due to the COVID-19 pandemic, the fieldwork will be pushed to autumn 2021. Fieldwork will be conducted in Italy to interview experts from the veterinary agencies.

Research work:
The start of 2021 was used to finalise the manuscript "Assessing Environmental Factors within the One Health Approach". I transcribed interviews that were conducted with public health and environmental experts from Italian institutes in autumn 2020. Additionally, I supported the NOVA project by analysing interviews. A survey on the governance of One Health was launched in March. Data gathering was completed in June 2021 and followed by analysis.
An edited book will be published by Oxford University Press and I was invited to write a chapter with Chris Degeling (University of Wollongong in Australia) on One Health and AMR in the European Union. This chapter is under construction and will be finalised in autumn.

Scientific output:
In March and June 2021 respectively, the following articles were published:

- "Assessing Environmental Factors within the One Health Approach". Medicina journal by MDPI. https://doi.org/10.3390/medicina57030240
- "One Health practices across key agencies in Sweden – Uncovering barriers to cooperation, communication and coordination". Scandinavian Journal of Public Health. https://doi.org/10.1177/14034948211024483
-

Teaching:
In spring 2021, I taught a Bachelor class on the topic of "Global governance Policy Area IV: Health" in the course "International Institutions and Global Governance".

Conferences:
I participated at the OHEJP Annual Scientific Meeting 2021 by engaging in the three minute competition and presenting my research at the conference (Lessons Learned from One Health Practices in Sweden and Italy). Additionally, I supported the organizing team in creating and presenting kahoot quizzes, which were held in the breaks of the conference, accessible for both the online and on-site participants.

8.1.4.1.11.2 Overview of the PhD project progress

The SUSTAIN PhD project is connected to WP7 of the OHEJP but it is not connected to specific tasks, deliverables or milestones.
Nevertheless, there has been progress. In 2021, data analysis was conducted as planned for the PhD project. This included transcribing interviews with experts from public health and environmental agencies and Italy. Data gathering for the interviews has been slowed down due to the Covid-19 pandemic. Due to travel restrictions, few interviews are left to be conducted with Italian veterinary experts. These remaining interviews are planned to be conducted in autumn 2021.
Data analysis was also conducted for the NOVA project "Surveillance barriers and opportunities as perceived by med-vet-food experts".
Further, a survey on the governance of One Health was launched in March. The survey is addressed to ministries, EU institutions, international organisations and high level members of staff from public health, veterinary, environment and food agencies.
Within the PhD project, a number of articles must be produced. In March and June 2021, two articles

were published (https://doi.org/10.3390/medicina57030240 and https://doi.org/10.1177/14034948211024483).
All teaching obligations (defined by Roskilde University) were completed after the spring semester 2020. However, I did teach a Bachelor class on the topic of "Global governance Policy Area IV: Health".

8.1.4.1.11.3 Progress of the research performed in the PhD project and key scientific results

The SUSTAIN PhD project is connected to WP7 of the OHEJP but it is not connected to specific tasks, deliverables or milestones.

Nevertheless, there has been progress. The start of 2021 was used to finalise the manuscript "Assessing Environmental Factors within the One Health Approach", which is a co-authored article with Alberto Mantovani. The article was published in March 2021.

Another focus was put on transcribing interviews that were conducted with public and environmental experts in Italy at the Istituto Superiore di Sanità and the Istituto Superiore per la Protezione e la Ricerca Ambientale in October 2020. This was concluded in April. The interviews will be analysed when all interviews will be gathered. The remaining interviews are planned to be conducted in autumn 2021 with veterinary experts from the Istituti zooprofilattici in Italy.

Additionally, I am supporting the NOVA project "Surveillance barriers and opportunities as perceived by med-vet-food experts". After having conducted the interviews and started transcription and coding in 2020, we (Maria-Eleni Filippitzi & I) started to analyse the data.

A survey on the governance of One Health was launched in March. The survey was addressed to ministries, EU institutions, international organisations and high level members of staff from public health, veterinary, environment and food agencies. The online survey examined policy networks for One Health by understanding policy- and decision-making processes of One Health. The survey investigated political constrains for turning One Health topics into policy (science to policy) to shed light on opportunities for One health policy integration. Data gathering was completed in July 2021 and followed by analysis.

An edited book by Olivier Rubin, Erik Bækkeskov and Louise Munkholm (tentative title: Steering against Superbugs – The Global Governance of Antimicrobial Resistance) will be published by Oxford University Press and I was invited to write a chapter with Chris Degeling (University of Wollongong in Australia). The chapter will be about One Health and AMR in the European Union. This chapter is under construction and will be finalised in autumn. It will use a literature review as well as data from the survey (described above).

Within the PhD project, a minimum of three articles must be produced, of which at least two have to be self-authored. In 2020, two co-authored articles were published (https://doi.org/10.1016/j.onehlt.2020.100146 & https://doi.org/10.1057/s41271-021-00277-y). In 2021, a co-authored and a self-authored article was published (https://doi.org/10.3390/medicina57030240 and https://doi.org/10.1177/14034948211024483).

All teaching obligations (defined by Roskilde University) were completed after the spring semester 2020. However, I did teach a Bachelor class in April 2021 on the topic of "Global governance Policy Area IV: Health".

Dissemination of the research was done by presenting at the OHEJP ASM 2021. At the conference in June, my PhD project was presented online at the 3MT. Additionally, I was able to present my research titled - Lessons Learned from One Health Practices in Sweden and Italy" on-site at the ASM.

8.1.4.1.11.4 Progress of the research project: milestones and deliverables

8.1.4.1.11.4.1 *Deliverables*

Deliverables of Roskilde University's PhD School:
Every 6 months, the status of the PhD progress is assessed by the half-yearly evaluation, which entails detailed description of the past 6 months in terms of status, changes in the time schedule or budget plan, the nature of supervision, research stays at other institutes, the assessment of all attended events (courses, conferences) as well as teaching hours.

Total of 30 ects for whole period of PhD From January to September 2021:
Attendance and presentation at conference

- OHEJP ASM

Courses

- Get your Article Published in a Social Science or Business Journal
- Work in Progress seminars (research group at Roskilde University)
- OHEJP Summer School

- Remaining ects will be mainly spent on presentations at conferences in 2021 and 2022

Fieldwork and change of research environment stay, 3- 6 months

- Remaining time of fieldwork will be spent in autumn 2021

Publications (Mandatory: three articles of which at least two are self-authored articles)

- Three co-authored articles published
- One single-authored article accepted and in the publishing process
- Remaining articles to be done in 2021 & 2022

8.1.4.1.11.4.2 *Milestones*

N/A

8.1.4.1.11.5 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Teaching at bachelor level: International Institutions and Global Governance	Global governance Policy Area IV: Health	19.04.21	Roskilde University
OHEJP Summer School 2021	Environmental Issues in One Health: from risk assessment to surveillance	26.07.-06.08.21	Istituto Superiore di Sanità
Course: Get your Article Published in a Social Science or Business Journal	Getting started (writing an abstract and an introduction)	18.05.21	Roskilde University
Virtual PhD Career Seminar	Understanding the job market, How do employers view PhDs as candidates for a position?, Competences and career paths as a PhD	12.04.21	Roskilde University

8.1.4.1.11.6 Publications and patents

Assessing Environmental Factors within the One Health Approach

- https://doi.org/10.3390/medicina57030240
- gold open access (no fees)

- Uploaded to Zenodo

Abstract for the OHEJP ASM 2021 - Lessons Learned from One Health Practices in Sweden and Italy

One Health practices across key agencies in Sweden – Uncovering barriers to cooperation, communication and coordination

- https://doi.org/10.1177/14034948211024483
- gold open access (no fees)
- Uploaded to Zenodo

8.1.4.1.11.7 Remarkable outcomes

Publications:

- Assessing Environmental Factors within the One Health Approach
  - https://doi.org/10.3390/medicina57030240

- One Health practices across key agencies in Sweden – Uncovering barriers to cooperation, communication and coordination
  - https://doi.org/10.1177/14034948211024483

8.1.4.1.11.8 Impact & relevance

The PhD project has a unique angle on One Health. It investigates the whole project in terms of One Health integration, implementation and how One Health is put into practice within public health, veterinary, food and environment Institutes. The results of this research can be used by all partners to evaluate and consider their coordination and collaboration efforts. It can be used to enhance disease surveillance nationally and internationally. It produces useful knowledge and examples for institutes on challenges for collaboration and how to overcome those. On a political level, it will provide insight into sharing and translating knowledge between scientists and politicians.

8.1.4.1.11.9 Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

The responses to previous ethical reviewers comments have been accepted and this is closed.

8.1.4.1.11.10 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay

Comments:

The Covid-19 pandemic has affected my PhD project, as there were changes in terms of fieldwork, conferences and courses. Conferences were postponed or rescheduled to online events, which impedes the ability to network.

My fieldwork was postponed or cancelled and cut short. In spring 2020, a research stay in Sweden at the Swedish Veterinary Agency was planned to conduct my fieldwork. However, due to the Covid-19 pandemic, I remained in Denmark and conducted my interviews online via Microsoft Teams and Skype instead of conducting face-to-face interviews.

A three month fieldwork stay in Italy at the Istituto Superiore di Sanità in the autumn semester 2020 was shortened to a one month stay. It is planned to finish the fieldwork in Italy in autumn 2021.

8.1.4.1.11.11 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

8.1.4.1.11.12 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

I am collaborating in the JPR: NOVA (WP1) on the project of *Surveillance barriers and opportunities as perceived by med-vet-food experts*. Here, I have assisted with conducting and analysing Interviews of Swedish, French and Norwegian experts.

8.1.4.1.11.13 List of dissemination and communication activities

Name of the activity:	*OHEJP ASM 2021– 3MT*		
Date:	*10.06.2020*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*YES*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*

Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	*20*
Industry	***0***	***Investors***	*0*
Civil Society	***0***	***Customers***	*0*
General Public	***0***	***Other***	*0*
Policy Makers	***0***		

Name of the activity:	*OHEJP ASM –Presentation (oral)*		
Date:	*10.06.2021*		
Place:	*Copenhagen, Denmark*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			

	Number		Number
Scientific Community (Higher Education, Research)	***550+***	***Media***	*20*
Industry	***0***	***Investors***	*0*
Civil Society	***0***	***Customers***	*0*
General Public	***0***	***Other***	*0*
Policy Makers	***0***		

8.1.5 Task 6.5: One-Health Continuing Professional Development (CPD) Module

The second CPD module was organised by the German Federal Institute for Risk Assessment (with support from the WP6 and communications teams based at the University of Surrey) and took place successfully as a virtual interactive event in M38. This event was open to consortium members only.

As with all WP6 previous events, a dedicated page on our website for this event was available as described above. The CPD module 2021 page can be found here. This page was used as a base for the promotional campaign on social media and other internal routes such as the internal mailing list, monthly Education and Training bulletin, and the OHEJP consortium newsletter. The promotional campaign took place between November 2020 and February 2021, and more information about the campaign is detailed in D6.9. The event website page and promotional branded tools for the event were produced by the OHEJP communications team.

The one-week module theme was Digital Innovation for One Health Practitioners, and centred around the use of innovative open-source software solutions supporting risk assessment, zoonotic outbreak investigations and data interoperability. The objective of this training module was to introduce new tools and technologies for One Health researchers and professionals. Specifically, solutions that support foodborne disease outbreak investigations, efficient surveillance data integration as well as the re-use of risk assessment models were introduced. The solutions presented have the potential to support the whole One Health community including national and international risk assessment agencies, risk managers and academic institutions.

The virtual training was delivered by senior research scientists from BfR and partners from our consortium, including SVA, DTU Food, ANSES, RKI and NVI and stakeholders, EFSA with an extensive track record in providing training for research scientists from different research domains. Scientists of the JRPs and JIPs ORION, COHESIVE and RaDAR also shared their knowledge and lessons learned from their projects. The CPD module provided joint plenary presentation sessions, moderated interactive workshops with practical exercises, as well as an e-learning platform, that can still be used by participants to further improve their knowledge.

Over 50 participants from early-career researchers and PhD candidates, up to senior scientists – from 15 European countries in our consortium attended the workshop. The event encouraged knowledge sharing and even allowed participants to present their digital innovation tools, which can be applied to scenarios of food safety, public health and animal health.

The online workshop received approved accreditation by the Academy of Veterinary Continuing Education (ATF), therefore participants from across Europe who attended and participated in the entire module, received 22 ATF hours, and could convert these to CE credits in their respective country.

Social media played a role in encourage conversations and interactions during and after the event. The event was well promoted on the One Health EJP Twitter and LinkedIn accounts which not only connected delegates on social media, but also gave them the opportunity to share their experiences

to a wide audience. This is not only important for building relationships and collaboration, but also increasing the awareness of the training opportunities that the One Health EJP offers.

The full report of the organisation, logistics and successes of the second CPD module can be found in D6.9 – report on the second CPD module which was submitted in M40.

Finally, the call to organise the fourth and final CPD module in 2022 was launched in M37. In addition, the call to organise the third CPD module was re-launched in M37, as no applications were received in 2020. There were no applications received for these calls, and both calls were extended to M47with the expectation that these events will take place in Y5. In addition to promoting these calls via the education and training monthly bulletin and newsletters, WP6 made specific requests to PMT, SSB and new consortium partners to forward to contacts in their respective institutes.

8.1.6 Task 6.6: Communications workshop and media training

This task was completed in Y3. Please refer to Summary Progress Report Y3 or D6.2 – report on the Communication and Media workshop.

8.2 Deliverables and Milestones

8.2.1 Deliverables

Del. Rel. No	Deliverable title	Est. Del. Month	Notification
D6.10	Report n°2 of the annual short term missions completed also uploaded onto the EJP webpage.	M38	Timely submitted
D6.9	Report of the second CPD module in one health	M40	Timely submitted
D6.11	Report n°3 on one workshop per year associated with ASM (with WP1,3,4 and 5)	M48	Under preparation
D6.12	Report of the third One health summer school	M48	Under preparation
D6.13	Report of the third CPD module in one health	M48	Under preparation

8.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS80	Launching of annual call for Short Term Mission n°4	M40	Completed in M37
MS85	Preparation and scheduling of annual training workshop programme associated with ASM n°4	M48	Calls to select ASM #4 organiser is open until M42
MS89	Preparation and launching of annual One-Health Summer Schools n°4	M48	Calls to select Summer School #4 organiser is open until M42
MS95	CPD Module & annual course n°4	M48	Calls to select CPD #4 organiser is open until M42

9 WP7 – Sustainability

9.1 Work carried out to date

9.1.1 Task 7.1: Gathering Stakeholders´ Needs and Expectations

The sustainability plan had to take into account the SWOT analysis and the developments of the Horizon Europe Programme: in this latter there is no dedicate initiative to OH, rather, OH issues are split into different partnership: AMR, Animal Health and Welfare, Food Safety from Farm to Fork and Pandemics.

In close interaction with WP5 (science-to-policy transfer) and WP2 (as regards gap analysis and new, unmet research needs) WP7 has launched two modules that involve scientists from OHEJP beneficiaries and scientists from outside (including SSB), representing inputs from the scientific world outside the OHEJP. Meanwhile, updates on WP7 have been always presented to Stakeholders committee, SSB and ESAB in order to gather critical inputs from the main external governance bodies.

The two modules are:

- **AMR module** (already launched, chairs: A. Kasebohrer and R. La Ragione) in order to develop a SRIA on the OH aspects of AMR in close interactions with the core group of the new AMR partnership. The discussion has evidenced areas of overlaps and possible collaboration, as well as different approaches: AMR activities on animals could be carried out in the oncoming Animal Health and Welfare partnership. A OHEJP SRIA on AMR is in preparation.
- **OH module** will be launched in June 2021, and its scope is to build a SRIA on OH-relevant activities on food safety and emerging threats, using the risk assessment paradigm, thus a cross-cutting module. Co-chairs are P. Jokelainen and A. Mantovani.

Therefore, due to the general disruption caused by the Covid pandemics, and in order not to postpone unduly the Workshop with stakeholders, D7.2 has been changed in the two modules. The Work of the two modules will be completed by M48.

9.1.2 Task 7.2: Strategic Research and Innovation Agenda 2021-2030 (SRIA 2021-2030)

A preliminary SRIA (D.7.3) -outlining the main points and priority issues of the final SRIA was timely submitted in M42.

The deliverable is called "OHEJP Strategic Research and Innovation Agenda 2021-2030, a strategy document for the sustainability of OHEJP" and it outlines the onstituent parts of the SRIA including:

- The past: background and context, introduction of the OHEJP
- The present: setting the scene based on today's situation and conditions
- The future: how this SRIA will address the actual needs of stakeholders in the future
- Vision and mission
- Objectives

a) The six OHEJP specific objectives (SRA)
b) Additional objectives of the SRIA

- Expected impacts
- Priority research and integrative topics/themes or R&I priority areas:
    - AMR: Introduction, rationale, challenges; R&I objectives; Activities that will ensure sustainability in the future; Synergies and external (outside OHEJP) links with JPIAMR, and other relevant initiatives; Outcomes; Expected Impacts;
    - One Health: Introduction, rationale, challenges; R&I objectives; Activities that will ensure sustainability in the future; Synergies and external (outside OHEJP) links with JPIAMR, and other relevant initiatives; Outcomes; Expected Impacts;

- The environment and climate change, preparedness, emerging threats and others;
- Cross cutting issues and horizontal activities

- Drivers and enablers
- Implementation plan or Road map for the SRIA
- Synergies and complementarities of the SRIA (in general, besides the specific ones under each topic area)
- Dissemination
- Next steps: what the SRIA can be used for

The draft SRIA will be finalized on Month 42 as foreseen.

9.1.3 Task 7.3: Making the EJP sustainable through other funding and/or legal basis

In Y3 WP7 has started a plan in collaboration with WP2, to assess the new opportunities for OHEJP sustainability provided by Horizon Europe and Partnerships. As well as the drivers that modulate the demands of stakeholders.
Therefore Task 7.3 is inclided in the work carried out Tasks 7.1 and 7.2

9.1.4 Task 7.4: Making the bridges between EJP's beneficiaries and stakeholders sustainable

For the PhD project SUSTAIN, interviews were collected during a stage at ISS under the tutoring of A. Mantovani, with experts of the ISS (Dept Food safety, Nutrition and Veterinary public, Environment and Health, Infectious Diseases, Center for Global Health) and Ministry of Health (next stage forseen on late 2021 will target Istituti Zooprofilattici, Ministry of Environment, and regional Environmental protection Agencies). The stage was performed on October 2020 (3 weeks): the PhD S. Humboldt-Dachroeden was granted by ISS with a office sfor her own use, in fulfilment of the precautions adopted for mitigation of Covid-related risks. In particular, due to the covid-19 pandemic all traveling was suspended; however, together with online interviews, a number of face-to-face interviews iat ISS were performed using due precautions (face masks and distance). . The interviews are part of a qualitative study on the institutionalisation of One Health. The interviews were transcribed, analysed and are compared with interviews previously conducted with experts in Sweden. The work was disseminated, as a,poster at World One Health Congress in Edinburgh that was postponed from June to the 30.10. – 03.11.2020 due to the covid-19 pandemic. A preliminary comparative analysis of findings in both countries is going to be presented at the OHEJP ASM 2021. As an additional result, the environmental aspects of One Health were pointed out by the interviews in both Countries as a priority issue for conceptual development and implementation: consequently the following paper has been publihed in an indexed journal primarily focussed on human health: Humboldt-Dachroeden S., Mantovani A. (2021) Assessing environmental factors within the One Health approach. Medicina (Kaunas) 57(3):240. doi: 10.3390/medicina57030240.
Finally, the teaching activities at the Roskilde University on global health governance and supervision of bachelor and master semester projects went on.
The 9M report of the PhD is included into the section 8.1.4.

9.2 Deliverables and Milestones

9.2.1 Deliverables

Del. Rel. No	Deliverable title	Est. Del. Month	Notification
D7.1	Report of the workshop on Stakeholders and users´ Needs and Expectations	M48	Due to Covid-19 pandemic the workshop has been cancelled. The deliverable D7.1 will be renamed to refer to the policy event to be held in Y5 and a new submission date will be

			requested in the next amendment to the GA.
D7.2	One Health SRIA 2021-2030 - Preliminary Outline	M42	Timely submitted

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
N/A			

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