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D1.7 Annual Work Plan for the third year

WP1 Coordination

Responsible Partner: ANSES

Contributing partners: all partners

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1 Glossary

APC: Article Processing Charges
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
FOC: Free Of Charge
JIP: Joint Integrative Projects
JRP: Joint Research Project
OHEJP: One Health European Joint Programme
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
T&S: Travel and Subsistence
WS: Workshop

2 Coherence with annex 1

2.1 AWP objectives for month 25 to 36 (year 2020)

The third year of the One Health EJP will be dedicated to ensuring the follow-up of the following OHEJP activities:

- The 13 JRPs and JIPs selected under first call: the three-year projects and the two-year projects having requested a 6 months extension will be supervised and monitored to ensure the implementation of the projects according to their objectives and work plan;
- The 16 JRPs and JIPs selected under the second call will start;
- The final evaluation of the first round two years JRPs will be carried out
- The 16 PhD programmes will be supervised and monitored to ensure the implementation of the PhDs according to their objectives and work plan;
- The 2020 Training and Education Activities: STMs, Workshop satellite to the ASM, CPD, CaM, Summer School will be implemented;
- The 2021 Training and Education Activities will be selected.
- The second Annual Scientific Meeting (ASM) will be organised in Prague during spring 2020;
- The One Health EJP website and social media accounts will be regularly updated. The annual report for stakeholder will be prepared and disseminated;
- The second series of integrative missions will be launched to build relations between partner institutes and allow additional partners to liaise with ongoing Joint Integrative Projects;
- Two cogwheel workshops will be arranged to continue to pinpoint projects and initiatives of strategic interest to the OHEJP and provide opportunities for interaction;
- Update of the overarching OHEJP data management plan will be made to ensure its alignment with the OHEJP dissemination plan;

- Supervision and support will be provided to the JRP and JIP leaders in their task to develop (new projects) or update (running projects) their data management plans;
- Dissemination of outcomes from the OHEJP to stakeholders to meet their needs and to support policy initiatives will be ensured as well as a broaden communication with stakeholders with a focus on international stakeholders and global visibility.

2.2 Expected impacts

The set of activities described in the Annual Work Plan (AWP) from month 25 to 36 will contribute towards the implementation of the third year of the OHEJP with the ongoing implementation of the scientific research and integrative projects and Training and Education activities. The expected impact described below takes into account the REA's comments on the preliminary version of the AWP of the third year presented by the CT to the REA Steering Group on 5th of July 2019.

2.2.1 WP1

In the third year, the Coordination Team (P01-Anses and P04-Sciensano) assisted by the Project Management Team will ensure that the AWP of the third year is correctly implemented. The CT will manage the both the technical and financial reporting, of the second year to the Research Executive Agency. The Support Team will support the organisation of PMT, PMC, SSB, POC and ESAB meetings. The website and social media will be regularly updated and the project results will be disseminated both within the consortium, the European and international scientific community and the wider public.
Together with WP7 a specific attention will be sought towards the reflection on the sustainability of the OHEJP, notably by involving the PCM and the POC into the reflection on the sustainability, at a joint meeting, where the Directors General of the OHEJP institutions (the PMC) will have the opportunity to provide their views to the line Ministries of the participating organisaitons (the POC), on what should become the OHEJP consortium beyond its H2020 cofunded period, the objective being to fit into the next Framework Programme Horizon Europe.

<u>Expected communication impacts:</u>
The implementation of the Communication Strategy in the Year 3 will be essential to ensure that the progress of the One Health EJP is disseminated both internally and externally to the One Health EJP consortium. The key impacts expected in Year 3 are as follows:

- Regularly updating the One Health EJP website with news, events and information will ensure that all of the up to date information is available for those internal and external to the consortium. This will subsequently encourage collaboration and involvement in the One Health EJP events and funding opportunities.
- Similarly, our social media presence will encourage participation from a range of scientists at difference career stages, stakeholders and the general public. This is essential to the Communication Strategy to ensure that our events, results and impact are disseminated widely.
- Google Analytics and the analytics tools available for Twitter and LinkedIn will be continually monitored. Google Analytics allows the tracking of successful pages on the website, the success of the One Health EJP newsletters, the referrals from social media, the audience of the website and the browsers they use. This not only allows the Communications Officer to tailor the user experience on the website, but also improve the user experience. Furthermore, tracking the audiences to the website will enable us to monitor the Communications Strategy and view increasing traffic to our website following advertisement on social media or at a particular event. This in turn allows the goals defined in the Communications Strategy to be monitored. The use of Twitter and LinkedIn analytics ensures that the external

communications are reaching the desired target audiences and that there is an increasing number of followers, impressions and engagements on these platforms each month. Close monitoring of these analytics tools will allow the Communications Team to monitor the success of the Communications Strategy and modify it appropriately if necessary, to ensure we are meeting the goals set out in the Strategy.

- Strengthening the relationships with the CCP network across the One Health EJP will improve internal communication and facilitate the involvement of all partners. This is especially important for the partners that have not yet been involved in the One Health EJP projects, events and activities.
- The internal and external newsletters will also improve the internal and external communication from the One Health EJP and ensure that all of our stakeholders are aware of our progress and achievements on a regular basis.
- Effective communication of the One Health EJP progress, news, events, funding opportunities and Education and Training events and their subsequent alumni will create a European One Health community of scientists who are experts in the One Health field. The use of our social media platforms and increasing our followers and engagements will also contribute to developing this community of scientists, stakeholders and the general public that have a been interest and passion for implementation of the One Health concept.
- The Communication Team will work with all of the One Health EJP Work Packages to ensure that our research outcomes and activities are effectively communicated with policy makers across the EU. This will be a multifaceted approach and will require an internal network, including the CCPs, within the consortium to ensure that the impact of our work is resulting in translation of science to policy.

Overall, the communication impacts for Year 3 will continue to inform our target audiences of the One Health EJP progress, in addition to improving and refining the Communication activities on social media and at One Health EJP events. Improving relationships with the CCPs will be a key goal in Year 3 to ensure that each consortium member is receiving the information of all related activities and funding opportunities.

2.2.2 WP2

The public version of the strategic research agenda (SRA) will be disseminated towards OHEJP partners, stakeholders and other EU projects. Strategic interactions with other EU projects and initiatives and initiatives such as JPIAMR and JAMRAI will be further explored and encouraged, with the aim to create synergies and avoid duplication (also through cogwheels workshops, WP4). Moreover, the web based database of relevant EU research projects and related initiatives will be updated.

2.2.3 WP3

The main task of WP3 Team is to see that all JRP deliver as expected. In Y3 most of the 2-year projects that started in Y1 will come to an end, albeit later than initially planned due to the extension most project leaders asked and have obtained. These final reports and the periodic reports of the 3-year projects plus the first reports for the second call projects will be collected. All output will be disseminated to the right WP in the OneHealth EJP for further processing, i.e. to WP2 and WP7 for further updating and developing the Strategic Research and Innovation Agenda and to WP5 regarding science to policy, the information of our stakeholders of the scientific and innovative progress made. WP7 needs to identify the added value OneHealth EJP can bring in the future, an important step in the sustainability process.

In addition, WP3 brings together scientists in the domains of foodborne zoonoses, antimicrobial resistance and emerging threats through the organisation of the Annual Scientific Meeting, thus supporting the creation of an OneHealth Community.
In accordance with the REA's recommendation, WP3 will give special attention to the use of machine-learning technologies and its application for food safety risk assessment and risk management. Currently, two Joint Research Projects (RaDAR and ListAdapt) have activities concerning Machine Learning in their planning. The WP3 Team will encourage together with WP6 implementation of a specific training or education event in this area intended for a broader community. On this latter point, a satellite workshop to the ASM2019 in Dublin has been organised, called Digital Innovation and Data Management, where Machine Learning was discussed.
Furthermore, to meet the recommendation concerning the fostering of the risk assesment expertise, the WP3 will be particularly attentive to emphasize the results of the JRP RADAR having a clear risk assessment approach.

2.2.4 WP4

During the third year of the OHEJP, the second round of JIP projects will be launched. A prime objective will be to ensure that they get a good start working towards the integrative needs of both co-funding MS as well as EU stakeholders (EFSA, ECDC, COM). The second annual report on JIP activities will be published and further document how the partners have worked towards the joint integrative objectives. Furthermore, in response to expressed concerns from partners and stakeholders regarding constraints to national data sharing across the Med-Vet interface, WP4 will support a specific JIP aimed at exploring opportunities for harmonised national implementation of GDPR. The objective is to reduce unnecessary barriers to data sharing that emanate from variability in how GDPR is interpreted across MS.
Two additional cogwheel workshops will contribute to the exchange with relevant One Health initiatives and projects across Europe. A second round of integrative missions will be launched, which will aid in the interaction and exchange between current JIP partners and the rest of the consortium, and specifically promote integration of partners who are not part of the consortia from the onset of the JIPs. Furthermore, a thematic workshop will be arranged to bring together scientists within the consortium to work on issues of joint interest.
During the year output from the OHEJP is made available and useful through the policy for open data management which is part of the overarching dissemination policy. Continuous support to help partners keep their data management plans up to date will increase the capacity of the consortium to contribute to open science.
In the same line as the efforts undertaken so far, the Joint Integrative Project Leaders will be encouraged to enlarge their ongoing consortium to include partners geographically spread over Europe, in the Med and Vet balance. The integrative measures are planned to include, during the life span of any JIP, new beneficiaries. Guidelines for Project Leaders to request for an enlargement procedure of ongoing projects is available.

2.2.5 WP5

During the third year, WP5 "Science to policy translation" will follow the established procedures for an efficient information flow to and from European stakeholders. It will ensure that output of research and integrative activities reaches national, EU and international stakeholders in a timely and targeted manner, focusing on closing the identified knowledge gaps as well as fulfilling needs for scientific support. In parallel, WP5 will keep up to date with the EU key stakeholders' needs mainly by direct communication, complemented indirectly via horizon scanning of stakeholders' published documents. Efficient dialogue and regular scanning of stakehodlers' documents will also contribute to avoid any duplication of work between OHEJP and key EU stakeholders.

Indeed, the engaged participation of both ECDC and EFSA in the OHEJP Stakeholders Committee and in workshops of some OHEJP projects ensures the continue liaison, avoiding data models overlap and guarantying the access for risk assessment. Both organisations clearly support any OHEJP initiative that builds on existing features such as databases, models and procedures. There is also a close and direct communication set up between ECDC, EFSA and the respective JIPs. The appointment by EFSA of a liaison officer for the JIP NOVA illustrates well this synergy. WP5 will continue to work towards strengthening this dynamic of dialogue.
Moreover, tools like the capacity map will have an additional role in informing national stakeholders as well as fostering internal communication and interaction. Through the actions described in the AWP, WP5 will also depict complementarity between activities inside the consortium and outside, supporting potential collaborations and interactions.

2.2.6 WP6

In the third year, Work Package 6 will coordinate, support and monitor the 16 PhD grants which were selected to be funded in first and second year. Each project has a dedicated webpage. PhD students will take part in the 3MT PhD spotlight platform at each ASM to showcase their projects and create a cohort of OHEJP PhD students, and they will also join students attending or participating in the remaining WP6 activities to build a new generation of 'One Health' scientists. PhD students will also submit an annual summary report in addition to the final thesis.

WP6 will monitor the progress of the Short Term Missions funded in second year, and will communicate this on the website. WP6 will also co-ordinate the call to fund up to a further ten Short Term Missions in fourth year.

In addition, WP6 will also coordinate the organisation of the following events in the third year, which were selected in second year:

- The second ASM Satellite Workshop
- The second Summer School
- The Communication and Media workshop
- The first CPD module
- The second CPD module

Among the above-mentioned activities, special attention will be accorded to the organization of Communication and Media Training. The aim of this training will be to equip researchers with the skills, knowledge and practices to be able to communicate their science appropriately and effectively for public media such as to the general public, stakeholders and media journalists. This type of skills are of paramount importance in the scientific community, thus to engage in clear communication with citizens at large on the impact/role of their scientific activities.

Simultaneously, WP6 will coordinate the calls and selection procedures for the organisation of the third satellite workshop, summer school and CPD module in fourth year.

Furthermore, in accordance with REA's recommendation to encourage capacity building in the field of risk assessment, WP6 will consider the possibilitie to set up a training to promote expertise for risk assessment in the domain of the EJP activities.

2.2.7 WP7

During the third year, the issue of sustainability will be addressed making avail of the proven capacity and relevance of EJP, as well as of the more refined knowledge of stakeholders needs and of the

different sustainability opportunities. Sustainability scenarios will be defined, with the help of SWOT analysis launched during the second year.
In particular,

- the results of the SWOT analysis will be presented and discussed with the main current stakeholders (public health/food safety agencies of participating Countries, EFSA, ECDC). The assessment of SWOT outcomes together with the discussions with the stakeholders will help to set up a targeted appraisal of strengths and opportunities of the EJP: this will be achieved through a follow-up survey with stakeholders, in order to investigate more in-depth critical aspects identified by the SWOT analysis; the outcomes of SWOT analysis and the survey will be discussed in a workshop. This analysis will specifically aim at the perspectives for implementation into public health and food safety policies of the OH conceptual framework, considering both the detailed appraisal of the EJP strengths and the identification of priority topics for research on OH beyond 2020.
- The institutional stakeholder list needs to be enlarged and go global. During the third year the final definition of the following sets of stakeholders will be achieved: national risk management (public health/food safety) authorities of i) EU Countries, ii) of surrounding countries of the Balkan, Mediterranean and Eastern European areas, iii) of main global partners of EU (Russian Federation, India, China, USA, Canada) and iv) international organizations (OIE, FAO and WHO): these latter will be involved on the basis of the increasingly global character and transferability of the EJP approach and activities, namely the EJP added value to support harmonization of approaches, methodologies, databases and procedures; a further group of stakeholders would be v) private partners such as industrial (e.g., food, feed sectors) and farmer associations as well as consumers organizations. The collection of needs from stakeholders beyond the EU will help to refine the sustainability goals during year 4.
- At the end of year 3, the outcome of these activities will make it possible to identify and assess: i) concrete funding opportunities for OH research activities at large- (EU) and smaller-scale (e.g., Horizon Europe missions and/or partnerships, LIFE programmes, national and inter-Country calls) levels, thus implementing the SRA developed by WP2; ii) challenges (already partly identified by the SWOT analysis) that need to be considered in view of a sustainable EJP, such as stronger involvement of human health and environmental health components; iii) a selection of different scenarios to make the EJP sustainable, including a potential legal basis of long-term EJP sustainability related to its potential leverage.

2.3 Correspondence with the description of work – Annex 1

The following actions are foreseen in the third year:

2.3.1 WP1

WP1 work will be dedicated to supervision of the overall activities undertaken within the OHEJP and the reporting activities, namely periodic report of the second year, SPR of the third year and AWP of the fourth year. Financial follow-up of activities will be of utmost importance in the third year of implementation in order to ensure a good budgetary trajectory for the second half of the project allowing to have spent up to the maximum grant amount at the end of the OHEJP. The ST will also prepare the third amendment to the Grant Agreement
Organisation support for the periodic meetings of the governance bodies as well as the Annual Scientific Meeting will be provided**.**

2.3.2 WP2

WP2 activities will be focused in the dissemination of the public version of the SRA to OHEJP partners, stakeholders and other EU projects. Strategic interactions with other EU projects and initiatives and initiatives such as JPIAMR and JAMRAI will be further explored and encouraged, with the aim to create synergies and avoid duplication (also through cogwheels workshops, WP4). Moreover, the web based database of relevant EU research projects and related initiatives will be updated. Furthermore, relevant scientific developments, including the emergence of zoonotic threats and opportunities for scientific innovations will be monitored. For this, the output from OHEJP projects (e.g. deliverables, scientific publications) will be screened as well as relevant information from external sources. This information will be used as input for the development of the strategic research and innovation agenda (SRIA) in WP7.

2.3.3 WP3

Within WP3 all joint research projects will be monitored: the 2-year projects that will come to an end, and the 3-year projects of the first call and all new projects (second call) that need to report through the summary and periodic reports. The final reports need to be evaluated by external scientists and this outcome will be discussed by the SSB members. Also the organisation of the third ASM will be done as planned.

2.3.4 WP4

During the third year, WP4 will support the JIPs that are starting on 1 January 2020 to get a good launch, and a startup meeting will be arranged jointly with WP3. An online questionnaire will also be administered to new project leaders to follow up on the start of their projects. The progress of the two running JIPs during Y2 will be summarised in the 2nd periodic report, and actions resulting from its conclusions will be implemented. Guidelines for evaluation of the final JIP reports will be drafted, and external evaluators will be recruited together with WP3. To facilitate data sharing across the Med-Vet sectors, a specific project aimed at exploring opportunities for harmonised national implementation of GDPR will be launched, and supported in a similar manner as the other JIPs.
WP4 will liaise with leaders of JIPs to gather input for the 2nd call for integrative missions, to facilitate interaction with ongoing JIPs. Two cogwheel workshops (#5 and #6) will be executed to support external integration, and one thematic workshop will be given to support internal integration across domains.
The overarching data management plan (DMP) will be updated, and WP4 will follow up on the update also of the DMPs of JRPs and JIPs from the 1st call. Support will be provided to ensure the JRPs and JIPs from the 2nd call have their draft DMPs in place by M34.

2.3.5 WP5

During the third year, the actions foreseen in WP5 include strengthening of communication and communication means between the OHEJP and various stakeholders. The stakeholder committee will be further broadened as planned. In the annual SC meeting information on OHEJP outcomes will be sharedand synergies identified to support consideration of OHEJP results in their policies. Furthermore, in a focused meeting with key EU stakeholder linked with the Annual Scientific Meeting, additionally awareness of upcoming research needs and the potential impact the OHEJP can provide will be discussed. WP5 will respond to the stakeholders' needs and support the closure of knowledge gaps. Moreover WP5 will disseminate results of research and integrative activities following a well-ordered, tailored approach to maximize uptake in policy initiatives.

2.3.6 WP6

During the third year, the WP6 will co-ordinate the following activities:

- All PhD grants will have started before M25. In the third year, Work Package 6 will coordinate, support and monitor the sixteen PhD grants which were selected to be funded in Y1 and Y2. WP6 will routinely liaise with the Principal Investigators (PIs) awarded the funding and their respective PhD students to ensure WP6 receives the relevant project progress updates, and any dissemination activities are reported on our website and are also reported to the coordination team via the Internal Events Survey. As the projects progress, PhD students will also be asked to participate in smaller activities including encouraging the use of the private space of the website, showcasing their projects at the ASM every year through various style of presentations,, getting involved in informal events at the ASM and contributing content to their dedicated project page on the website and for the OHEJP social media channels to raise the profiles of the PhD student cohort and their achievements. The PhD students will join students attending or participating in the remaining WP6 activities to build a new generation of 'One Health' scientists. To report the progress of the project, PhD students will submit a summary report to WP6 on an annual basis.

- WP6 will also monitor the progress of the Short Term Missions (STMs) selected in Y2. These missions will take place throughout Y3, and once the missions are completed, individual reports will be produced. These reports will be used to inform the content for both Deliverable 6.10 and the webpage dedicated for STMs in 2020.

- WP6 will also review and amend the protocol (if necessary) for selecting the STMs for Y4. WP6 will then co-ordinate this call following the validated steps, and will select the successful awardees of the STM funding. This will be communicated with the consortium as described on the protocol, and the website will be updated with a webpage dedicated for STMs in 2021.

- The organisers for the second ASM Satellite Workshop and Summer school, both CPD modules and the Communication and Media Workshop were selected in Y2. These events will be held in the third year.

- WP6 will also review and amend the protocols (if necessary) for selecting the organisers of the third satellite workshop, Summer School and CPD module for Y4. WP6 will then co-ordinate the call ensuring the validated steps described in the protocol are followed.

- WP6 will produce the deliverable reports for D6.2, D6.5, D6.6, D6.7, D6.8, and D6.9

2.3.7 WP7

The main activities of WP7 will include i) a follow-up and a workshop in order to integrate the inputs from the SWOT analysis and assess the stakeholders needs regarding the EJP; ii) the finalized mapping the relevant stakeholders at national (EU and non-EU), European and international levels. The analysis of stakeholder's needs will help focus the current strengths and the areas to be strengthened within the EJP activities. For WP7 team will work on a method to identify and to share appropriate calls for funding in the scope of the EJP. Horizon Europe negotiations will be monitored.

2.4 Annual Work Plan

2.4.1 Timing of the different programmed activities and their components

WP	Task	Subtask	Title	M25	M26	M27	M28	M29	M30	M31	M32	M33	M34	M35	M36
WP1	1.1		Management of EC contractual obligations									1.10 1.11			
	1.2		Project management	MT	SSB		MT		MT		SSB	PMC	POC		
	1.3		Organisation of EJP management and governance meetings												
	1.4		Communication tools												
		1.4.0	Communication groups												
		1.4.1	Web site/interface												
		1.4.2	Internal communications	1.8											
		1.4.3	External communications: Press releases												
		1.4.4	External communications: Professional social networks												
		1.4.5	Special issue on One Health-Zoonoses for a scientific journal												
		1.4.6	External communications: Merchandise and branding												
		1.4.7	Annual and final report					1.9							
	1.5		Ethics		1.24										
	1.6		Declaration of Cofund												
WP2	2.1	2.1.1	Identification and updating of priority research and integrative topics												
		2.1.2	Development and updating of the strategic research agenda												
	2.2	2.2.1	Identification of relevant EU-projects and initiatives												
		2.2.2	Strategic interactions with EU projects and initiatives												
WP3	3.1		Guidlines for project coordinators to report + request extension (JRP&JIP)												
			Updated guidelines for Submission and selection of project proposals (JRP&JIP)												
			Guidelines for WP3/WP4 to monitor projects (JRP&JIP) (at start, periodic and end-												
			Updated guidelines for Evaluation of proposal + Selection of projects (JRP&JIP)												
			Guidelines for WP3/WP4 on the evaluation of final reports												
	3.2	3.2.2	Follow up of recently started projects								3.14				
		3.2.3	Monitoring: mid- and end-term reports	3.11											
		3.2.4	Evaluation of JRP reports by external experts: final report							3.13					
	3.3		Second call for projects: Evaluation of full proposals												
	3.4	3.4.1	Protocol for Annual Scientific Meeting organisation												
		3.4.2	Abstract book for Annual Scientific Meeting (ASM)				3.12								
WP4	4.1	4.1.1	Guidelines for submission and selection of JIP proposals												
		4.1.2	Instructions for reporting on, and monitoring of, JIP progress												
		4.1.3	Guidelines for final evaluation of JIPs										4.19		
	4.2	4.2.1	Start-up support	4.16											
		4.2.2	Project monitoring	4.17											
		4.2.3	Final evaluations												
	4.3	4.3.1	Alignment with strategic initiatives at EU level				4.18						4.20		
		4.3.2	Support function for integration of additional partners in ongoing JIPs												
		4.3.3	Scientific meetings to enhance integration												4.21
	4.4	4.4.1	Call for letters of intent												
		4.4.2	Prepare output from external peer review for project selection												
	4.5	4.5.1	Development of the Data Management Plan												
		4.5.2	Guidance and support for the implementation of the DMP												
		4.5.3	Open data access point												
WP5	5.1	5.1.1	Communication links with international stakeholders												
		5.1.2	Communication links with national stakeholders												
	5.2	5.2.1	Platform for interaction												
		5.2.2	Systematic screening												
		5.2.3	Consolidation of results												
	5.3	5.3.1	Capacity map												
		5.3.2	Scientific support to enhance exploitation of results												
	5.4	5.4.1	Dissemination strategy												
		5.4.2	Targeted communication												5.8
		5.4.3	Stakeholder conference												
WP6	6.1		Short term missions												6.7
	6.2		Workshop programme												6.8 6.2
	6.3		One health' Summer School for medical and veterinary science undergraduates												6.9
	6.4		Doctoral training programme												
	6.5		One-Health CPD Module		6.6										
	6.6		Communications workshop and media training												
WP7	7.1		Collection of end user Stakeholders' Needs and Expectations.												7.1
	7.2		Definition of the One Health SRIA 2021-2030												
	7.3		Development of governance model and related financial plan												
	7.4		Definiton of the road map for institutionalisation of One Health across the EJP												

2.4.2 Detailed work description

2.4.2.1 Annual work plan activities

2.4.2.1.1 WP1-Coordination and Management

Overarching activity		**WP1: Coordination and Management**				Lead Beneficiary			**P1-Anses**	
						Deputy Lead Beneficiary			**P4-Sciensano**	
Start month		**M1**				End month			**M60**	
Participant	**1**	**2**	**4**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	Anses	**Ages**	**Sciensano**	**NDRVMI**	**SZU**	**VRI**	**BfR**	**FLI**	**RKI**	**DTU**
PM	**47.5**	**0,5**	**6.5**	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**
Participant	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
	SSI	**UT**	**VFL**	**INIA**	**UCM**	**MVNA**	**INRA**	**IP**	**APHA**	**PHE**
PM	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**		**0,5**	**0,5**	**0,5**	**0,5**
Participant	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
	UoS	**OKI**	**NUIG**	**TEAGASC**	**ISS**	**IZSAM**	**IZSLER**	**RIVM**	**WbvR**	**FHI**
PM	**26.60**	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**
Participant	**33**	**34**	**35**	**36**	**37**	38	**39**	**40**	**41**	
	NVI	**PIWET**	**INIAV**	**INSA**	**IISPV**	IC	**SLV**	**FOHM**	**SVA**	
PM	**0,5**	**0,5**	**0,5**	**0,5**	**0,5**		**0,5**	**0,5**	**0,5**	

Objectives

T1.1: To carry out the EC contractual obligations regarding legal, financial and administrative management and coordination of the EJP.
T1.2: To ensure efficient project management
T1.3: To organize EJP management meetings of all the governance and management bodies of the EJP:
T1.4: To set up and put in place and manage efficient communication tools

Description of work

Task 1.1: Management of EC contractual obligations
The Coordination Team (CT) will supervise the preparation and submission of the deliverables due from M25 to M36 to the Research Executive Agency (REA). At the beginning of the period, the CT will carry out the annual technical and financial reporting. Also, the CT will coordinate the

preparation, compilation and submission to the EC of the Summary Progress Report (SPR) for third year and the Annual Work Plan (AWP) for fourth Year. At the end of the period, the Support Team will achieve the third amendment to the Grant Agreement.

Task 1.2 Project management
The Coordination Team will exchange on a weekly basis via teleconferences to ensure that administrative, financial and contractual requirements are met. The CT and the PMT will have monthly teleconference to ensure a smooth management and coordination of the WP activities. The CT will also regularly liaise with the REA project officers and report any issue that may arise.

Task 1.3: Organisation of EJP management and governance meetings
The ST will ensure the organisation of all the project governance bodies meetings in regards to the identified planning. There will be 2 SSB, 3 PMT, 1 POC and 1 PMC meetings organised.

Task 1-4: Communication tools
Subtask 1.4.0: Communication Groups
Stronger working relationships with the CCP network will be established, this will be achieved by implementing the Communication Contact Person mandate detailed in the Communication Strategy, in addition to organising a face-to-face meetings with the CCPs at the University of Surrey. It is essential that we have strong relationships with the CCPs in each of the One Health EJP institutes to ensure that information is disseminated effectively throughout and outside the consortium.

Subtask 1.4.1 Web site/interface: The website will continue to be developed throughout third Year. It will be monitored to ensure the most up to date information is available regarding events, news and project updates. The Work Package 6 pages will also be updated with information regarding the Short Term Missions, Summer School testimonials, PhD updates, publications and information to advertise any upcoming events. The Joint Research and Joint Integrative projects will also be regularly updated with key information, events and publications. Furthermore, the second round of funded projects will also have their pages created and available on the front end of the website. The Communication Officer will continue to encourage use of the private space of the website and will assist consortium members in their use of the website. Where necessary, individual pages on the website will be improved and we hope to improve the user experience of the website to ensure that consortium members can make the most of the interface provided by the One Health EJP.

Subtask 1.4.2 Internal communications: Newsletters: The consortium newsletters will be disseminated to the consortium every three months. These newsletters will provide One Health EJP updates regarding events, meetings, research projects, funding opportunities and any other information communicated to the Communications Officer. These newsletters will be sent using MailChimp to monitor the success of each newsletter. Additionally, these consortium newsletters will be available on the website and advertised on social media to increase the influence of the One Health EJP. The external newsletters will be disseminated every six months in January and June 2020. This newsletter will be sent to the consortium, in addition to the external mailing list generated on the One Health EJP website. These newsletters will be targeted to a wider audience, including the general public and therefore will have less technical information and more engaging content.

Subtask 1.4.3 External communications: Press releases: Press releases for One Health EJP events will be published on the One Health EJP website and advertised on the social media platforms. Press releases for the ASM and Work Package 6 activities will be the responsibility of the hosting institute,

however the Communications Officer can assist if requested and will facilitate this information being disseminated by the One Health EJP.

Subtask 1.4.4 External communications: Professional social networks: The One Health EJP social media platforms will continue to grow, this growth will be monitored closely to ensure that the Communication Strategy is effective. The key objective for the social media networks is to increase the number of followers, impressions and engagements with our content. This will work towards creating a One Health Community on social media. Advertising and live tweeting of One Health EJP events such as the ASM, meetings, events and funding opportunities will raise the profile of the One Health EJP and encourage engagement online.

Subtask 1.4.5 Special issue on One Health-Zoonoses for a scientific journal: not applicable- due in fifth Year.

Subtask 1.4.6 External communications: Merchandise and branding: The One Health EJP branding has been rolled out across the whole consortium, however the use of the correct templates and logos etc. will continue to be monitored in third Year. The One Health EJP merchandise will be refined and expanded according to the needs of the consortium. We have a selection of items available to the consortium. The Communication Officer will contact each institute to offer a package of One Health EJP merchandise which can be distributed at our partner institutes. For events such as the ASM, more engaging merchandise such as Giant Microbes will be explored to improve the aesthetics of the Communication Desk.

Subtask 1.4.7 Annual and final report: The annual report for second Year will be completed in this period.

Task 1.5 Ethics
In third year the project leaders will indicate how they have complied with the recommendations drafted by the Ethics Advisors. Based on these ethics reports plus the 24-months scientific reports, the Ethics Advisors will be able to evaluate all ethics aspects.
Another ethical review of the projects of the second internal call will be performed, and where clarifications are foreseen, recommendations will be provided to the concerned Project Leaders.

Deliverables

Ref	Title	Due month
D1.8	Annual report on the internal and external newsletter produced during the second year	M29
D1.9	Complete version of annual report for stakeholders n°2	M30
D1.10	Summary progress report of the third year	M33
D1.11	Annual work plan of the fourth year	M33
D1.24	Ethical review report of the second year	M26

Milestones

Ref	Title	Due month
MS5	SSB Meeting n°4	M26
MS6	SSB Meeting n°5	M32
MS14	PMC/POC/ESAB & SC Annual meeting n°3	M33

2.4.2.1.2 WP2-Strategic Research Agenda

Overarching activity		**WP2: Strategic Research Agenda**				Lead Beneficiary			**P30-RIVM**	
						Deputy Lead Beneficiary			**P17-UCM**	
Start month		**M1**				End month			**M60**	
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	**17**	18	19	20	21	22
	SSI	UT	VFL	INIA	**UCM**	MVNA	INRA	IP	APHA	PHE
PM					**2.25**					
Participant	23	24	25	26	27	28	29	**30**	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	**RIVM**	WbvR	FHI
PM								**2.25**		
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives

T2.1: Providing input for development of the strategic research and innovation agenda (SRIA) directed by WP7
T2.2: Updating the database of EU-projects/initiatives and establishing strategic interactions with other EU-projects/initiatives

Description of work

Task 2.1: Development of the SRA
Task Leader: RIVM
Deputy Task Leader: UCM
Participants: all partner organisations

The public version of the SRA will be distributed to the Scientific Community and Stakeholders trough different channels and it will be the starting point to develop, under the leadership of WP7, the development of the strategic research and innovation agenda (SRIA). To provide input for the SRIA, WP2 will monitor relevant scientific developments, including the emergence of zoonotic threats and opportunities for scientific innovations. For this, the output from OHEJP projects (e.g.

deliverables, scientific publications) will be screened as well as relevant information from external sources.

Task 2.2: Strategic interactions with EU projects and initiatives
Task Leader: UCM
Deputy Task Leader: RIVM
Participants: Anses, Sciensano, BfR, SVA

The web based database of relevant EU research projects and related initiatives will be regularly updated. Strategic interactions with other EU projects and initiatives, such as JPIAMR (Joint Programming initiative on Antimicrobial Resistance) and JAMRAI (Joint Action Antimicrobial Resistance Healthcare-Associated Infections) will be further explored and encouraged, with the aim to create synergies and avoid duplication (also through cogwheels workshops, WP4).

Deliverables

Ref	Title	Due month
N/A	N/A	

Milestones

Ref	Title	Due month
N/A	N/A	

2.4.2.1.3 WP3-Management of Joint Research Projects

Overarching activity		**WP3: Joint Research Projects management**				Lead Beneficiary			**P4-Sciensano**	
						Deputy Lead Beneficiary			**P13-SSI**	
Start month		**M1**				End month			**M60**	
	1	2	**4**	6	7	8	9	10	11	12
Participant	Anses	Ages	**Sciensano**	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM			**14**							
	13	14	15	16	17	18	19	20	21	22
Participant	**SSI**	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM	**1**									
	23	24	25	26	27	28	29	30	31	32
Participant	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
	33	34	35	36	37	38	39	40	41	
Participant	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives

Task 3.1: Drawing up of guidelines for submission, selection and evaluation of JRP proposals as well as request of extension of accepted JRPs.
Task 3.2: Supervision of the JRP in the first round of projects
Task 3.3: Organisation of a second round of projects and their supervision.
Task 3.4: Organisation of annual scientific meetings (ASM) where results from JRP are presented.

Description of work

Task 3.1: Drawing up of guidelines for submission, selection and evaluation of JRP proposals as well as request of extension of accepted JRPs. Task with input from WP4 (Joint Integrative Projects) and with WP6 (Education and Training)

All guidelines concerning WP3 have been delivered in Y1 and Y2

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

Subtask 3.2.1 Periodic follow up of activities in the JRP consortia

As in Y1 and Y2, WP3 team will continue to have a close formal and informal contact with project leaders, i.e. through videoconferences or via their consortium meetings. This subtask helps in creating constructive collaborations among partners and thus facilitate integration.
Special attention will be paid to the new projects of the second call that will start in January 2020.

Subtask 3.2.2 Follow-up of recently started projects
About two months after the launch of the second call projects a quick inventory of the status (started in time, specific needs, collaborations) will be made by means of a short online questionnaire. This will be reported to the EJP PMT and to the SSB.

Subtask 3.2.3: Mid- and end-term evaluations; monitoring of the KPI, deliverables and milestones detailed in the annual JRP reports
As each year, project leaders of first and second call projects will be requested to submit reports at 9 and 12 month of Y3, following templates that WP3 make available. The 9 month reports will serve as an input for the WP3 part of the EJP Summary Progress Report of Y3. Similarly, the consolidated 12 month reports of all projects will be used to feed into the Periodic OneHealth EJP Report of Y3. However, this 12 month reports will also serve to draft the WP3 and the Ethics reports of Y3. These reports will consequently be discussed in the SSB and validated by the Programme Managers Committee in the autumn of Y3.

Subtask 3.2.4: End-term evaluations by external experts: final report
In Y3 eight JRP will come to an end and Project Leaders will submit a final report. Almost all PLs have requested an extension of 6 months, which will delay the external evaluation by the experts.
The evaluation reports will be discussed by PMT and SSB and validated by the Programme Managers Committee in the autumn of Y3.

Task 3.3: Organisation of a second round of projects and their supervision. Task with help from WP4.
This task and its subtasks is finalised in Y2.

Task 3.4: Organisation of annual scientific meetings (ASM) where results from JRP are presented

Subtask 3.4.1: Protocol for organisation of annual scientific meetings
This subtask is finalised in Y1.

Subtask 3.4.2: Organisation of ASM
The organisation of the ASM2020 started in June 2019 and most of the practical issues will be arranged in Y3. It will be organised by the Czech partners P07-SZU and P08-VRI and will take place in Prague during the last week of May 2020.

Deliverables

Ref	Title	Due month
D3.11	2nd periodic report on ongoing JRP	M25
D3.12	Abstract book for 2nd Annual Scientific Meeting (ASM)	M28
D3.13	Final JRP reports, after expert evaluation (#1)	M31
D3.14	Report on the recently started projects, 2nd call	M32

Milestones

Ref	Title	Due month
MS34	Kick-off meeting (2nd call projects)	M26
MS35	Final reports n°1	M27
MS36	Feedback questionnaire n°2	M27
MS37	Second Annual Scientific Meeting	M28
MS38	Preparation of final evaluation report (#2)	M33
MS39	Interim and final reports preparation n°2	M35

2.4.2.1.4 WP4-Management of Joint Integrative Projects

Overarching activity		**WP4: Joint Integrative Projects**				Lead Beneficiary			**P41-SVA**	
						Deputy Lead Beneficiary			**P36-INSA**	
Start month		**M1**				End month			**M60**	
Participant	1	2	**4**	6	7	8	9	10	11	12
	Anses	Ages	**Sciensano**	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM			**1.25**							
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	23	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
Participant	**33**	34	35	**36**	37	38	39	40	**41**	
	NVI	PIWET	INIAV	**INSA**	IISPV	IC	SLV	FOHM	**SVA**	
PM	**1**			**3.9**					**9.42**	

Objectives

T4.1: To develop guidelines for evaluation of final reports and keep already produced guidelines on JIP reporting up to date.
T4.2: To follow and support the launch and implementation of the JIPs from the 1st and 2nd call.
T4.3: To support the alignment of EJP activities with two external EU initiatives/projects, and to support internal integration through the arrangement of integrative missions and a thematic workshop.
T4.4: n/a
T4.5: To provide support and follow the implementation of the Data Management Plan (DMP), and ensure alignment with the overarching dissemination plan.

Description of work

Task 4.1 Development of procedures and guidelines for submission and selection of JIP proposals, and for reporting and evaluation.

Subtask 4.1.1 Guidelines for submission and selection of JIP proposals
n/a

Subtask 4.1.2 Instructions for reporting on, and monitoring of JIP progress

- Revise and update D4.1 Guidelines for reporting on JIPs (M26).

Subtask 4.1.3 Guidelines for evaluation of JIPs

- Prepare D4.19 Guidelines for final evaluation of Joint Integrative Projects (M34).

Task 4.2 Supervision of JIPs

Subtask 4.2.1 Start-up support

- Arrange start-up meeting for 2nd call projects (jointly with WP3), and report on its completion (D4.16 (M25))
- Update and administer questionnaire following up the launch of 2nd call projects (jointly with WP3) (M27).

Subtask 4.2.2 Project monitoring

- Continue to follow-up on milestones and deliverables of ongoing JIPs.
- Produce the 2nd annual report on the progress of the JIPs (D4.17 (M25))

Subtask 4.2.3 Final evaluations

- Recruit external evaluators for COHESIVE and ORION (jointly with WP3).
- Collect information from EJP partners to determine the overall status of integration of project outputs (M36).
- .

Task 4.3 Integrative support

Subtask 4.3.1 Alignment with strategic initiatives at EU level

- Arrange two cogwheel workshops (#5 and #6) for alignment of activities with external strategic initiatives (D4.18 (M28) and D4.20 (M34)).
- Identify the target initiative for cogwheel workshop #6 and #7 (M28 and M34, respectively).

Subtask 4.3.2 Support function for integration of additional partners in ongoing JIPs

- Liaise with JIPs to identify an integrative mission programme for Y3 (M29).
- Oversee the implementation of integrative missions aimed at facilitating internal integration between COHESIVE and ORION partners and OHEJP partners not participating in the JIPs.

Subtask 4.3.3 Scientific meetings to enhance and leverage integration

- Arrange one thematic meeting related to a theme identified by partners/experts within the consortium (to be identified during Y3) (D4.21 (M36)).

Subtask 4.3.4 Data Sharing Initiative

- Launch a call for participation in the initiative.
- Supervise implementation of the initiative.

Task 4.4 Organisation of call for additional JIPs for the period Y3-Y5

n/a

Task 4.5 Open data management

Subtask 4.5.1 Development of the Data Management Plan

Subtask 4.5.2 Guidance and support for the implementation of the DMP

- Update overarching Data Management Plan (DMP)
- Support partners in updating project-specific DMPs
- Support new project leaders in drafting first version of project-specific DMP

Subtask 4.5.3 Open data access point

- Follow the implementation of the OHEJP dissemination policy to ensure the open data access point is functional and facilitates access to open resources.

Deliverables

Ref	Title	Due month
D4.16	Report from supportive start-up meeting, 2nd round	M25
D4.17	2nd periodic report on ongoing JIPs	M25
D4.18	Report from 5th cogwheel workshop	M28
D4.19	Guidelines for evaluation of final reports	M34
D4.20	Report from 6th cogwheel workshop	M34
D4.21	Report from thematic meeting III	M36

Milestones

Ref	Title	Due month
MS56	Four cogwheel workshops concluded and reported	M26
MS57	Online questionnaire administered to new coordinators	M27
MS58	Draft evaluation guidelines for JIPs presented to SSB	M32
MS59	All projects from 2nd call have started implementing the DMP	M34
MS60	External evaluators for JIPs recruited and internal assessment conducted	M36

2.4.2.1.5 WP5-Science to policy translation

Overarching activity		**WP5: Science to policy translation**				Lead Beneficiary			**P9-BfR**	
						Deputy Lead Beneficiary			**P13-SSI**	
Start month		**M1**				End month			**M60**	
Participant	1	2	4	6	7	8	**9**	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	**BfR**	FLI	RKI	DTU
PM							**13.33**			
Participant	**13**	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM	**12**									
Participant	23	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives

- To broaden the interaction with stakeholders, covering national, EU and international interests
- To follow the established procedures for a good information flow and interaction between the OHEJP projects and national, EU and international stakeholders to support complementarity and synergy of activities and exploitation of results
- To regularly update research and integrative needs of stakeholders identified in policy processes and to keep the interaction with the OHEJP consortium
- To regularly integrate available science and activities and support alignment for closure of knowledge gaps of international stakeholders
- To support identification of potential for synergies between OHEJP activities and other international projects by supporting communication and disseminating actively results
- To foster dissemination of results from activities within the OHEJP following the targeted dissemination policy initiatives addressing the protection of consumers health

Description of work

Task 5.1: Identification of the stakeholders and establishment of communication links

Subtask 5.1.1 Establish communication links with EU and international stakeholders

- The consolidation of communication links will be achieved by the Stakeholders Committee Meeting (SCM, subtask 5.4.2), which takes place twice during Y3, and by inviting stakeholders' representatives to join meetings of selected JIPs and JRPs as well as the ASM.
- WP5 will actively update key EU stakeholders regarding the scientific and integrative output of the consortium though the Targeted Reports (subtask 5.4.2).
- Through a helpdesk, ad hoc communications will be supported.
- Additional effort will be made to foster communication with international stakeholders such as WHO, OIE and FAO.

Subtask 5.1.2 Establish communication links to national stakeholders

- Interaction with national stakeholders will be consolidated by applying the capacity map (subtask 5.3.1), and by communicating outcomes and achievements of the OHEJP as well as identified synergies and activities complementary to the OHEJP.

Task 5.2: Identification of the research needs of EU stakeholders

Subtask 5.2.1 Platform for interaction

- WP5 will actively collaborate with the stakeholders to improve the way in which the needs and support requests are identified and communicated, in order to make the process more flexible.
- Dynamic and timely communication will be continued through the use of the virtual groups of the OHEJP website.
- Consolidation of research and integrative needs of the EU stakeholders will be continued to provide specific support and input for the SRIA.
- Stakeholders will have the possibility to communicate newly emerged needs through the "helpdesk" (subtask 5.4.1)

Subtask 5.2.2 Systematic screening

- Regular and systematic screening of stakeholders' documents will be carried on in order to:
    - Identify synergies and potential overlaps with OHEJP research and address complemental activities.
    - Identify stakeholders' potential needs.
- Requests via the helpdesk will be screened for additional needs to be considered

Subtask 5.2.3 Consolidation of results

- Scientific output of the OHEJP will be collected and mapped against the outcomes of the systematic screening. The result will be regularly discussed with the stakeholders (task 5.1 and task 5.4.2).
- Stakeholders' identified research and integrative needs will be used to update the Strategic Research Agenda.
- Action to close knowledge gaps will be discussed within the consortium and taken as appropriate.

Task 5.3: Linking of the scientific capacity available in the EJP with the stakeholders' identified needs: closure of knowledge gaps

Subtask 5.3.1 Capacity map

The capacity map is an important tool for highlighting expertise and selected technical infrastructure within the consortium, dissemination of results of the various activities within the consortium (e.g. research projects, integrative activities), and depicting to some extent complementarity with activities outside the OHEJP. The capacity map targets not just the key EU stakeholders, but also national stakeholders, and supports internal collaboration and dissemination. During the 3rd year focus will be put on:

- Content of the capacity map will be updated and presented following the recommendations from ongoing discussions with the stakeholders and feedback within the consortium.
- To ensure timely identification of potential overlaps and synergies of OHEJP activities with similar activities of key EU stakeholders, WP5 will screen stakeholders' documents (subtask 5.2.2) and accordingly incorporate relevant information in the capacity map.
- Indicators developed for assessing the impact of the OHEJP will be validated and updated if necessary

A strategy for methodically adapting the capacity map to additional needs, as well as an approach to easily integrate newly emerging outcomes of the OHEJP to keep the content up to date will be established.

Subtask 5.3.2:Scientific support to stakeholders

- WP5 will improve the strategy to achieve maximum exploitation of scientific results and integrative activities fulfilling the policy needs of various stakeholders. This will include the development of specific strategies addressing interests of national, EU and international stakeholders.
- Procedures to provide scientific support will be further developed and improved
- Allocation of resources to support specific stakeholders' needs with specific actions will be continued and optimized.

Task 5.4: Dissemination of new knowledge, tools and materials

Subtask 5.4.1 Dissemination strategy

WP5 will further develop the dissemination of science and integrative outputs to meet the stakeholders' identified needs:

- A tool in the form of a "helpdesk" will be optimized, through which the stakeholders have a direct and easy way to communicate their newly emerged needs for scientific support and to request information about integrative and research activities of the OHEJP.
- The capacity map (subtask 5.3.1) will be developed further to list scientific updates in a tidy and well-ordered way.
- Targeted reports (subtask 5.4.2) will be regularly distributed to the stakeholders.
- The dissemination strategy will be discussed biannually in the SCM (subtask 5.4.2.

Subtask 5.4.2 Targeted communication

- Targeted reports will be drafted and distributed to the stakeholders
- Focus will be given on those topics of highest interests for stakeholders
- Stakeholders Committee Meeting (SCM) will be organized biannually, one in autumn with the full SC, and one linked with the ASM with the key EU stakeholders.
    - During Y3 the SCM OHEJP outcomes will be shared and synergies identified to support consideration of OHEJP results in their policies
    - The SCM will also be used to discuss the dissemination strategy, the procedures to meet various stakeholders' needs, and options for the sustainability of the activities and procedures.
    - Topics and points of discussion will be incorporated in the agenda as needs emerge.

Subtask 5.4.3 Stakeholder conference

- During Y3, first activities will be started to prepare the stakeholders conference, scheduled for Y5.

Deliverables

Ref	Title	Due month
D5.8	Third annual report on dissemination activities to international stakeholders	M36

Milestones

Ref	Title	Due month
MS70	New knowledge gaps and research needs identified n°2	M36
MS74	Scientific support provided n°2	M36

2.4.2.1.6 WP6-Training and Education

Overarching activity		**WP6: Training and Education**				Lead Beneficiary			**P23-UoS**	
						Deputy Lead Beneficiary			**P31-WbvR**	
Start month		**M1**				End month			**M60**	
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	**23**	24	25	26	27	28	29	30	**31**	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	**WbvR**	FHI
PM	**14**								**1.5**	
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives

T6.1: Short Term Missions

- WP6 will manage and support the reporting requirements from the missions which took place in Y2. From these reports, WP6 will compile D6.6.
- WP6 will coordinate, support and monitor the Short Term Missions selected through the second call and will manage and support the reporting requirements. All missions will be uploaded onto the One Health EJP website.
- WP6 will coordinate the call to select the Short Term Missions to fund in Y4

T6.2: Workshop programme (satellite to Annual Scientific Meetings)

- WP6 will coordinate and support the organisation of the second satellite workshop ; and will manage and support the reporting requirements.
- WP6 will coordinate the call to select the organisers of the third ASM Satellite Workshop

T6.3: One health' Summer School for medical and veterinary science undergraduates

- WP6 will coordinate and support the organisation of the second Summer School ; and will manage and support the reporting requirements.

- WP6 will coordinate the call to select the organisers of the third Summer School

T6.4: Doctoral Training Programme

- WP6 will coordinate, support and monitor the 17 PhD grants which were selected to be funded in Y1 and Y2.

T6.5: One-Health Continuing Professional Development (CPD) Module

- WP6 will coordinate and support the organisation of CPD modules (Y2 and Y3) and will manage and support the reporting requirements.

T6.6: Communications workshop and media training

- WP6 will coordinate and support the organisation of the Communications and Media workshop and will manage and support the reporting requirements.

Description of work

Task 6.1: Short Term Missions

WP6 will manage and support the reporting requirements from each of the missions from the first short term mission call i.e. the missions that took place in Y2. WP6 will then compile the official deliverable for D6.6, which is due in M26. All missions will be uploaded onto the One Health EJP website.

WP6 will coordinate, support and monitor the Short Term Missions selected through the second call. The missions will all take place between M25 and M36. WP6 will manage and support the reporting requirements from each of the missions for D6.10 (due in M38). All missions will be uploaded onto the One Health EJP website.

WP6 will coordinate the call to select the Short Term Missions to fund in Y4 following the validated procedure.

Task 6.2: Workshop programme (satellite to Annual Scientific Meetings)

WP6 will coordinate and support the organisation of the second satellite workshop, which will be hosted alongside the ASM in Prague. After the event, WP6 will manage and support the reporting requirements of D6.7.

WP6 will coordinate the call to select the organisers of the third ASM Satellite Workshop following the validated procedure.

Task 6.3: 'One health' Summer School for medical and veterinary science undergraduates

WP6 will coordinate and support the organisation of the second Summer School. After the event, WP6 will manage and support the reporting requirements of D6.8.

W6 will coordinate the call to select the organisers of the third Summer School following the validated procedure.

Task 6.4: Doctoral Training Programme

WP6 will coordinate, support and monitor the 17 PhD grants selected for funding in Y1 and Y2. All PhDs will have started by M25.

Task 6.5: One-Health Continuing Professional Development (CPD) Module

WP6 will coordinate and support the organisation of CPD modules (Y2 and Y3).

An ammendment was made to the deliverable due for the CPD module in Y2. This CPD event will now take place in Y3. After the event, WP6 will manage and support the reporting requirements of D6.5.

WP6 will coordinate and support the organisation of the second CPD module. After the event, WP6 will manage and support the reporting requirements of D6.9.

WP6 will coordinate the call to select the organisers of the third CPD module following the validated procedure.

Task 6.6: Communications workshop and media training

WP6 will coordinate and support the organisation of the Communications and Media workshop. After the event, WP6 will manage and support the reporting requirements of D6.2.

Deliverables

Ref	Title	Due month
D6.2	Report on communications workshop and media training	M36
D6.6	Report n°2 of the annual short term missions completed also uploaded onto the EJP webpage.	M26
D6.7	Report n°2 on one workshop per year associated with ASM	M36
D6.8	Report n°2 of the four, 4-week One health summer school	M36
D6.5	CPD module in one health to 15 participants (1st session)	M24
D6.9	CPD module in one health to 15 participants (2nd session)	M36

Milestones

Ref	Title	Due month
MS79	Launching of annual call for Short Term Missions n°2	M28

2.4.2.1.7 WP7-Sustainability

Overarching activity		**WP7: Sustainability**				Lead Beneficiary			**P27-ISS**	
						Deputy Lead Beneficiary			**P20-IP**	
Start month		**M1**				End month			**M60**	
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	17	18	19	**20**	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	**IP**	APHA	PHE
PM								**1.5**		
Participant	**23**	24	25	26	**27**	28	29	30	**31**	32
	UoS	OKI	NUIG	TEAGASC	**ISS**	IZSAM	IZSLER	RIVM	WbvR	FHI
PM					**4.5**					
Participant	33	34	35	36	37	38	39	40	**41**	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	**SVA**	
PM									**6**	

Objectives

T7.1: Gathering Stakeholders´ Needs and Expectations
T7.2: Strategic Research and Innovation Agenda (SRIA) 2021-2030 (SRIA 2021-2030)
T7.3: Making the EJP sustainable through other funding and/or legal basis
T7.4: Making the bridges between EJP's beneficiaries and stakeholders sustainable

Description of work

Task 7.1: Gathering Stakeholders´ Needs and Expectations
Task leader Bfr/participants: ANSES, IP, ISS
The results of the SWOT analysis will be presented and discussed with the main current stakeholders (punlic health/food safety agencies of participating Countries, EFSA, ECDC). The assessment of SWOT outcomes together with the stakeholders will help to set up a targeted appraisal of strenghts of opportunities of the EJP: this will be achieved through a survey (mail questionnare and phone meetings) with stakeholders and a workshop.

Subtask 7.1.1: Mapping the stakeholders at national, European and international stages
The final definition of the following sets of stakeholders will be achieved: national risk management (public health/food safety) authorities of i) EU Countries, ii) of surrounding countries of the Balkan, Mediterranean and Eastern European areas, iii) of main global partners of EU (Russian Federation, India, China, USA, Canada) and iv) international organizations (OIE, FAO and WHO):
Subtask 7.1.2: identification and analysis of key documents issued by the stakeholders
In this phase key documents issued by current stakeholders (authorities of EU MS, EFSA, ECDC) will be the (already available)A m outcomes of SWOT analysis, as well as the replies to the survey.These documents will be analysed in order to evaluate ii) needs and expectations of stakeholders; ii) strengths and areas to be strengthened of the OHEJP as preceived by stakeholders

Task 7.2: Strategic Research and Innovation Agenda (SRIA) 2021-2030 (SRIA 2021-2030)
Task leader: ISS / Participants: RIVM, ANSES, BfR, IP, SVA UCM.
The SRA produced by WP2 is complemented with the information from task 7.1. At the end of year 3 task 7.2 will identify and assess concrete funding opportunities for OH research activities at large (EU) and smaller-scale (e.g., national, inter-Country networks, LIFE programmes) levels. At the same me, Task 7.2 will identify challenges for the current OHEJP that need to be considered in view of a successful and competitive SRIA for the next decade. Therefore, Task 7.2 is intended as a work in progress to be devekoped and updated throughout the course of the EJP.
Subtask 7.2.1: Definition of the SRA in cooperation with WP2
The SRA is jointly developed by WP2 and by WP7 with the support of collection and analysis of the inputs from stakeholders. Points of strengths and areas to be strengthened or included in the SRA will be identified.
Subtask 7.2.2: definition of the innovation activities to be included in the agenda, taking into account the SRA and the results of task 7.1
Based on task 7.2.1, task 7.2.2 will define the help define the specific innovation actions to be included in the agenda. Specifically, a close monitoring of the shaping of Horizon Europe will be developed in collaboration with WP2

Task 7.3: Making the EJP sustainable through other funding and/or legal basis
Task leader: IP / Participants: all beneficiaries
The EJP mission and objectives will be evaluated considering the next EU and international calls, to attract other funding thanks to the EJP's leverage effect.
FP9 missions, LIFE programme, national and inter-Country calls will be analysed to support the competitive development and update of the EJP objectives and activities continuity beyond H2020.
Subtask 7.3.1: SRIA cofunded implementation program
To be started in Year 4
Subtask 7.3.2: Funding opportunities
To be started in Year 4
Subtask 7.3.3: Legal basis for sustainability
A selection of different scenarios to make the EJP sustainable, including a potential legal basis of long-term EJP sustainability related to its potential leverage.

Task 7.4: Making the bridges between EJP's beneficiaries and stakeholders sustainable

Task Leader: SVA / Participants: all beneficiaries

Year 3 will draft a detailed plan (to be implemented in year 4) for a EU-wide analysis of current work processes, shared infrastructures, reference documents and guidelines across the multifacted OH interface (environmental health-animal health-public health), with identification of legal, financial, cultural and political barriers and drivers for long-term maintenance, implementation and updating. This work is conducted partly in the form of a PhD project in collaboration with Roskilde University, Department of Social Sciences and Business.

Subtask 7.4.1 Application of framework for evaluation of One Health
to be implemented from Year 4

Subtask 7.4.2 Institutional analysis
to be implemented from Year 4

Subtask 7.4.3 Road map for institutionalisation of One Health
to be implemented from Year 4

Deliverables

Ref	**Title**	**Due month**
D7.1	Report of the end user Stakeholders´ Needs and Expectations	M36

Milestones

Ref	**Title**	**Due month**
MS99	Launching the Strategic Research and Innovation Agenda (SRIA) 2021-2030	M36
MS100	Report on institutionalisation analysis of the EJP	M36

2.4.2.1.8 JRP01-R1-AMR1-IMPART

Reference to the Stategic Research Agenda (please refer to D2.7)	**AMR 1: Development and harmonisation of phenotypic methods**									
Research Project Title	**Improving phenotypic testing of AMR by development of sensitive screening assays for emerging resistances and setting missing ECOFFs**									
Research Project Acronym	**IMPART**									
Leading Organisation	**P31-WbvR**					Deputy Leading Organisation			**P1-Anses**	
Project Leader	**Kees Veldman**					Deputy Project leader			**Sophie Granier**	
Project Start month	**M1**					Project End month			**M30**	
Participant	**1**	2	4	6	7	8	**9**	10	11	**12**
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	**BfR**	FLI	RKI	**DTU**
PM	**3.83**						**3.60**			**0**
Participant	**13**	14	15	16	17	18	19	20	**21**	**22**
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	**PHE**
PM	**0**								**1.87**	**1.05**
Participant	23	24	25	26	27	28	29	**30**	**31**	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	**RIVM**	**WbvR**	FHI
PM								**1.85**	**2.75**	
Participant	**33**	**34**	35	36	37	38	39	40	**41**	
	NVI	**PIWET**	INIAV	INSA	IISPV	IC	SLV	FOHM	**SVA**	
PM	**3.30**	**1.90**							**4.42**	

Objectives

Work package 1:

Develop, improve and validate common methods to isolate, characterize and confirm resistance to colistin in relevant Gram negative bacteria.

Work package 2:

To develop a sensitive and validated culturing method for the detection of relevant carbapenemase-producing Enterobacteriaceae (CPE) in meat samples and caecal samples from animals.

Work package 3:

Development of epidemiological cut-off values (ECOFFs) of antimicrobials for veterinary pathogens.

Work package 4:
To develop and standardize a disk diffusion method for antimicrobial susceptibility testing of *Clostridium difficile* followed by testing of a large collection of *C. difficile* isolates resulting in inhibition zone diameter distributions of a relevant set of antimicrobials.

Work package 5:
To coordinate the four work packages and the knowledge dissemination within and beyond IMPART.

Description of work

WP1: Selective isolation, detection and characterization of colistin-resistant Enterobacteriaceae
WP1 start month: M1
WP1 end month: M30

WP1 Leader: Sophie Granier (1, Anses)
Deputy WP1 Leader: (junior scientist to be hired at 1, Anses)
WP1 participants: Anses (1), BfR (9), DTU (12), SSI (13), APHA (21), PHE (22), WbvR (31), RIVM (30), NVI (33), PIWET (34), SVA (41), IZSLT (Ext 1)

Description of the WP:
Work package 1 continues based upon what was achieved during the first and second annual period of the EJP.
The first description of a transferable colistin resistance mechanism (Liu *et al* 2015), urged antimicrobial resistance laboratories world-wide to quickly implement methods to confirm or reject the presence of transferable colistin resistance genes. Since then, variants of *mcr-1* and *mcr-2* genes have been described without enough knowledge of their epidemiological relevance. Due to the public health concerns about resistance to a last resort antimicrobial to treat life threatening human infections, each laboratory empirically set their own methods relying on recent literature.
The time has come to share, compare and evaluate, to "IMPART", our experience and knowledge in order to evaluate and standardize methodologies to:

- Selectively isolate colistin-resistant Enterobacteriaceae
- Phenotypically characterize colistin resistance
- Confirm the presence of transferable colistin resistance mechanisms

IMPART-WP1 will ensure that all participants will implement the same harmonized method for detection and characterization of colistin-resistant Enterobacteriaceae.
At the end of IMPART, the participants will have raised their expertise on colistin resistance identification in general. IMPART will raise us all to the same level in order to create a network of laboratories throughout Europe able to monitor this potential threat to public health, using similar harmonized methodologies.

Task 1.7: Publication in peer-reviewed journal
Task start month: M25
Task end month: M30

Task Leader: Sophie Granier (1, Anses)
Deputy Task Leader: Kees Veldman (31, WbvR)
Task Participants: Anses (1), BfR (9), DTU (12), SSI (13), APHA (21), PHE (22), WbvR (31), RIVM (30), NVI (33), PIWET (34), SVA (41), IZSLT (Ext 1)

Description of the task:
T1.7 takes place over the third annual period of the EJP. The WP1 will generate a publication, in an open access peer-reviewed journal, containing a science-based advice for a reliable and standardized method to isolate, detect and confirm colistin resistance in relevant Enterobacteriaceae from meat or caecal samples.

Task 1.8: Plan joint implementation
Task start month: M25
Task end month: M30

Task Leader: Sophie Granier (1, Anses)
Deputy Task Leader: Kees Veldman (31, WbvR)
Task Participants: Anses (1), BfR (9), DTU (12), SSI (13), APHA (21), PHE (22), WbvR (31), RIVM (30), NVI (33), PIWET (34), SVA (41), IZSLT (Ext 1)

Description of the task:
T1.8 takes place over the third annual period of the EJP. After the evaluation of the ring trial, when the methodology is established, an amount of available samples will be investigated for colistin-resistant Enterobacteriaceae at each lab. The final stage of IMPART–WP1 will be to plan the joint implementation of the harmonized surveillance and to write proposal(s) for future projects.

WP2: Selective isolation, detection and characterization of carbapenemase-producing Enterobacteriaceae
WP2 start month: M1
WP2 end month: M30

WP2 Leader: Jannice Schau Slettemeås (33, NVI)
Deputy WP2 Leader: Marianne Sunde (33, NVI)
WP2 participants: Anses (1), BfR (9), DTU (12), SSI (13), APHA (21), PHE (22), WbvR (31), RIVM (30), NVI (33), PIWET (34), SVA (41), IZSLT (Ext 1)

Description of the WP:
Work package 2 continues based upon what was achieved during the first and second annual period of the EJP.
The main goal for WP2 is the development and harmonization of a sensitive culturing method to detect and characterize carbapenemase-producing Enterobacteriaceae (CPE) in caecal and meat samples from animals. WP1 and WP2 are of the same character, addressing the importance of establishing the most sensitive culturing method to detect colistin-resistant and carbapenemase-producing Enterobacteriaceae (CPE) in both human and veterinary laboratories. WP2 fits very well to the scope of the call description regarding AMR-1: Development and harmonizing phenotypic methods sharing data and/or bacterial strains of the participating veterinary and medical institutes. IMPART-WP2 will give the best methods to:

- Selectively isolate carbapenemase-producing Enterobacteriaceae
- Phenotypically characterize carbapenemase production
- Confirm the presence of transferable carbapenem resistance mechanisms

IMPART-WP2 will gather information on methods already available, perform a ring trial between the participants in the consortium and evaluate the results to give the best available method to be used in both monitoring programs as well as in routine diagnostic labs in human and veterinary sector.

Task 2.7: Publication in peer-reviewed journal
Task start month: M25
Task end month: M30

Task Leader: Jannice Schau Slettemeås (33, NVI)
Deputy Task Leader: Kees Veldman (31, WbvR)
Task Participants: Anses (1), BfR (9), DTU (12), SSI (13), APHA (21), PHE (22), WbvR (31), RIVM (30), NVI (33), PIWET (34), SVA (41), IZSLT (Ext 1)

Description of the task:
T2.7 takes place over the third annual period of the EJP. WP2 will generate a publication in an open-access peer-reviewed journal, containing a science-based advice for the best method to isolate and detect carbapenemase-production in relevant Enterobacteriaceae from meat and caecal samples.

Task 2.8: Plan joint implementation
Task start month: M25
Task end month: M30

Task Leader: Sophie Granier (1, Anses)
Deputy Task Leader: Kees Veldman (WbvR)
Task Participants: Anses (1), BfR (9), DTU (12), SSI (13), APHA (21), PHE (22), WbvR (31), RIVM (30), NVI (33), PIWET (34), SVA (41), IZSLT (Ext 1)

Description of the task:
T2.8 takes place over the third annual period of the EJP. After the evaluation of the ring trial, when the methodology is established, an amount of available samples will be investigated for carbapenemase-producing Enterobacteriaceae at each lab. The final stage of IMPART–WP2 will be to plan the joint implementation of the harmonized surveillance and to write proposal(s) for future projects.

WP3: Establishing epidemiological cut-off values (ECOFFs)
WP3 start month: M1
WP3 end month: M30

WP3 Leader: Kees Veldman (31, WbvR)
Deputy WP3 Leader: Els Broens (31, WbvR)
WP3 participants: Anses (1), BfR (9), SSI (13), APHA (21), PHE (22), WbvR (31), RIVM (30), NVI (33), PIWET (34), SVA (41), IZSLT (Ext 1)

Description of the WP:
Work package 3 continues based upon what was achieved during the first and second annual period of the EJP.
This work package will accelerate the development of epidemiological cut-off values (ECOFFs) for veterinary pathogens and thereby support the process defining animal-specific clinical breakpoints for veterinary antimicrobials, which is essential for the prudent use of antimicrobials in animals.
WP3 is aiming to set ECOFFs for a defined list of bacterial species-antimicrobial agent combinations by collecting a sufficient number of MIC distributions from different laboratories.

Task 3.4: Analysis of the data and publication of ECOFFs
Task start month: M25

Task end month: M30

Task Leader: Kees Veldman (31, WbvR)
Deputy Task Leader: Els Broens (31, WbvR)
Task Participants: WbvR (31)

Description of the task:
T3.4 takes place over the third annual period of the EJP. Data will be analysed by the WP leader under supervision of the EUCAST subcommittee on MIC distributions and ECOFFs. Finally, defined ECOFFs will become available on the EUCAST antimicrobial wild type distribution website (http://mic.eucast.org/Eucast2/).

WP4: Developing and optimizing a disk diffusion method for antimicrobial susceptibility testing of *Clostridium difficile*
WP4 start month: M4
WP4 end month: M30

WP4 Leader: Sven Maurischat (9, BfR)
Deputy WP4 Leader: Mirjam Grobbel (9, BfR)
WP4 participants: ANSES (1), BfR (9), SSI (13), APHA (21), RIVM (30), PIWET (34), SVA (41)

Description of the WP:
Work package 4 continues based upon what was achieved during the first and second annual period of the EJP.
This work package will develop and standardize a disk diffusion test for antimicrobial susceptibility testing (AST) for *C. difficile*. This will improve the AST of this pathogen using the disk diffusion method. By collecting data on inhibition zone diameter distributions of relevant antibiotics from different classes analysing at least 500 *C. difficile* isolates from different origin (human, animal, food) and different European countries, we will be able to propose inhibition zone based cut-offs and thereby distinguish between wild type and resistant strains.
Finally, the aim of WP4 is to facilitate the usage of the disk diffusion method for a broad spectrum of antimicrobials and support its application for routine diagnostic purposes as alternative to the agar dilution and commercially available gradient strips for minimal inhibitory concentration (MIC) testing of *C. difficile*.

Task 4.3: Performance of a ring trial study
Task start month: M22
Task end month: M27

Task Leader: Sven Maurischat (9, BfR)
Deputy Task Leader: Mirjam Grobbel (9, BfR)
Task Participants: ANSES (1), BfR (9), SSI (13), APHA (20), RIVM (31), PIWET (35)

Description of the task:
T4.3 takes place between the second and third annual period of the EJP. A ring trial study will be conducted to determine the interlaboratory reproducibility and repeatability of the method. A selected subset of isolates of task 4.2 will be distributed to the participating laboratories and analysed by each partner according to the proposed protocol.

Task 4.4: Producing inhibition zone diameter distributions and proposing cut-off values for *C. difficile*
Task start month: M17
Task end month: M30

Task Leader: Sven Maurischat (9, BfR)
Deputy Task Leader: Mirjam Grobbel (9, BfR)
Task Participants: BfR (9)

Description of the task:
T4.4 continues based upon what was achieved during the first and second annual period of the EJP. Using the disk diffusion method established in task 4.1, inhibition zone diameter distributions are established analysing at least 476 *C. difficile* strains from task 4.2 towards clindamycin, erythromycin, imipenem, metronidazole, moxifloxacin, rifampicin, tetracycline and vancomycin. Based on these distributions, cut-offs will be proposed by statistical analysis. For certain antimicrobials and a subset of the strain collection, the correlation of inhibition zone diameters and MICs produced by agar dilution or Etest will be determined in parallel to verify the classification by comparing the proposed cut-off values and the published MIC-based ECOFFs. Furthermore, the WP4 leader will publish the data in an open-access peer-reviewed scientific journal.

WP5: Coordination of the four work packages and knowledge dissemination both internally within and externally beyond the IMPART consortium
WP5 start month: M1
WP5 end month: M30

WP5 Leader: Kees Veldman (31, WbvR)
Deputy WP5 Leader: Sophie Granier (1, Anses)
WP5 participants: Anses (1), BfR (9), WbvR (31), NVI (33)

Description of the WP:
Work package 5 continues based upon what was achieved during the first and second annual period of the EJP.

Task: T5.1: Organization of IMPART
Task start month: M1
Task end month: M30

Task Leader: Kees Veldman (31, WbvR)
Deputy Task Leader: Sophie Granier (1, Anses)
Task Participants: Anses (1), BfR (9), WbvR (31), NVI (33)

Description of the task:
T5.1 continues based upon what was achieved during the first and second annual period of the EJP. Under the supervision of the project coordinator, each of the four WPs will run in parallel with the common goal on improving and standardizing phenotypic methodologies to survey antimicrobial resistance in various ecosystems. Work plans will be shared and interactions between WPs will be encouraged in order to be as cost effective as possible. Work package leaders and the project coordinator constitute the coordination team of IMPART through T5.1.

T5.2: Communication within IMPART
Task start month: M1

Task end month: M30

Task Leader: Sophie Granier (1, Anses)
Deputy Task Leader: Kees Veldman (31, WbvR)
Task Participants: Anses (1), BfR (9), WbvR (31), NVI (33)

Description of the task:
T5.2 continues based upon what was achieved during the first and second annual period of the EJP. IMPARTs main goal is to improve the methodologies on phenotypic characterization of antimicrobial resistance. All partners have agreed on a list of priority issues from their daily lab practice that will be addressed together. All knowledge generated by the scientific WPs will be shared on a special web-based platform with controlled access, containing meeting notes, protocols, reports and Video tutorials (using Windows Movie Maker and Camtasia). Two meetings are planned, Kick-off and Final, in between Video conferences will be organised on regular basis.

T5.3: Communication beyond IMPART
Task start month: M1
Task end month: M30

Task Leader: Sophie Granier (1, Anses)
Deputy Task Leader: Kees Veldman (31, WbvR)
Task Participants: Anses (1), BfR (9), WbvR (31), NVI (33)

Description of the task:
T5.2 continues based upon what was achieved during the first and second annual period of the EJP. Outputs from IMPART will be shared with the scientific community beyond MedVet EJP. The improved or developed methods, and the results of the ring trials, will be presented at international conferences and published in open-access peer-reviewed scientific journals. IMPART will also contribute to the MedVet EJP Website, on a regular basis, following the general MedVet EJP communication strategy.

Deliverables

Ref	Title	Due month
D-JRP1-1.7	Publication in an open-access peer-reviewed journal	M30
D-JRP1-1.8	Proposal(s) for epidemiological study to monitor resistance to colistin	M30
D-JRP1-2.7	Publication in an open-access peer-reviewed journal	M30
D-JRP1-2.8	Proposal(s) for epidemiological study to monitor resistance to carbapenems	M30
D-JRP1-3.3	Publication of ECOFFs on EUCAST website	M30
D-JRP1-4.3	Performance of a ring trial study	M27
D-JRP1-4.4	Publication in an open-access peer-reviewed journal	M30
D-JRP1-5.3	IMPART News online on MedVet EJP website	M30
D-JRP1-5.6	Final meeting notes sent to participants	M30

Milestones

Ref	Title	Due month
M-JRP1-11	Final meeting (notes of the meeting)	M30

2.4.2.1.9 JRP02-R1-AMR2-ARDIG

Reference to the Stategic Research Agenda (please refer to D2.7)	**AMR 2: Epidemiological studies into the dynamics of AMR in human and animal populations and the environment including horizontal gene transfer and selection of AMR**									
Research Project Title	**Antibiotic Resistance Dynamics: the influence of geographic origin and management systems on resistance gene flows within humans, animals and the environment**									
Research Project Acronym	**ARDIG**									
Leading Organisation	**P21-APHA**					Deputy Leading Organisation			**P11-RKI**	
Project Leader	**Muna Anjum**					Deputy Project leader			**Tim Eckmanns**	
Project Start month	**M1**					Project End month			**M36**	
Participant	**1**	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM	**2.71**						8		27	
Participant	13	14	15	16	**17**	18	19	**20**	**21**	**22**
	SSI	UT	VFL	INIA	**UCM**	MVNA	INRA	**IP**	**APHA**	**PHE**
PM					**28**			**4**	**14.21**	**9**
Participant	**23**	24	25	26	27	28	29	30	**31**	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	**WbvR**	FHI
PM	**6.04**								**3.48**	
Participant	**33**	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM	**10**									

Objectives

- Work-Package 1:
  - To compare AMR prevalence and AB usage in six different European regions collected through national surveillance, including EU and other surveillance programmes, from humans, animals, food and the environment.
  - To correlate resistance trends with antibiotic sales or usage (available through research project or industry data and by collecting primary data), ecological factors and management practices.
  - To provide recommendations for improved One Health surveillance.

- Work-Package 2:
  - Performing longitudinal studies to determine prevalence of ESBL/AmpC/carbapenemase producing or *mcr-1 and -2*/PMQR-carrying Enterobacteriaceae in pig, poultry, cattle farms or hospitals and their environments.
  - Comparing national AMR prevalence data by considering herd/hospital level prevalence.
  - To set up a database integrating data from WP1 and 2 and accessible to all partner of the project.

- Work-Package 3:
  - To characterise antimicrobial resistance mechanisms in isolates of human and animal origin, and the fitness of multidrug resistant isolates in different environment.
  - To understand the flux of antimicrobial resistance determinants and platforms, especially of those genes associated with antimicrobials that are used as human and animal therapeutics.

Description of work

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.
WP start month: 1 (first annual period)
WP end month: 36 (third annual period)
WP Leader: Bernd-Alois Tenhagen (9, BfR, DE)
Deputy WP Leader: Marianne Sunde (33, NVI, NO)
WP participants:
APHA (20): Pablo Alarcon
BfR (9): Bernd-Alois Tenhagen
NVI (33): Marianne Sunde
WbvR (30): Michael Brouwer
ANSES (1): Jean-Yves Madec
UCM (16): Bruno Gonzalez-Zorn
PHE (21): Neil Woodford
RKI (11): Tim Eckmanns
IP (19): Philipe Glaser
UoS (22): Roberto La Ragione

Description of the WP1:
WP1 will continue based upon achievements during the first and second annual period of the EJP. This WP will explore and use epidemiological data available from humans, animals (pig, poultry and cattle), food (including from retail sector), and the environment, and link data (geographically) to investigate emerging trends and possible associations between AMU and AMR, and with potential risk factors beyond usage. We will have a special focus on resistance to important antimicrobial agents such as extended spectrum cephalosporins, colistin and fluoroquinolones focusing on *E. coli*. However, as much as possible, data on other bacteria (e.g. *Salmonella* spp or *Campylobacter* spp.), will also be collected to validate results across bacterial species.
It is expected that this work package will also provide data supporting the selection of relevant farms/hospitals to be included in the longitudinal study in WP2 and samples/isolates to be included in WP3

Task 1.2: Investigation of trends, associations and risk factors
Task start month: 9 (first annual period)
Task end month: 30 (third annual period)
Task Leader: Pablo Alarcon (20, APHA)
Task Participants: BfR (9), NVI (33), APHA (20), RKI (11) and PHE (21)

Description of the task:
This task will be conducted during the first year of the EJP through to the 3rd year. It is based on the data collection in Task 1.1 but can already start by discussion on methodology issues before task 1.1 is finished.
Using the dataset obtained in task 1.1, a description of trends for specified AMR prevalence/frequency (primarily at the phenotypic level, and genotypic level where data is available, see WP3) at population/country/regional level will be carried out. Spatial analysis will be performed to assess and compare the coverage of the available data between countries, and investigate possible regional clusters of AMR and AMU in animals and humans. The spatial analysis done will allow for the identification of specific regions where available data will help perform in depth analysis. These will then be used to assess potential associations between AMR and AMU in livestock and humans in regional populations. The geographical regions identified will be used to investigate for risk factors for AMR beyond usage. This will consider ecological aspects like population density, urbanization, livestock density (or livestock to human relation), geographical features, but also livestock management practices (outdoor/indoor farms, farm size and distance between farms, level of integration, waste management, etc.). Existing datasets with potential risk factors that could be linked to the AMR/AMU data will be explored. Multilevel regression analyses will be performed to account for country and time (year) correlation.

Task 1.3: Develop recommendations for improved “One Health” surveillance strategies
Task start month 25 (third annual period)
Task end month 36 (third annual period)
Task Leader: Madeleine Norstrom (33 NVI)
Task Participants: BfR (9), NVI (33), APHA (20), RKI (11) and PHE (21)

Description of the task:
The results obtained in task 1.1 and 1.2 will be combinedwith results from WPs 2 and 3 to provide recommendations for improved “One Health” surveillance. Development of surveillance target zones maps will be performed using (and extrapolating) the results from the AMR and AMU trends observed in humans and animals, and the risk factors found associated to these. The comparison of data between countries will help to understand the existing differences and problems, which would help to formulate recommendations to improve harmonization of surveillance activities.

WP2: Longitudinal studies of persistence ESBL/AmpC/carbapenem/mcr-1/PMQR producing Enterobacteriaceae on farms or hospitals
WP2 start month 1 (first annual period)
WP2 end month 36 (third annual period)
WP Leader: Philippe Glaser (19, IP)
Deputy WP Leader: Michael Brouwer (30, WbvR)
WP participants:
BfR (9): Bernd-Alois Tenhagen
ANSES (1): Jean-Yves Madec
UCM (16): Bruno Gonzalez-Zorn
IP (19): Philipe Glaser
APHA (20): Francesca Martelli

PHE (21): Neil Woodford
UoS (22): Roberto La Ragione
WbvR (30): Michael Brouwer
NVI (33): Marianne Sunde

Description of the WP2[vii]:
WP2 will continue based upon achievements during the first and second annual period of the EJP. This work-package will study the persistence of ESBL/*AmpC*/carbapenemase/*mcr-1and -2*/PMQR producing Enterobacteriaceae at individual farm/slaughterhouse and hospital levels by collecting data on management and antibiotic usage as well as consecutive samples of faeces/meat and environmental samples through time. Furthermore, data from previous longitudinal studies that meet these criteria are present in the collection from several partners and will be included for an international comparative analysis. Retrospective and prospective collections will include samples from human, pig, poultry and cattle and related environmental isolates from all countries represented in the consortium.
Data and samples collected are expected to follow the national trends as analysed in WP1 but longitudinal samples from the same farms/hospitals and in certain cases from the same animals/patients will give a much higher resolution of the prevalence, spread, persistence and risk factors involved for resistant Enterobacteriaceae. An estimate of 100-1000 samples will be collected and analysed per project partner, taking into account variations between study designs. Prevalence of resistance over time in specific farms/hospitals will provide information on the seasonal trends, effects of age of the subjects and individual management practices such as cleaning or disinfection, in which sampling from environmental/waste water samples will be included in farm and hospital studies, where possible. Isolates from consecutive batches of animals will be collected in a selection of pig and poultry farms to investigate if the same clone/plasmid persists at the farms or if new strains are introduced with each batch/time point. In beef and dairy farms, as well as in hospitals, where no 'all in, all out' policy is present, persistence of resistant Enterobacteriaceae within the same cohort of animals/patients overtime will be investigated by longitudinal sampling of the same animals/patients.
While all samples collected will be analysed phenotypically for ESBL/*AmpC*/carbapenem/*mcr-1and -2*/PMQR producing Enterobacteriaceae, a representative number of samples will also be selected for detailed genotypic characterisation of resistance genes and associated plasmids using molecular methods and WGS, as described in WP3. For this in-depth analysis, samples will be chosen to maximise variability and to include infrequent types like CPE and *mcr-1/2* encoding isolates to address specific questions on the genetic environment of these resistance genes and on possible specificities of the corresponding lineages.

Task 2.2: Isolation of resistant Enterobacteriaceae on farms
Task start month 1 (first annual period)
Task end month 30 (Third annual period)
Task leader: Michael Brouwer (30, WbvR)
Task Participants ANSES (1), UCM (16), APHA (20), WbvR (30) and NVI (33)
Description of the task:
Task 2.2 will be performed in the first, second and third year of the EJP. Longitudinal sampling will be performed at broiler, turkey or cattle farms/slaughterhouses and will be performed by all listed participants. Depending on individual study design, each site will be visited 2 to 40 times. Data on management and antibiotic usage over the period of the study will be recorded and faecal/meat swabs/samples will be analysed for the presence of resistant Enterobacteriaceae in these environments. Phenotypic data will be recorded by established methods such as disc diffusion or broth dilution to determine the MIC of the isolates for panels of antibiotics. Methods for genotypic characterisation of the isolates, as well as plasmid composition will include genetic determination

of the resistance genes via WGS, PCR, microarray, as well as other molecular methods such as PBRT, S1-PFGE, RFLP and NGS, as described in WP3. All information related to isolates on their origin, genotype and phenotype will be stored in a common database accessible through the internet to all member of the consortium.

Task 2.3: Isolation of resistant Enterobacteriaceae in hospitals and care facilities
Task start month 1 (first annual period)
Task end month 30 (third annual period)
Task leader Philippe Glaser (19, IP)
Task Participants UCM (16), IP (19), PHE (21) and UoS (22)
Description of the task:
Task 2.3 will be performed in the first, second and third year of the EJP. Longitudinal sampling will be performed at hospitals and/or care facilities and will be performed by all listed participants for this Task. Data on patients including gender, age, disease, treatment and antibiotic treatment, as well as data on management will be recorded and faecal samples/swabs will be analysed for the presence of resistant Enterobacteriaceae. Phenotypic data will be recorded by established methods such as disc diffusion or broth dilution to determine the MIC of the isolates for panels of antibiotics. Methods for genotypic characterisation of the isolates, as well as plasmid composition will include genetic determination of the resistance genes via WGS, PCR, microarray, as well as other molecular methods such as PBRT, S1-PFGE, RFLP and NGS, as described in WP3. All information related to isolates on their origin, genotype and phenotype will be stored in a common database accessible through the internet to all member of the consortium.
Informed consent will be requested following national regulation and all data on patient will be anonymised.

Task 2.4: Data analysis of collected resistant Enterobacteriaceae on national levels
Task start month 22 (second annual period)
Task end month 34 (third annual period)
Task leader: Francesca Martelli (20, APHA)
Task Participants ANSES (1), BfR (9), RKI (11), UCM (16), IP (19), APHA (20), PHE (21), UoS (22), WbvR (30) and NVI (33)
Description of the task:
Task 2.4 will be performed over the second and third years of the EJP. The phenotypic and genotypic data on prevalence, spread and persistence of resistant Enterobacteriaceae collected in Tasks 2.1, 2.2 and 2.3 will be analysed per sample source by each of the listed participants. Analysis will be performed according to predetermined criteria as determined in D-2.1 by all contributors to WP2 to ensure compatibility of the results.
A risk factor analysis on relation between management of farms/hospitals and presence/persistence of resistance will be performed. Persistence of resistance will be compared for conditions of between batch and within batch persistence. Phenotypic data will be complemented with the genotypic characterisation performed in WP3. Trends will be analysed in prevalence of resistance genes and associated plasmids.

Task 2.5: Comparative analysis of collected isolates on a Europe-wide level
Task start month 30 (third annual period)
Task end month 36 (third annual period)
Task leader Francesca Martelli (20, APHA)
Task Participants ANSES (1), BfR (9), RKI (11), UCM (16), IP (19), APHA (20), PHE (21), UoS (22), WbvR (30) and NVI (33)
Description of the task:

Task 2.5 will be performed in the third year of the EJP. All listed participants that have provided data for the longitudinal studies will contribute to the comparative analysis of resistant Enterobacteriaceae by taking advantage of the project isolate database. This analysis will include all data collected from the retrospective and prospective studies and compare national versus European-wide results of resistant Enterobacteriaceae. It will also incorporate the WGS data generated in WP3 defining clonal groups and their respective prevalence in the farms and hospitals and to deduce correlation between specific lineage and AMR profile.

WP3: AMR characterization, transmission of plasmids and fitness of MDR isolates.
WP3 start month 6 (first annual period)
WP3end month 36 (third annual period)
WP3 Leader Bruno Gonzalez-Zorn (16, UCM)
Deputy WP3 Leader: Roberto La Ragione (22, UoS)
WP3 participants:
ANSES (1): Dr Jean-Yves Madec
UCM (16): Bruno Gonzalez-Zorn
IP (19): Philippe Glaser
APHA (20): Muna Anjum
PHE (21): Neil Woodford
UoS (22): Roberto La Ragione
WbvR (30): Michael Brouwer
NVI (33): Marianne Sunde
BfR (9): Jens-Andre Hammerl

Description of the WP3:
To understand the epidemiology of antimicrobial resistance in bacteria it is essential to understand the flux of antimicrobial resistance determinants, especially of those genes associated with antimicrobials that are used as human and animal therapeutics. This is particularly important as several different genes/alleles can encode for the same antibiotic resistance, but can be from varied sources and be associated with different mobile elements.
WP3 will continue based on achievements during the first and second annual period of the EJP. In WP3, in-depth molecular characterisation of AMR genes present in a subset of isolates collected in WP1 and WP2 will be performed using WGS to add details of the mechanism of resistance present in isolates collected national and longitudinal studies. The prevalent circulating clones and/or plasmids which may be present in data sets from different compartments will be determined from the WGS data and compared between animal, human and environmental isolates from the same region and across different regions to determine their distribution. Conjugation experiments will be performed in vitro in the laboratory under standard culture conditions; in-vitro pig and poultry gut models and in-vivo animal models to determine transmission. Fitness studies performed experimentally in the laboratory both in-vitro (in culture) and in-vivo (in mice) will help determine the likely spread and persistence of dominant clones over time, which can then be compared with the on farm/hospital longitudinal data and national surveillance data gathered from different years. Such studies will not only help improve isolate characterization, which can be incorporated into future strategies, but will ultimately help in the accurate prediction of emergence and transmission of existing and novel AMR determinants across human and animal populations. It will also help understand why certain clones may be more successful than others and why some plasmids remain largely stable in their structure while others are highly variable and constantly evolving.

<u>Task 3.3: Fitness cost of AMR and stability of plasmids in different host strain backgrounds</u>
Task start month 18 (second annual period)
Task end month 30 (third annual period)

Task Leader: Jean Yves Madec (1, ANSES)
Task Participants: ANSES (1), UCM (16), APHA (20), PHE (21), UoS (22), WbvR (30), NVI (33), IP (19), BfR (9)
Description of the task:
Fitness and survival of isolates harboring plasmids with both high and low AMR transfer rates, selected from Tasks 3.1 and 3.2, will be compared under different conditions, to determine the contribution they make in host survival and AMR dissemination. Combination of these experiments with WGS will identify the plasmid and host chromosome factors that determine plasmid stability, spread, and adaptation to new bacterial hosts and conditions. Bioinformatical analyses will provide an overview on the genome composition (i.e. genemaps, synteny plots) and the gene content of mobile genetic elements (transfer and mob-genes, resistances, stability factors). Studying plasmid stability and plasmid host adaptation in presence of subinhibitory concentrations of antibiotics / biocides, will determine the conditions that elicit emergence of High Risk clones or plasmids that ultimately spread successfully between humans, animals and the environment.

Task 3.4: Measuring AMR plasmid dissemination in mouse and Galleria, and chicken and pig in-vitro models
Task start month 24
Task end month 36
Task Leader: Muna Anjum (20, APHA)
Task Participants: UCM (16), IP (19), ANSES (1), APHA (20)
Description of the task:
At this stage, prevalent plasmids will have been identified, and characterized in vitro from Tasks 3.1, 3.2 and 3.3. With the knowledge acquired *in vitro*, the consortium will be able to assess conjugation and plasmid-host adaptation experiments in vivo. For this purpose, a chicken and/or a pig *in-vitro* gut model or *Galleria* model will be used, to identify the host factors that influence plasmid-host adaptation and conjugation rates. Furthermore, mouse conjugation models are set in place, and will be used to analyze transfer and adaptation of plasmids in the multicellular conditions of the mouse gut. WGS and analysis of the microbiome will help to identify plasmid-modifications and effects on the resident microbiota.

WP4: Project coordination and management.
WP4 start month 1 (first annual period)
WP4 end month 36 (third annual period)
WP4 Leader: Muna Anjum (20, APHA)
WP Participants: ALL
Description of the WP4
In WP4 the aim will be for the steering committee, with at least one member from each Institute, to ensure timely delivery of the Project. They will meet at least every 3 months to assess progress of the Project, whether virtually videoconference or physically, by each partner involved in the Project. Any problems or concerns that Partners or Scientists may have will be communicated to, and discussed by, the steering committee at the earliest opportunity. A kick-off and annual meetings of consortium members will be arranged in different countries. All publications and communications concerning the Project will be approved by members of the steering committee to ensure awareness and fair representation of all facts.

Task 4.1: Steering committee quarterly meeting
Task start month 1 (first annual period)
Task end month 36 (third annual period)
Task Leader: Muna Anjum (20, APHA)
Task Participants: APHA (20), BfR (9), NVI (33), WbvR (30), ANSES (1), PHE (21), RKI (11),

IP (19), UoS (22), UCM (16)

Description of the task:
To meet at least every 3 months between month 1 to month 36 of the project, to assess progress of the Project by each partner involved in the Project and discuss and address possible problems, risks, technical issues, project communication and publications.

Task 4.2: Consortium members annual meeting
Task start month 1 (first annual period)
Task end month 36 (third annual period)
Task Leader: Muna Anjum (20, APHA)
Task Participants: APHA (20), BfR (9), NVI (33), WbvR (30), ANSES (1), PHE (21), RKI (11), IP (19), UoS (22), UCM (16)

Description of the task:
This Task will comprise of four annual meetings for the consortium partners and include a kick off meeting (Month 1); two progress meetings (Months 12 and 24); and a concluding meeting of the program (Month 36). The meetings will be organized in different countries and will include representatives from all partners.

Task 4.3: Reporting and communication
Task start month 1 (first annual period)
Task end month 36 (third annual period)
Task Leader: Muna Anjum (20, APHA)
Task Participants: APHA (20), BfR (9), NVI (33), WbvR (30), ANSES (1), PHE (21), RKI (11), IP (19), UoS (22), UCM (16)

Description of the task:
A summary annual communication from each WP for distribution to relevant organisations or stakeholders such as national governments, EU, international agencies and relevant industry/health partners. It is expected that members will also publish scientific papers and other communications from the results obtained in this study.

Deliverables

Ref	Title	Due month
D-1.4	A report of the assessment of risk factors for AMR and AMU	34
D-1.5	Recommendations and target zones for improved "One Health" surveillance	36
D-2.4	Data analysis of all collected isolates on a national level.	34
D-2.5	Comparative analysis of results from the various national studies.	36
D-2.6	Comparative analysis of strains persistence in farms and hospital through longitudinal studies	30
D 3.3.	Predictive modelling of plasmid spread	30
D-3.4	Validation of plasmid threats in animal models	36
D-4.3	Annual communication to stakeholders	36

Milestones

Ref	Title	Due month
M-AMR2.ARDIG.8	Assessment of ecological and management factors associated with AMR and Antimicrobial usage (from WP1)	30
M-AMR2.ARDIG.9	Recommendation for improved One Health surveillance	36

M-AMR2.ARDIG.10	Collecting of samples and veterinary data, phenotypical testing of resistant isolates from farms and slaughterhouses.	30
M-AMR2.ARDIG.11	Collecting of samples and clinical data, phenotypical testing of resistant isolates from hospitals and care homes.	30
M-AMR2.ARDIG.12	Data analysis of all collected isolates on a national level.	34
M-AMR2.ARDIG.13	Comparative analysis of results from the various national studies (WP2).	36
M-AMR2.ARDIG.14	Identification of plasmid and host chromosome factors that determine plasmid stability, spread, and adaptation to new bacterial hosts and conditions.	36
M-AMR2.ARDIG.15	Identification the host factors that influence plasmid-host adaptation and conjugation rates using animal models	36
M-AMR2.ARDIG.16	Identification of plasmid-modifications and effects on the resident microbiota through WGS.	36

2.4.2.1.10 JRP03-R1-AMR3-RADAR

Reference to the Stategic Research Agenda (please refer to D2.7)	**AMR 3: Risk Assessment AMR**									
Research Project Title	**Risk and Disease burden of Antimicrobial Resistance**									
Research Project Acronym	**RaDAR**									
Leading Organisation	**P30-RIVM**				Deputy Leading Organisation			**P12-DTU**		
Project Leader	**Eelco Franz**				Deputy Project leader			**Tine Hald**		
Project Start month	**M1**				Project End month			**M30**		
Participant	**1**	2	4	6	7	8	**9**	10	11	**12**
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	**BfR**	FLI	RKI	**DTU**
PM	**11.50**						**15**			**2**
Participant	13	14	15	16	17	18	19	20	**21**	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	PHE
PM									**3.10**	
Participant	23	24	25	26	**27**	28	29	**30**	**31**	32
	UoS	OKI	NUIG	TEAGASC	**ISS**	IZSAM	IZSLER	**RIVM**	**WbvR**	FHI
PM					**3.75**			**19.42**	**1.14**	
Participant	**33**	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM	**4.35**									

Objectives

- Development of harmonized modelling strategies
- Systematic integration of top-down epidemiological and bottom-up risk assessment information from diverse modalities to obtain best estimates for attribution, risk factors and health effects.
- Determining the most effective strategies for controlling transmission and to mitigate risks

Description of work

<u>WP0: Coordination and communication</u>

WP1 takes place over the first and the second annual period of the EJP
WP start month 01

WP end month 30
WP Leader **31-RIVM**
Deputy WP Leader **12-DTU**
WP participants ALL
Description of the WP:
This WP will This task takes responsibility for and effective project coordination. This will be facilitated by conducting conference calls with WP leaders every 2 months. We will organize 3 consortium meetings (a M1 kick-off meeting, a M12 mid-term meeting and a M24 final meeting) to promote synchronized decision making and development within the project as well as exchange of knowledge. This WP also makes keeps track of timely provisioning of partner's deliverables. Further it will manage the creation of yearly project reports.

Task 0.1: Coordination and project management

Task start month 01
Task end month 024
Task Leader: 31-RIVM
Deputy Task Leader: 12-DTU
Task Participants: ALL
Description of the task:
A Project Management Team will be formed including the Coordinator, the deputy coordinator, and the WP leaders. Throughout the project, the group will have regular meetings (quarterly or more often, if needed) to communicate across WPs and ensure that the work flow within and between WP proceed as expected. This task will monitor the progress of the project (i.e. milestones and deliverables) as a whole by organizing regular teleconferences / Webex-meetings.

Task 0.2: Consortium meetings

Task start month 01
Task end month 30
Task Leader: 31-RIVM
Deputy Task Leader: 12-DTU
Task Participants: ALL
Description of the task: This tasks will organize 3 consortium meetings aimed at establishing corporation, common direction, and critical issues. These meetings will take place at M 1, 12 and 24. Minutes of meeting reports will be produced for all these 3 meetings.

Sub-Task 0.2.1: Final meeting

Sub-Task start month: 30
Sub-Task end month: 30
Sub-Task Leader: 31-RIVM
Deputy Sub-Task Leader: 12-DTU
Sub-Task Participants: ALL

Task 0.3: Annual reports

Task start month 01
Task end month 30
Task Leader: 31-RIVM
Deputy Task Leader: 12-DTU
Task Participants: ALL
Description of the task: This tasks will produce two annual reports (for each annual period one report) summarizing the project progress as a whole. ,

Sub-Task 0.3.2: Third annual report

Sub-Task start month: 28
Sub-Task end month: 30
Sub-Task Leader: 31-RIVM
Deputy Sub-Task Leader: 12-DTU

Sub-Task Participants: ALL

WP1: New genomic information to feed AMR transmission models

WP1 takes place over the first and the second annual period of the EJP
WP start month 1
WP end month 30
WP Leader **Michel-Yves Mistou (Anses)**
Deputy WP Leader **Camilla Sekse (NVI)**
WP participants **Anses, NVI, DTU, NCOH, ISS**

Description of the WP: Theoretically, access to the whole genomic information makes it possible to identify genetic mechanism of AMR and predict the resistance phenotype of isolates without culturing. However, the move from theory to practice requires additional biological knowledge. WP1 is about the development of new bioinformatics tool to retrieve information from genomic data relevant for modelling AMR risk and transmission. It focuses on plasmid content and mutations associated with AMR and on the impact they have on the phenotype. Genomic data in combination with phenotypic information will be linked with epidemiological data and integrated into appropriate transmission models to have full impact on public health. WP1 will collaborate closely with WP6 where new ways to combine information different by nature are developed. Extensive exchange of data with the modelling tasks of WP3 and WP5 will be promoted. In this WP, Anses will develop bioinformatics tools to analyse (meta-) genomic data and retrieve information relevant for AMR and its transmission. The other partners will be involved in biological interpretation of data and integration of the new information into transmission models.

Task 1.1 Build collections of high throughput sequencing (HTS) data needed for bioinformatics developments and knowledge advancement
Task start month: 1
Task end month: 18
Task Leader **Pierre-Emmanuel Douarre (Anses)**
Deputy Task Leader: **Camilla Sekse (NVI)**
Task Participants: Anses, DTU, NVI
Sub-Task 1.1.1: WP1 addresses questions related to the exploitation of genomic data with a particular attention to plasmids. For benchmarking the new assembly pipeline (**T1.2**) two kind of data will be used: First, all complete plasmids and associated metadata available in public databases will be retrieved and curated to create a comprehensive plasmid database. The analysis of this database will gave us a global overview of plasmid diversity and classification and will provide information on plasmid host range and transmission routes. The manuscript summarizing these results is currently being prepared and should be submitted for publication.
This database will be used for further bioinformatics developments (Task 1.2) and new environmental datasets characterization (Subtask 1.1.2). In a second step experimental raw reads from well-studied bacterial species with reference genomes and annotated plasmids will be gathered for pipeline validation.

Sub-Task 1.1.2: Field HTS data relevant to various AMR challenges will be collected and organised in subprojects on pilot species (e.g. *Salmonella*, *E. coli*), antimicrobials (e.g. cephems, carbapenems, colistin and fluoroquinolones) and origins (human, animal-food sectors, environment).
Examples of available WGS datasets:

- Salmonella surveillance network (2000-2016) from all food, animal and environmental sectors, a particular focus will be on colistin resistance and on the plasmidome in relation with certain serovar (*S*. infantis). (Salmonella network Anses Maisons-Alfort/Ploufragan)
- QREC (quinolone resistant *E. coli*) from animals (poultry, pigs, cattle) in Norway
- Cephalosporin resistant *E.coli* (containing blaCMY-2) from poultry in Norway

Sub-Task 1.1.3: The pig faecal metagenomics data collection obtained in the frame of the EFFORT H2020 project (https://academic.oup.com/jac/article/74/4/865/5289505 ; ENA: PRJEB22062) and the metagenomics dataset of urban sewages under the H2020 COMPARE project (doi: 10.1038/s41467-019-08853-3.; ENA: ERP015409) are metagenomics dataset relevant to the AMR dissemination problem have been made publicly available recently. They will be screened against the plasmid database.

The curated collection of HTS data associated to AMR metadata will be organised in a dedicated DB accessible to partners. The DB data will be used in **T1.2**, **T1.3 and T1.4** for testing and validating the methods.

Task 1.2 Develop an innovative automated bioinformatic pipeline integrating *de novo* plasmid reconstruction and identification

Task start month: 1
Task end month: 24
Task Leader: **Nicolas Radomski (Anses)**
Deputy Task Leader: **Arnaud Felten (Anses)**
Task Participants: Anses, NVI

Sub-Task 1.2.1:
Plasmid assembly is particularly challenging from short read sequencing reads due to the presence of numerous repetitive elements. An automated pipeline will be developed by integrating plasmid assembly programs, annotation tools and reference-based identification. PlasmidSPAdes will perform plasmid *de novo* assembly based from the coverage and the assembly graph. All the reconstructed plasmids will be annotated using Prokka and resistance genes searched against the Resfinder database. Finally, our comprehensive database will be integrated into the MOB-suite program for plasmid characterization. The outputs of the pipeline will be designed for handling by microbiologists and modellers to facilitate biological interpretation (**T1.3**) and integration into transmission models (**WP6**).
***Sub-Task 1.2.2*:** The pipeline will be tested and parameterised for analysis of genomics data.

Task 1.3 Plasmidome: Biological annotation and risk assessment

Task start month: 12
Task end month: 30
Task Leader: **Pierre-Emmanuel Douarre (Anses)**
Deputy Task Leader: **C. Sekse (NVI)**
Task Participants: Anses, NVI, DTU, NCOH, ISS

Sub-Task 1.3.1: HTS data gathered from **T1.1** will be run through the *in silico* validated pipeline (**T1.2)**. Outputs will be examined and curated by microbiological experts and phenotypic characteristics such as MIC values will be used for verifying the genomic findings. The knowledge will be used to predict risk factors associated to different plasmids/plasmid genes /AMR profiles and to inform the exposure assessment modelling of (**WP3 T3.3**), as well as the evidence-based synthesis attribution in **WP5**.

Task 1-4 Methods to identify genetic traits associated to AMR

Task start month: 12
Task end month: 30
Task Leader: : **Pierre-Emmanuel Douarre (Anses)**
Deputy Task Leader: **C. Sekse (NVI)**
Task Participants: Anses, NVI, DTU, NCOH, ISS

Sub-Task 1.4.1: Whole-genome association studies (WGAS to identify SNP's and Indels statistically associated to AMR traits whether plasmid-borne or not) will be performed on sets of HTS data issued of epidemiologically comparable population (with tools like Clonal Frame and linear mixed modelling).

Sub-Task 1.4.2: A second approach will be through regression model that will focus on the presence/absence of gene belonging to the accessory genomes (chromosomal and plasmid-borne, ***Sub-Task* 1.2.2**). Variations in traits important for survival (e.g. secretome, heat tolerance) and infection (virulence factors) are considered useful in WGS-based risk assessment models, where the "conventional" predictive modelling is not applicable. Genome wide association tests will be performed using a recently described statistical tool for determining the SNPs or kmer that influence bacterial phenotypes of interest (AMR resistance). Population structure and genome-wide linkage disequilibrium will be controlled with linear mixed model. The Bonferroni method will be implemented to consider multiple testing and Manhattan plots will be used to summarize results.

T1.4.3 The results of the analysis performed in ***Sub-Tasks 1.3.1***, ***1.4.1*** and ***T1.4.2*** will be used to predict risk factors associated to different genetic markers linked to plasmids and/or AMR profile and to inform the exposure assessment modelling of WP3.3, as well as the evidence-based synthesis attribution in WP5.

WP2: Pharmacodynamics and transmission models

WP start month: 01
WP end month: 24
WP Leader **Robin Simons (APHA)**
Deputy WP Leader **Martin Bootsma (NCOH)**
WP participants **ANSES, BfR43, DTU, ISS, NCOH, RIVM**
Description of the WP:

Resistance clone and/or gene dissemination (spread) is a multi-factorial phenomenon influenced by a variety of processes, with antimicrobial usage (AMU) in livestock production being a driving factor for antimicrobial resistance selection. The relative importance and dependence of these processes on e.g. production practices, national policies and consumption patterns is unknown. Additionally, transmission between distinct animal/human populations, underpinned by these processes, remains poorly quantified. All of this hinders effective control, with WP2 responding to this call to action.

Task 2.1: On-farm transmission models
Task start month: 01
Task end month: 30
Task Leader: Robin Simons
Deputy Task Leader: Pascal Sanders
Task Participants: APHA, ANSES
Description of the task: Using selected case studies and existing data from primarily pigs, we will develop models to explore the association between AMU and AMR at the herd level, assess the relative importance of AMU and clonal dissemination for resistance occurrence and simulate the within-farm transmission dynamics of AMR. Previous research has applied pharmacokinetic/pharmacodynamic modelling (PK/PD) – the relationship between drug concentration and effect – to the veterinary field to predict drug resistance and drug optimisation (dosing regimen, green antibiotics, etc…) as well as in vitro activity of antibiotics against pathogens. We will explore the use of this methodology here. Few PK/PD models describe the drug distribution in the body, in the gut and in excreta for pigs or poultry. The potency of the drug against veterinary pathogens and commensals could be described, but a fit between data and prediction requires some experimental results of good quality to set the parameter values and then an approval of datasets used for modelling will be necessary. Coupled with a population pharmacokinetic approach, PK/PD models can be used to estimate the range of effects for different levels of susceptibility of bacteria and so define the antimicrobial resistance selective window and compartment. Few theoretical models simulate the adaptation process in bacterial populations leading to resistance. During the project, a set of models will be reviewed and applied to available data sets produced by other WPs (after their release) and/or previous data obtained via avenues such as a literature search of peer reviewed publications. The analysis will be performed to identify the data gaps for the parameter value settings. We will then develop a strategy to implement future experimental studies able to fill the gaps (1. methodology for acquisition of the data and release of data sets intended for modelling) and derive a generic modelling approach to cover different scenarios (2. Harmonized methodology for PK/PD modelling).
We will also develop an on-farm model for transmission of AMR bacteria in the pig livestock sector and parameterise it for at least one type of AMR bacteria (e.g. ESBL *E. coli*). While a number of transmission models have previously been developed for bacteria such as *Salmonella*, there has been little development regarding AMR transmission and AMU usage, a gap we propose to address with this model. The model will also be designed to be able to assess the effectiveness of control measures with regards to reducing the prevalence and/or level of AMR bacteria in slaughter age pigs.

Sub-Task 2.1.1: PK/PD model to assess relationship between animal exposure and change in antimicrobial resistance
Sub-Task start month: 01
Sub-Task end month: 26
Sub-Task Leader: Jerome Henri
Deputy Sub-Task Leader:
Sub-Task Participants: ANSES, APHA
There are both experimental and clinical evidence to support that dosing schedule may influence resistance development and that dosing regimen may be optimized by proper use of PK/PD. The investigations made by Eagle *et. al.* were the first demonstration that minimum inhibitory concentration (MIC) is not enough to describe bacterial activity and that the pharmacokinetics of the antibiotics need to be taken into account to have a better understanding of the antibiotics activity and to define precise targets. Vogelman et al. and Craig investigated the PK/PD relationship of antibiotics and defined three PK/PD indices based on a measure of the drug exposure and the

MIC of the bacteria. The terminology of these PK/PD indices have been standardized into fAUC/MIC, fCmax/MIC and fT>MIC. PK/PD indices are summary endpoints based on the MIC, which do not provide any information on the time course of antimicrobial activity. Therefore a lot of information is lost in the process of generating these endpoints, such as the regrowth of bacteria that can be observed after an initial decay although the antibiotic concentration is kept constant, and suggesting a changing effect with time. These complexities can be analyzed by a modelling approach that allows description of the full time course of antimicrobial activity. A review of these PK/PD structural models describing the time course of resistance levels in the gut/excreta of the host (animal) will be necessary during the project to develop a generic PK/PD model. This PK/PD model will be developed according to a population approach (nonlinear mixed effect model) in order to associate the inter-animal variability to the structural model and then to get PK/PD relationship for each host (individual values) of the entire herd (population values). The identifiability of the model's parameters will be assessed according to collected datasets after their qualification. The model will allow testing of methodologies for (a) acquisition and qualification of data intended for PK/PD modelling purposes and (b) implementation, calibration and validation a generic PK/PD model of AMR in a herd for a couple AMD/bacteria.

Sub-Task 2.1.2: Assess relative importance of AMU and clonal dissemination for resistance occurrence

Sub-Task start month: 01
Sub-Task end month: 26
Sub-Task Leader: Michel Laurentie
Deputy Sub-Task Leader:
Sub-Task Participants: ANSES, APHA

Once the variability is defined at the host level in the trio AMD/bacteria/host, simulations will be made in order to integrate variability factors relating to (i) the AMD and (ii) the bacteria.

(i) AMU's variability will be studied according to "Green AMDs", a concept recently developed by Toutain et al. and then simulations of the population PK/PD model (with the same structure) will be realized for the same AMDs but with different pharmacokinetic properties (different parameters' mean values and standard deviations) due to changes of formulations (long-acting formulations, i.e. a short elimination half-life with a limited volume of distribution and an appropriate plasma clearance AND eco-friendly properties, i.e. a clearance by hepatic metabolism is ideal, provided the metabolites are inactive but a high renal clearance is acceptable provided the eliminated active AMD is rapidly degraded in the environment or immobilized by physical sorption) in order "to make them greener" and to see the positive impact on AMR levels in the gut of the animals.

(ii) Clonal dissemination depends on resistance mechanism. In this part we will study if the variability of these processes can be simulated in changing values of the PD parameters of the PK/PD model or if structural adaptations of the generic model are necessary depending on the studied bacterial species.

This will allow us to identify data gaps in order to experimentally check the simulations and modelling hypotheses done in this sub-task. The connection between the PK/PD model and the transmission model will also be elaborated in collaboration with sub-task 2.1.3 participants

Sub-Task 2.1.3: Development of on-farm transmission model

Sub-Task start month: 01
Sub-Task end month: 30
Sub-Task Leader: Robin Simons
Deputy Sub-Task Leader:
Sub-Task Participants: APHA, ANSES

The on-farm model will use Monte-Carlo simulation techniques to allow incorporation of variability about model parameters and follow individual pigs on a farm over a period of time (e.g. one year); both with regards to susceptible and resistant bacteria status, using a modified Susceptible-Infected-Recovered (SIR) approach, and pig production factors such as moving pens while transitioning between production stages (i.e. from grower to weaner to finisher). The model will consider the risk posed from environmental contamination (e.g. from ingestion of faeces in the pig pen) and incorporate the effect of farm management factors such as cleaning of pens between batches. AMU on farm will be a primary consideration; The model will simulate when pigs are given antibiotics and the incorporation of equations to model the pharmacodynamics will enable simulating the effect of antimicrobials on the numbers of resistant gut bacteria. It is anticipated that the PK/PD models developed in this task (sub tasks 2.1.1 & 2.1.2) will inform this and we will investigate the feasibility of directly integrating them into the on-farm model. The model will simulate the levels of resistant and susceptible bacteria and antimicrobials consumed by the pigs and the resulting levels present in the faeces of infected pigs. The model will be designed to allow for relatively simple re-parameterisation for other (resistant and susceptible) bacteria in the future.

Sub-Task 2.1.4: Scenario analysis to assess hypothetical on-farm intervention measures.

Sub-Task start month: 06
Sub-Task end month: 30
Sub-Task Leader: Robin Simons
Deputy Sub-Task Leader:
Sub-Task Participants: APHA, ANSES

The on-farm model will be designed to help assess the impact of on-farm interventions in controlling prevalence and spread of AMR bacteria. Discussion with policy colleagues in the first year of the project will identify potential intervention measures that can be incorporated into the on-farm model. Potential control measures to consider include reducing antimicrobial usage. It is anticipated that in the future data will be available to paramaeterise the model for specific interventions, e.g. from the AMR-2 EJP project, but are unlikely to be available during the timeframe of this task. As such, here we will conduct scenario analyses to assess the effect of hypothetical interventions to demonstrate the proof of principle for when real world data are available and inform as to the level of effectiveness necessary for an intervention to have a meaningful impact. The scenario analyses will also be used to investigate the impact of uncertainty about the model parameters on the results.

Sub-Task 2.1.5: Communication of results

Sub-Task start month: 18
Sub-Task end month: 30
Sub-Task Leader: Robin Simons
Deputy Sub-Task Leader: Pascal Sanders
Sub-Task Participants: APHA, ANSES

In addition to being reported inside the project, results will be communicated through appropriate standard scientific outlets (e.g. peer-reviewed publications, scientific meetings).

Task 2.2: Models for transmission between livestock and human populations

Task start month: 01
Task end month: 30
Task Leader: Martin Bootsma (NCOH)
Task Participants: BfR43, CVI, NCOH, RIVM

T2.2 takes place over the first and the second annual period of the EJP. Its aim is to understand better large-scale transmission between populations, assess infection risk and set the stage for designing intervention measures and assessing them in silico. This modelling study will first use a massive sample library available within the consortium and counting 30,000 samples from human and animal populations and the environment. The study will be adapted using project- generated

data as these become available. Models developed in T2.1 and expert knowledge from WP3 will play significant roles in intervention assessment.

Sub-Task 2.2.1: Development of mathematical models for source-attribution

Sub-Task start month: 01
Sub-Task end month: 28
Sub-Task Leader: NCOH
Sub-Task Participants: CVI, NCOH, RIVM

T2.2.1 takes place over the first and the second annual period of the EJP. Its scope is to model transmission of resistance mechanistically and, in this manner, understand the relative importance of human, animal and environmental transmission routes. The focal point will be ESBL's, because of relevance and genetic data availability, but the methods developed here will be more widely applied.

Prior dynamical models left these routes underexplored by focusing on within-host AMR dynamics. Recent statistical studies, on the other hand, clustered human and animal populations into epidemiological units using the relative prevalence of AMR-encoding plasmids. Here, we will use these analyses to fit data to several transmission models – compartmental models (CM), next generation matrices (NGM), system dynamics (SD) - and to assess goodness of fit. This will allow estimation of AMR dissemination levels between human/animal reservoirs, food and the environment, thus directing efficient intervention design in T2.2.2. It will also pinpoint important transmission routes insufficiently resolved due to data gaps, thus aiding the design of further data collection.

Two significant data sources will feed into this task: longitudinal data on AMR prevalence in different reservoirs and strain data from them. We will explore and integrate multiple modeling approaches due to problem complexity, starting with a limited number of reservoirs afforded high-quality data (e.g., hospitals and extramural community, as well as farm animals and farmers) and gradually incorporating more reservoirs, in one overall model, as we test and refine our methodology.

Sub-Task 2.2.2: Assessment of intervention measures

Sub-Task start month: 13
Sub-Task end month: 28
Sub-Task Leader: RIVM
Sub-Task Participants: BfR43, CVI, NCOH, RIVM

T2.2.2 will be underpinned by model building from T2.1.3 - T2.2.1 and expert input from WP3. It will focus on investigating systematically how strain prevalence relates to transmission, so as to quantify changes in prevalence brought about by transmission-attenuating measures. An additional point of interest will be the (lack of) robustness of the transmission network to disruptive attacks, so that high-impact interventions can be designed.

Interventions targeting single transmission routes will impact all reservoir prevalences nonlinearly, necessitating a systematized approach. A first step will be a univariate sensitivity analysis quantifying relative importance of AMR transmission pathways. We will follow up by computing the basic reproduction number R0 between reservoirs under various intervention scenarios, to reveal intervention opportunities within the current operational model. This part of the work will employ simplified model representations using the next generation matrix (NGM) methodology (validated against the full dynamical model). Combining interventions should substantially reduce the effective reproduction number and significantly limit transfer of resistance traits between key reservoirs. Input from T2.1 and WP3 will be critical in obtaining realistic estimates for the magnitude of interventions. An analysis of the transmission network characteristics will provide additional guidance, utilizing recent advances on understanding robustness/vulnerability of various types of dynamic networks.

Sub-Task 2.2.3: Communication of results

Sub-Task start month: 18

Sub-Task end month: 30
Sub-Task Leader: Martin Bootsma (NCOH)
Sub-Task Participants: BfR43, CVI, NCOH, RIVM
This work will form part of a Ph.D. project within NCOH and will thus extend past the two-year project duration. In addition to reporting in the project, we will communicate results through the resulting Ph.D. thesis and standard scientific outlets (peer-reviewed publications, scientific meetings).

WP3: Transmission through the food chain
WP start month: 1
WP end month: 30
WP Leader: BfR43
Deputy WP Leader: CVI
WP participants: BfR43, CVI, DTU, NVI, RIVM
Description of the WP:
This workpackage will aim on reviewing available methods to model the change of prevalence and concentration of bacteria with phenotypic resistance or specific resistance determinants along the food chain for exposure assessments. This will increase accessibility and exchangeability of these models. Building on that and in collaboration with WP2, generic models will be developed and implemented where different exposure pathways as well as different food chains can be evaluated and compared with respect to risk. These models will be translated into a standardised format according to the recently (at BfR) developed/proposed Food Safety Knowledge (FSK) Framework which aims at facilitating access and exchange of models. This should support rapid assessments when new patterns arise or specific measures needs to be assessed.
The work will contribute to progress beyond the present state of art and enable rapid exchange of models and information between researchers, countries and public & animal health institutes in the future.
Hence, the objectives of the WP are:

- To create an inventory of available exposure assessment models and to translate them into a standardised format according to the Food Safety Knowledge Framework
- To establish generic exposure assessment models for the chicken, pork and mussels production chain
- To establish a generic exposure assessment model based on WCS/metagenomics data

This WP is closely linked with WP2, where specific on farm models are developed and with WP6, where overarching analysis will take place. The close linkage with these two WPs is guaranteed in staffing, since the team of deputy WP 3 leader is also actively involved in WP2, and the team of the WP3 leader is actively involved in WP6.
BfR43 will lead WP3 and task 3.1, focusing on an inventory of models following FSK Standard and contribute to task 3.2. by developing a generic exposure assessment model for the chicken production chain.
CVI will assist leading WP3, and contribute to interaction and integration of results which link WP2 and WP3. As such, they will participate as consultant/advisor in each subtask.
RIVM will lead task 3.2 and contribute to task 3.2 by improving the exposure assessment model for the pork production chain (subtask 3.2.2).
NVI will lead task 3.2.3 and contribute to this task by developing a generic model for the mussels production chain.
DTU will lead task 3.3 and contribute to tasks 3.1, 3.2.1 and 3.2.2.
Task 3.1: *Inventory of available exposure assessment models and related data and transfer to FSK Standard*
Task start month: 01

Task end month: 30
Task Leader: BfR43
Task Participants: BfR43, CVI, DTU, NVI, RIVM
Description of the task: An inventory will be made on the models/ elements for exposure assessment which are available among partners. Details on bacterial species, phenotypic and resistance traits, animal species and production stages, processing steps, transmission pathways (foodborne, direct contact), spread (horizontal vs. vertical) as well as risk factors (AMU, biosecurity, trade, other factors) considered will be collected and summarised. Relevant data on regional/ local patterns for food processing and consumption habits linked to or relevant for the models will be collected.

Sub-Task 3.1.2: Transfer of available exposure assessment models developed in R (or matlab) to FSK Standard for at least one type of AMR bacteria and at least one animal (chicken, pig or mussels)
Sub-Task start month: 10
Sub-Task end month: 30
Sub-Task Leader: BfR43
Sub-Task Participants: BfR43, CVI, DTU, NVI, RIVM

Task 3.2: Exposure assessment models for different production chains
Task start month: 01
Task end month: 30
Task Leader: RIVM
Task Participants: BfR43, CVI, DTU, NVI, RIVM
Description of the task:
Exposure assessment calculations for selected AMR determinants (e.g. ESBL *E. coli*, Colistin resistance gene *mcr-1*) will be performed for broiler, pig and mussels. Task 3.1 will provide an inventory of available models. Based on this, possibly further improvement of the available farm-to-fork models will be necessary for the calculations in Task 3.2. A close link to WP2 where transmission models within farms will be addressed is envisaged. It is expected that the main part of the model parameters will be micro-organism-aspecific and reusable for specific AMR (e.g. ESBL *E. coli*) calculations. As for the AMR specific (e.g. ESBL *E. coli)* parameters, the main challenge will be to obtain data the concentrations along the food chain. For parameter values in general, data made available by partners or collected in collaborating consortia (e.g. FBZ-2, FBZ-3, FBZ-4, AMR-2) will be useful. Possibly assumptions, expert estimates or scenario studies will have to fill remaining data gaps. Given the large variation in farming systems, slaughter processes and consumption habits across the EU, it will be necessary to select one or a limited number of countries or cluster(s) of similar countries to come to a realistic amount of work. For comparative exposure assessment used for attribution (e.g. between food animals, farming systems, specific food products within food animals, etc.) relatively simple exposure models can be applied. As a next step, the ambition is to create the ability to calculate the impact of intervention measures, but possibly scenarios studies will prove to be the highest achievable.

Sub-Task 3.2.1: Exposure assessment model for the chicken production chain
Sub-Task start month: 13
Sub-Task end month: 30
Sub-Task Leader: BfR43
Sub-Task Participants: BfR43, DTU
BfR-43 will improve a model for the chicken production chain with a main focus on the spread of newly emerging resistance determinants, e.g. mcr-1 and/or specific plasmidic ESBL-genes and impact of intervention at different processing steps. Implementation will following the FSK-standard and will aim to develop a generic model. In this workpackage we will work close together

with WP2 which focuses on farm transmission. Variability about model parameters will be integrated. Where real-world data are missing, different scenarios will be assessed to feed in future research needs and decisions towards preliminary risk management decisions.

Sub-Task 3.2.2: Exposure assessment model for the pork production chain
Sub-Task start month: 13
Sub-Task end month: 30
Sub-Task Leader: RIVM
Sub-Task Participants: BfR43, CVI, DTU, RIVM
Work conducted in this workpackage will built on previous work related to modelling Salmonella in the farm-to-fork pork chain. These approaches will be modified to allow assessment of exposure by emerging resistance determinants and the impact of different processing steps as well as intervention measures. In this workpackage we will work close together with WP2 which focuses on farm transmission.
Sub-Task 3.2.3: Exposure assessment model for the mussel production chain
Sub-Task start month: 01
Sub-Task end month: 30
Sub-Task Leader: NVI
Sub-Task Participants: BfR43, NVI,
This workpackage will first assess different cooking procedures of mussels and their effect on the load on emerging resistance determinants of the indicator bacteria *E.coli*. An exposure assessment model will be developed which will thereby use data regarding resistance frequencies from surveillance of mussels and data from the experiments to assess the probability of consumption of emerging resistance determinants from mussels.

Task 3.3: *Generic comparative exposure assessment model*
Task start month: 13
Task end month: 30
Task Leader: DTU
Task Participants: BfR43, CVI, DTU, NVI, RIVM
Description of the task:
In interaction with WP2 we will determine the potential for deriving or creating generic methods for exposure assessments for different production types, different bacteria, plasmids, and genes. These generic methods may be more crude, but will allow for combining the risks in the different (sub)- categories, which is otherwise never totally acceptable and correct. Thus we expect them to create a more complete picture of the AMR problems throughout.

WP4: Machine learning methods for quantification of risk and health effects
WP start month 01
WP end month 30
WP Leader **Thomas Selhorst (BfR33)**
Deputy WP Leader **Madelaine Norström (NVI)**
WP participants **NVI, RIVM**
The development of antibiotic resistance is a complex process influenced by a multitude of risk factors, and this process is still not understood in all parts. It is undoubted that the occurrence of multiple resistance patterns is a key feature of antibiotic resistance and the aim of risk factor analysis should be the disclosure of resistance patterns and potential risk factors. Perhaps, we might not have understood the process of antimicrobial resistance, because we have not used the appropriate statistical methods for the analysis of our observation.
In order to contribute to the understanding of antimicrobial resistance, a repository of state of the art statistical methods collectively referred to as ‘machine learning’ methods will be compiled. The

aim of this WP is the systematic identification and recording of the known methods and their comparison. In particular, a comparison with the classical methods (e.g. logistic regression) of data analysis is employed. The logistic regression model will be taken as the baseline comparator, because it is the de facto standard model for the analysis of relations of a binary (and also multinomial) outcomes and observed influential factors. Its popularity is based on its availability in statistical software programs and its adaptability to specific tasks (Dasgupta et al.; 2014). But, good predictions and effect size estimation from a logistic model requires to guess the true data generating model, i.e. guessing the predictors (risk factors) and their interactions. If the model is mis-specified, both predictions and effect size estimates may be more than slightly in error (Dasgupta et al.; 2014).
All documentation and program code will be produced with the help of R and especially knitR with is used today for the creation of reproducible scientific documents. Additionally, we will use github for versioning.
WP is subdivided into 4 tasks:
Task4.2 Methods for testing model -validity, -sensitivity and -robustness
Task start 13
Task end 27
Task Leader BfR 33, Thomas Selhorst:
Deputy Task Leader NN: Madelaine Norström (NVI)
Task Participants: **NVI, RIVM**
Description of the task: in this task we will analyze model validity, model sensitivity, model robustness, the influence of test and training data selection, and the influence of correlated, biased, blurred, missing influential variables on model predictions. Comparison to the baseline comparator will be performed. This task can be performed only in the presence of data. A preliminary investigation has revealed that records on antimicrobial resistance were freely available in the past (for example Ludwig et al, 2013), and it can be expected that the situation has and will improve in the future. The use of these records ensures the implementation of Task 4.2. Nevertheless, WP 4 will be happy to use records that are generated as part of AMR-3 or have been generated by the project partners in the past.
Task 4.2 is subdivided in 3 subtasks:
Sub-Tasks T4.2.1: Selection of test data set(s) to be used (at least one taken from the internet will be provided) includes literature review and description of data sets provided by partners of project AMR-3. A list of possible datasources will be compiled.

Sub-Task start month: 13
Sub-Task end month: 20
Sub-Task Leader: BfR 33, Thomas Selhorst:
Deputy Sub-Task Leader: Madelaine Norström (NVI)
Sub-Task Participants: **NVI, RIVM**
Sub-Tasks T4.2.2: will define a work bench for assessing model validity, model sensitivity, model robustness, the influence of test and training data selection, and the influence of correlated, biased, blurred, missing influential variables on model predictions.

Sub-Task start month: 15
Sub-Task end month: 23
Sub-Task Leader: BfR 33, Thomas Selhorst:
Deputy Sub-Task Leader: Madeleine Norström (NVI)
Sub-Task Participants: **NVI, RIVM**
Sub-Tasks T4.2.3: will conduct analysis on model validity, model sensitivity, model robustness, the influence of test and training data selection, and the influence of correlated, biased, blurred,

missing influential variables on model predictions using example data sets. A comparison with the baseline comparator is conducted.

Sub-Task start month: 18
Sub-Task end month: 27
Sub-Task Leader: BfR 33, Thomas Selhorst:
Deputy Sub-Task Leader: Madelaine Norström (NVI)
Sub-Task Participants: **NVI, RIVM**

Task4.3 Methods for the quantification of effect sizes and calculation of population attributable fractions (PAFs)
Task start 22
Task end 33
Task Leader BfR 33, Thomas Selhorst:
Deputy Task Leader NN: Madelaine Norström (NVI)
Task Participants: **NVI, RIVM**
Description of the task: we will analyze and develop methods for the quantification of effect sizes. This is an important issue because ML methods do not necessarily provide estimates for the effect size. But, quantified effect sizes are essential for the calculation of risks and PAFs (population attributable fractions) and are related to burden of disease calculations. Thus quantified effect sizes are necessary condition for further calculations. The baseline comparator, i.e. logistic regression models, naturally provide quantified effect sizes and alternative methods are compared with the baseline comparator.

Task 4.3 is sub-divided into three subtasks.
Sub-Task 4.3.1 Literature review of methodologies and compilation of the selected methods
Sub-Task start month 22
Sub-Task end month 30
Sub-Task Leader: BfR 33, Thomas Selhorst:
Deputy Sub-Task Leader: Madelaine Norström (NVI)
Sub-Task Participants: NVI, RIVM
Sub-Task 4.3.2 Programming of the selected methods, inclusion in the collection of methods and creation of methods Description.
Sub-Task start month 24
Sub-Task end month 33
Sub-Task Leader: BfR 33, Thomas Selhorst:
Deputy Sub-Task Leader:
Sub-Task Participants: NVI, RIVM
Sub-Task 4.3.3 Application of selected methods to the available data, comparison of the results including comparison with baseline comparator and creating recommendations.
Sub-Task start month 26
Sub-Task end month 33
Sub-Task Leader: BfR 33, Thomas Selhorst:
Deputy Sub-Task Leader: Madeleine Norstrom (NVI)
Sub-Task Participants: NVI, RIVM

Task4.4: ML and causality. Does it fit together?
Task start 28
Task end 36
Task Leader BfR 33, Thomas Selhorst:
Deputy Task Leader NN: Madelaine Norström (NVI)

Task Participants: NVI, RIVM
Description of the task: Causality is a fundamental prerequisite for the calculation of source attribution, risk factor profiling, health impact assessment of antimicrobial resistance, and finally for decision making, and control. But, often it is assumed that machine learning and causality preclude each other.
Despite this assumption, advances were made in machine learning for tackling problems in causality (e.g. distinguish cause from effects, infer the effect of interventions). Modern machine learning methodologies provided efficient methods for causal structure learning and powerful tools for conditional independence test, which is a key component in traditional constraint-based causal discovery. This task will review the literature, setup a repository of methods and finally apply these methods to available data sets.
Sub-Task 4.4.1 Literature review of methodologies and setup of a repository for the selected methods
Sub-Task start month 28
Sub-Task end month 36
Sub-Task Leader: BfR 33, Thomas Selhorst:
Deputy Sub-Task Leader: Madelaine Norström (NVI)
Sub-Task Participants: NVI, RIVM
Sub-Task 4.4.2 Implementation of methods in R, including a description and a sample application.
Sub-Task start month 30
Sub-Task end month 36
Sub-Task Leader: BfR 33, Thomas Selhorst:
Deputy Sub-Task Leader: Madelaine Norström (NVI)
Sub-Task Participants: NVI, RIVM
Sub-Task 4.3.3 Application of selected methods to the available data, comparison of the results including comparison with baseline comparator and creating recommendations.
Sub-Task start month 33
Sub-Task end month 36
Sub-Task Leader: BfR 33, Thomas Selhorst:
Deputy Sub-Task Leader: Madelaine Norström (NVI)
Sub-Task Participants: NVI, RIVM

WP5: The burden of disease caused by AMR exposure
WP start month 1
WP end month 30
WP Leader Tine Hald (DTU)
Deputy WP Leader Marie-Josee Mangen (RIVM)
WP participants DTU, RIVM
Description of the WP: In December 2015, WHO published the first estimates of the global burden of foodborne diseases including 12 bacterial infections (1,2). These estimates, however, did not address AMR, and the overall contribution of AMR to the human disease burden and specifically the proportion attributable to livestock and food remains unknown. The overall objective of this WP is to develop source attribution models for AMR based on both geno- and phenotypic data, and to propose a new paradigm for surveillance of AMR. Attribution estimates from this WP will be compared with estimates of exposure to AMR through different transmission routes (as estimated by other WPs in this project and culminating in WP6) to estimate the incidence of each health outcome in the population, as well as the burden of this disease in terms of Disability Adjusted Life years, DALYs (WP6).

Task: T-5.3. Application of AMR disease burden framework to urinary tract infections.
Task start month:18

Task end month: 30
Task Leader: Scott McDonald (RIVM)Deputy Task Leader: Eelco Franz (RIVM)
Task Participants: DTU, RIVM
During years 1–2 a methodological framework was developed for computing the burden of disease attributable to AMR (the so-called "excess burden method"), measured in disability-adjusted life-years (DALY). In year 3 we will finish applying this methodology to estimate the excess disease burden attributable to ESBL-producing *E coli* experienced by patients with urinary tract infection in the Netherlands. A systematic literature review to obtain country-specific DALY parameters will be completed, which will allow the disease burden model to be parameterized to the Netherlands healthcare situation.

Task: T-5.4 Source attribution of AMR for attribution of disease burden to sources
Task start month:4
Task end month:30
Task Leader: Lapo Mughini Gras (RIVM)eputy Task Leader: Ana Sofia Duarte (DTU)ask Participants: DTU, RIVM
Sub-Task 5.3.1. Attribution of community-acquired ESBL/pAmpC-EC. We developed a methodology to estimate contributions of several putative sources to community-acquired ESBL/pAmpC-EC intestinal carriage through the application of an established source attribution model for foodborne pathogens based on nationally representative ESBL/pAmpC-EC gene frequencies, prevalence and exposure data. We made modifications to existing source attribution models by including humans as a source itself. The attribution will be based on ESBL genotypes encountered in human carriage and different livestock reservoirs and the environment. In the second year a manuscript has been finished and submitted to Lancet Planetary Health. In the third year we will finish an extended source attribution model, in which exposure weights based on exposure assessments are used to weigh transmission routes and statistical and conceptual aspects will be reconsidered.
Sub-Task 5.3.2. AMR source attribution based on metagenomics. In Y2, the developed attribution model based on metagenomics was explored further by including human as a reservoir. This work will be continued and finalised in Y3. Work will consist on gathering additional human resistome data or metagenomic data that may represent human exposure to antimicrobial resistance (e.g. from samples of sewage/human waste) and use the model to predict the sources of AMR.

Task: T-5.5. Propose and assess a new paradigm for AMR surveillance in pigs.
Task start month:18
Task end month: 30
Task Leader: Ana Sofia Duarte (DTU)
Deputy Task Leader: Tine Hald (DTU)Task Participants: DTU, RIVM
In Y2, faecal samples collected under Denmark's AMR surveillance program (DANMAP), representing two past periods of 5 years (2000-2004) and 4 years (2015-2018) were metagenomically sequenced. Additionally, historical data of antimicrobial usage (AMU) in pigs and results of minimum inhibitory concentration assays performed under DANMAP were gathered. In Y3, the resistomes will be compared to the percentage of resistance among isolates of different bacteria species selected for surveillance in the past and to AMU records. Isolates will include indicator bacteria such as *Escherichia coli*, and pathogens such as *Salmonella enterica*. Genotypic- and phenotypic AMR profiles, and AMU data will be analysed in parallel for trends and associations. The aim is to assess the potential of metagenomics for the surveillance of AMR.

WP6: Integration of information by Bayesian Evidence Synthesis
WP start month 01
WP end month 30
WP Leader **Eelco Franz (RIVM)**

Deputy WP Leader: **Thomas Sellhorst (BfR)**
WP participants **BfR, DTU, NVI**
Description of the WP:
To estimate the public health impact caused by AMR, data from various origins must be combined. These include occurrence of resistance (genetic factors, or carrier species) in environmental compartments (including food and water), transmission of resistant microbes in and between animal and human populations, and the relation between exposure to resistant microbes and health effects. Combining these sources of information not only requires dealing with different sources of varying accuracy, but also different modalities. Occurrence of a resistance gene in a food ingredient cannot be directly translated into human exposure to a resistant pathogen, or to an associated health risk. As an analogy, the fraction of the reported incidence of urinary tract (UT) infections caused by resistant strains may not be simply proportional to the carriage of these strains in the general population. In work packages 1 – 5 such relations are described by means of mathematical models. Integration of the information contributed by these work packages must not only consist of combining model outputs, but should also include metrics of the model performance, to allow information to be weighted by accuracy. This approach is similar to meta-analysis, but it is more general, in that information from different modalities is translated into a common metric (de Angelis et al. 2014; Spiegelhalter 2003). A highly simplified example is shown in Figure 1: resistant bacteria may be enumerated in samples from surface swabs. The sampling distribution (Poisson, or some compound distribution to model microbial aggregation) may be used to characterize the concentration of these bacteria, and an exposure model may be employed to predict human exposure, for instance, the transmission of infectious bacteria via the hand to cause urinary tract infection. A dose response model is needed to translate exposure into UT infection, or another relevant health endpoint as may be observed in hospital studies. Thus, the two bottom rectangles represent observed data: the occurrence of (AB resistant) bacteria on surfaces, and incident cases of UT infections. The connection between these observations are described by the model framework, possibly informed by other data sources (not shown here). The Bayesian evidence synthesis framework allows a joint analysis of the relationships between parameters and data sources in an information network of the type shown in Figure 1 to be conducted, and thus aids in providing consensus estimates (in this case, of the dose response parameters α and β, and/or parameters of the exposure model). This framework also ensures that uncertainty associated with all parameters is correctly propagated to the parameters for which inference is desired. Absence of convergence indicates inconsistencies, allowing identification of potentially incorrect (model) assumptions and/or possibly biased data sources. Thus, the integrative model may help in finding conflicting evidence, and thus provides a means for correcting the analysis.

Task 6.3: Update evidence network for information developed in the other work packages

Task start month 12
Task end month 24
Task Leader: RIVM
Deputy Task Leader: RIVM
Task Participants: BfR, NVI, DTU
Description of the task:
Continuous, iterative work, use project meetings (to be organized by the project lead and WP leaders) to keep up to date with WP results, and select results that can be used to improve the Bayesian evidence synthesis model. If possible, early versions of the evidence synthesis models may guide selection of problem areas: identify critical variables that are poorly known, or conversely, variables that exert little influence, e.g. on health burden estimates. This may help prioritizing data acquisition and identify critical data gaps.

Task 6.4: Define endpoint for the current project and report results for the evidence synthesis model in its endpoint state.

Task start month 18

Task end month 30
Task Leader: RIVM
Deputy Task Leader: RIVM
Task Participants: BfR, NVI, DTU
Description of the task:
The evidence synthesis as described here is a model that is built and then continually revised, through updating both the data inputs and the model structure with information as it becomes available. The endpoint may be reached when future iterations are expected to lead to small improvements (diminished returns) after reaching a certain stage. Alternatively, available information may be exhausted and/or there may be logistic conditions that limit further development. The end report of WP6 includes a policy-targeted description of the evidence synthesis model, and a detailed motivation of the endpoint choices.
References
De Angelis D, Presanis AM, Conti S, Ades AE. Estimation of HIV burden through Bayesian evidence synthesis. Statistical Science 2014;29(1):9–17).
Spiegelhalter DJ, Best NG. Bayesian approaches to multiple sources of evidence and uncertainty in complex cost-effectiveness modelling. *Stat Med* 2003;22(23):3687-709.

Deliverables

Ref	Title	Due month
D-0.3.2	Third annual report	30
D-1.1.2	Establishment of a database of field genomic data	18
D-1.2.2	Test and parameteriZation of the assembly pipeline for metagenomics data	24
D-1.3.1	Biological annotations of plasmid identified in the pipeline	24
D-1.4.1	WGAS-based method for genomic data analysis	30
D-1.4.2	Development of regression model for genomic data analysis	30
D-1.4.3	Integration of genetic traits associated to AMR and plasmid content information into models (WP6)	30
D-2.1	Report/draft paper on generic PK/PD model	M30
D-2.2	Report/draft paper on on-farm model	M30
D-2.3	Report/draft paper on transmission model	M30
D-2.4	Report/draft paper on intervention strategies	M30
D-3.1	Inventory with models and related data in FSK standard	30
D-3.2	Scientific report on a generic model for the chicken production chain	30
D-3.3	Scientific report on an adapted model for the pork production chain developed	30
D-3.4	Scientific report on an model for the exposure assessment of AMR through mussels	30
D-3.5	Scientific report on a generic comparative exposure assessment model	30
2	Recommended methods for risk profiling in investigations on antibiotic resistance available	24
D5.4	The'excess burden' approach for computing the burden of disease attributable to AMR: Application to urinary tract infection	24
D5.5	A proposal for a new paradigm for AMR surveillance	30

Milestones

Ref	Title	Due month
M 0.2.2	Final meeting	30

M-AMR3-1.2.1	Test and validation of assembly pipeline on synthetic and reference genomic data	18
M-AMR3-1.2.2	Analysis of HTS field data with assembly pipeline	24
M-AMR3-1.4.1	Analysis of field genomic data with WGAS-based method	30
M-AMR3-1.4.2	Analysis of field genomic data with regression model	30
M AMR3.2	Develop model frameworks for PK/PD and on-farm model (ANSES, APHA)	M8
M AMR3.3	Produce case study (collected datasets) baseline results for PK/PD (ANSES)	M15
M AMR3.4	Establish connection between the PK/PD model and the on-farm-model (ANSES, APHA)	M24
M AMR3.5	Establish connection between the PK/PD model and the on-farm-model (ANSES, APHA)	M24
M AMR3.6	Simulations of the PK/PD model for green AMDs (ANSES)	M24
M AMR3.7	Produce case study baseline model results for on-farm model (APHA)	M28
M AMR3.8	Structural adaptations of the PK/PD model according to AMR mechanisms (ANSES)	M28
M AMR3.9	Assess hypothetical intervention measures using on-farm model (APHA)	M30
M AMR3.10	Data gaps in PK/PD modelling and propositions of experimental studies with a defined methodology (ANSES)	M30
M-AMR3.12	Formulate CM/NGM (CVI, NCOH) and SD (RIVM) transmission models	M24
M-AMR3.13	Calibrate models using data library (CVI)	M24
M-AMR3.14	Formulate/investigate interventions (RIVM, NCOH, CVI, BfR43)	M27
M-AMR3.15	Assimilate project-generated data (NCOH)	M28
M-AMR3.16	Make recommendations to fill data gaps (RIVM, NCOH, CVI)	M30
M-3.2	Concept for an improved model for the chicken production chain developed	24
M-3.3	Concept for an adapted model for the pork production chain developed	24
M-3.4	Concept for an model for the exposure assessment of AMR through mussels	24
M-3.5	Concept on a generic comparative exposure assessment model	27
M4.2	Model development and model assessment completed	21

2.4.2.1.11 JRP12-R2-AMRSH5-FARMED

Reference to the Stategic Research Agenda (please refer to D2.7)	**AMR-SH 5: Development of new tools for early (real-time) detection of resistant pathogens in humans and animals, as well as new diagnostic tools, in particular on-site tests for humans and animals**									
Research Project Title	**Fast Antimicrobial Resistance and Mobile-Element Detection using metagenomics for animal and human on-site tests**									
Research Project Acronym	**FARMED**									
Leading Organisation	**P21-APHA**				Deputy Leading Organisation			**P31-WbvR**		
Project Leader	**Manal AbuOun (Nick Duggett)**				Deputy Project leader			**Michael Brouwer**		
Project Start month	**M25**				Project End month			**M54**		
Participant	1	2	**4**	6	7	8	**9**	10	11	**12**
	Anses	Ages	**Sciensano**	NDRVMI	SZU	VRI	**BfR**	FLI	RKI	**DTU**
PM			**9.8**				**6**			**5**
Participant	**13**	14	15	16	**17**	18	19	20	**21**	22
	SSI	UT	VFL	INIA	**UCM**	MVNA	INRA	IP	**APHA**	PHE
PM	**6**				**20**				**11.81**	
Participant	23	24	25	26	**27**	**28**	29	30	**31**	32
	UoS	OKI	NUIG	TEAGASC	**ISS**	**IZSAM**	IZSLER	RIVM	**WbvR**	FHI
PM					**2.6**	**13**			**5.27**	
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives

- Work Package 1: Assess feasibility of Long-read metagenome sequencing on exemplar matrices. Investigate the use of Hi-C metagenomics.
- Work Package 2: Bioinformatics tools to analyse long-read metagenome samples
- Work Package 3: Implementation of on-site protocols for long-read metagenomic DNA sequencing
- Work Package 4: Project management, coordination and training workshop

Description of work

JRP16-WP1 - Assess feasibility of Long-read metagenome sequencing on exemplar matrices. Investigate the use of Hi-C metagenomics.

JRP16-WP1 takes place over the first and the second year of the project.

WP start / end month: M25 – M44
WP Leader: Sigrid De Keersmaecker (4, Sciensano, BE)
Deputy WP Leader: Josephine Grützke (9, BfR, GE)
WP participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), ISS (27), IZSAM (28), WbvR (31)
Description of the WP:
WP1 will test and compare long-read sequencing to short-read sequencing to characterise the metagenome (including bacterial species) within 'simple' and 'complex' matrices. The aim will be to implement these methods for use with medical or veterinary practice. The ONT MinION, is a portable real-time device (100 grams), generating, in real-time, up to 30Gb of DNA sequencing. The longer lengths (>100kb) of DNA sequence data enable the user to find associations with bacteria, the plasmid and AMR genes, in real-time, without the need for traditional whole genome sequencing. Long-read sequencing technology has not been used broadly for metagenomics, so it will be compared to the current gold standard (short-read sequencing). A limited number of samples will also be compared to PacBio sequencing. Current laboratory based DNA extraction protocols will be used to establish if long-read sequencing is suitable for metagenome analysis.
A new sequencing technology, Hi-C that fuses DNA that is in close structural proximity during the DNA isolation and DNA-library preparation, will be investigated. This method uses short-read sequencing technology but during the analysis, this method will reveal information of the genetic context in which AMR genes are present. Together with long-read sequencing data, this information will be used to determine which bacterial species (pathogen and commensal) the AMR gene is present in, whether it is harboured on the chromosome or a plasmid.

JRP16-WP1-T1 - Assess feasibility /perform long-read metagenomics MinION from 'defined' microbial community.
JRP16-WP1-T1 takes place over the first and the second year of the project.
Task start / end month: M25-M40
Task Leader: Sigrid De Keersmaecker (4, Sciensano, BE)
Deputy Task Leader: Josephine Grützke (9, BfR, GE)
Task Participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), ISS (27), IZSAM (28), WbvR (31)
Description of the task[1]: In the first instance, we will use DNA isolated, using standard techniques, from a defined bacterial community containing isolates from various bacterial species (Gram negative and Gram positive, with available whole genome sequencing) to demonstrate that long-read sequencing is able to resolve the community. We will then use MinION sequencing to analyse various sample types of interest such as faeces (human and animal), saliva, milk, urine, environmental (e.g. water, dust) and food/feed samples spiked with characterized microorganisms (including uncommon bacteria harbouring AMR genes on extrachromosomal elements or in the chromosome for which we already have WGS data). Different concentrations of bacteria will be tested to determine limits of detection, and evaluate the accuracy of bacterial species and its association with genetic characteristics (such as AMR and mobile genetic elements) prediction. The long-read sequencing output will be compared to i) the known spiked AMR/species content and ii) the output of short read sequencing. A selection of samples will also be analysed using PacBio sequencing.

JRP16-WP1-T2 - Assess feasibility /perform long-read metagenomics MinION from 'simple' sample matrices.
JRP16-WP1-T2 takes place over the first and the second year of the project.
Task start / end month: M27-M42
Task Leader: Sigrid De Keersmaecker (4, Sciensano, BE)
Deputy Task Leader[1]: Soren Persson (27, SSI, DK)
Task Participants: Sciensano (4), DTU (12), SSI (13), UCM (17), APHA (21), IZSAM (28)
Description of the task[1]: We will use current standard DNA isolation techniques, on a selection of 'simple' sample matrices (i.e. a limited number of bacteria expected, such as water, urine, blood), to will evaluate the capability and practical feasibility of long-read sequencing to analyse the microbiome, and compare this to short-read sequencing. 'Simple' sample matrices present challenges such a potentially low concentration of the bacterial community, which could affect ability of sequencing to resolve the community, to a level that is diagnostically beneficial to detect bacterial species and AMR determinant for the clinician or veterinarian. Different spiking concentrations of bacteria will be tested to determine limits of detection, and evaluate the accuracy of bacterial species and its association with genetic characteristics (such as AMR and mobile genetic elements) prediction.

We will compare the sensitivity and specificity for species and subspecies identification, as well as the acquisition of information regarding the genetic context of AMR genes.

JRP16-WP1-T3 - Assess feasibility/perform long-read metagenomics MinION from 'complex' sample matrices.
JRP16-WP1-T3 takes place over the first and the second year of the project.
Task start / end month: M29-M44
Task Leader: Josephine Grützke (9, BfR, GE)
Deputy Task Leader: Sigrid De Keersmaecker (4, Sciensano, BE)
Task Participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), IZSAM (28), WbvR (31)
Description of the task: For a selection of 'complex' sample matrices (i.e. either multiple bacteria present, or difficult DNA extraction, such as faeces (human and animal), milk, environmental (e.g. dust, wastewater, boot swabs) and food/feed samples), we will evaluate the capability and practical feasibility of long-read sequencing to analyse the microbiome, and compare this to short-read sequencing. 'Complex' sample matrices present several challenges such as the large microbial load as well as the presence of contaminants, which could inhibit DNA sequencing and the presence of non-bacterial DNA. We will compare the sensitivity and specificity for species and subspecies identification, as well as the acquisition of information regarding the genetic context of AMR genes. Amongst others, already microbiologically well-characterized poultry faecal samples collected from in vivo animal experiments, carried out during the EFFORT project for which short-read sequencing data which is already available, will be analysed.

JRP16-WP1-T4 - Perform Hi-C metagenomics.
JRP16-WP1-T4 takes place over the first and the second year of the project.
Task start / end month: M33-M42
Task Leader: Josephine Grützke (9, BfR, GE)
Deputy Task Leader: Sigrid De Keersmaecker (4, Sciensano, BE)
Task Participants: Sciensano (4), BfR (9), UCM (17), WbvR (31)
Description of the task: A 'defined' bacterial community, containing various bacterial species (with known complete sequences), and harbouring different AMR plasmids will be prepared, and analysed using Hi-C library preparation and short-read sequencing. This analysis will reveal the feasibility, specificity, and sensitivity of Hi-C. Available bioinformatics tools will be used to analyse

the sequencing data, although these tools may require adaptation, which will take place in WP2-T1 and WP2-T2. In the next step, spiked samples (T1.1) and samples from the EFFORT project (T1.3) will be analysed by this approach, compared to long-read and conventional short-read metagenomic sequencing. Additionally, where possible, approaches for a combined analysis of Hi-C and long-read data (currently in development by Phase Genomics) will be explored to improve the analysis. Although Hi-C metagenomics is currently not ready for on-site use, it will be included in the comparison to cover the current spectrum of available metagenomics approaches for early detection of resistant pathogens.

WP2 - Bioinformatics tools to analyse the sequencing data and defining the characteristics within the sample.

JRP16-WP2 will take place over first, second and third year of the project.
WP start / end month: M28 – M45
WP Leader: Frank Aarestrup (12, DTU, DK)
Deputy WP Leader: Bruno Gonzalez-Zorn (17, UCM, Spain)
WP participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), ISS (27), IZSAM (28), WbvR (31)
Description of the WP:
WP2 will implement a ready-to-use package (MGMapper) to assess, analyse, and perform reference-based taxonomic assignment of next generation sequence data. The package can also carry out a post-processing analysis to produce reliable taxonomic annotation for species, and strain resolution, of both metagenomics and single isolate samples. MGMapper also employs a novel mapping method, KMA (*k*-mer alignment), which outperforms any currently available method in both accuracy and speed, without increased computational demands. The package and the mapping method are already in use in our routine daily analyses at DTU and will be available to analyse the data generated in WP1. These bioinformatics tools will markedly improve taxon detection, AMR and mobile element characterisations, and microbial diversity within metagenomics samples. The current high performance computing (HPC) based tools will be established as a laptop version for further validation and testing.

JRP16-WP2-T1 - Development/adaptation of a pipeline that can predict species within sample/matrix

JRP16-WP2-T1 will take place over first and second year of the project.
Task start / end month: M28-M45
Task Leader: Frank Aarestrup (12, DTU, DK)
Deputy Task Leader: Josephine Grützke, (9, BfR, GE)
Task Participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21)
Description of the task: The pipelines (MGMapper) will be tested, evaluated, and improved based on data submitted from the project participants. Performance of the MGMapper to produce reliable taxonomic annotation will be assessed using long-read sequencing data from defined mock communities and spiked samples as well as 'real' samples, established in WP1 and WP3. Bioinformatics tools for analysis of Hi-C/short-read sequencing will also require testing and optimisation to allow integration with MGMapper.

JRP16-WP2-T2 - Development/adaptation of a Resfinder-'like' pipeline for identification of AMR for long-read sequences

JRP16-WP2-T2 will take place over year the first, second and third year of the project.
Task start / end month: M30-M51
Task Leader: Frank Aarestrup (12, DTU, DK)
Deputy Task Leader: Bruno Gonzalez-Zorn (17, UCM, Spain)
Task Participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), ISS (27)

Description of the task: The MGMapper tool will be developed and tested for use with long-read sequencing to map and identify AMR, virulence and plasmid genes as well as their genetic origin with a metagenomics sample. Bioinformatics tools for analysis of Hi-C/short-read sequencing will also require testing and optimisation to allow integration with MGMapper.

WP3 - Implementation of on-site protocols for long-read metagenomic DNA sequencing

JRP16-WP3 will take place over first, second and third year of the project.
WP start / end month: M25 – M54
WP Leader: Mike Brouwer (31, WbvR, NL)
Deputy WP Leader: Soren Persson (27, SSI, DK)
WP participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), IZSAM (28), WbvR (31)
Description of the WP:
WP3 will focus on the necessary protocols and technologies to prepare samples in an on-site setting such as on a farm or in a hospital ward. To allow for on-site DNA sequencing and analysis, all steps will need to be carefully considered, including the DNA isolation from various sample matrices (WP1), sequencing library preparation, and downstream analysis. DNA isolations for on-site molecular applications have previously been described but the DNA quality and yield are likely to be low in many protocols as other molecular methods often rely on amplification of nucleic acids, for which low amounts of starting material is needed. For the on-site preparation of the sequencing libraries, two strategies will be investigated. The first will rely on the VolTRAX automated sample preparation unit. The second strategy will include investigation of the feasibility of the standard DNA extraction protocols used in WP1 to be adopted for on-site usage.

JRP16-WP3-T1 - Literature search & Harmonisation of on-site DNA isolation
JRP16-WP3-T1 will take place over first year of the project.
Task start / end month: M25-M29
Task Leader: Mike Brouwer (31, WbvR, NL)
Deputy Task Leader: Soren Persson (27, SSI, DK)
Task Participants: Sciensano (4), SSI (13), APHA (21), IZSAM (28), WbvR (31)
Description of the task: To perform next-generation sequencing (NGS), the quality of the input DNA is of great importance. As on-site methods for DNA isolation have previously been described and several partners have experience with such protocols, these protocols will be compared and assessed for suitability with NGS methods. In addition, a review of scientific literature on the topic will be performed in order to determine what DNA isolation methods have been described previously that could potentially be adopted for on-site use. During this task, a suitable method for isolation of DNA from different 'simple' and 'complex' sample matrices will be considered. Although a universal DNA isolation method would be preferable, it could very well be imaginable that different methods will need to be pursued for different sample matrices.

JRP16-WP3-T2 - Investigate the use of Voltrax
JRP16-WP3-T2 will take place over first and second year of the project.
Task start / end month: M30-M41
Task Leader: Manal AbuOun (21, APHA, UK)
Deputy Task Leader: Mike Brouwer (31, WbvR, NL)
Task Participants: Sciensano (4), DTU (12), SSI (13), UCM (17), APHA (21), WbvR (31)
Description of the task: The use of the newly released ONT VolTRAX V2 will be examined for its capacity to extract high quality DNA from the sample matrices tested in WP1. The VolTRAX is a

small, portable, and automated sample preparation device, which uses magnetic beads for DNA library preparation using magnetic beads, checks DNA quality, and has multiple reagent ports. In addition to a harmonised on-site DNA extraction protocol, the VolTRAX provides the option for on-site DNA library preparation so it can be directly loaded onto the MinION for sequencing.

JRP16-WP3-T3 - Assess DNA isolation methods for suitability, test on matrices (faeces, blood, dust, (environmental), milk).
JRP16-WP3-T3 will take place over first and second year of the project.
Task start / end month: M33-M54
Task Leader: Giuliano Garofolo (28, IZSAM, Italy)
Deputy Task Leader[1]: Sigrid De Keersmaecker (4, Sciensano, BE)
Task Participants: Sciensano (4), DTU (12), SSI (13), UCM (17), APHA (21), IZSAM (28), WbvR (31)
Description of the task: DNA isolation methods that have been determined most suitable for on-site application in the literature study of Task WP3-T1, will be assessed for 'simple' and 'complex' matrices as described in WP1. These experiments will be evaluated whilst replicating on-site conditions, within the laboratory, to determine which methods are most suitable for downstream NGS library preparation. Numerous parameters will be taken under consideration such as DNA yield, absence of contaminants and host DNA, limitations on us of specialised equipment, ease of use by trained but non-expert personnel, and cost.

WP4 - Project management, coordination and training workshop.

JRP16-WP4 will take place over first, second and third year of the project.

WP start / end month: M25 – M54
WP Leader: Manal AbuOun (21, APHA, UK)
Deputy WP Leader: Mike Brouwer (31, WbvR, NL)
WP participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), ISS (27), IZSAM (28), WbvR (31)
Description of the WP:
This work package will manage and steer the project, all institutes will contribute with at least one member present on the management committee, to ensure successful and timely delivery. Four videoconference meetings per year and one annual physical meetings, between all institutes will assess progress of the project. The annual meetings will take place at three different participating institutes. Any issues or concerns raised by any participating institutes will be communicated to the project lead and deputy lead, to allow rapid resolution. The management committee will also be in regular email contact with WP leaders, deputy leaders and task leaders, and project participants in general to ensure awareness and transparency, as well as to approve all documents and communications about the FARMED project.

JRP16-WP4-T1 - Annual physical project meetings

JRP16-WP4-T1 will take place over first, second and third year of the project.

Task start / end month: M30-M54
Task Leader: Mike Brouwer (31, WbvR, NL)
Deputy Task Leader: Manal AbuOun (21, APHA, UK)
Task Participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), ISS (27), IZSAM (28), WbvR (31)
Description of the task: Three 'physical' annual meetings will take place to enable an open exchange of ideas and project outcomes between FARMED partners. These annual meetings will be organised in different countries and will include representatives from all partners. The meetings will take place in Month 30, Month 41 and a concluding meeting in Month 50.

JRP16-WP4-T2 - Teleconferences will be organised every 3 months, between partners

JRP16-WP4-T2 will take place over first, second and third year of the project.
Task start / end month: M25-M54
Task Leader: Nick Duggett (21, APHA, UK)
Deputy Task Leader: Bruno Gonzalez-Zorn (17, UCM, Spain)
Task Participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), ISS (27), IZSAM (28), WbvR (31)
Description of the task: All project partners/management committee will meet or communicate every quarter, to assess progress of the project by each partner. This will also allow partners to discuss and address possible problems, risks, technical issues, project communication, and publications. Partners will be encouraged to continue email communication, contacting the management committee at the earliest opportunity, as and when required, especially if any issues arise.

JRP16-WP4-T4 - Annual reports
JRP16-WP4-T4 will take place in the final 2 months of first, second and third year of the project.
Task start / end month: M36-M54
Task Leader: Manal AbuOun (21, APHA, UK)
Deputy Task Leader: Mike Brouwer (31, WbvR, NL)
Task Participants: Sciensano (4), BfR (9), DTU (12), SSI (13), UCM (17), APHA (21), ISS (27), IZSAM (28), WbvR (31)
Description of the task: An annual report will be complied by project partners to provide updates on progress of work packages and tasks. These communications will be distributed to relevant organisations or stakeholders such as national governments, EU, international agencies, and relevant industry/health partners. On-site DNA extraction and sequencing protocols, as well as bioinformatics pipeline/tools generated from this project will be published in appropriate scientific journals and coding repositories.

Deliverables

Ref	Title	Due month
D-JRP16-WP3.1	Review on current scientific literature and overview of commercially available methods for on-site DNA isolation.	M30
D-JRP16-WP4.1	Annual communication to external stakeholders and OH EJP coordinators	M36

Milestones

Ref	Title	Due month
M-JRP16-01	Review on current scientific literature and overview of commercially available methods for on-site DNA isolation presented to FARMED consortium.	M29

2.4.2.1.12 JRP13-R2-AMRSH5-WORLDCOM

Reference to the Stategic Research Agenda (please refer to D2.7)			**AMR-SH 5: Development of new tools for early (real-time) detection of resistant pathogens in humans and animals, as well as new diagnostic tools, in particular on-site tests for humans and animals**							
Research Project Title			**Development of new tools for real-time detection of zoonotic bacteria and antimicrobial resistance in veterinary, human and environmental sources**							
Research Project Acronym			**WorldCom**							
Leading Organisation			**P25-NUIG**			Deputy Leading Organisation			**P23 UoS**	
Project Leader			**Prof. Terry Smith**			Deputy Project leader			**Prof. R. La Ragione**	
Project Start month			**M25**			Project End month			**M54**	
Participant	1	2	4	6	7	8	9	**10**	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	**FLI**	RKI	DTU
PM								**6.8**		
Participant	13	**14**	15	16	**17**	18	19	20	21	22
	SSI	**UT**	VFL	INIA	**UCM**	MVNA	INRA	IP	APHA	PHE
PM		**16**			**23.3**					
Participant	**23**	24	**25**	26	27	28	29	30	31	32
	UoS	OKI	**NUIG**	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM	**12.8**		**31.35**							
Participant	33	34	35	**36**	37	38	39	40	41	
	NVI	PIWET	INIAV	**INSA**	IISPV	IC	SLV	FOHM	SVA	
PM				**27.4**						

Objectives

- Analysis of publically available sequences for antimicrobial resistance genes.
- Targeted and whole genome *de novo* sequencing of phenotypically characterised isolates from various settings.
- Development of machine learning algorithms for the prediction of anti-microbial resistance.
- Development of assays for selected pathogens and AMR targets.

WP: 1 Generation of up to date sequence information for selected pathogens and antimicrobial resistance genes

WP start month: Month 25
WP end month: Month 36
WP Leader: VISAVET Health Surveillance Centre UCM
Deputy WP Leader: University of Tartu
WP participants: VISAVET Health Surveillance Centre UCM, University of Tartu, University of Surrey, NUI Galway, Friedrich-Loeffler-Institut, National Institute of Health Dr. Ricardo Jorge (INSA).
Description of the Work-Package: Generation of up to date sequence information for selected pathogens and antimicrobial resistance genes.
Task 1 (JPR15-AMR2.1-WP1-T1): Analysis of publically available sequences for antimicrobial resistance genes associated with *Salmonella, Campylobacter and E. coli*.
Task start month: 25
Task end month: 28
Task Leader: VISAVET Health Surveillance Centre UCM
Deputy Task Leader: University of Tartu
Task Participants: VISAVET Health Surveillance Centre UCM, University of Tartu, University of Surrey, NUI Galway, Friedrich-Loeffler-Institut, National Institute of Health Dr. Ricardo Jorge (INSA).
Description of the task: Publicly available sequence information for pathogens including *Salmonella, Campylobacter and E. coli* and anti-microbial resistance genes including CTX-M-15, NDM-5, KPC-2, OXA-48 and MCR-1 will be analysed. Particular emphasis will be place on using already existing data from projects such as COMPARE where reference genomes for both *E. coli* and *Salmonella* are available. The project will also link to the EJP ARDIG project and the HECTOR consortium (JPIAMR). Publicly available genomic information on *Campylobacter* species is available from a number of sources including UK Food Standards Agency report (Project code: FS101087) where sequences generated have been deposited in European Nucleotide Archive (ENA) databases at the European Bioinformatics Institute (EBI). Use of existing sequence information will ensure that in WP 1 Task 2 there will be a focus on generating information from new sources thus avoiding any duplication of work.
Task 2 (JPR15-AMR2.1-WP1-T2): Targeted and whole genome *de novo* sequencing of phenotypically characterised isolates from various settings.
Task start month: 25
Task end month: 30
Task Leader: VISAVET Health Surveillance Centre UCM
Deputy Task Leader: University of Tartu
Task Participants: VISAVET Health Surveillance Centre UCM, University of Tartu, University of Surrey, NUI Galway, Friedrich-Loeffler-Institut, Instituto Nacional de Saúde Doutor Ricardo Jorge.
Description of the task: Whole genome and targeted sequencing of bacterial pathogens (*E. coli*, *Salmonella* and *Campylobacter* spp.) and antimicrobial resistance genes (CTX-M-15, NDM-5, KPC-2, OXa-48 and MCR-1) from phenotypically characterised organisms isolated from various settings including pig and chicken farms, environmental sources including recreational and drinking water and clinical settings. A sequence database will be assembled to collate the data generated. The sequence data will be analysed and together with publicly and consortium available sequence information (Task 1) will form the basis on which the design of a suite of molecular diagnostics assays will be carried out. The addition of newly generated of new sequence information will ensure that the most up to date and relevant sequences are used in work-package 2 for assay development. Additionally, the new sequences generated as part of this task will be used in the development of machine learning algorithms in work-package task 3.
Task 3 (JPR15-AMR2.1-WP1-T3): Development of machine learning algorithms for the prediction of anti-microbial resistance. (University of Surrey, VISAVET Health Surveillance Centre UCM)

Task start month: 30
Task end month: 36
Task Leader: University of Surrey
Deputy Task Leader: VISAVET Health Surveillance Centre UCM
Task Participants: VISAVET Health Surveillance Centre UCM, University of Surrey.
Description of the task: The sequence collection generated in tasks 1 and 2 of this work-package will be used in this task to develop and train novel machine learning algorithms for the prediction and detection of antimicrobial resistance from the genomes of clinically relevant bacterial species (*E. coli*, Salmonella and *Campylobacter*). Where possible the project will link with other projects using Machine Learning approaches.

Deliverables

Ref	Title	Due month
D-JPR15-AMR2.1-WP1.1	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
D-JPR15-AMR2.1-WP1.2	Novel machine learning algorithms for the prediction and detection of AMR from genomic sequences.	36

Milestones

Ref	Title	Due month
M-JPR15-AMR2.1-01	Generation of sequence information for pathogens and resistance genes of interest.	30
M-JPR15-AMR2.1-02	Transfer of relevant sequence information for development and training of novel machine learning algorithms.	36

2.4.2.1.13 JRP14-R2-AMR2.1-FULL-FORCE

Reference to the Stategic Research Agenda (please refer to D2.7)	**AMR 2.1: Dynamics of AMR selection, clonal spread and horizontal gene transfer in humans, animals and the environment, including epidemiology of resistant microorganisms and antimicrobials in the environment and their (environment-mediated) spread**									
Research Project Title	**Full-length sequencing for an enhanced EFFORT to map and understand drivers and reservoirs of antimicrobial resistance**									
Research Project Acronym	**FULL_FORCE**									
Leading Organisation	**P4-Sciensano**					Deputy Leading Organisation			**P31-WbvR**	
Project Leader	**Pieter-Jan Ceyssens**					Deputy Project leader			**Michael Brouwer**	
Project Start month	**M25**					Project End month			**M54**	
Participant	**1**	2	**4**	6	7	8	**9**	10	11	**12**
	Anses	Ages	**Sciensano**	NDRVMI	SZU	VRI	**BfR**	FLI	RKI	**DTU**
PM	**5**		**12.2**				**6.5**			**6.9**
Participant	**13**	14	15	**16**	17	18	**19**	20	**21**	22
	SSI	UT	VFL	**INIA**	UCM	MVNA	**INRA**	IP	**APHA**	PHE
PM	**8**			**4**			**9.1**		**6.2**	
Participant	23	24	25	26	**27**	28	29	**30**	**31**	32
	UoS	OKI	NUIG	TEAGASC	**ISS**	IZSAM	IZSLER	**RIVM**	**WbvR**	FHI
PM					**8.5**			**3.7**	**11.47**	
Participant	**33**	**34**	**35**	**36**	37	38	39	40	**41**	
	NVI	**PIWET**	**INIAV**	**INSA**	IISPV	IC	SLV	FOHM	**SVA**	
PM	**3**	**8.2**	**11.3**	**2.4**					**7.8**	

Main Objectives

In the first year of the FULL_FORCE project, an important emphasis will be on EU-wide technical implementation of SMRT sequencing among public health and veterinary reference laboratories. This will include the standardization/harmonization of protocols and techniques where possible, or the establishment of common quality criteria where needed (cfr. ongoing discussion in SOLIDNESS). This will be complemented with the organization of workshops (dry/wet lab) and a proficiency test.

A second major milestone is the enabling of ENA's AMR hub, developed during COMPARE, as central depository for all partners, and organize a webinar to explain its function and data submission. Work packages 2-4 will also take an onset, most of them with either the selection of AMR strains with associated short-read data and/or predominant MGEs for initial typing. The modelling work will commence with the model design and parametrization of AMR spread in the broiler production pyramid.

Description of work

WP 0: *PROJECT MANAGEMENT*

WP start/end month: M25 – M54. This WP takes place over the three annual years of the project.
WP Leader: Pieter-Jan Ceyssens (SCI); **WP participants**: SSI, ANSES, INSA, BfR, DTU, APHA, INIA, INRA, ISS, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+UG, UU, IZSLT)
This overarching WP ensures proper coordination at both the overall project and the individual WPs, as well as timely reporting of results and budgets according to the formal EU requirements. It will also involve knowledge transfer for data storage at ENA from the COMPARE project, and the development of a data management plan.

Task 0.1: MEETINGS AND TELCALLS
Start/end month: M25-M54
Leader: Pieter-Jan Ceyssens (SCI); Task Participants: SSI, ANSES, INSA, BfR, DTU, APHA, INIA, INRA, ISS, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+UG, UU, IZSLT)
This task takes place over the three annual periods of the EJP. In the first year, the FULL_FORCE consortium will physically meet at the SMRT sequencing workshop at Copenhagen. Here, detailed task overviews will be presented by the WP leaders for the coming project year as well as task achievements that will be disseminated by both WP leaders and relevant project participants.
Apart from these, the project coordinator and deputy coordinator will perform teleconferences with all WP leaders at the start of every quarter to ensure momentum of the overall project. Dates for teleconferences will be determined using the Doodle web tool. In addition, WP leaders will perform WP teleconferences with relevant WP members according to the individual requirements and deliverables.

Task 0.2: REPORTING
Start/end month: M25-M54
Leader: Pieter-Jan Ceyssens (SCI); Task Participants: WP leaders (SSI, DTU, APHA, INRA, SVA)
This task takes place over the three annual periods of the EJP.The project coordinator will collect detailed reports on deliverables and milestones from WP leaders according to the work plan outlined below. Also, budget information will be collected at the end of each project year from all participants and reported according to the requirements of EU.

Task 0.3: CENTRAL DATA REPOSITORY
Start/end month: M25-M36
Leader: Pieter-Jan Ceyssens (SCI); Task Participants: SSI, ANSES, INSA, BfR, DTU, APHA, INIA, INRA, ISS, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+UG, UU, IZSLT, Sébastien Matamoros, creator ENA AMR hub)
T-0.3 takes place over the first year of the EJP project. FULL_FORCE will use a centralized data repository to upload sequence- and metadata which are generated during WP1 and WP2. Upon consultation with experts from the European COMPARE Consortium and the European Nucleotide Archive (ENA), we will create password-protected subgroups for data storage at ENA using the AMR data hub. This online infrastructure is built to store and share structured phenotypic AMR data linked to bacterial genome sequences. By default, this data will be stored at 'pre-publication' state for 24 months, a period which can be manually extended if deemed necessary. To ensure proper

implementation and data upload, we will organize a webinar with the AMR hub creator in M25 (See letter of support). A follow-up meeting with ENA to evaluate the use by EJP members is foreseen at the end of 2020.

Task 0.4: DATA MANAGEMENT PLAN
Start/end month: M25-M54
Leader: Pieter-Jan Ceyssens (SCI); Task Participants: SSI, ANSES, INSA, BfR, DTU, APHA, INIA, INRA, ISS, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI.
This task takes place over the three annual periods of the EJP. To create a sound Data Management plan (DMP), we will use the DMPonline tool which was originally developed by the UK's Digital Curation Centre and which is now available under a GNU Affero General Public License. Sciensano has access via https://dmponline.be/, and FULL_FORCE will be supported by EJP WP4 responsible Valerie Dewaele. The first version of the DMP will be discussed at the first annual meeting in Denmark, and annual updates will be published if necessary.

Deliverables		
D-JRP19-WP0.D1	Start-up meeting report	M27
D-JRP19-WP0.D2	Financial and activity report Y3	M36
D-JRP19-WP0.D3	Recorded webinar tutorial ENA AMR data hub	M27
Milestones		
M-JRP19-M1	Creation of specific data hubs in ENA AMR hub	M27
M-JRP19-M5	Publication of first version of data management plan	M30

WP 1: *SMRT IMPLEMENTATION*
WP start/end month: M25 – M36. This WP takes place in the first annual year on FULL_FORCE.
WP Leader: Henrik Hasman (SSI); **WP participants**: SCI, ANSES, INSA, BfR, DTU, APHA, INIA, INRA, ISS, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+UG, UU, IZSLT)

FULL_FORCE will initiate with EU-wide **SMRT Implementation**. In the first months of the project, we will organize a three-day workshop on practical implementation of long-read sequencing in Copenhagen. All participants will bring their expertise and challenges, both from the wet and dry lab. Institutes with the most expertise in SMRT sequencing (SSI, DTU, APHA, WbvR, SCI, BfR) will prepare course material and take the lead during this workshop. Experts from the SOLIDNESS project will participate in preparatory teleconferences, and will be invited to attend our workshop. Each institution's proficiency in SMRT sequencing will be assessed afterwards using a proficiency test, organised and coordinated by SSI.

Task 1.1 METHODOLOGY FOR MGE SEQUENCING
Start/end month: M25-M27
Leader: Leo Schouls (RIVM); Task Participants: SCI, ANSES, INSA, BfR, DTU, APHA, INIA, INRA, ISS, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+UG, UU, IZSLT)
This task comprises the definition of the consensus protocol or (at minimum) quality parameters for DNA isolation and purification, the protocol to create libraries to perform multiplex sequencing using the ONT MinION, run conditions, data collection and base calling to obtain sufficiently long reads that can be used to reconstruct large bacterial plasmids (and if needed chromosomes). Strong input is expected from final recommendations from the SOLIDNESS project (UG).
In two rounds of consultation, we will assess which software is most suitable for sequence assembly based on combined Illumina and ONT sequencing (hybrid assembly, e.g. UniCycler) and ONT

sequencing alone (e.g. Canu). The selected software will be made available for all participants that will run the SMRT sequencing. Furthermore, the hardware requirements to run the software, incl. software for additional data analyses, such as annotation tools, will be defined. This will ensure all partners, who are going to perform SMRT sequencing, have the bioinformatic infrastructure in place locally before the start of task T1.3.

Task 1.2 SMRT SEQUENCING WORKSHOP
Start/end month: M28-M30
Leader: Henrik Hasman (SSI); Task Participants: ANSES, INSA, BfR, DTU, APHA, INIA, INRA, ISS, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+ IZSLT, UG, UU)
This task takes place after completion of T1.1, where consensus protocols for DNA purification, sample preparation, SMRT sequencing and sequencing analysis have been developed. A 3-days workshop on the practical implementation of SMRT sequencing will be organized and one representative from each relevant partner institution will be invited to participate. Partners with the most expertise in SMRT sequencing (SSI, DTU, APHA, WbvR, SCI) will prepare course material and take the lead during this workshop. Experts from the SOLIDNESS project will participate in preparatory teleconferences and will be invited to attend our workshop. The workshop will cover 1) hands-on laboratory work for DNA purification, 2) labelling and SMRT (ONT MinION) sequencing, theory on the ONT MinION technology with focus on data quality/quantity and troubleshooting and 3) plenum presentation tools required for sequence analysis using hybrid assemblies of mobile genetic elements (MGEs).

Task 1.3 PROFICIENCY TEST FOR MGE SEQUENCING
Start/end month: M31-M36
Leader: Henrik Hasman (SSI); Task Participants: ANSES, INSA, BfR, DTU, APHA, INIA, INRA, ISS, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+ IZSLT, UG, UU)
After completion of T1.2, all relevant partners will be invited to participate in a dry-lab proficiency test, where Illumina and MinION sequencing data from a set of isolates carrying well-characterized MGEs are distributed for the participants to analyse using protocols from T1.1 and methods presented during the workshop (T1.2). The results of the proficiency tests will be reported to, and discussed with, participants individually and furthermore aggregated into an anonymized final report.

Deliverables

D-JRP19-WP1.D1	Teleconference to assess required protocols and infrastructure	M25
D-JRP19-WP1.D2	Teleconference to discuss proposed protocols and infrastructure (follow-up)	M26
D-JRP19-WP1.D3	Completion of final protocol for SMRT sequencing	M27
D-JRP19-WP1.D4	Invitation to workshop delivered to all participating institutions	M28
D-JRP19-WP1.D5	Completion of workshop organization plan including selection of course material	M29
D-JRP19-WP1.D6	Selection of proficiency test data	M32
D-JRP19-WP1.D7	Delivery of analysis results of proficiency test by partners to SSI	M33
D-JRP19-WP1.D8	Completion of final report of proficiency tests	M36

Milestones		
M-JRP19-M6	3-days workshop on SMRT sequencing event	M30
M-JRP19-M7	Shipment of proficiency test data and strains	M32
M-JRP19-M15	Analysis of proficiency test data by all partners	M35
M-JRP19-M16	Individual reports of proficiency test sent to partners	M36

WP 2: *GENOME STUDIES*

WP start/end month: M25 – M54. This WP takes place over the three annual years of the project.

WP Leader: Muna anjum (APHA); **Deputy Leader:** Pieter-Jan Ceyssens (SCI); **WP participants**: SSI, ANSES, INSA, BfR, DTU, INIA, INRA, ISS, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+UU, IZSLT)
The general goal of this WP is to apply SMRT sequencing technology on six selected case studies, from cross-sectional and longitudinal studies from research and surveillance projects including EU projects such as EFFORT, COMPARE, ENGAGE and ARDIG, as well as national and EU surveillance activities, for which short read Illumina sequences may already be available. Using this additional layer of information, we will be able to compare specific MGEs and their possible evolution in different environments and geography.

Task 2.1 MGE evolution in longitudinal sample sets (ARDIG, ABRES)
Start/end month: M25-M54. This task takes place over the three annual years of the project.
Leader: Muna Anjum (APHA); Task Participants: NVI, ANSES, RIVM, WbvR

The focus of this task will be to characterise the evolution of MGEs, such as plasmids, in longitudinal datasets from livestock (e.g. extended spectrum beta-lactamase (ESBL) *Escherichia coli* from livestock) and human samples (RIVM dataset). In the first year, isolates collected from longitudinal studies from both human and livestock that are available in partner collections will be selected for further characterisation. Short read Illumina WGS will be performed on the isolates by each partner, mostly in the budgetary context of other proposals; up to 500 isolates will be selected per partner. Preliminary analysis will be performed on the WGS, if not available, to characterise the antimicrobial resistance (AMR) gene content. The AMR genes will be characterised using pipelines such as DTU ResFinder or the APHA SeqFinder, and isolates grouped at the AMR family level according to their resistance genotypes. Using the resistance genotype profiles, a subset of isolates (up to 20 per partner) from each profile will be selected for long read sequencing using the harmonised methods from WP1.

Task 2.2 MGE evolution in cross-sectional data sets (EFFORT, ENGAGE & National Surveillance)
Start/end month: M28-M54. This task takes place over the three annual years of the project.
Leader: Jens A. Hammerl (BfR); Task Participants: WbvR,INIA, INSA, PIWET, PHAS, SSI, DTU, RIVM, APHA, SCI, (IZSLT)

The focus of this task will be to characterise the modular structure of selected plasmid types from different sectors (isolates from human, livestock, and environment), with a similar approach as Task 2.1. On the basis of the available WGS datasets of the involved partners, important plasmid types (i.e. AMR/virulence) that are prevalent in isolates of different sources will be identified and subjected to further analyses over the whole project time. In the first year, relevant plasmid types in WGS datasets are identified and a final sets of cross-sectional samples for SMRT sequencing are selected, according to methodology described above.

Task 2.3 *Klebsiella pneumoniae*: the canary in the coalmine
Start/end month: M28-M54. This task takes place over the three annual years of the project.
Leader: Petra Edquist and Alma Brolund (FOHM); Task Participants: ISS, INSA, SCI, INIA, NVI, SSI, BfR, (IZSLT)

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.
In this task we will perform long read sequencing of ~40 *K. pneumoniae* from each contributing site. *K pneumoniae* from different sectors will be compared, such as human patients, environment and infected animals as well as geographic origin of the strains. We will also be able to compare *K. pneumoniae* strains with different antibiotic resistance genes, i.e. CPO vs. ESBL.
During year 1, the isolate population will be defined between the task participants. Preparations for long read sequencing of the selected isolates will take place. Time lines defined. After the project workshop the sequencing pipeline will be set-up and the sequencing can start by the end of Y1.

Task 2.4 ESBL-producing *Enterobacteriaceae* in horses – A separated epidemiology of plasmids?
Start/end month: M28-M54. This task takes place over the three annual years of the project.
Leader: Stefan Borjesson (SVA); Task Participants: ANSES, INRA, RIVM, (UU, IZSLT)

This task objective is to investigate the spread of ESBL-producing Enterobacteriaceae in horses in Europe. The focus will be on $bla_{CTX\text{-}M\text{-}1}$ and $bla_{SHV\text{-}12}$ genes. To date, isolates carrying $bla_{CTX\text{-}M\text{-}1}$ 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+ $bla_{CTX\text{-}M\text{-}1}$ appears to be almost exclusively linked to horses. Less is known of the epidemiology of $bla_{SHV\text{-}12}$, which is often associated with IncX3-plasmids. In total around 200 isolates from horses, both commensals and clinical isolates, from different parts of Europe will be included to investigate clonal and horizontal transmission.
In Year 1, partners will be identifying and selecting isolates for inclusion in the study and collecting metadata related to the isolates. After the project workshop the SMRT sequencing will be initiated and continued through year 2.

Task 2.5 Salmonella Infantis and S. Kentucky across reservoirs: role of MGEs
Start/end month: M28-M54. This task takes place over the three annual years of the project.
Leader: Laura Villa (ISS); Task Participants: ISS, SCI, INRA, INSA, PHAS, PIWET, RIVM, SVA, (IZSLT)

The focus of this task is characterization of MGEs in *S*. Infantis and *S*. Kentucky associated with ESBL, colistin resistance and eventually to other ARGs from human and animal sample. In the first year the repertoire of the MGEs (plasmids, transposable elements…) available from the genomes already present in partner collections will be selected for further characterisation. Illumina WGS will be performed on the isolates by each partner, if these are not already available. SMRT technology will be performed on a subset of isolates, interesting for the complexity of MGEs encoding antimicrobial resistance. Up to 200 isolates of *S.* Infantis and *S.* Kentucky per partner will be analysed from human and animal origin, if available.

Task 2.6 Evaluation of publicly available and in-house tools for MGE typing
Start/end month: M30-M54. This task takes place over the three annual years of the project.
Leader: Jannice Schau Slettemeås (NVI); Task Participants: ANSES, Bfr, DTU, APHA, INSA, (UU, IZSLT)
The focus of this task will be to assess the proficiency of publicly available and in-house developed tools to analyze genome data, with focus on MGE. In the first year, publicly available and in-house

developed tools will be identified and explored. The tools to be assessed include the plasmid pipeline designed in the context of the RaDar project, the TransposonFinder tool of DTU, Phaster (http://phaster.ca/) and others. These tools will be used during the second year to explore the full-length genomes from WP2.

Deliverables		
D-JRP19-WP2.D1	Submission of sequence- and metadata of longitudinal samples at ENA hub	M33
D-JRP19-WP2.D2	Submission of sequence- and metadata of cross-sectional samples at ENA hub	M33
D-JRP19-WP2.D3	Submission of sequence- and metadata of *K. pneumoniae* samples at ENA hub	M33
D-JRP19-WP2.D4	Submission of sequence- and metadata of *S. enterica* samples at ENA hub	M33
D-JRP19-WP2.D5	Submission of sequence- and metadata of horse-related samples at ENA hub	M33
D-JRP19-WP2.D6	List of relevant publicly available and in-house developed tools.	M36
Milestones		
M-JRP19-M10	Final selection of longitudinal samples for SMRT sequencing	M34
M-JRP19-M11	Final selection of cross-sectional samples for SMRT sequencing	M34
M-JRP19-M12	Final selection of *K. pneumoniae* samples for SMRT sequencing	M34
M-JRP19-M13	Final selection of *S. enterica* samples for SMRT sequencing	M34
M-JRP19-M14	Final selection of horse-related samples for SMRT sequencing	M34

WP 3: *CULTURE-INDEPENDENT TYPING AND METAGENOMICS*

WP start/end month: M30 – M54. WP3 takes place over Y3, Y4, and Y5 of the EJP.
WP Leader: Frank Aarestrup (DTU); **WP participants**: WbvR, APHA, NVI, INIA, (UU, IZSLT)

At least an 80% of bacteria present in complex samples such as faeces and soil are uncultivable under routine microbiological procedures. The development of culture-independent methods for detect, quantify and identify bacterial plasmids carrying antimicrobial resistance genes is greatly encouraged for future surveillance efforts. This WP will focus on (i) enhanced mining of existing metagenomics data, including those from the EFFORT and COMPARE projects, and correlate this to AMR-gene abundance, and (ii) development of diagnostic tools for direct identification of MGE/plasmid identifications from various sample types.

Task 3.1 MGE ANALYSES IN METAGENOMIC DATASETS

Start/end month: M30-54. This task will take place in the three annual years of the project.
Leader: Frank Aarestrup (DTU); Task Participants: WbvR, APHA, NVI, (UU, IZSLT)

This task takes place over the three years of FULL_FORCE. In the first annual year, we will develop, evaluate and make available a database of MGEs for use in detection of single isolates and metagenomics datasets. This will be based on known sequences of transposons, integrative elements, and plasmid replicons, and will be updated with full-length MGEs coming from WP2.
In subsequent years, this database will be updated and implemented in the evaluation of bioinformatics pipelines for quantification of MGE in metagenomics datasets, and in determining the presence and abundance of MGEs in public and de novo generated metagenomics datasets.

Task 3.2 CULTURE INDEPENDENT METHODS FOR PLASMID IDENTIFICATION
Start/end month: M30-54. This task will take place in the three annual years of the project.
Leader: Fernando Esperón (INIA); Task Participants: WbvR, (IZSLT)

In this task, we will develop two novel methods for the study of plasmid-mediated ARGs on environmental samples without the need of previous culture. These methods will be tested on field samples taken in the context of EFFORT. Two partial objectives are involved:

- Developing a panel for multiple detection and quantification of ARGs for high throughput real time PCR analysis in nanoliter scale (e.g. OpenArray®, SmartChip®). The panel is comprised by up to 31 relevant plasmid-mediated ARGs primer sets plus a primer set targeting 16S rRNA.
- Adapting methods for SMRT sequencing for identifying plasmids in different types of sample without previous culture (e.g. from faeces, food, soil and water). It comprises three main steps: 1) Adaptation of plasmid DNA purification procedures on different sample types (faeces, soil and water); 2) development of a protocol for Nanopore sequencing of purified plasmid DNA, and 3) bioinformatics analysis of sequences.

During the Y3 the main objective is to develop a universal, simple method for plasmid DNA isolation from environmental samples. The efficacy recovery of the methods will be tested by quantification by real time PCR spiked control samples.

Deliverables		
D-JRP19-WP3.D1	Database construction tailored at MGEs	M36
D-JRP19-WP3.D2	Protocol for plasmid DNA extraction from environmental samples	M34
Milestones		
M-JRP19-M17	Successful adaptation of plasmid purification protocol to field samples	M36

WP 4: *FUNCTIONAL CHARACTERIZATION OF AMR MOBILE GENETIC ELEMENTS (MGE)-CARRYING AMR GENES AND BACTERIAL HOST ASSOCIATIONS*

WP start/end month: M25 – M54.
WP Leader: Benoit Doublet (INRA, 19); **Deputy WP Leader:** Laura Villa (ISS, 27); **WP participants:** ANSES, INSA, BfR, DTU, APHA, INIA, SCI, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+UU, UG, IZSLT)
The overall goals of this WP are to (i) gain knowledge on molecular mechanisms of spread and persistence of main MGEs carrying critically/highly important antimicrobial resistances, (ii) identify key molecular interactions between AMR-MGEs and bacterial host important for dissemination and maintenance, and (iii) provide first line evidences for the development of novel exploratory strategies to curb the spread of major AMR-MGEs. WP4 takes place over Y3, Y4, and Y5 of the EJP.

Task 4.1 SELECTION of MGEs and HOST STRAINS FOR DETAILED CHARACTERIZATION
Start/end month: M25-42. This task will take place in the first and second annual year of the project.
Leader: Laura Villa (ISS); Deputy Task Leader: Benoît Doublet (INRA); Task Participants: ANSES, INSA, BfR, DTU, APHA, INIA, SCI, PIWET, PHAS, SSI, RIVM, SVA, WbvR, NVI (+UU, UG, IZSLT)
The initial selection contains plasmids such as pESI of *S.* Infantis, pKpQIL of KPC-producing *Klebsiella pneumoniae*, IncX3-SHV and IncHI1-CTX-M plasmids from horses, IncI1-AmpC/ESBL plasmids, *Salmonella* genomic island 1, IncC plasmids of *S*. Kentucky ST198 and IncX4, IncI2, IncHI2 and ColE-like plasmids carrying *mcr* genes in *Salmonella* and *Escherichia coli*.
Based on current knowledge of AMR-MGEs that will be analysed in subtasks of WP2 (Genome studies) and in WP3 (Metagenomic and culture-independent detection of MGEs and resistance), we will refine the initial selection (see below) of epidemic plasmids and integrative elements, defined as MGEs found in different bacteria, at distant geographical regions and/or associated with emerging clones. During the first months of the project and until the 3-day SMRT sequencing

workshop, all participants will be invited to exchange by e-mails and teleconferences on the initial selection. The following selection criteria are/will be discussed:

- Host strains belonging to pathogens, opportunistic and commensals linked to WP2.
- Carrying critically/highly-important AMR genes that encode extended-spectrum β-lactamases (ESBLs: CTX-M, SHV, TEM, PER), carbapenemases (NDM, OXA-48-type, VIM, IMP, KPC), mobile colistin resistance determinant (MCR-type).

This task will take advantage of the outcomes of WP 2 and 3 to identify successful genomic variants of AMR-MGEs to be studied in task T4.2 and specific host associations to be analysed in task T4.3. We will define sets of type-materials (MGEs and strains) to be shared between involved partners for task T4.2 implementation.

Task 4.2 FUNCTIONAL CHARACTERIZATION of MGEs

Start/end month: M31-54. This task will take place in the three annual years of FULL_FORCE.
Leader: Benoît Doublet (INRA); Deputy Task Leader: Michael Brouwer (WbvR); Task Participants: ANSES, SCI, INRA, ISS, WbvR, INSA, IZSLT

AMR-MGEs considered of interest will be investigated by molecular functional analysis to identify both the MGE-encoded factors and the bacterial host factors important for dissemination, stability and maintenance. Based on the selection performed in T4.1, experimental works will be divided between involved participants in regard of their expertise. Briefly, *in vitro* conjugation studies will be conducted with selected type- MGEs and host strains. Conjugation assays will be performed from different genetic backgrounds (*Salmonella*, *Klebsiella*, *E. coli*, *Proteus*, etc) of donor strains and recipient strains using protocols harmonized across participants. AMR-MGEs will be introduced in different host strains and studied for their ability to persist over time in the bacterial host without antimicrobial selective pressure. Genetic factors from MGEs and the host that may influence persistence will be investigated. Beyond the known incompatibility between plasmids of the same Inc group, the capacity of different AMR-MGEs "to live together" in the same host will be also studied. We will share approaches, protocols, molecular tools between involved participants. The initial involvement of participants is defined on different AMR-MGEs, as follow:

- *Salmonella* Genomic Island 1 family and IncC plasmids in S. Kentucky ST198 and beyond: role of the host-encoded SOS response and other SGI1-encoded fitness properties outside AMR (**INRA**, SCI, IZSLT)
- Assessment of horizontal transfer and persistence of IncHI1-CTX-M plasmids in different *E. coli* genetic backgrounds from horses: impact of prebiotic feed additives (**Anses**, INRA, IZSLT)
- Assessment of horizontal transfer of IncX4, IncI2, IncHI2 and ColE-like plasmids carrying *mcr* plasmids (**ISS**, IZSLT, INSA)
- pKpQIL plasmids of KPC-producing *Klebsiella pneumoniae*: plasmid-host associations of pKpQIL plasmids and multiple independent transposition events in KPC-producing *K. pneumoniae* strains (**INSA**, SCI).
- Horizontal transfer capacity of pESI plasmid from *S.* Infantis to other *Salmonella* serovars and other microbial species as *E. coli*, and fitness advantages to the host bacteria (**IZSLT**, SCI, ISS).

Task 4.3 HOST ASSOCIATION STUDIES

Start/end month: M43-54.
Leader: Valeria Bortolaia (DTU); Task Participants: DTU, (UU, IZSLT)
This task will take place in the second and third annual year of the EJP (see related work plans).

Deliverables		
D-JRP19-WP4.D1	First collection of type-materials (MGEs and strains) to be shared between involved partners	M30

Milestones		
M-JRP19-M3	Selection of MGEs and host strains to be studied T4.2	M28
M-JRP19-M8	Sharing of protocols, recipient- and host-strains, molecular tools	M33

WP 5: *MODELLING*
WP start/end month: M25 – M54. WP5 takes place over Y3, Y4, and Y5 of the EJP.
WP Leader: Stefan Widgren (SVA, 41); **WP participants:** RIVM, BfR, UU - Farm Animal Health
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
Start/end month: M25-42. This task will take place in the first and second annual year of the project.
Leader: Stefan Widgren (SVA, 41); Task Participants: RIVM, BfR, UU - Farm Animal Health

First, we will focus on the design for model of the **spread in the broiler production** pyramid, including **implementation in SimInf (M25-M33).** SVA will lead the simulation of the spread of AMR within production chains using their SimInf modelling R package. Design of a model for and parameterization of the transmission dynamics in the broiler production pyramid will be used to focus, because of the relatively simple structure and high level of data. The model design will be coupled to assessment of crucial transmission points in the production chain by elasticity and scenario analyses. Scenario analyses will incorporate structural changes in the model structure (i.e. elimination of hatcheries in the production chain), where elasticity analysis will focus on the relative contribution of specific parameters in the existing production chain (i.e. the effect of reducing contamination in the hatchery).
Next, SVA will lead the **parameterization of the model** using ABC methodology (**M34-42**). Estimating model parameters from observed data is a major challenge in stochastic modelling. However, it is a necessary and critical step in order to evaluate a model's explanatory power. Parameterization is preferably conducted within a Bayesian framework, when prior knowledge is present, as in the case of transmission in a production chain. In WP5 we will focus on Approximate Bayesian computation (ABC), a recent computational approach for parameterisation of complex problems, for example, in epidemiology. ABC is appropriate as we can use the SimInf-simulator as a so-called generative model. However, since ABC is computationally challenging it is important to explore how to efficiently perform parameter inference within an AMR transmission simulation framework. There are several open source software tools available for ABC analysis that potentially can be used in combination with the SimInf modelling R package. In WP5 we will investigate approaches and tools for using ABC methodology for parameter inference in order to facilitate complex epidemiological research of AMR transmission in the broiler production chain. The methodology developed within the WP5 will be made publicly available - it will thus become possible to adapt the modelling to other similar applications.

Task 5.2 EXPOSURE ASSESSMENT of HORIZONTAL and VERTICAL TRANSMITTED AMR
Start/end month: M25-54. This task will take place in the three annual years of the project.
Leader: Eric Evers (RIVM); Task Participants: SVA, UU - Farm Animal Health

In the first annual year, RIVM will lead an attempt to **quantify the fraction contribution** of horizontal vs. vertical transmission to human AMR exposure (**M25-39**). These fractions might vary between the different stages in the food chain. Information for this will be gained from discussions with experts, mainly within this work package but also outside, and from literature study.

Deliverables		
D-JRP19-W5.D1	Source code of the implementation of a SimInf model designed for pAMR transmission.	M33
Milestones		
M-JRP19-M2	A teleconference or physical meeting on horizontal vs. vertical AMR transmission	M27
M-JRP19-M4	A teleconference or physical meeting on input/output relationship between SimInf and sQMRA	M29
M-JRP19-M9	An implementation of a SimInf model designed for pAMR transmission to be studied in T5.1	M33

2.4.2.1.14 JRP15-R2-AMR2.1-FED-AMR

Reference to the Stategic Research Agenda (please refer to D2.7)		**AMR 2.1: Dynamics of AMR selection, clonal spread and horizontal gene transfer in humans, animals and the environment, including epidemiology of resistant microorganisms and antimicrobials in the environment and their (environment-mediated) spread**							
Research Project Title		**The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain**							
Research Project Acronym		**FED-AMR**							
Leading Organisation		**P2-AGES**			Deputy Leading Organisation			**P36-INSA**	
Project Leader		**Wögerbauer**			Deputy Project leader			**Canica**	
Project Start month		**M25**			Project End month			**M54**	
Participant	1	**2**	4	6	**7**	8	**9**	**10**	11	12
	Anses	**Ages**	Sciensano	NDRVMI	**SZU**	VRI	**BfR**	**FLI**	RKI	DTU
PM		**11.44**			**14.1**		**1.75**	**8.4**		
Participant	**13**	**14**	15	16	17	18	19	**20**	21	22
	SSI	**UT**	VFL	INIA	UCM	MVNA	INRA	**IP**	APHA	PHE
PM	**8**	**16**						**1.25**		
Participant	**23**	24	**25**	26	27	28	29	30	31	32
	UoS	OKI	**NUIG**	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM	**12.96**		**18**							
Participant	**33**	**34**	35	**36**	37	38	39	40	41	
	NVI	**PIWET**	INIAV	**INSA**	IISPV	IC	SLV	FOHM	SVA	
PM	**2.81**	**9.6**		**55**						

Objectives

- Establishment of harmonized and standardized sampling and testing protocols in alignment with the EFFORT project approach
- Establishment of a Scientific Supervisory Board (SB)
- Facilitating inter-institutional communication, exchange of knowledge and expertise
- Organization of kick-off and final project meetings. Support of interim meeting
- Establishing regularly held Webinars and ad hoc Skype Meetings to solve acute problems and allow a smooth progress in project plan

- Development of a data and protocol management plan
- Establishment of a data, results and protocol repository accessible by the partner institutions via internet.
- Support reporting and publishing

Description of work

<u>WP1:</u> Project Management and Communication

WP1 takes place over the first, the second year and the third year of the project (Y3, Y4, Y5)

- Task 1.1, subtasks sT1.1.2, sT1.1.2, task T1.2 and task 1.3 take place over the first, the second year and the third year of the project (Y3, Y4, Y5)
- subtask sT1.1.1 takes place in the first year of the project (Y3)

WP start month: M25
WP end month: M54
WP Leader: Dr. Markus Wögerbauer
Deputy WP Leader: Prof. Dr. Allerberger
WP participants: 2-AGES, 7-SZU, 9-BfR, 10-FLI, 13-SSI, 14-UT, 23-UoS, 20-IP, 25-NUIG, 33-NVI, 34-PIWET, 36-INSA
Description of the WP: WP 1 will consist of 3 primary tasks: Task 1.1. will organize the scientific management of the project. Task 1.2. describes the measures applied for managing administrative and legal activities. Task 1.3. deals with data and protocol management. A Supervisory Board (SB) constituted by a single representative of each of the 13 participating organizations will control all FED-AMR scientific and administrative activities and decides about all key issues concerning project execution and exploitation of the results. Management is organized by 2-AGES, all remaining institutes nominate a representative responsible for communicating management issues to and from their local institutions.

Task: JRP17-R2-**<u>WP1-T1</u>**: *Scientific Management*
Task start month: M25
Task end month: M54
Task Leader: Dr. Markus Wögerbauer
Deputy Task Leader: Prof. Dr. Mark Chambers
Task Participants: 2-AGES, 7-SZU, 9-BfR, 10-FLI, 13-SSI, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 34-PIWET, 36-INSA
Description of the task: The scientific management of the FED-AMR project will be organized by an AGES senior expert who has experience in the management of scientific projects for over 20 years (MW). He will be supervised and assisted by the head of the AGES business area “Public Health” (Prof. Allerberger) which facilitates the access to the organizational infrastructure of this business area. The other partners nominate a representative for the Scientific Supervisory Board. The SB will meet in regular intervals to control the progress of the project.
Sub-Task: JRP17-R2-**<u>WP1-T1.1</u>**: Coordination of sampling, laboratory experiments and building a database.
Sub-Task start month: M25
Sub-Task end month: M28
Sub-Task Leader: Dr. Alexandre De Menezes
Deputy Sub-Task Leader: Prof. Mark Chambers
Sub-Task Participants: 2-AGES, 23-UoS, 25-NUIG
Description of the task: In collaboration with the SB within one month after the initial kick off meeting the scientific manager will provide unified sampling and experimental protocols based on

the DPMP guidelines devised by the data manager at AGES, including an interactive checklist concerning quality control steps for sampling, experiments, analyses and data processing.

Sub-Task: JRP17-R2-**WP1-T1.2**: Webinar forum and Skype meetings for instant scientific interactions
Sub-Task start month: M25
Sub-Task end month: M50
Sub-Task Leader: Dr. Monica Oleastro
Deputy Sub-Task Leader: Dr. Markus Wögerbauer
Sub-Task Participants: 2-AGES, 36-INSA
Description of the task: To facilitate interaction and problem solving at regular intervals Webinars will be organized as well as ad hoc Skype meetings. These activities will be co-ordinated by 2-AGES and 36-INSA.

Sub-Task: JRP17-R2-**WP1-T1.3**: Project Meetings
Sub-Task start month: M25
Sub-Task end month: M52
Sub-Task Leader: Dr. Manuela Canica
Deputy Sub-Task Leader: Dr. Markus Wögerbauer
Sub-Task Participants: 2-AGES, 36-INSA
Description of the task: Project meetings will be held in Vienna (M26, M52) and Lisbon (M41) trying to bring as many members of the FED-AMR consortium together. However, the meetings will be broadcast vis Adobe Connect to those who cannot attend. 2-AGES and 36-INSA will organize the events.

Task: JRP17-R2-**WP1-T2**: *Administrative Management*
Task start month: M25
Task end month: M54
Task Leader: Dr. Juliane Pichler
Deputy Task Leader: Mag. Christine Feiertag
Task Participants: 2-AGES
Description of the task: The administrative management will be 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 will be coordinated by AGES (see above). Additionally, each partner will appoint an administrative representative who will be in direct contact with the AGES administrative manager (AM).

Task: JRP17-R2-**WP1-T3**: *Data and Protocol Management.*
Task start month: M25
Task end month: M52
Task Leader: Dr. Manuela Canica
Deputy Task Leader: Dr. Tanel Tenson
Task Participants: 2-AGES, 7-SZU, 9-BfR, 10-FLI, 13-SSI, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 34-PIWET, 36-INSA
Description of the task: The SB of the FED-AMR consortium will start to develop a Data and Protocol Management Plan (DPMP) at the Kick-off Meeting and aims to have a final DPMP available within the following 1 months. The DPMP will determine which data will be collected, processed or produced, which techniques and standards will be used, whether data and/or protocols will be shared and made publicly available, whether and how they will be made accessible for verification and/or reuse, and how they will be curated and stored (even after the end of the project period). The database will be curated and updated at regular intervals.

Deliverables		
Ref	**Title**	**Due month**
D-JRP17-FED-AMR - WP1.1	Scientific Supervisory Board (SB) installed. Local administrative representatives nominated (T1, T2)	M25
D-JRP17- FED-AMR - WP1.2	Unified sampling and experimental protocols (T1.1.)	M27
D-JRP17- FED-AMR - WP1.3	Data and protocol management plan (T3)	M27
D-JRP17- FED-AMR - WP1.4	Webinars (T1.2.)	M30
D-JRP17- FED-AMR - WP1.5	Annual project report	M36
Milestones		
Ref	**Title**	**Due month**
M-JRP17- FED-AMR -01	Kick off meeting	M26
M-JRP17- FED-AMR -02	Database repository active	M27
M-JRP17- FED-AMR -03	Webinar forums started	M30

WP2: Field experiments: Determination of the naturally occurring ARG background load and microbial biodiversity in the tested environmental compartments

Objectives

- Determination of the origins and transmission dynamics of resistant bacteria over various environmental compartments of an open air agricultural testing catchment (HOAL)
- Determination of the microbial biodiversity and naturally occurring ARG background load along the food/feed chain in an open air agricultural testing catchment (HOAL)
- Determination of the environmental resistome encoded by extracellular DNA isolated from various environmental compartments (HOAL)
- Determination of ARG concentration dynamics over a complete crop growing period in various environmental compartments in the catchment area (annual longitudinal study)
- Determination of the clonal spread of resistant bacteria versus dissemination of resistance by HGT via comparison of metagenomic data from DNA samples retrieved from the catchment area
- Determination of geographical differences and trends in AMR in relation to animal husbandry and land use techniques by comparing catchment area results with results obtained from similar compartments in Northern, Eastern and Southern regions of Europe
- Determination of multidrug and emerging resistances via metagenomics (NGS) and qPCR
- Identification of naturally transformable bacteria in the tested environmental compartments (NGS)

Description of work

WP2: Field experiments: Determination of the naturally occurring ARG background load and microbial biodiversity in the tested environmental compartments – Longitudinal study over a crop growing season (1 year).

WP2 takes place over the first and second year of the project (Y3 and Y4). Tasks 2.1. and 2.2. take place in the first year (Y3). Tasks 2.3. , 2.4. and 2.7. take place over the first and second year of the project (Y3 and Y4). Tasks 2.5. and 2.6. take place over the second year (Y4)

WP start month: M25

WP end month: M48
WP Leader: Dr. Manuela Caniça
Deputy WP Leader: Dr. Markus Wögerbauer
WP participants: 2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 36-INSA
Description of the work package: In this WP the prevalence, quantity and movement of AMR via free exDNA will be monitored along different compartments of the food/feed chain within the HOAL catchment (refer to Figure 1 in work plan description): "human/animal gut -> manure -> soil -> crop -> drainage -> surface water -> groundwater -> human/animal". All matrices (pig faeces, manure, agricultural soil, crop plants, drainage, surface and ground-water) will be analyzed for the presence of clinically relevant ARGs [focus on *tet*(M), *tet*(W), *tet*(Z), $bla_{\text{TEM-type}}$, $bla_{\text{SHV-type}}$, $bla_{\text{CTX-M-type}}$, carbapenemase-encoding genes, *sul*1, *sul*2, *sul*3, *erm*-like genes, PMQR-encoding genes, *mcr*-variants and *intI*1] encoded on free exDNA by ARG-specific qPCR taking into special account antimicrobial treatments of the pig herds. DNA extractions will be performed with the PowerSoil DNA kit applying a modified procedure that allows the differentiation between extra- and intracellular DNA. Illumina amplicon and shotgun sequencing will be applied to establish the bacterial biodiversity in the tested compartments and to search for competence genes. As ARG sequences from complex environmental matrices like soil are usually underrepresented in metagenomic analyses, a gene enrichment strategy with gene capture probes will be applied. This approach also allows the identification of the most prevalent naturally transformable species in agricultural soils and the monitoring of their fate in different environmental compartments. Concentrations of relevant antimicrobials will be determined in all of the compartments (see WP4). Culturable, resistant soil and gut bacteria will be characterized with standard microbiological methods. The results will be compared with data obtained from similar testing locations and environmental compartments from Northern (EE, NO) and Southern European (PT) regions. WP2 will provide sample material for WP3 (isolated C. difficile strains), WP4 (selection pressure determination) and WP5 (soil microcosms).WP2 is the core element of the FED-AMR project.

Task: JRP17-R2-**WP2-T1**: *Assemble list of sampling compartments and points. Determination of test areas representative for the European regions (North, West, East, South).*
Task start month: M25
Task end month: M26
Task Leader: Dr. Tanel Tenson
Deputy Task Leader: Dr. Markus Wögerbauer
Task Participants: 2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 36-INSA
Description of the task: In this task a list of sampling compartments and points will be compiled by the Task Leader in co-operation with the Deputy Task Leader and presented at the Kick-off meeting. All participants discuss the list online before and decide at the Kick-off meeting. The final list will be made available to the participants one week after the Kick-off meeting via the data repository. This list supports harmonization of testing procedures and enhances comparability of the results obtained from different regions of Europe. 14-UT and 2-AGES provide preparatory work; the remaining participants provide input and advice according to their expertise and take part in voting and the final decision.

Task: JRP17-R2-**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*
Task start month: M25
Task end month: M26
Task Leader: Dr. Manuela Caniça
Deputy Task Leader: Dr. Markus Wögerbauer
Task Participants: 2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 36-INSA

Description of the task: In this task common protocols for sample collection, DNA extraction from various matrices (soil, faeces, manure, water etc.) including the application of special procedures to selectively extract free extracellular DNA (according to Zinger et al., 2016), qPCR and 16S amplicon sequencing will be prepared by the Task and Deputy Task leader. These common protocols support harmonization of testing procedures and enhances comparability of the results obtained from different regions of Europe and will be made available one week after the Kick off meeting via the data repository. 36-INSA and 2-AGES provide preparatory work; the remaining participants provide input and advice according to their expertise and take part in voting and the final decision.

Task: JRP17-R2-**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.*
Task start month: M27
Task end month: M48
Task Leader: Dr. Manuela Caniça
Deputy Task Leader: Dr. Tanel Tenson
Task Participants: 2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 36-INSA
Description of the task: In this task the bacterial biodiversity and the naturally occurring ARG background load in the tested environments – with a focus on free extracellular DNA – is determined. This facilitates the classification of ARGs as environmental pollutants and the identification of clinically relevant resistance determinants. Samples will be drawn from various – but interconnected - environmental compartments using harmonized protocols as decided in **WP2-T1** and **WP2-T2** over a crop growing period of one year. Total and free extracellular DNA will be extracted. 16S targeted amplicon and shotgun sequencing (only in year 4: M40-M50. Relevant information there) will be performed in single batches to save costs. Sequence comparisons will be performed in year 4 (M40-M50) (25-NUIG). The cultivable fraction of environmental bacteria will be characterized on complete and minimal medium with a focus on human and animal pathogens whose AMR profiles will be determined using MIC standard methods (all partners). The results will be compared between the different participating regions to identify predominant ARG carrier strains and potential ARG variability. This approach identifies environmental hot spots of resistance and potential intervention points to reduce AMR spread from environmental sources. The results will be used for WP5 and WP6. Appropriate bacterial isolates will be made available for WP3.

Sub-Task: JRP17-R2-**WP2-T3.1**: Shot gun sequencing and bioinformatic analyses of AMR genes and MGEs
Will be performed in year 4. Please see relevant information there.

Sub-Task: JRP17-R2-**WP2-T3.2**: Gene enrichment with gene capture probes
Sub-Task start month: M31
Sub-Task end month: M42
Sub-Task Leader: Dr. Alexandre De Menezes
Deputy Sub-Task Leader: Prof. Mark Chambers
Sub-Task Participants: 23-UoS, 25-NUIG
Description of the sub-task: Gene enrichment for certain ARGs is beneficial in NGS shot gun approaches on samples with complex communities and various sources of DNA (e.g. soil). An AMR gene probe database will be established using ARG-ANNOT, Resfinder, NCBI Lahey Clinic beta-lactamase, and similar sources. The INTEGRALL gene database will be used for intl1 capture probes. Agilent SureSelect baits covering a wide variety of ARG and intl1 sequences will be used to capture ARG sequences from the isolated DNA. Gene capture will be performed using Agilent's SureSelect

protocol recommendations, followed by post-capture PCR amplification and Illumina HiSeq sequencing. The results obtained will be integrated into WP5 and WP6.

Task: JRP17-R2-**WP2-T4**: *Quantify clinically relevant ARGs in the tested compartments (qPCR; qPCR arrays).*
Task start month: M27
Task end month: M42
Task Leader: Dr. Manuela Caniça
Deputy Task Leader: Dr. Markus Wögerbauer
Task Participants: 2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 36-INSA
Description of the task: In this task the same samples as retrieved in **WP2-T3** will be used. ARG qPCRs will be performed at the sampling institutes following agreed standardized protocols (preferred gold standard: TaqMan qPCR), but can be executed to some extent collectively at 2-AGES in case of urgent needs and to relieve partnering institutes from acute work burden. 2-AGES offers qPCR training for interested partner institutes to support harmonisation of the approach and increase comparability of the results. This exercise identifies high risk environmental ARG sources and interventions points to decrease AMR spread via free exDNA. The results will be compared between different European regions to pinpoint potentially different ARG hot spot and are used for WP5 and WP6.

Task: JRP17-R2-**WP2-T5**: *Identify naturally transformable bacterial species in the tested compartments (NGS).* Will be performed in year 4. Please see relevant information there.

Task: JRP17-R2-**WP2-T6**: *Assess clonal/lineage diversity in selected ARB species.* Will be performed in year 4. Please see relevant information there.

Task: JRP17-R2-**WP2-T7**: *Isolate and assess quantity, diversity and stability of free extracellular ARG encoding DNA in the tested environments. Sequence comparisons.*
Task start month: M27
Task end month: M40
Task Leader: Dr. Markus Wögerbauer
Deputy Task Leader: Dr. Manuela Caniça
Task Participants: 2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 36-INSA
Description of the task: In this task free, extracellular DNA will be extracted from the samples based upon the methodology as from Zinger et al., 2016, and optimized to the respective matrix. Aqueous solutions will be extracted according to Liang and Keely, 2013, and/or a modified CTAB procedure (Corinaldesi et al., 2013). The DNA will be quantified by Qubit dsDNA-specific assays. Metagenomic 16S and shotgun sequencing will be performed as described in **WP2-T3** and qPCR analyses will be performed as described in **WP2-T4.** The results will give clear information about the relevance of free DNA as a reservoir for AMR and whether free DNA is prone to cross ecosystem boundaries. The obtained data will facilitate the risk assessment of extracellular DNA borne ARGs. The results will be forwarded to WP5 and WP6. The role of the partners is the same as already described in the preceding tasks.

Deliverables

Ref	Title	Due month
D-JRP17-FED-AMR-WP2.1	List of sampling compartments, points and European test areas and harmonized protocols in alignment with EFFORT project protocols available in data repository (T2.1, T2.2)	M26
D-JRP17-FED-AMR-WP2.2	Preliminary data collection on ARG prevalence and ARG background load in the compartments analysed so far (T2.4)	M36

D-JRP17-FED-AMR-WP2.3	Quantity and stability of free extracellular DNA observed in environmental compartments tested so far (T2.7)	M36
D-JRP17-FED-AMR-WP2.4	Annual report for Y3 (WP2)	M36

Milestones

Ref	Title	Due month
M-JRP17-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

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

Objectives

- Evaluation of *C. difficile* as a zoonotic agent and it's transmission networks
- Evaluation of the zoonotic versus anthropogenic AMR transmission using *C. difficile* as a model organism

Description of work

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

WP3 takes place over the third and the fourth year of the project

WP start month: M25

WP end month: M49

WP Leader: 36-INSA

Deputy WP Leader: 13-SSI

WP participants: 9-BfR, 10-FLI, 14-UT, 20-IP

Description of the WP3: *C. difficile* is one of the most important causes of healthcare-associated infections worldwide, and is capable of causing significant enteric disease in various animal species, including farm animals. There is increasing evidence that C. difficile may have a foodborne or zoonotic etiology. In addition, AMR is frequently reported in epidemic *C. difficile* strains and is thought to play a major role in the infection and dissemination of this pathogen. C. difficile has also been suggested as a reservoir/receptor of resistance genes that might be transferred to other species in the host gut as well as in the environment. The existence of indistinguishable ribotypes and toxin gene profile is extensively described in the literature suggesting that zoonotic or anthropogenic transmission of *C. difficile* may be occurring, although the true extent of genetic overlap between these populations and the environment remains to be determined. Characterising the overlap of *C. difficile* genotypes in different reservoirs can improve our understanding of possible transmission routes of this pathogen and AMR associated determinants. This WP is organized into four tasks that will address the epidemiology of zoonotic *C. difficile*, the genetic overlap between human and non-human *C. difficile* lineages, as well as its role as an ARG transfer platform over ecosystems boundaries.

Task: FED-AMR2.2-WP3-T1 - Epidemiological survey of zoonotic ribotypes across participant countries.

Task start month: M25

Task end month: M36

Task Leader: 36-INSA

Deputy Task Leader: 13-SSI
Task Participants: 9-BfR, 10-FLI, 14-UT, 20-IP
Description of the task: The aim of this task is to identify zoonotic types of *C. difficile* across participant countries. The task will comprise collection of genomic data (WGS reads) and associated metadata available from consortium partners on potential zoonotic types of toxigenic *C. difficile* isolates from various sources (human, animal and environment). Metadata includes demographic and epidemiological data, as well as strain type, namely ribotype, toxin profile and AMR profile, when available. Discrimination between zoonotic and non-zoonotic types will be based on *C. difficile* ribotypes and other genetic markers described in the literature. Previously developed genomic platforms (projects COMPARE (http://www.compare-europe.eu/); INNUENDO (https://www.efsa.europa.eu/en/supporting/pub/en-1498) will be used to share metadata and genomic data.
When possible, institute partners should collect new isolates from other sources in order to generate a One Health collection of *C. difficile* strains per partner, meeting the objectives of the task.
The participant institutes involved in this task gathers conditions to support it through their national reference, surveillance and investigation activities, providing access to *C. difficile* strains collections and respective genomic, phenotypic and epi-data, either from public health (13-SSI, 36-INSA, 14-UT, 20-IP), from veterinary health, including farm and pet animals (10-FLI, 36-INSA, 20-IP) and from food and environment (9-BfR, 14-UT). Furthermore, through collaborations with other sectors, partners have the opportunity to have access to isolates from other sources.

Task: FED-AMR2.2-WP3-T2 - WGS and AMR characterization of human and non-human *C. difficile* isolates
Task start month: M31
Task end month: M44
Task Leader: 36-INSA
Deputy Task Leader: 13-SSI
Task Participants: 9-BfR, 10-FLI, 14-UT, 20-IP
Description of the task: WGS and AMR characterization will be carried out for the zoonotic isolates identified among the consortium in task 3.1, and not previously characterized, as well as for the isolates obtained within the project, specifically in task 3.4. For determination of AMR profile, the outputs of project JRP1 IMPART (https://onehealthejp.eu/projects/jrp1-impart/), regarding the development of a suitable and cost-effective method for AMR surveillance of *C. difficile*, will be used to harmonise methods between the consortium participants. WGS will be performed by each participating partner.

Deliverables

Ref	Title	Due month
D-JRP17–FED-AMR-WP3.1	Database of zoonotic *Clostridium difficile* isolates across participant countries (task 3.1)	M36

Milestones

Ref	Title	Due month
M-JRP17–FED-AMR-25	Completed database with zoonotic types	M36

WP4: *Determination of the selection pressures in the tested compartments of human, animal and environmental ecosystems*

Objectives

The overall objective of the WP4 is the determination of the selection pressures in the tested compartments of human, animal and environmental ecosystems

The specific objectives are:

- **O4.1** To evaluate the presence and quantify the most commonly used in veterinary practice antimicrobials in selected environmental samples (water, soils, manure, faeces)
- **O4.2** To quantify of herbicides in agricultural soli
- **O4.3** Measurement of the concentration of trace elements in environmental samples gathered across participant countries

Description of work

WP4: *Determination of the selection pressures in the tested compartments of human, animal and environmental ecosystems*

WP4 takes place over the first, second and third year of the project (Y3, Y4, Y5). Tasks 4.1. and 4.2. take place in the first year (Y3). Tasks 4.3 - 4.7. take place over the first, second and third year of the project (Y3, Y4, Y5).

WP start month: M25. WP end month: M50. WP Leader: Dr. Martin Brandtner (2-AGES). Deputy WP Leader: *Dr.* Anna Gajda (34-PIWET). WP participants: 2-AGES (sample collection), 7-SZU (sample collection), 14-UT (sample collection), 23-UoS (sample collection), 25 NUIG (sample collection), 34-PIWET (testing), 36-INSA (sample collection)

In this WP selection pressures will be determined by the quantification of residues of antimicrobials (like tetracyclines, sulfonamides, macrolides, fluoroquinolones), herbicides, and heavy metals. Measurements of the concentration of trace elements in environmental samples from participant countries will reveal their relationship to induction of competence in naturally transformable bacteria. The analysis of antibiotics in animal faeces is important to obtain more insight in the possible formation of bacterial resistance in the animals' gut. The quantification of antimicrobials will be performed with an Agilent Series 1200 HPLC system connected with an API 4000 triple quadropule mass analyser with a TurboIonSpray source (AB Sciex, Canada; liquid chromatography - tandem mass spectrometry - LC/MS/MS).

Task 4.1 Selection of essential antimicrobials to be quantified in the tested compartments (published antibiotic consumption data, farmers' questionnaire, personal experience, expert interviews (veterinarians)

Task start month: M25. Task end month: M26. Task Leader: Dr. Martin Brandtner (2-AGES). Deputy Task Leader: Dr. Anna Gajda (34-PIWET). Task Participants: 2-AGES, 23-UoS, 25 NUIG, 34-PIWET.

In task 4.1. harmonized protocols for sample collection, transport and antimicrobials, herbicide and trace element detection will be established in co-operation with WP1.

Task T4.2 Quantification of three antimicrobial classes (tetracyclines, macrolides, sulphonamides) in aqueous matrices (water)

Task 4.2 start month: M27. Task 4.2 end month: M50. Task Leader: Dr. Anna Gajda (34-PIWET). Deputy Task Leader: Dr. Martin Brandtner (2-AGES). Task Participants: 2-AGES (sample collection), 23-UoS (sample collection), 25 NUIG (sample collection), 34-PIWET (testing), 36-INSA (samples collection)

In task T4.2 samples of water (drinking, ground, surface, river) will be collected. The number of references samples analysed for the presence of antimicrobials from M25 to M50 will be about 40. Three classes of antimicrobials most common used in veterinary practice (tetracyclines, sulfonamides and macrolides) will be analyzed with a sensitive technique, allowing the detection of trace amounts of tested compounds. Antimicrobial analysis in water will be performed by liquid chromatography - tandem mass spectrometry (LC-MS/MS) method using an Agilent Series 1200 HPLC system connected with an API 4000 triple quadropule mass analyser with a TurboIonSpray source (AB Sciex, Canada). In water samples analysis, antibiotics will be isolated by sodium acetate with purification of the extracts using SPE Strat X columns. Before LC injection, samples will be

additionally purified with syringe filters. The Analyst 1.6 software will controlled the LC-MS/MS system and process the data.

Task T4.3 Quantification of three antimicrobial classes (tetracyclines, macrolides, sulphonamides) in manure

Task start month: M27. Task end month: M50. Task Leader: Dr. Anna Gajda (34-PIWET). Deputy Task Leader: Dr. Martin Brandtner (2-AGES). Task Participants: 2-AGES, 23-UoS, 25 NUIG, 34-PIWET.

In the tasks 4.3, 4.4., 4.5. samples of manure, faeces and soil will be taken from farms and from the areas of nearby farms. The number of references samples analysed for the presence of antimicrobials in tested material will be about 200. Three classes of antibacterials most common used in veterinary practice (tetracyclines, sulfonamides, and macrolides) will be analyzed by liquid chromatography - tandem mass spectrometry (LC-MS/MS) method using an Agilent Series 1200 HPLC system connected with an API 4000 triple quadropule mass analyser with a TurboIonSpray source (AB Sciex, Canada). A sample preparation step in multiclass method for the analysis of antimicrobial residues in faeces and manure will involve the addition of Na_2EDTA and extraction with solution of formic acid in acetonitrile, and next with solution of ammonia in acetonitrile followed by filtration by SPE column, further evaporation and reconstitution before LC injection. The chromatographic separation will be performed on C18 column with gradient elution mode. For the soil and sediments samples, selected compounds will be extracted with addition of acetonitrile, citric acid and sodium acetate. The clean-up step will be performed on Oasis HLB cartridges. Mass spectral acquisitions were performed under selective multiple reaction monitoring mode by triple quadrupole mass spectrometry detector. Before LC injection, samples will be additionally purified with syringe filters.

Task T4.4 Quantification of three antimicrobial classes (tetracyclines, macrolides, sulphonamides) in faeces

Task start month: M30. Task end month: M50. Task Leader: Dr. Anna Gajda (34-PIWET). Deputy Task Leader: Dr. Martin Brandtner (2-AGES). Task Participants: 2-AGES, 23-UoS, 25 NUIG, 34-PIWET.

For technical details on the execution of this task see task 4.2.

Task T4.5 Quantification of three antimicrobial classes (tetracyclines, macrolides, sulphonamides) in soil

Task start month: M27. Task end month: M50. Task Leader: Dr. Anna Gajda (34-PIWET). Deputy Task Leader: Dr. Martin Brandtner (2-AGES). Task Participants: 2-AGES, 23-UoS, 25 NUIG, 34-PIWET.

For technical details on the execution of this task see task 4.2.

Task 4.6 Quantification of herbicides in agricultural soil

Task start month: M27. Task end month: M50. Task Leader: Dr. Anna Gajda (34-PIWET). Deputy Task Leader: Dr. Martin Brandtner (2-AGES). Task Participants: 2-AGES, 23-UoS, 25 NUIG, 34-PIWET.

In this task a representative list of herbicides will be quantified by an AGES associated sister company according to an established in-house protocol.

Task 4.7 Measurement of the concertation of trace elements in environmental samples gathered across participants countries

Task start month: M27. Task end month: M50. Task Leader: Dr. Anna Gajda (34-PIWET). Deputy Task Leader: Prof. Mark Chambers (2-AGES). Task Participants: 2-AGES, 23-UoS, 25 NUIG, 34-PIWET.

In this task the sample preparation involves replicate procedures and different chemical methods depending on the 'nature' of the sample. All bio-materials (fluids/tissues, plants) are dried at 80˚C or 6 hours (to constant weight). The samples will be analysed by an Agilent 7700 inductively coupled plasma mass spectrometer. The resultant data is checked for accuracy and precision by Excel and the subsequent sample data is calculated (blank and internal standard checked) to produce the final database of elemental values for statistical analysis

Deliverables		
Ref	**Title**	**Due month**

D-JRP17-FED-AMR-WP4.1	Standardize protocols for sampling and testing of environmental samples	M26
Milestones		
Ref	**Title**	**Due month**
M-JRP17-FED-AMR-30	Starting the selection of essential antimicrobials to be quantified in the tested compartments	M25
M-JRP17-FED-AMR-31	Starting the analysis of antimicrobials in aqueous matrices	M27
M-JRP17-FED-AMR-32	Starting the analysis of antimicrobials in manure	M31
M-JRP17-FED-AMR-33	Starting the analysis of antimicrobials in faeces	M29
M-JRP17-FED-AMR-34	Starting the analysis of antimicrobials in soil	M27
M-JRP17-FED-AMR-35	Starting the quantification of herbicides in agricultural soil	M27
M-JRP17-FED-AMR-36	Starting the measurement of the concentration of trace elements in environmental samples	M27

WP5: Identification of environmental conditions modulating transformation frequencies in soil microcosms and an *in vitro* porcine gut model (poGutMo) (laboratory studies).

Objectives

- Evaluate the effect of different environmental conditions on ARG HGT rates using *Acinetobacter* spp. (transformation) and *Clostridium* (conjugation) as prototype gene-transfer platforms.
- Identify conditions favouring induction of competence in recipient bacteria in agricultural soils.

Description of work

WP5 takes place over the first, the second and the third year of the project.

- T5.1 takes place over the first year of the project.
- T5.1 is split into 4 sub-tasks, each lasting 3 months.
- T5.2 takes place over the second and the third year of the project.
- T5.2 is split into 3 sub-tasks, each representing a block of experiments that build iteratively with input from WP4 and input from, and output to, WP6. The first 2 sub-tasks take place in the second year and the final sub-task takes place in the third year.

WP5: Identification of environmental conditions modulating transformation frequencies in soil microcosms and an *in vitro* porcine gut model (poGutMo) (laboratory studies).
WP start month M25
WP end month M52
WP Leader Professor Mark Chambers (MC)
Deputy WP Leader Professor Roberto La Ragione (RLR)
WP participants MC, RLR, PDRA
Description of the WP: The focus of this work package will be novel work to assess the environmental effects on transformation and conjugation frequencies in a porcine gut model (poGutMo). The experiments described in **Task 1** will be performed in the absence of the selective pressure of antibiotics or herbicides to establish baseline levels of HGT in the model organisms (*Acinetobacter* and *Clostridium*) arising from transformation and conjugation, respectively. As we are also interested in the role of trace/heavy metals in driving HGT, we shall subject a portion of each sample taken from the poGutMo to ICP-MS (WP4) so we can relate the frequency of HGT by transformation and conjugation to the concentration of metals within the sample. Work undertaken in WP4 and WP6 will identify the most likely drivers of HGT through transformation and the emergence of AMR. We suspect these will be a combination antibiotic/herbicide and heavy metals, including other environmental conditions that favour the development of transformation

competence. These candidate drivers will be evaluated in the gut model in **Task 2** using the approaches described for T1, but this time supplementing the poGutMo with appropriate concentrations of antibiotic, herbicide, cation. MC will take responsibility for delivery of this WP to time and to budget. Should he be absent for any reason, this responsibility will be undertaken by RLR. The laboratory work will be undertaken by the PDRA who will meet weekly with MC and/or RLR to review progress. The PDRA will be supported in the laboratory by other PDRAs working the gut models in the group of RLR.

Task: FED-AMR#-WP5-T1
Task start month 25
Task end month 36
Task Leader: Mark Chambers
Deputy Task Leader: Roberto La Ragione
Task Participants: PDRA

Description of the task: At the University of Surrey we use an *in vitro* gut model for chicken (avGutMo) [1] and more latterly have developed similar models of the horse (eqGutMo) and pig (poGutMo) gut. Sterile stirred batch culture fermentation vessels will be prepared and aseptically filled with sterile basal nutrient medium and adjusted to pH 7.0 before autoclaving. Once in the fermentation vessels, the sterile medium will be sparged with O_2-free N_2 overnight to maintain anaerobic conditions. Faecal slurry obtained from pig farms (sampled under WP2) will be mixed with pre-reduced sterile phosphate-buffered saline (PBS) (to yield a 10%, w/v, faecal slurry). Each vessel will be inoculated with the freshly prepared faecal slurry. The temperature of the fermentation vessels will be held at 37 °C. The pH within each vessel will be held between 6.4 and 6.6 by the addition of 0.5 M NaOH or HCl, controlled via pH meter controllers. Anaerobic conditions will be maintained by sparging the vessels with O_2-free N_2.

For studies of transformation within the gut model, we shall use *A. baylyi* as it exhibits very a high level of competence during normal growth. Specifically, we shall use a BD413 reference strain (trpE27) as it is spontaneously rifampicin resistant and is known to develop competence during growth [2]. This is tagged with a chromosomal copy of the *ecfp* gene (cyan fluorescent protein; *Aequorea Victoria*). Based on studies conducted *in vivo* in rodents, we anticipate that the basal level of transformation within the gut model will be relatively low [3]. Therefore, to maximise the likelihood of detecting transformants we aim to achieve a level of colonisation of the gut model with *A. baylyi* strain BD413 not less than 10^7 CFU per gram of content. The poGutMo will be allowed to stabilise for 24 hrs before the introduction of different quantities of *A. baylyi* strain BD413 (starting with 10^6 CFU). Samples will be removed from the poGutMo prior to inoculation (control) and then at regular intervals over the following 48 hrs and the concentration of *A. baylyi* strain BD413 within the poGutMo determined either by plating on LB agar-plates supplemented with rifampicin or using our quantitative real-time PCR to quantify *A. baylyi* directly. Efforts to maximise the concentration may include the addition of 0.2% lactate as this is known to stimulate growth and transformation of *A. baylyi in vitro* [4]. In this way **we shall determine the optimal growth parameters for cultivating *A. baylyi* strain BD413 within the porcine gut model and the time after inoculation at which its concentration is maximal (ST1)**.

We shall then use PCR amplicons as ARG donors to the poGutMo in tightly controlled pilot experiments as this will allow us to control the length (and the sequence) of the DNA fragments of interest **(ST2).**

Studies will then progress to the introduction of chromosomal DNA derived from *A. baylyi* strain KTG. This strain was derived through the insertion of the selectable kanamycin resistance gene (*nptII*) into the chromosome of strain BD413 of *A. baylyi* [4]. Chromosomal DNA will be isolated from a bacterial culture of strain KTG grown in LB medium supplemented with rifampicin and kanamycin and the concentration of DNA determined using a UV-spectrophotometer. Lysates of strain KTG will also be prepared by heat-treating an overnight culture at 80°C for 15 min, as per Nordgard *et al.* [3].

Fifty µg of chromosomal DNA from strain KTB will be introduced into the poGutMo at the point at which either the growth rate or concentration of *A. baylyi* strain BD413 is maximal **(ST3)**.
In the final sub-task, **chromosomal DNA will be replaced with lysate of strain KTG to see if this influences the recovery of transformed *A. baylyi* (ST4)**. Total CFU of *A. baylyi* strain BD413 and transformants of the strain in samples from the gut model will be determined by serial dilution and plating on LB agar supplemented with rifampicin for enumeration of total CFU of *A. baylyi* strain BD413 and on plates with rifampicin and kanamycin for transformant CFU determination. Calculated transformation frequencies will be given as the number of CFU growing on transformant selective plates divided by the number of CFU on recipient-selective plates. Controls will include the incubation of chromosomal DNA and bacterial lysate with DNAse before introducing them into the poGutMo.
For studies of conjugation-mediated HGT within the gut model we shall use *C. difficile* as conjugation is a common method of HGT in Clostridia; being demonstrated to occur both *in vitro* and *in vivo* [5]. The conjugal transfer machinery carried by clostridial plasmids is encoded by the *tcp* region. We shall use different *C. difficile* strains as plasmid donor and recipient, respectively. The strains will be cultivated in a suitable medium, such as tryptose sulphite cycloserine (TSC) broth. **Their ability to serve as plasmid donor and recipient will be confirmed by performing *in vitro* mating using the filter-mating method (ST1)** described by Farrow *et al.* [6].
Growth of these strains will be first determined in the gut model in the absence of any selective pressure (e.g. antibiotic). The poGutMo will be allowed to stabilise for 24 hrs before the introduction of different quantities of each strain individually or in combination. Samples will be removed from the poGutMo prior to inoculation (control) and then at regular intervals over the following 48 hrs and the concentration of each strain within the poGutMo determined by plating on TSC agar plates supplemented with the appropriate antibiotic. **In this way we shall determine the optimal growth parameters for cultivating the clostridial strains within the gut model (ST2).**
Conjugation-mediated HGT between the clostridial donor and recipient strains within the gut model will be determined using a seeding density for each strain that results in as close to equal concentration of each strain over a 48 hr period as possible. Rates of transconjugation will be calculated by taking samples from the gut model and plating on TSC agar plates supplemented with the appropriate antibiotics **(ST3)**.
Transconjuagtes will be characterised by whole-genome sequencing to examine the movement of plasmids and chromosomal elements using the approach of Lacey *et al.* [5]. Plasmid transfer rates via bacterial conjugation will be calculated using the endpoint method of Simonsen *et al.* [7] **(ST4)**.

[1] Card RM, Cawthraw SA, Nunez-Garcia J, Ellis RJ, Kay G, Pallen MJ, Woodward MJ, Anjum MF mBio. 2017; 8(4): e00777-17.
[2] Palmen R, Hellingwerf KJ. Gene. 1997;192(1):179-90.
[3] Nordgård L, Nguyen T, Midtvedt T, Benno Y, Traavik T, Nielsen KM. Environ Biosafety Res. 2007; 6(1-2):149-60.
[4] Nielsen KM, Bones AM, Van Elsas JD. Appl Environ Microbiol. 1997; 63(10):3972-7.
[5] Lacey JA, Keyburn AL, Ford ME, Portela RW, Johanesen PA, Lyras D, Moore RJ. Appl Environ Microbiol. 2017; pii: AEM.01814-17.
[6] Farrow KA, Lyras D, Rood JI. Microbiology. 2001; 147(Pt 10):2717-28.
[7] Simonsen L, Gordon DM, Stewart FM, Levin BR. J Gen Microbiol. 1990; 136(11):2319-25.

Sub-Task: FED-AMR#-WP5-T1-ST1
Sub-Task start month 25
Sub-Task end month 27
Sub-Task Leader: Mark Chambers
Deputy Sub-Task Leader: Roberto La Ragione
Sub-Task Participants: PDRA

Sub-Task: FED-AMR#-WP5-T1-ST2

Sub-Task start month 28
Sub-Task end month 30
Sub-Task Leader: Mark Chambers
Deputy Sub-Task Leader: Roberto La Ragione
Sub-Task Participants: PDRA

Sub-Task: FED-AMR#-WP5-T1-ST3
Sub-Task start month 31
Sub-Task end month 33
Sub-Task Leader: Mark Chambers
Deputy Sub-Task Leader: Roberto La Ragione
Sub-Task Participants: PDRA

Sub-Task: FED-AMR#-WP5-T1-ST4
Sub-Task start month 34
Sub-Task end month 36
Sub-Task Leader: Mark Chambers
Deputy Sub-Task Leader: Roberto La Ragione
Sub-Task Participants: PDRA

Deliverables

Ref	Title	Due month
D-JRP17-FED-AMR-WP5.1	Clostridial strains demonstrated to be suitable plasmid donor and recipient, respectively	M26
D-JRP17-FED-AMR-WP5.2	Optimal growth parameters for cultivating *A. baylyi* within the porcine gut model and the time after inoculation at which its concentration is maximal determined	M27
D-JRP17-FED-AMR-WP5.3	Optimal growth parameters for cultivating the clostridial strains within the gut model determined	M28
D-JRP17-FED-AMR-WP5.4	Results from pilot experiments using PCR amplicons as ARG donors	M30
D-JRP17-FED-AMR-WP5.5	Conjugation-mediated HGT between the clostridial donor and recipient strains within the gut model determined	M31
D-JRP17-FED-AMR-WP5.6	Results from using chromosomal DNA derived from *A. baylyi* as ARG donor	M33
D-JRP17-FED-AMR-WP5.7	Results from using lysate of *A. baylyi* as ARG donor	M36
D-JRP17-FED-AMR-WP5.8	Clostridial transconjuagtes characterised by whole-genome sequencing	M36

Milestones

Ref	Title	Due month
M-JRP17-FED-AMR-37	Bacterial strains supplied to UofS	M25
M-JRP17-FED-AMR-38	Porcine gut model set up using faecal samples obtained through WP2, samples stored for trace element analysis (WP4) – experiments can start	M26
M-JRP17-FED-AMR-39	Samples from gut model experiments stored for trace element analysis (WP4)	M30
M-JRP17-FED-AMR-40	Samples from gut model experiments stored for trace element analysis (WP4)	M31

M-JRP17-FED-AMR-41	Samples from gut model experiments stored for trace element analysis (WP4)	M33
M-JRP17-FED-AMR-42	DNA sent for whole-genome sequencing	M33
M-JRP17-FED-AMR-43	Samples from gut model experiments stored for trace element analysis (WP4)	M36

WP 6: Probabilistic and mechanistic models of the links between antimicrobial usage in animals, AMR in the environment, and the risks for public health.

Objectives

- Developing probabilistic models for predicting the antimicrobial phenotypes and analysis of genomic sequences
- Developing mechanistic models to elucidate environmental driven spatio-temporal changes observed in microbiological communities and how these are linked with AMR
- Use the models for informed risk assessment for public health

Description of work

- WP6 takes place over the first, the second and the third year of the project. It consists of two tasks (T6.1 and T6.2).

WP: Probabilistic and mechanistic models of the links between antimicrobial usage in animals, AMR in the environment, and the risks for public health.
WP start month: M25
WP end month: M54
WP Leader: Dr Giovanni Lo Iacono (GL)
Deputy WP Leader: Professor Payam Barnaghi (PB)
WP participants: GL, PB
Description of the WP: In line with Objective O5, WP6 is intrinsically multidisciplinary. Accordingly, we will use mathematical modelling and machine learning as a powerful tool to:
i) *Detect relevant variables (predictors) which can predict observed appearance and transmission of ARGs in different environmental compartments (Task 6.1).* To address Objective 4, in Task 6.1 we will use a machine learning approach, trained with data provided by WP4 and WP5 to identify the most relevant drivers of AMR emergence by analysing phenotypic and genotypic markers of AMR in association with environmental conditions, such as use of antibiotic and/or herbicide and trace element concentrations.
ii) *Gain insight of the mechanisms underlying transmission dynamics of resistant bacteria (Task 6.2).* Microbiological communities (either within host or in the natural environment) are affected by the external environmental drivers (e.g. temperature and pH) and by mutual influences within the community (e.g. competition for resources). Both factors impact on the spatiotemporal patterns of the community, potentially leading to the extinction/establishment of specific (e.g. resistant) microorganisms. In Task 6.2, we will develop and apply models to investigate if and how the resilience of microbiological communities changes when subjected to: i) different external drivers and ii) internal drivers represented, for instance by richer biodiversity; this last point will provide a theoretical testing of Hypothesis H2. Furthermore, we will investigate how perturbation of the environmental drivers (which we can reproduced in the porcine gut model, Task 5.1) can critically reduce/enhance the population of specific microorganism (*1*). This will lead to a better understanding of the mechanism, at population level, of extinction/emergence and transmission dynamics of resistant microorganisms (in line with objective O1 in WP2). The WP will also help in understanding some macro-ecological relationships observed in the gut (*2*) and potentially relevant to other systems.

iii) Assess and identify associated risks to public health and strategies to mitigate these (Task 6.1 and 6.2).

The WP will identify relevant environmental drivers (i.e. risk factors) of observed AMR. It will elucidate the possible mechanism leading to the observed spatiotemporal dynamics (increasing/decreasing the risk) of the microbiological communities. The modelling approach can be formulated and applied to within-host microbiological communities (e.g. in the gut) or in the natural environment (e.g. in soil). In the latter case, as these dynamics depends on environmental drivers which are varying geographically, the task is relevant to objective O3. Insights from the model will provide crucial information for risk management options for public health (WP6).

Finally, understanding how environmental perturbations affect the extinction or establishment of a pathogen will provide risk management options for the control of disease (e.g. developing a protocol which maximizes efficiency in the use of antibiotics by identifying optimal temporal patterns in their deployment (*1*)).

Task: FED-AMR#-WP6-T1
Task start month 25
Task end month 37
Task Leader: Payam Barnaghi (PB)
Deputy Task Leader: Dr Giovanni Lo Iacono (GL)
Task Participants: PB

Description of the task: **A Machine Learning Approach for AMR phenotype prediction and gene sequence analysis**. This tasks will focus on developing mathematical and machine learning models that will shorten the time and will significantly reduce the cost and resources required for the identification of AMR. This will be performed by analysing large volumes of genomic data and by detecting and interpreting the complex associations between the underlying AMR phenotypes. We will create methods that will allow to identify and predict which genes will contribute AMR.

Overall machines learn in two ways: by example or by experience. To create effective machine learning models in this domain we will create classification models that will predict AMR phenotypes by learning the genetic signatures. The existing models in this domain reply on probabilistic models such as Support Vector Machines (SVM) or Deep Learning models such as deep convolutional neural networks. The probabilistic models usually have several parameters that need to be set appropriately to allow the model perform at its best and this usually make it difficult to train models that can deal with changes in the data or work effectively with noisy data and missing values (which is common in real world datasets). The deep learning models often require large number of samples, can become overfit and their findings is not easy to interpret (as their do not provide any strong theoretical foundation for their outcomes). In this task we will create semi-supervised machine learning models that will use large volume of unlabelled data to learn and extract the features and complex interplays underlying these features and will then create probabilistic classifiers to learn from the existing labelled data (i.e. example) to predict the genomic signatures that cause AMR.

Sub-Task: FED-AMR#-WP6-T1-ST1

Data Integration, Annotation and Association Analysis. This sub-task will focus on integration of genomic and AMR data from multiple sources. We will use entropy based models and mutual information measures to identify the associations and determine AMR related phenotypes.

Sub-Task start month 25
Sub-Task end month 37
Sub-Task Leader: PB
Deputy Sub-Task Leader: GL
Sub-Task Participants: PB

Task: FED-AMR#-WP6-T2
Task start month M25

Task end month M54
Task Leader: GL
Deputy Task Leader: PB
Task Participants: GL, MAC, PDRA
Description of the task: T6.2 takes place over the first, the second and the third year of the project. T6.2 is split into 2 sub-tasks, representing two broad specific scientific questions identified in point (*ii)* in the description of the WP6 ("Gain insight of the mechanisms.."); these issues will be investigated in parallel with point (*iii)* to identify which mechanisms and how they are potentially responsible for increasing the risk for public health. Sub-tasks T6.2.1 takes place over the first year of the project for 15 months. Sub-tasks T6.2.2 takes place over the second-half of the second year for the remaining 15 months to the end of the project.
Sub-Task: FED-AMR#-WP6-T2.1
In this subtask we will address two questions:
a) What is the resilience of microbial communities in the different situations (e.g. environmental conditions, geographical compartments, etc.)
b) How is resilience affected by the diversity and 'richness' of the microbial community? (H1)
Resilience is the ability of a community to recover from perturbation. Example of perturbations are the introduction of an antibiotic targeting particular bacteria in the gut, or fluctuations in temperature affecting the survival of free-living bacteria in the soil. Resilience is expected to depend on environmental drivers and also on the richness and diversity of the community.
Inspired by models of ecological communities (*3*), we will develop a dynamic model for the population of the micro-organism (either within host or in the environment) as a set of differential equations, to track the changes in the abundance of each microbiological subpopulation (e.g. bacterial species or aggregated species if their number is too large). Important interaction mechanisms, such as competition for shared resources, will be included. Resilience will be studied by investigating the stability of solutions in different settings similar to what has been done previously (*4*).
GL will supervise the task while an appointed PDRA will lead the work. We will interact with relevant member of the consortium (e.g. MAC, PA) for refinement of scientific questions, formulation of assumptions, feedback on the modelling approach and results etc.
Sub-Task start month M25
Sub-Task end month M37
Sub-Task Leader: GL
Deputy Sub-Task Leader: PB
Sub-Task Participants: GL, PB, PDRA

1. G. Lo Iacono, F. van den Bosch, C. A. Gilligan, *PLos Comp. Bio.* **9**, e1002870 (2013).
2. B. W. Ji, R. U. Sheth, P. D. Dixit, D. Vitkup, *bioRxiv*. **1**, 370676 (2018).
3. R. M. (Robert M. May, *Stability and complexity in model ecosystems* (Princeton University Press, second., 2001).
4. G. Lo Iacono *et al.*, *Proc. Natl. Acad. Sci.*, 201803264 (2018).

Deliverables

Ref	Title	Due month
D-JRP17-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
D-JRP17-FED-AMR-WP6.2	Findings presented at one international conference and one national conference.	M36
D-JRP17-FED-AMR-WP6.3	Update of codes and documentations in public repository (e.g. GitHub).	M36

Milestones		
Ref	**Title**	**Due month**
M-JRP17-FED-AMR-53	Literature review on concept of resilience and modelling in microbial communities.	M27
M-JRP17-FED-AMR-54	Identification of relevant available data. Formulation and implementation of the model for the microbiological community within-host. Conditioned to data availability, potential extension of the model to natural environment (e.g. soil).	M30
M-JRP17-FED-AMR-55	Application of the model to address specific questions and dissemination of findings in conferences, preparation of draft papers.	M36

2.4.2.1.15 JRP05-R1-ET1-TOXdetect

Reference to the Stategic Research Agenda (please refer to D2.7)	**ET 1: Development and harmonisation of non-NGS-based methods for detection of FBZ agents and emerging threats**									
Research Project Title	**Development and harmonization of innovativemethods for comprehensive analysis of food-borne toxigenic bacteria, ie. Staphylococci, Bacillus cereus and Clostridium perfringens**									
Research Project Acronym	**TOX-Detect**									
Leading Organisation	**P1-Anses**					Deputy Leading Organisation		**P1-Anses**		
Project Leader	**JA Hennekinne**					Deputy Project leader		**Yacine Nia**		
Project Start month	**M1**					Project End month		**M36**		
Participant	**1**	2	**4**	6	7	8	**9**	10	11	12
	Anses	Ages	**Sciensano**	NDRVMI	SZU	VRI	**BfR**	FLI	RKI	DTU
PM	**32.76**		**5.5**				**10**			
Participant	13	14	15	16	17	18	**19**	**20**	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	**INRA**	**IP**	APHA	PHE
PM							**22**	**9.3**		
Participant	23	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
Participant	**33**	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM	**3**									

Description of work

<u>WP0</u> Coordination, management and communication
WP0 continues based upon what was achieved during the first and second annual period of the EJP WP start month: M0
WP end month: M36
WP Leader: Jacques-Antoine Hennekinne
(P1) Deputy WP Leader: Frédéric Auvray
(P1) WP participants: P1, P4, P9, P18, P19

Description of the WP:

General management strategy

The overall purpose of the management structures 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. This will ensure that all tasks and research objectives are realised effectively and at a high quality level within time and budget. Due to the structure of the work packages and the relatively low number of partners, a very lean, thus flexible management structure and a clear decision-making process was chosen to avoid creating an unduly hierarchical system or an unnecessary administrative burden.

The project management will be subdivided into several sub-domains as follows:
Administrative and scientific management: Administrative management is the responsibility of the Project Coordinator (PC) who will delegate tasks directly to project partners. The scientific management of each of the work packages is the responsibility of the relevant WPL who reports to the PC.
Financial and legal management: Overall, the financial management of the budget will also be the responsibility of the PC, who will, should the need arise, formulate any required redistribution of the overall budget in cooperation with the WPL. Financial management of the individually allocated parts of the budget and all required reporting will be the sole responsibility of the individual institution.
Overall legal management will be coordinated by the PC. Management of intellectual property rights (IPR) is the primary responsibility of the data or intellectual property creator.
Although direct communication between all consortium members is preferred and strongly encouraged, project-internal communication will be ensured through the establishment of clear communication channels and reporting structures within each WP by the WPL and between all project partners by the PC. All information materials like flyers, leaflets etc. as well as scientific publications like posters, presentations or journal articles will be made available to partners for approval. Each partner has also the right to veto its name being mentioned on any publication.

Management structures

Project coordinator (PC)

The PC is named in the contract with the EJP and acts as the intermediary between the contractors and the EJP.
The main responsibilities of the Coordinator are the following:

- General coordination of the project
- Submit reports and deliverables to the EJP, in particular periodic scientific and management reports
- Participate in and be responsible for coordination of meetings
- Ensure the timely interaction and communication between the WPs
- Ensure the necessary information and document flow between the partners
- Ensure that all necessary legal and ethical responsibilities and obligations are known to the consortium institutions and that they are acted upon
- Overall responsibility for financial management, including budgeting, resource allocation and distribution and financial controls
- Ensure external communication, information and dissemination of knowledge.

Work Package Leader (WPL)

Each WP has one WPL. Deputy WPL can be nominated. In case any replacement should become necessary, a new WPL will be appointed by partners. The main responsibilities of the WPLs are the following:

- Ensure the timely implementation of their WP

- Attain all deliverables
- Communicate whenever milestones are reached or delayed
- Suggest solutions on how delays can be avoided or their effects minimised
- Issue a detailed project work plan.

Meetings and reports

Although electronic communication will be used extensively for the implementation of the WPs and the management of the project, face-to-face meetings are nevertheless an essential part of research and the running of any project. They further team building, help to develop mutual respect as well as confidence and, in many situations, provide the most effective mechanism for trouble-shooting of arising difficulties.

Consortium meetings with partners will take place at the beginning (kick-off meeting, meeting 0), after 18 mounts (meeting 1), after 24 months (meeting 2) and at the end of the project (wrap-up meeting, meeting 3).
Minutes and reports on all formal meetings and decisions will be distributed to all partners. Great importance will be attached to regular reporting during the whole project period. Where appropriate work package leaders will provide internal reports to the PC twice yearly. These will contain information on work completed during the period, work planned for the next period and work behind schedule.
Although the coordinator has overall responsibility for the preparation of the financial reports, it is the sole responsibility of each participating institution to ensure proper accounting and timely reporting to the PC and the EJP. As link between the project contractors and the EJP, the coordinator will be responsible for scientific reporting to the EJP.

Risk management

Changes necessary in order to deliver milestones on time as well as delays and problems in achieving the milestones will be reported in advance of partner meetings by the WPL, including a suggestion for alternative strategies. Modifications to the project made possible by newly available materials and techniques will be discussed, evaluated and, if considered appropriate, implemented in a coordinated manner, as will possible changes in emphasis based on developments in perceived threats and newly published work appearing in the literature.

Task 0.1: General coordination and management of the project (administrative and financial)

- T0.1 continues based upon what was achieved during the first and second annual period of EJP.

Task 0.2 to 0.5: Organisation of four face-to-face meetings with all partners.

- T0.5 Meeting 3: results of technical WP4 and WP5 and overall results of the project
- T0.5 takes place over the third annual period of the EJP.

Task 0.6: mandatory reports on network activities: interim activity report, final report

- T0.6 continues based upon what was achieved during the first and second period of the EJP.

<u>WP2.</u> Characterization of toxins/virulence factors

WP2 continues based upon what was achieved during the first and second annual period of the

EJP. WP start month: M4

WP end month: M30

WP Leader: **Nalini Rama-Rao** (P18)

Deputy WP Leader: **Valérie Fessard**

(P1) WP participants: P1, P18, P19

Description of the WP:

Background and objectives

Although the main causes of pathogenicity for toxin-producing bacteria such as Bacillus cereus and Clostridium perfringens are well known today, the mechanisms responsible for the expression of this virulence need, however, more investigations.

For B. cereus, because there was a tendency in the past to consider all B. cereus strains as potentially pathogen, food microbiological diagnostics were focused on species determination using conventional culture-based methods. However the pathological potential of the strains ranges from neutral to highly virulent. Therefore, in order to characterize pathogenic stains isolated from food poisoning outbreaks, methods have been developed to detect toxin genes using PCR-based diagnostic tools for the three main enterotoxins (Nhe, Hbl and CytK) as well as for the emetic toxin (cereulide). However, only the emetic strains (which carry the ces gene) and rare diarrheal strains harboring the cytK1 gene could be assigned as clearly pathogenic while the other factors contributing to B. cereus-induced gastroenteritis remain unknown.

Similarly for C. perfringens, even if 17 different toxins have been described, this bacterial pathogen is currently classified in five toxinic types (A to E) only based on the production of 4 major toxins (alpha, beta, epsilon and iota). The C. perfringens type A that produces CPE toxin is a major cause of food-borne gastroenteritis (FBGE) whereas the role of other virulence factors remains poorly investigated.

Several data also indicate that, for these two pathogens, the level of toxin production could play a major role in determining the pathogenic potential of a particular strain, while the presence of specific toxin genes appears to be less discriminating. In addition, although cytotoxicity assays have already been used for classifying bacterial strains according to their toxic potency, further investigation on the toxicity mechanisms involved has been rarely carried out.

Approach

For toxigenic bacteria such as B. cereus and C. perfringens whose pathogenic potential remains to be further defined, two complementary approaches will be used in parallel. On one hand, candidate toxins and/or virulence factors will be characterized using automated fluorescence microscopy and in vitro and cellular tests. In order to correlate the results with a “true” toxicity of the different strains at the intestinal level, toxicity testing will be conducted on a human intestinal cell line for viability measurement as well as for identification of the molecular mechanisms of toxicity (apoptosis, DNA damage, pro-inflammatory effects, oxidative stress and mitochondrial membrane potential) using High Content Analysis. This method is based on automated image acquisition and analysis, thereby rapidly generating large amounts of quantitative biological data from cellular imaging. On the other hand, reverse-transcriptase (RT)-PCR and general transcriptomic assays will also be used to assess virulence and toxin gene expression, from bacteria grown as pure cultures. The expression patterns of bacterial toxins/virulence factors will then be correlated to the identification of specific toxicity profiles, which could provide a toolbox for the prediction of the toxicity of the bacterial strains.

Task 2.3. Correlation of specific toxicity profiles with expression patterns of bacterial toxins/virulence factors

Task start month: M25

Task end month: M30

Task Leader: Nalini Rama Rao (P18)

Deputy Task Leader: Valérie Fessard

(P1) Task Participants: P1, P18, P19

Description of the task:

The specific toxicity profiles identified in T2.1. will be correlated with the expression patterns of bacterial toxins/virulence factors identified in T2.2. in order to provide a toolbox for the prediction of the toxicity of specific strains of the different bacterial species investigated. This work will allow the identification of gene expression signature to distinguish pathogenic strains.

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

WP3 continues based upon what was achieved during the first and second annual period of

the EJP WP start month: M4

WP end month: M30

WP Leader: Mirjana Andjelkovic

(P4) WP participants: P1, P4, P18,

P19

Description of the WP:

Background and objectives

The aim of this WP is to ensure the development and/or optimisation of LC-MS/MS methods for detection of bacterial toxins. The main objective is to deliver dedicated methods using different proteomic approaches (bottom-up, top down and targeted quantification) for the detection of toxins of interest. To accept the results in food control purposes it will be necessary to work on the harmonization of the quality criteria. These criteria are well defined in the legal frameworks describing the monitoring and controlling of the food safety. The new proteomic approaches do not have either specific guidelines or standards about the acceptable quality criteria enabling the food safety authorities to make decisions. The outcome of the WP3 should be adoption of the acceptance criteria as decided on the basis of the results obtained from the developed and tested methods.

Another aspect that will be elaborated in the WP3 is the sample preparation techniques and optimization of all steps of the workflow (protein extraction, LC separation including column length, type and gradient, MS method). This may be detrimental for the analyses of the toxins in highly different and sometimes highly protein content food matrices.

In particular different mass-spectrometry based proteomics approaches will be used to

target Detection of toxins including:

- currently undetectable enterotoxins produced by S aureus (eg SEG, SEH, SEI),
- known toxins from B cereus (cereulide),
- known toxins from C. perfringens (CPE)

Detection of virulence factors of B. cereus in order to distinguish between pathogenic and nonpathogenic strains

Approach

In this WP, two different mass-spectrometry based proteomics approaches will be used to identify and quantify toxins and virulence factors of S. aureus, B. cereus, and C. perfringens and separate pathogenic vs non-pathogenic strains.

- Targeted approach (bottom-up LC-MS/MS) will be undertaken in discovery mode. The objective here is to achieve a comprehensive analysis of the different bacterial proteomes and assess the presence of toxins, virulence factors, as well as protein biomarkers differentiating pathogenic and non-pathogenic strains. The analyses will be performed on state-of-art equipment. The bottom-up approach relies on the analysis of proteins after enzymatic digestion (usually trypsin). Shotgun LC-MS/MS experiments on bacterial samples can lead to the confident identification, in routine, of thousands of proteins.
- Non targeted approach (top-down proteomics) will be used in discovery mode. Top-down proteomics is an emerging technology based on the analysis of intact proteins using high-resolution mass spectrometry. A great advantage of top-down proteomics is its ability to address protein variations and characterize proteoforms arising from alternative splicing, allelic variation, or post-translational modification (PTM). Top-down proteomics provides a bird's eye view of all proteoforms and thus information closely connected to complex disease phenotypes. By studying intact proteins rather than peptides, top-down proteomics also neatly sidesteps the so-called “inference problem” that is inherent in the bottom-up workflow (the same peptide can be shared by several proteins which can lead to ambiguous identification).

Task T3.1: development of Mass Spectrometry-based methods for the detection of new enterotoxins (eg SEG, SEH, SEI) from S. aureus

T3.1 continues based upon what was achieved during the first and second annual period of

the EJP Task start month: M4

Task end month: M27

Task Leader: Mirjana Andjelkovic

(P4) Task Participants: P1, P4

Description of the task:

To date, more than 20 S. aureus enterotoxins (SEs) or enterotoxin-like proteins (SEIs) have been identified. However, recognised detection methods are available for only a few of them (SEA to SEE), and are limited to ELISA based techniques or confirmation by PCR analyses. Direct link between the toxin concentration present in a sample and its toxic potential is thus interrupted. Therefore, task T3.1 will provide a strategy platform for detection of other enterotoxins and offer possibility to estimate the toxic dose using bottom-up proteomics. Three enterotoxins (SEG, SEH, SEI) were selected according to their estimated high prevalence in staphylococcal food poisoning outbreaks. The major output of the task T3-1 will be the optimisation and assessment of the analytical method for SEG, SEH and SEI enterotoxins for which no commercial tests are yet available.

Task T3.2: development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from B. cereus

T3.2 continues based upon what was achieved during the first and second annual period of the

EJP. Task start month: M4

Task end month: M27

Task Leader: Julia Chamot-Rooke

(P19) Task Participants: P1, P4, P19

Description of the task:

The approach that will be used for detection of B. cereus toxins and virulence factors will be searched using a combination of bottom-up and top-down proteomics approaches. In this task, the current LC-MS and MALDI-ToF MS methods will be evaluated together with available reference materials and standards, as well as their applicability in the detection purposes. The major answers to be given with the results is how to distinct the pathogenic from non-pathogenic strains in various sample types from B. cereus related food intoxications; and at which level these toxins may be detected in the sample. Specific targets starting from our current experience will be the emetic toxin (cereulide) and any possible analogues that would arise from the discovery analyses. We will also try to assess the presence of other toxins such as the major enterotoxins HBL, NHE and T.

Task T3.3: development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from C. perfringens

T3.3 continues based upon what was achieved during the first and second annual period of the EJP.

Task start month: M4

Task end month: M27

Task Leader: Michel Hebraud (P18)

Task Participants: P1, P18, P19

Description of the task:

A bottom-up proteomic approach will be performed on state-of-art equipment installed in partner
P18). Shotgun LC-MS/MS experiments on bacterial samples can lead to the confident identification,
in routine, of thousands of proteins. Specific focus will be done on the C. perfringens enterotoxin (CPE) responsible for causing the gastrointestinal symptoms of several C. perfringens food- and nonfood-borne human gastrointestinal diseases.

Task T3.4. Transfer of LC-MS/MS methods

T3.4 continues based upon what was achieved during the second annual period of the EJP

Task start month: M24

Task end month: M30

Task Leader: Mirjana Andjelkovic (P4)

Task Participants: P1, P4, P18, P19

Description of the task:

The new LC-MS/MS methods developed will be in-house validated. For each analytical method a detailed analytical protocol and validation report will be written. These protocols will include

all QA criteria which have to be fulfilled for a sensitive and reliable analysis. Among others, these QA parameters are scope definition, analytical range, chromatographic separation/selectivity, sensitivity - Control of LOD and LOQ, control of the calibration standards and exchange of standard solutions, recovery of the isotopically labelled internal standards (if available), matrix additions experiments at regular intervals, duplo analysis at regular intervals, control of the blank concentrations/ blank samples, quality control during analyses. Once validated the methods will be transferred to the partners of the WP3 in order to test applicability of the methods before further implementation in WP5. As an important step towards harmonisation of technical approaches, performance criteria for those developed analytical techniques will be defined. This will be requirement set up for WP5 participants.

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

WP4 continues based upon what was achieved during the first and second annual period of the EJP.

WP start month: M4

WP end month: M33

WP Leader: Alexandra Fetsch (P9)

Deputy WP Leader : Michel Gohar (P18)

WP participants: P1, P4, P9, P18, P33

Description of the WP:

Background and objectives

The available immune-enzymatic methods do not cover the entire spectrum of SEs as only five SE serotypes (namely SEA, SEB, SEC, SED, and SEE) out of 23 SE types are currently detectable. Moreover, epidemiological data indicate that there is an increase of human cases caused by emerging S. aureus strains producing other types of toxins such as SEG, SEH, and SEI. On the other hand, also a variety of B. cereus specific toxins currently lack appropriate detection methods hampering valid characterization of the pathogenicity, in particular of diarrheal strains. Therefore, the aim of this WP is to facilitate the investigation of foodborne outbreaks and toxigenic S. aureus and B. cereus strains by enlarging the scope of already available diagnostic tests.

Approach

We plan to develop (quantitative) immunoassays fit for purpose to detect staphylococcal and B. cereus specific virulence factors. More precisely, these assays will cover targets allowing for the detection of at least two SE-types other than SEA-SEE, e.g. SEG, SEH, and/or SEI, and for at least three B. cereus specific toxins, e.g. Hbl (HblL1, HblL2, HblB), Nhe (NheA, NheB, NheC), CytK2, the phospholipases (PlcA, PlcB, Smase), and/or any new virulence factor or toxin variant specific to diarrheal strains. For B. cereus, these assays will be different from the assays commercially available today for Hbl, Nhe and CytK, which do not all allow for their quantification.

Our approach foresees the cloning of the various targeted toxins of interest in expression vectors, their overproduction in Escherichia coli and subsequent purification. Polyclonal, and/or (preferably) monoclonal antibodies raised against these toxins will then be selected for their affinity, specificity and compatibility with different food matrices (e.g. milk/milk products, meat/meat products)

Moreover, for the development of immunoassays to detect SE we plan to run a ‘cell-free’ approach in comparison to the ‘classical’ approach as described before at least for one of the three SE types SEG, SEI and SEH, resp. In this procedure, the constituents of the cell are used to produce a certain

target protein. The basic principle of the cell-free reaction is the same. Crude extracts are generated from cultured cells and depleted of endogenous DNA and mRNA, and the lysate is subsequently supplemented with energy components and free amino acids. The translational process is initiated by the addition of a suitable template (linear or circular DNA or mRNA), and carried out at an appropriate temperature for the chosen system.

WP4 will operate in close collaboration with WP2 dedicated to the characterization of (i) known virulence factors (Nhe, Hbl, CytK, SEG, SEH and SEI) and (ii) new virulence determinants highlighted using cytotoxic assays coupled to qRT-PCR and transcriptomic approaches (see WP2).

Moreover, the feasibility of the developed assays will be tested in a ring trial between the project partners (see WP5) using artificially and naturally contaminated food or culture supernatants allowing for the immediate set-up in routine analyses.

Task T4.1. Development of quantitative immunoassays for five known S. aureus and B. cereus toxins and virulence factors

T4.1 continues based upon what was achieved during the first and second annual period of the

EJP. Task start month: M4

Task end month: M32

Task Leader: Alexandra Fetsch (P9)

Deputy Task Leader: Michel Gohar

(P18) Task Participants: P9, P18

Description of the task:

2 SE-encoding genes from CPS (e.g. SEG, SEI, SEH) and 3 toxin-encoding genes from B. cereus (e.g. Hbl, Nhe, CytK2) will be selected and genetic tools allowing protein overexpression will be constructed. Production of proteins will be performed using (i) a classical approach for 2 SEs from CPS and 3 toxins from B. cereus, and (ii) a 'cell-free' approach for 1 SE from CPS. Polyclonal or monoclonal antibodies will be produced and immuno-enzymatic assays will be designed. The results obtained with antibodies derived from classical and cell-free approaches will be compared.

Sub-Task **sT4.1.4. Design of immunoenzymatic assays**

sT4.1.4 continues based upon what was achieved during the second annual period of the

EJP Sub-Task start month: M17

Sub-Task end month: M32

Sub-Task Leader: Alexandra Fetsch (P9)

Deputy Sub-Task Leader: Michel Gohar

(P18) Task Participants: P9, P18

Task T4.2. Development of a quantitative immunoassay on a new B cereus toxin or virulence factor

T4.2 continues based upon what was achieved during the second annual period of the

EJP. Task start month: M18

Task end month: M32

Task Leader: Michel Gohar

(P18) Task Participants: P18

Description of the task:

Further to the identification and characterization of new B. cereus candidate virulence factors in WP2 and to NGS in WP1, one of these candidate factors will be selected for the development of a dedicated immuno-enzymatic assay.

Sub-Task **sT4.2.1 Construction of a genetic tool for protein overexpression**

sT4.2.1 continues based upon what was achieved during the second annual period of the EJP

Sub-Task start month: M18

Sub-Task end month: M26

Sub-Task Leader: Michel Gohar (P18)

Sub-Task Participants: P18

Sub-Task **sT4.2.2. Protein production**
sT4.2.2 continues based upon what was achieved during the second annual period of the EJP

Sub-Task start month: M24

Sub-Task end month: M27

Sub-Task Leader: Michel Gohar (P18)

Sub-Task Participants: P18

Sub-Task **sT4.2.3. Development of specific Ab (poly/monoclonal)**

Sub-Task start month: M25

Sub-Task end month: M28

Sub-Task Leader: Michel Gohar (P18)

Sub-Task Participants: P18

Sub-Task **sT4.2.4. Design of the immunoenzymatic assay**

Sub-Task start month: M27

Sub-Task end month: M32

Sub-Task Leader: Michel Gohar (P18)

Task Participants: P18

WP5. Inter-laboratory ring trial scheme

WP start month: M25

WP end month: M34

WP Leader: Yacine Nia

(P1) Deputy WP Leader:

/

WP participants: P1, P4, P9, P18, P19,

P33 Description of the WP:

Background and objectives

The inter-lab comparison tests will enable the transfer of the methods developed in this project to different partners within the consortium and will allow the identification of success factors critical for bacterial strains characterization and detection of their toxins. They will also help in identification of "best practices" for the analysis of these toxins. Based on the results and evaluation of the inter-lab tests, an identification of critical gaps in the detection technology, both under qualitative and quantitative aspects will be carried out. These ring tests are prerequisite for the use of the developed methods in routine analysis in the longer term.

Eight inter-laboratory comparison tests will be organized in order to check performance of (i) MALDI-ToF based analysis for a selection of reference bacterial strains as established in the frame of the WP1, and (ii) Mass Spectrometry methods and immuno-enzymatic assays developed in the framework of WP3 and WP4, respectively.

Approach

The inter-lab tests will be organized in order to transfer and assess the methods established or developed in WP1, WP3, and WP4. In total, 8 inter-lab tests will be organized within the framework of WP5 and more than 8 laboratories from the EJP project will participate. As the methods tested will be developed by a few laboratories from a limited number of instruments, the inter-lab tests will be performed at a larger scope using various additional instruments, with some variations expected according to the method. The differences in the results obtained from the participating laboratories will be analyzed in depth in order to optimize the interpretation procedures of raw data and develop more robust methods. This will also be a basis for harmonization of the methods by establishing the general criteria. Results of the inter-lab tests will not be used therefore as a certification of the participating laboratory's proficiency.

The 8 inter-lab tests will be organized and planned in year 3 of the project, after the complete transfer and the successful implementation of the methods by the participating laboratories. For MALDI-ToF and Mass Spectrometry based methods, the inter-lab comparison tests will be organized to evaluate the methods performance for detection of the three pathogens (i.e. CPS, Bc and Cp) and their toxins (10 to
15 strains or culture supernatants in total per pathogen) while for immuno-enzymatic assays, the interlab comparison tests will be organized to evaluate the detection capabilities of the participants for two CPS toxins and three B. cereus toxins. The final selection of the toxins to be assessed in WP5 will be confirmed after the deliverables from linked WPs (i.e. WP3 and WP4).

These inter-lab tests will be prepared according to the regulation ISO/IEC 17043 (inter-lab test announcement, tests schedules, instructions for the participants, samples preparation and identification, homogeneity and stability studies, reporting forms…). The WP5 leader will prepare the inter-lab tests documents according to this regulation, and task leaders will adapt them to the specific characteristics of each method. For each inter-lab test, an organizer and a list of participants have been identified. The organizer will prepare the inter-lab items, perform the homogeneity and stability tests, and organize the dispatch of items. The task leader and the organizer will prepare together the inter-lab evaluation report. If needed, possibly further collaborators within the EU-RL/NRLs network or other laboratories will be invited to participate to the trials. Finally, a final report including the results and assessment of the eight inter-lab tests will be prepared by the WP5 leader.

Task T5.1. Inter-lab test on Maldi-ToF for species identification

Task start month: M25

Task end month: M29

Task Leader: Taran Skjerdal (P33)

Deputy Task Leader: /

Task Participants: P1, P4, P9, P19, P33

Description of the task:

For Maldi-Tof, the inter-laboratory comparison testing will be organized for the three pathogens: S.
aureus, B. cereus and C. perfringens (10 to 15 strains in total/pathogen).

Task T5.2. Inter-lab test on LC-MS/MS

Task start month: M28

Task end month: M33

Task Leader: Mirjana Andjelkovic (P4)

Deputy Task Leader: /

Task Participants: P1, P4, P18, P19

Description of the task:

For Mass Spectrometry, the inter-laboratory comparison testing will be organized for the three
pathogens: S. aureus, B. cereus and C. perfringens (10 to 15 culture supernatants in total/pathogen).

Task T5.3. Inter-lab test on immuno-enzymatic assays

Task start month: M30

Task end month: M34

Task Leader: Yacine Nia (P1)

Deputy Task Leader: /

Task Participants: P1, P4, P18, P33

Description of the task:

For immuno-enzymatic assays, the inter-laboratory comparison testing will be organized for two S.
aureus toxins, and three bacillus toxins/factors (contained within culture supernatants).

WP6. Dissemination, protection and exploitation of results

WP6 continues based upon what was achieved during the first and second annual period of the EJP.

WP start month: M0

WP end month: M36

WP Leader: Jacques-Antoine Hennekinne (P1)

Deputy WP Leader: Michel Gohar (P18)

WP participants: P1, P4, P9, P18, P19, P33

Description of the WP:
As described in WP0, the partners will set up a committee, composed of at least one representative from each partner, which will be in charge of evaluating the results, and suggesting appropriate means of protection and valorization of the results. For results concerning fundamental research or public health, the results are meant to be freely published through both oral presentations and publications in open access journals. If some of the results may constitute an invention which can be protected by a patent application, publication will be delayed until the

proper protection is sought. If results might be commercially exploited, the committee will be in charge of suggesting plans for their exploitation (choice of a party in charge of valorization, actions for finding one or several commercial exploitation partners). Exploitation, ruled by a commercial exploitation license, will include a fair financial return for the (co)owners. If an application is predicted, the patents service of the institutions hosting the research teams associated to the discovery will also be consulted, to obtain help with the administrative tasks linked to filing a patent. A consortium agreement will also be prepared by the partners that will detail the rules concerning intellectual property rights, classification of results stemming from the project and their valorization.
Partners, through their respective valorization departments, will take appropriate decisions to protect results and to file a patent regarding the developed assays. The scientific and technico-economic outcome of these findings will be assessed by realizing a technico-economic study of the trade environment, of the technological and legal constraints, and of the market expectations. An analysis of the freedom to operate will be performed, in which the filed patent dependence upon patents held by third parties will be examined. Partners will also search for industrial partners, by elaborating and distributing technological offers to targeted private firms. Terms and conditions of the technology transfer (license or license option according to development stage of the invention) will be negociated with industrial partners. A support group will be organized to follow up the administrative and financial issues of the contractant, and to keep the invention on the forefront of the technology. Results not directly involved in the patent filing will be published in open access peer-reviewed journals and presented in international meetings.

Task T6.1: dissemination of information within the partners

T6.1. continues based upon what was achieved during the first and second annual period of the EJP

Task start month: M0

Task end month: M36

Task Leader: Jacques-Antoine Hennekinne (P1)

Deputy Task Leader: Michel Gohar (P19)

Task Participants: all participants involved

Description of the task:

Based on the developed methods, an exchange of standard operating procedures and tools will be initiated, wherever possible.

Task T6.2: dissemination of information to the outside.

T6.2. continues based upon what was achieved during the first and second annual period of the EJP

Task start month: M0

Task end month: M36

Task Leader: Jacques-Antoine Hennekinne (P1)

Deputy Task Leader: Michel Gohar (P19)

Task Participants: all participants involved

Description of the task:
Presentation of network activities at international conferences (talks, posters, flyer) and to respective national and international decision makers in different networks and institutions
Publication of results in open access peer-reviewed journals

Deliverables		
Ref	**Title**	**Due month**
D0.4	Report of the meeting 2	M25
D0.5	Report of meeting 3	M36
D0.6	Final report	M36
D2.3.	Report on correlation between RNAseq data analysis and toxicity assays (including toolbox for toxicity prediction)	M30
D3.1	Report on Mass Spectrometry-based methods for the detection of new enterotoxins (eg SEG, SEH, SEI) from S. aureus	27
D3.2	Report on Mass Spectrometry-based methods for the detection of cereulide analogs and enterotoxins from B. cereus	27
D3.3	Report on Mass Spectrometry-based methods for the detection of CPE and virulence factors from C. perfringens	27
D3.4	Report on the performance criteria for method harmonisation	30
D4.1.	Report on the immuno-enzymatic assays	M33
D5.1.	Inter-lab tests documents according to ISO/IEC 17043 regulation, adapted to each method	M25
D5.2	Final report including results from the eight inter-lab tests	M34
D6.1	Dispatch of SOPs	M33
D6.2	Dissemination of results (publications, conferences…)	M36
Milestones		
Ref	**Title**	**Due month**
MS2.5	Correlation between toxicity profiles and expression patterns assessed.	M30
MS3.3	Methods transferred to partners	30
MS4.3	Immuno-enzymatic assays designed for known S. aureus and B. cereus toxins	M32
MS4.4	Genetic tools constructed for over-expression of a new B. cereus toxin/virulence factor	M26
MS4.5	Antibodies produced against new B. cereus toxin/virulence factor overproduced	M32
MS4.6	Immuno-enzymatic assays designed for a new B. cereus toxin/virulence factor	M33
MS4.7	Immuno-enzymatic assays transferred to partners	
MS5.1	Dispatch of inter-lab tests documents (according to ISO/IEC 17043 regulation) adapted to each method	M25
MS5.2	Dispatch of samples and evaluation report to be filled by partners	M27
MS5.3	Samples analysed by partners	M34
MS5.4	Dispatch of final report	M34
MS6.1	Final report dispatched	M36

2.4.2.1.16 JRP16-R2-ET2.2-TELE-Vir

Reference to the Stategic Research Agenda (please refer to D2.7)	**ET 2.2: Development of a toolkit to characterize emerging threats by combining genomic and phenotypic information**									
Research Project Title	**Point-of-incidence toolbox for emerging virus threats**									
Research Project Acronym	**TELE-Vir**									
Leading Organisation	**P13-SSI**					Deputy Leading Organisation			**P16-INIA**	
Project Leader	**Anders Fomsgaard**					Deputy Project leader			**Jovita Fernández-Pinero**	
Project Start month	**M25**					Project End month			**M54**	
Participant	**1**	2	**4**	6	7	**8**	9	10	11	12
	Anses	Ages	**Sciensano**	NDRVMI	SZU	**VRI**	BfR	FLI	RKI	DTU
PM	**2**		**7**			**0**				
Participant	**13**	14	15	**16**	17	18	19	20	21	22
	SSI	UT	VFL	**INIA**	UCM	MVNA	INRA	IP	APHA	PHE
PM	**29**			**3.1**						
Participant	**23**	24	25	26	27	**28**	**29**	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	**IZSAM**	**IZSLER**	RIVM	WbvR	FHI
PM	**9.7**					**7.5**	**1**			
Participant	**33**	**34**	35	**36**	37	38	39	40	**41**	
	NVI	**PIWET**	INIAV	**INSA**	IISPV	IC	SLV	FOHM	**SVA**	
PM	**1.33**	**7**		**26.31**					**6**	

Objectives

The overall objective is to develop a very fast point-of-incidence (poi) toolbox for identification and characterization of potentially all emerging virus threats for humans and/or domestic and wildlife animals.

Description of work for the first year of the TELE-Vir project

<u>WP1: Coordination and data management</u>
WP start month: M25
WP end month: M54
WP Leader: Maiken W. Rosenstierne, SSI

Deputy WP Leader: Miguel Ángel Jiménez-Clavero, INIA
WP participants: All
Description of the WP1: WP1 is the coordination WP and takes place over the first, the second and the third year of the TELE-Vir project.
WP1 is divided into 6 tasks:
WP1-T1: Coordination and project management
WP1-T2: Data management
WP1-T3: Kick-off-meeting at IZSAM, Italy
WP1-T4: 1st TELE-Vir meeting at Sciensano, Belgium
WP1-T5: 2nd TELE-Vir meeting at SSI, Denmark
WP1-T6: Poi-Toolbox workshop

Description of the WP1-Tasks for the 1st year of the TELE-Vir project:
WP1-T1: Coordination and project management
Task start month: M25
Task end month: M54
Task Leader: Maiken W. Rosenstierne, SSI
Deputy Task Leader[1]: Miguel Ángel Jiménez-Clavero, INIA
Task Participants: All
Description of the WP1-T1: WP1-T1 takes place over the first, the second and the third year of the project. The main tasks of WP1-T1 are;
1) Overall project management including contact to OHEJP, reporting to OHEJP etc.
2) Coordinate regular telecoms between the participating laboratories
3) Write project newsletters to participants

WP1-T2: Data management
Task start month: M25
Task end month: M54
Task Leader: Maiken W. Rosenstierne, SSI
Deputy Task Leader[1]: Miguel Ángel Jiménez-Clavero, INIA
Task Participants: All
Description of the WP1-T2: WP1-T2 takes place over the first, the second and the third years of the project. The main tasks of WP1-T2 are;
1) Development of a TELE-Vir data management plan (DMP)
2) Continuously updating of the TELE-Vir DMP

WP1-T3: Kick-off-meeting at IZSAM, Italy
Task start month: M25
Task end month: M26
Task Leader: Alessio Lorusso, IZSAM
Deputy Task Leader[1]: Maiken W. Rosenstierne, SSI
Task Participants: ALL
Description of the WP1-T3: WP1-T3 takes place over the first year of the project. The main task of WP1-T3 is;
1) Organize and host a Kick-off meeting of the TELE-Vir project in Italy in January 2020

WP2: Development of a Bioinformatics tool-kit for POI data analysis
WP start month: M25
WP end month: M54
WP Leader: Vítor Borges, INSA
Deputy WP Leader: Daniel Horton, UoS

WP Participants: INSA, UoS
Description of the WP2: WP2 is the bioinformatics WP and takes place over the first, second and third year of the project.
The goal is to develop an open-sourced bioinformatics platform that allows the user-friendly handling of high-throughput sequencing data (second and third generation) for detection and monitoring of viral emerging threats.
WP2 is divided into 5 tasks:
WP2-T1: Survey and collection of databases for genotype-phenotype associations
WP2-T2: Development of bioinformatics modules for third-generation sequencing analysis and pathogen identification
WP2-T3: Development of bioinformatics modules for sequence curation and phenotypic association
WP2-T4: Development of bioinformatics modules for genomic and metadata integration towards enhanced surveillance
WP2-T5: Development of a user- and surveillance-oriented web-based interface

Description of the WP2-Tasks for the 1st year of the TELE-Vir project:
WP2-T1: Survey and collection of databases for genotype-phenotype associations
Task start month: M25
Task end month: M36
Task Leader: Maiken W. Rosenstierne, SSI
Deputy Task Leader[1]: Vítor Borges, INSA
Task Participants: All
Description of the WP2-T1: WP2-T1 takes place over the first year of the project.
The main task of WP2-T1 is:
1) Collate available databases to make an inventory of phenotypically informative genetic traits (e.g., virus-specific mutation databases for antigenicity and drug susceptibility prediction). This will be prioritized for two model viruses where data are available. This task will also be primed at the project's kick-off meeting by an all partners' brainstorming session.

WP2-T2: Development of bioinformatics modules for third-generation sequencing analysis and pathogen identification
Task start month: M25
Task end month: M39
Task Leader: Vítor Borges, INSA
Deputy Task Leader[1]: Daniel Horton, UoS
Task Participants: INSA, UoS
Description of the WP2-T2: WP2-T2 takes place over the first and second year of the project.
The main tasks of WP2-T2 are:
1) Upgrade existing platform for third generation sequencing data (from reads quality assessment and improvement to de novo assembly- and reference-based analysis);
2) Implement methods for automatic pathogen identification/confirmation and *in silico* traditional sub-typing (when applicable), for viral pathogens other than influenza;

WP2-T3: Development of bioinformatics modules for sequence curation and phenotypic association
Task start month: M25
Task end month: M42
Task Leader: Vítor Borges, INSA
Deputy Task Leader[1]: Daniel Horton, UoS
Task Participants:

Description of the WP2-T3: WP2-3 takes place over the first and second year of the project.
The main tasks of WP2-T3 are:
1) Upgrade existing platform with functionalities for robust gene/genome sequence curation/analysis (i.e., generation of consensus sequences, SNP/indel identification, and minor variants detection) based on both reference- and/or de novo assembly. This will involve the expansion of the existing modules for influenza virus available at the platform INSaFLU, so that the toolbox can deal with multiple virus
2) Develop tools for screening/validation of phenotypically informative mutations (by querying, for instance, virus-specific mutation databases for antigenicity and drug susceptibility prediction). We will investigate the feasibility and reliability of inferring biochemical and immunological properties of mutations using existing molecular dynamics models and machine learning approaches that are already being tested at UoS.

WP2-T4: Development of bioinformatics modules for genomic and metadata integration towards enhanced surveillance
Task start month: M25
Task end month: M54
Task Leader: Daniel Horton, UoS
Deputy Task Leader[1]: Vítor Borges, INSA
Task Participants:
Description of the WP2-T4: WP2-4 takes place over the first, second and third year of the project.
The main tasks of WP2-T4 are:
1) Upgrade existing platform with functionalities for detailed comparative genomics against genome sequences from known viruses (by querying virus-specific gene- or genome-based databases)
2) Develop bioinformatics functionalities for surveillance-oriented phylogeny (e.g., for disclosing transmission chains and/or source attribution) and for temporal and geographical metadata integration/sharing.

WP2-T5: Development of a user- and surveillance-oriented web-based interface
Task start month: M25
Task end month: M54
Task Leader: Daniel Horton, UoS
Deputy Task Leader[1]: Vítor Borges, INSA
Task Participants:
Description of the WP2-T5: WP2-5 takes place over the first, second and third year of the project.
The main task of WP2-T5 is:
1) To optimize the existing web-based user interface so that it can provide final graphical outputs (e.g., phylogeography charts enriched with associated sample metadata and relevant viral genotypic and phenotypic features) that can be easily interpreted by a broad audience, not only bioinformaticians, but also other scientists (e.g., virologists, epidemiologists) and decision makers with little computational background.

WP3: Development of a protocol for POI MinION sequencing
WP start month: M25
WP end month: M48
WP Leader: Maiken W. Rosenstierne, SSI
Deputy WP Leader: Jovita Fernandez Pinero, INIA
WP Participants: SSI, INIA, SVA, Sciensano, NVI, IZSAM, PIWET

Description of the WP3: WP3 is the lab-based WP and takes place over the first and the second year of the project.
The goal is to develop a poi toolbox (size of a suitcase) that only contains a battery-driven heater, a MinION device, a MinIT/laptop and a cool box with reagents.
WP3 is divided into 3 tasks:
WP3-T1: Development and validation of a S.O.P for sample handling & pre-treatment
WP3-T2: Development and validation of a S.O.P for sample NA purification
WP3-T3: Development and validation of a S.O.P for NGS library preparation & MinION sequencing (DNA & RNA)

Description of the WP3-Tasks for the 1st year of the TELE-Vir project:
WP3-T1: Development and validation of a S.O.P for sample handling & pre-treatment
Task start month: M25
Task end month: M48
Task Leader: Maiken W. Rosenstierne, SSI
Deputy Task Leader[1]: Artur Rzeżutka, PIWET
Task Participants: SSI, INIA, PIWET
Description of the WP3-T1: WP3-T1 takes place over the first and second year of the project. The main tasks of the WP3-T1 are;
1) Validate inactivation of different virus (e.g. Influenza A virus, African swine fever virus and Hepatitis A virus etc.) using 1:1 volume of MPLB-buffer which has previously been shown to inactivated Ebolavirus (Rosenstierne et al., 2016), Orthopoxvirus (Vinner et al., 2007) and HIV (unpublished data).
2) Adapt the existing S.O.P for sample inactivation of blood samples using MPLB-buffer to other sample materials (e.g. swabs and tissue) using a minimum of equipment (Rosenstierne et al., 2016).
3) Explore the utility of combining virus inactivation by collecting samples on FTA cards with their viability for further application in the MinION device.

WP3-T2: Development and validation of a S.O.P for sample NA purification
Task start month: M25
Task end month: M48
Task Leader: Maiken W. Rosenstierne, SSI
Deputy Task Leader[1]: Lihong Liu, SVA
Task Participants: SSI, SVA, INIA, Sciensano, INSA
Description of the WP3-T2: WP3-T2 takes place over the first and second year of the project. The main tasks of the WP3-T2 are;
1) Adapt the existing S.O.P for sample NA purification of MPLB-buffer inactivated blood samples to other sample materials (e.g. swabs and tissue) for field use using a minimum of time and equipment (Rosenstierne et al., 2018)
2) Explore the viability of establish an automated NA purification using the recently launched VolTRAX device for field use (https://nanoporetech.com/products/voltrax).
3) Validate the methodology and the different S.O.Ps for NA purification in the laboratory using a wide variety of human and animal biological specimens e.g.: Samples from the ongoing 2018-2019 African swine fever virus outbreak in wild boar in Belgium, to validate the NA purification methodology for tissue and other relevant sample types in different decomposition stages, Hepatitis E virus positive samples from pigs and Influenza A positive sample (human and avian) etc.

WP3-T3: Development and validation of a S.O.P for NGS library preparation & MinION sequencing (DNA & RNA)
Task start month: M25
Task end month: M48

Task Leader: Lihong Liu, SVA
Deputy Task Leader[1]: Maiken W. Rosenstierne, SSI
Task Participants: SSI, SVA, INIA, Sciensano, NVI, IZSAM
Description of the WP3-T3: WP3-T3 takes place over the first and second year of the project. The main tasks of the WP3-T3 are;
1) Develop and adopt existing S.O.Ps for NGS library preparation for field use using a minimum of time and equipment.
2) Adopt existing MinION sequencing technology for field use using a minimum of time and equipment. E.g. Explore the viability of establish an automated Library preparation using the recently launched VolTRAX device for field use (https://nanoporetech.com/products/voltrax).
3) Validate the methodology and the different S.O.Ps NGS library preparation & MinION sequencing for DNA and RNA viruses in the laboratory using a wide variety of human and animal biological specimens e.g.: Samples from the ongoing 2018-2019 African swine fever virus outbreak in wild boar in Belgium, to validate the NA purification methodology for tissue and other relevant sample types in different decomposition stages, Hepatitis E virus positive samples from pigs and Influenza A positive sample (human and avian) etc.

Deliverables

Ref	Title	Due month
D-JRP#-WP1.1	Kick-off meeting in Italy (IZSAM)	M25
D-JRP#-WP1.2	1st version of the DMP	M30
D-JRP#-WP3.1	1st version of a poi S.O.P for sample handling & pre-treatment	M36
D-JRP#-WP3.2	1st version of a poi S.O.P for sample NA purification	M36
D-JRP#-WP3.3	1st version of a poi S.O.P for NGS library preparation & MinION sequencing	M36

Milestones

Ref	Title	Due month
M-JRP#-01	Collected and curated databases for genotype-phenotype associations	M36

2.4.2.1.17 JRP17-R2-ET2.2-IDEMBRU

Reference to the Stategic Research Agenda (please refer to D2.7)			**ET 2.2: Development of a toolkit to characterize emerging threats by combining genomic and phenotypic information**							
Research Project Title			**Identification of emerging Brucella species: new threats for human and animals**							
Research Project Acronym			**IDEMBRU**							
Leading Organisation			**P1-ANSES**			Deputy Leading Organisation			**P35-INIAV**	
Project Leader			**C Ponsart**			Deputy Project leader			**AC Ferreira**	
Project Start month			**M25**			Project End month			**M54**	
Participant	**1**	2	4	**6**	7	8	**9**	**10**	11	12
	Anses	Ages	Sciensano	**NDRVMI**	SZU	VRI	**BfR**	**FLI**	RKI	DTU
PM	**21.15**			**19**			**10**	**8.4**		
Participant	13	14	15	16	17	18	19	20	**21**	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	PHE
PM									**9.97**	
Participant	23	24	25	26	27	**28**	29	30	**31**	32
	UoS	OKI	NUIG	TEAGASC	ISS	**IZSAM**	IZSLER	RIVM	**WbvR**	FHI
PM						**12.25**			**1.1**	
Participant	33	34	**35**	**36**	37	38	39	40	41	
	NVI	PIWET	**INIAV**	**INSA**	IISPV	IC	SLV	FOHM	SVA	
PM			**25.30**	**12**						

Objectives

- Creation of a "one health" common database to share information about emerging *Brucella* and reservoirs among the consortium partners;
- Harmonisation and implementation of "one health" sampling protocols and collections (serum, samples, strains) focusing on emerging *Brucella* species and related reservoirs;
- Standardisation and implementation of protocols for detection, identification and phenotyping of emerging *Brucella* species;
- Definition and redaction of the data management plan;
- Organisation of the first annual workshop including exchanges between partners and inputs from external stakeholders.

WP 1-Recording the situation of brucellosis in emergent wild and environmental reservoirs

- WP1 takes place over the first and the second year of the project

- T1 takes place over the first year of the project
- T2 takes place over the first and the second year of the project

WP start month: 25
WP end month: 44
WP Leader: Ana Cristina Ferreira
Deputy WP Leader: Hristo Dalaskov
WP participants : ANSES, BfR, FLI, INIAV, IZSAM, NDRVMI–BFSA

Description of the WP[1]: A large part of the interactions within the network at the project start will be devoted to define the best sampling strategy, taking into account both methodological requirements and epidemiological relevance. It is important to probe the presence and diversity of *Brucella* isolates from different wildlife, such as amphibians, bats, cervidae, marine mammals, rodents, suidae. This WP involves the mapping of existing data and resources on emerging *Brucella* spp., additional sampling from emerging reservoirs and the creation of a common database to share both epidemiological data and results obtained during the project.

INIAV and NDRVMI–BFSA will be executive parties for the WP1. INIAV is the State Laboratory that develops research activities in the agricultural and veterinary fields. Its National Reference Laboratories perform official analysis in the fields of Animal Health, Plant Health, Food and Feed Safety. The Institute cooperates with related scientific and technological institutions, both at national and international level, and is equipped with a necropsy room, BSL3 facility and epidemiological platforms (such as ArcGIS Platform). NDRVMI–BFSA is responsible for laboratory testing of samples from all regions of the country for Brucellosis. The laboratory for brucellosis is playing role of National reference laboratory and coordinating all activities and training in this area. As a part of Bulgarian Food Safety Agency NDRVMI have the opportunity to organise the collection of samples from food producing animal farms, all slaughterhouses, game farms and wild game and the environment. NDRVMI–BFSA has laboratory facilities for serological studies, including facilities for necropsy. Sampling and analytical protocols will be harmonized among partners and shared. Collection of appropriate samples (animal and environmental) will be agreed and performed for detection and isolation of emerging *Brucella* and reservoirs by each involved partner. All strains will be characterized according to conventional microbiological methods and further investigated in WPs 3 to 5.

Task **JRP19-WP1-T1**: Mapping of existing data on emerging *Brucella* spp.
Task start month: 25
Task end month: 30
Task Leader: Ana Cristina Ferreira
Deputy Task Leader: Hristo Dalaskov
Task Participants: ANSES, APHA, BfR, FLI, INIAV, IZSAM, NDRVMI–BFSA

Description of the task: Mapping of the relevant data on emerging *Brucella* species will be implemented from existing or upcoming surveillance efforts and research projects within partners. The temporal and spatial data, host(s), type of samples, phenotype and genotype data will be gathered and organised. At the end of this task, a shared database will be created to maximize the sharing and exchange of information on the current status of knowledge and resources concerning emerging *Brucella* species between partners. Each involved partner will map emerging *Brucella* species from existing data in their country.

Task **JRP19-WP1-T2**: Sampling and analytical strategy according to previous epidemiological information from the different partners

Task start month: 29
Task end month: 44
Task Leader: Ana Cristina Ferreira
Deputy Task Leader: Hristo Dalaskov
Task Participants: ANSES, APHA, BfR, FLI, INIAV, IZSAM, NDRVMI–BFSA

Description of the task: This task is devoted to sampling and analytical protocols. It will enable the creation of new biological collections (serum, samples, and strains) including emerging *Brucella* species and related reservoirs. Selection of countries, regions, targeted animal species and their surrounding environment will be based on practical possibilities related to relevant contexts in terms of natural landscape, livestock demographics, and wildlife populations of each participating country. Sampling and analytical protocols will be harmonized among partners and shared. Collection of appropriate samples (animal and environmental) for detection and isolation of emerging *Brucella* spp. as agreed by the consortium will be performed. All strains will be characterized according to conventional microbiological methods. When available, blood samples will be collected to characterise serological responses using rough and smooth antigens based methods. Each involved partner will collaborate in producing the sampling and the analytical protocols and undertaking collection of biological samples.

Deliverables

Ref	Title	Due month
D-JRP19-WP1.Del1	Creation of a common database to share information about emerging *Brucella* and reservoirs among the consortium partners	30

Milestones

Ref	Title	Due month
M-JRP19-M6	Information on worldwide emerging Brucellae (shared database)	30
M-JRP19-M7	Definition of sampling and testing protocols (molecular, bacteriology and serology)	30

WP2 - Recording the situation of brucellosis in human

- WP2 takes place over the first and the second of the project
- T1 takes place over the first year of the project
- T2 takes place over the first and second year of the project

WP start month: 25
WP end month: 44
WP Leader: Ana Pelerito
Deputy WP Leader: Sascha Al Dahouk
WP participants: ANSES, BfR, INSA

Description of the WP: This work package will cover investigations in human populations, potentially in contact with emerging *Brucella* and related reservoirs. This will include the implementation of a surveillance network for human brucellosis, additional sampling design according to selected emerging reservoirs and characterisation of serological responses. The analysis of data collected should allow the identification of geographic distribution, contributing to the better understanding of emerging brucellosis epidemiology. By the end of the work package, the new collected *Brucella* strains and serums will be shared among partners for further investigations in WPs 3 to 5. Both leading organisations (INSA and BfR) will ensure coordination, implementation of the surveillance network for human brucellosis and harmonisation of protocols applied in this work package. Sampling and analytical protocols will be harmonized among partners and shared. Partners will contribute through collection of appropriate samples, dissemination of

the questionnaire (BfR, INSA) and / or through existing strain collections of emerging *Brucella* and reservoirs (ANSES). All strains will be characterized by microbiological methods and molecular biology.

Task **JRP19-WP2-T1**: Creation of a surveillance network dedicated to human brucellosis
Task start month: 25
Task end month: 30
Task Leader: Ana Pelerito
Deputy Task Leader: Sascha Al Dahouk
Task Participants: ANSES, BfR, INSA

Description of the task: This task aims to improve human brucellosis surveillance in order to achieve a "One Health" approach. Mapping of existing data will be the initial step in order to establish an existing strains and samples collection from human populations suspected of emerging *Brucella* infections. INSA, BfR and involved partners will contact hospital, human laboratories and European related institutes (ECDC and EFSA) to establish a common network. Collection of metadata including risk factors will be integrated mediated by an epidemiological questionnaire that will be elaborated within the surveillance network. Many emerging *Brucella* spp. isolates have initially been mis-identified by culture methods, particularly by widely used diagnostic microbiology platforms. Therefore an important aspect of this survey will be identifying how frequently those bacterial species for which *Brucella* spp. are commonly mis-identified occur in human health laboratories. This will provide valuable information regarding the potential scale for mis-identification of emerging *Brucella* by bacterial culture. Isolates identified through this survey will be used in JRP19-WP2-T3.

Task **JRP19-WP2-T2**: Sampling and analytical strategy according to epidemiological information from the surveillance network
Task start month: 29
Task end month: 44
Task Leader: Ana Pelerito
Deputy Task Leader: Sascha Al Dahouk
Task Participants: ANSES, BfR, INSA

Description of the task: This task is devoted to sampling and analytical protocols. It will enable the creation of human biological collections (serum, samples, and strains) including emerging *Brucella* species and related reservoirs. In order to set up "One Health" approach, selection of countries and regions will be based on information derived from WP1, response to the epidemiological questionnaire, practical possibilities and ethic constraints. Blood samples will be collected by INSA from potentially exposed people for blood culture and direct diagnosis. Methods for blood culture and molecular protocols will be standardised and shared among partners. When available, serum samples will be collected to characterise serological responses using rough and smooth antigens based methods. As isolates from atypical *Brucella* have a very different lipopolysaccharide (LPS), the seropositivity may be less reliable compared to direct approaches. ANSES and BfR have expertise in isolation and molecular detection of this agent and INSA uses all these techniques routinely.

Deliverables		
Ref	**Title**	**Due month**
D-JRP19-WP2.Del1	Set-up of a human brucellosis network	30
Milestones		

Ref	Title	Due month
M-JRP19-M1	Mapping of existing human *Brucella* species (shared database)	28
M-JRP19-M5	Creation of an epidemiological questionnaire	29
M-JRP19-M8	Definition of sampling and testing protocols (serology, bacteriology and molecular biology)	30

WP3 - Genomic characterisation of *Brucella* detected from samples and selected isolates

- WP3 takes place over the first and the second year of the project
- T1 takes place over the first and the second year of the project
- T2 takes place over the first year of the project
- T3 takes place over the first and the second year of the project

WP start month: 25
WP end month: 48
WP Leader: Roland Ashford
Deputy WP Leader: Giuliano Garofolo
WP participants: ANSES, APHA, BfR, FLI, INIAV, INSA, IZSAM, WbvR

Description of the WP: Molecular typing of *Brucella* spp. organisms has proved critical for characterising the diversity of the genus and identifying emerging species and isolates. However, there is a need to ensure that harmonised molecular typing approaches are in place to capture and describe the expanding diversity of the genus. The objectives of the WP are (i) to maximise the amount of molecular typing data that can be generated for emerging *Brucella* spp. by optimising methods for the direct typing of bacterial DNA in complex sample matrices (JRP19-WP3-T1); (ii) to ensure that protocols are available for generating comparable whole genome sequencing based molecular typing between all partner institutes (JRP19-WP3-T2); (iii) to ensure that existing molecular typing schemes remain applicable to the full diversity of the genus, and to develop discriminatory typing assay(s) to identify emerging and atypical *Brucella* spp. strains (JRP19-WP3-T3).

Task **JRP19-WP3-T1**: Optimisation of methods for generating molecular typing data from complex samples
Task start month: 29
Task end month: 46
Task Leader: Roland Ashford
Deputy Task Leader: Giuliano Garofolo
Task Participants: ANSES, APHA, BfR, FLI, INIAV, INSA, IZSAM, WbvR

Description of the task: In order to address this challenge within the current project we will seek to establish optimal protocols for the extraction of pathogen DNA from complex matrices (e.g. clinical and environmental samples). This will enable us to apply existing molecular typing methods (e.g. qPCR, HRM, MLVA, and MLST) to samples directly, without the need to isolate organisms. Identification of clinical and environmental samples of most relevance will be informed by WP 1 (JRP19-WP1-T1) & WP2 (JRP19-WP2-T1). The diagnostic sample collection will additionally be tested using discriminatory single nucleotide polymorphisms (SNPs) retrieved from the whole genome sequencing datasets (JRP19-WP3-T3).
Initial work under JRP19-WP3-T1 will primarily be undertaken by the WP3 Lead institute (APHA), with input from Deputy WP Lead institute (IZSAM). Once identified, optimal protocols will be disseminated amongst other partner institutes (ANSES, BfR, FLI, INIAV, INSA, WbvR), and applied to relevant sample sets generated under WPs 1 & 2.

Task **JRP19-WP3-T2**: Harmonisation and standardisation of protocols for whole genome sequencing
Task start month: 25
Task end month: 36
Task Leader: Roland Ashford
Deputy Task Leader: Giuliano Garofolo
Task Participants: ANSES, APHA, BfR, FLI, INIAV, INSA, IZSAM, WbvR

Description of the task: We will standardise the performance of library preparation and sequencing procedures undertaken in partner institutes (quality; number of reads, coverage, etc.). In order to do this a representative panel of *Brucella* spp. isolates will be identified, and (inactivated) bacterial suspensions will be shared with partner institutes. In the case of bioinformatics protocols we will benchmark assembly and alignment metrics, to ensure data are comparable between partner institutes, using shared samples and reference strains. Task JRP19-WP3-T2 will be led by the WP3 lead institute (APHA) with input from Deputy WP Lead institute (IZSAM). Standardised bioinformatics protocols will be shared with all other participating institutes (ANSES, BfR, FLI, INIAV, INSA, WbvR) to ensure that data generated under JRP19-WP3-T3 can are comparable. Data generated using standardised whole genome sequencing and bioinformatics protocols will be applied in WP4 and WP6.

Task **JRP19-WP3-T3**: Identification of emerging *Brucella* species: adaptation of molecular tools
Task start month: 31
Task end month: 46
Task Leader: Roland Ashford
Deputy Task Leader: Giuliano Garofolo
Task Participants: ANSES, APHA, BfR, FLI, INIAV, INSA, IZSAM, WbvR

Description of the task: Molecular typing methods such as multiple locus VNTR analysis (MLVA), multi-locus sequence typing (MLST) and specific gene sequencing (e.g. 16S) are widely applied to molecular typing of *Brucella*. We will apply whole genome sequencing using next generation sequencing platform to the emerging *Brucella* collection obtained from the activities related to WP1 and WP2. We will generate *in silico* 9 & 21 locus MLST, core genome (cg)MLST and whole genome SNP typing data; *in silico* 16-locus MLVA profiles, supported by conventional fragment analysis approaches where necessary. Using expanded genomic databases (incorporating data generated under this project); we will identify discriminatory SNP typing assay(s) to identify atypical *Brucella* spp. strains. The WP3 lead institute (APHA) with input from Deputy WP Lead institute (IZSAM) will lead JRP19-WP3-T3. Data will be generated using harmonised protocols (JRP19-WP3-T2) and shared amongst partner institutes contributing to the work package (ANSES, BfR, FLI, INIAV, INSA, WbvR).

Deliverables

Ref	Title	Due month
D-JRP19-WP2.Del1	Standard operating procedures for the extraction of *Brucella* DNA from complex matrices	36
D-JRP19-WP2.Del2	Harmonised whole genome sequencing and bioinformatic protocols	36

Milestones

Ref	Title	Due month
M-JRP19-M15	Harmonisation of protocols for whole genome sequencing	35
M-JRP19-M16	Optimised protocols for the extraction of Brucella DNA from complex matrices	36

WP4 - Phenotypic characterisation of *Brucella* detected from samples and selected isolates

- WP4 takes place over the first, the second and the third year of the project

- T1 takes place over the first and the second year of the project
- T3 takes place over the first, the second and the third year of the project

WP start month: 25
WP end month: 54
WP Leader: Falk Melzer
Deputy WP Leader: Sascha Al Dahouk
WP participants: ANSES, APHA, FLI, BfR, IZSAM, INIAV,INSA

Description of the WP: The major aim of WP4 is to identify diagnostically relevant features to characterize and differentiate novel emerging *Brucella* spp. from the classical species. This includes conventional phenotyping procedures as well as commercial biotyping. We will test and adapt existing AMR protocols in this project to comparatively analyse antibiotic resistance in the novel emerging strains of *Brucella*. The differences in metabolic activity between classical and emerging species shall be characterized using a combined RNASeq and peptidomic (mass spectrometry) approach after inactivation of the living agent. We will also analyse whether the detection of RNA in clinical samples which has a higher abundance is more sensitive than the detection of genomic *Brucella* DNA. For this purpose, the efficacy of RNASeq and 16S metagenomics for the detection of *Brucella* in clinical samples will be compared.
Initial work will primarily be undertaken by the WP4 Lead institute (FLI) together with Deputy WP Lead institute (BfR). Once identified, optimal protocols will be disseminated amongst other partner institutes (ANSES, APHA, IZSAM, INIAV, INSA), and applied to relevant sample sets generated under WPs 1, 2 and 3.

Task **JRP19-WP4-T1**: Phenotyping of novel emerging *Brucella* spp.
Task start month: 25
Task end month: 48
Task Leader: Falk Melzer
Deputy Task Leader: Sascha Al Dahouk
Task Participants: ANSES, APHA, FLI, BfR, IZSAM, INIAV, INSA

Description of the task:
JRP19-WP4-T1-ST1: Emerging *Brucella* species that have been recently described or that are isolated in the context of this proposal (WP1; WP2) will be phenotyped using all appropriate conventional methods under biosafety level 3 conditions. Phenotypic results from partners will be used for overall characterization. Lab work will start after the arrival of the first isolates at FLI.
JRP19-WP4-T1-ST3: Previous studies have shown that the atypical *Brucella species B. microti* exhibits expanded metabolic activities, clearly distinguishable to those of classical *Brucella* species like *B. abortus* and *B. melitensis*. The Micronaut™ test developed by BfR provides valuable information about the presence of distinct substrate utilizing enzymes and will contribute not only to the functional characterization of the emerging *Brucella* species but also to their discrimination and classification into biovars/subspecies. Therefore, we will analyze *B. microti* and other emerging *Brucella* species that have been recently described or will be isolated from wildlife in the context of this proposal (WP1; WP2) with this assay. In the context of this proposal, we will analyse with this assay *B. microti* and other emerging *Brucella* species that have been and will be isolated from wildlife (WP1; WP2).

Task **JRP19-WP4-T3**: RNA sequencing of a representative panel of classical and novel *Brucella spp*.
Task start month: 25
Task end month: 54
Task Leader: Sascha Al Dahouk

Deputy Task Leader : Dirk Hofreuter
Task Participants: ANSES, APHA, FLI, BfR, IZSAM, INIAV,INSA

Description of the task :
JRP19-WP4-T3-ST1: For our initial studies we will perform and compare RNA-Seq analyses of *in vitro* cultured classical and emerging *Brucella* species. Since, the quality of isolated RNA depends on the host bacteria and the isolation methods, our focus lies on the evaluation of commercial RNA isolation kits for their suitability to gain RNA from different *Brucella* species in high quality.
JRP19-WP4-T3-ST2: *Brucella* species exhibit genome sequence identities of more than 90%. Still, their biology seems to be significantly different as manifested in strikingly distinct host preferences. Such diverse host tropisms might result from the occurrence of species-specific genes and variations in the expression of conserved genes. Therefore, we will determine by RNA-Seq the transcriptomic profiles of emerging *Brucella* strains cultivated under different conditions (low pH and starvation).

Deliverables

Ref	Title	Due month

Milestones

Ref	Title	Due month
M-JRP19-M9	Drafted RNASeq protocols to identify regulatory differences between classical species and emerging *Brucella* strains	30

WP5 - Zoonotic potential and virulence

- WP5 takes place over the first, the second and the third year of the project
- T1 takes place over the first and the second year of the project
- T2 takes place over the first and the second year of the project

WP start month: 35
WP end month: 54
WP Leader: Alexander Umanets
Deputy WP Leader: Luca Freddi
WP participants: ANSES, APHA, BfR, FLI, IZSAM, WbvR

Description of the WP:
Understanding the zoonotic potential and virulence of a pathogen is necessary to assess risk to the public and for the development of appropriate preventive and control measures. The most reliable approach is the collection of historical and epidemiological data, but unfortunately this cannot be applied to an emerging disease. Several laboratory animal species, cell lines of human and animal origin, in combination with genomics have been used to understand *Brucella* pathogenesis. However, with the exception of *B. microti*, the virulence of these emerging *Brucella* strains has not been investigated. Hereby we propose to develop a pipeline for evaluation of virulence and zoonotic potential mediated by *in vitro* and *in vivo* infection models.
WbvR and ANSES will be executive parties for WP5. Collaboration between WbvR and ANSES will allow the harmonization of *in vitro* and *in vivo* protocols and provide access to all necessary facilities for successful development, execution and benchmarking of the proposed pipeline. All involved partners will provide selected emerging *Brucella* strains from their own strain bank.

Task **JRP19-WP5-T1**: Develop an *in silico* pipeline for preliminary assessment of overall virulence and zoonotic potential.
Task start month: 35
Task end month: 44

Task Leader: Alexander Umanets
Deputy Task Leader: Luca Freddi
Task Participants: ANSES, APHA, BfR, IZSAM, WbvR

Description of the task: Common and unique virulence factors will be identified by genome wide comparison of a novel emerging *Brucella* isolate with reference panel of *Brucella* species. A panel of classical and emerging *Brucella* species will be selected according to their zoonotic potential based on historical, genomic and phenotypic data. Data generated in the course of WP3 and from publicly available databases will be used for selection and benchmarking of bioinformatics tools for virulence factor detection, with the purpose of creating a reliable *in silico* pipeline for rapid preliminary zoonotic potential assessment. WbvR will be in charge of selecting bioinformatics tools for identification and comparison of virulence factors, as well as creating an automatic/semi-automatic pipeline for virulence assessment of novel isolates based on their genomes. ANSES will set up *Brucella* genomes database from publicly available and novel isolates (WP3). All involved partners will support the creation of *Brucella* genomes database and bioinformatics pipeline development.

Task **JRP19-WP5-T2**: Develop an *in vitro* protocol to investigate zoonotic potential based on macrophage cell lines originated from human and animals.
Task start month: 35
Task end month: 48
Task Leader: Alexander Umanets
Deputy Task Leader: Luca Freddi
Task Participants: ANSES, APHA, BfR, IZSAM, WbvR

Description of the task: Common and unique virulence factors will be identified by genome wide comparison of emerging *Brucella* isolates with the reference panel of classical *Brucella* species (selected according to their zoonotic potential based on historical, genomic and phenotypic data). As the next step an *in vitro* infection model based on macrophage cell lines derived from human and animals will be developed. WbvR will start to acquire and maintain animal and human derived macrophages. ANSES will start the identification of representative virulence genes. Finally, IZSAM will insure reproducibility of *in vitro* infection protocol.

Deliverables

Ref	Title	Due month

Milestones

Ref	Title	Due month

WP7 - Coordination, management and communication

- WP7 takes place over the first, the second and the third year of the project
- T1 takes place over the first, the second and the third year of the project
- T2 takes place over the first, the second and the third year of the project
- T3 takes place over the first, the second and the third year of the project
- T4 takes place over the first, the second and the third year of the project

WP start month: 25
WP end month: 54
WP Leader: Claire Ponsart

Deputy WP Leader: Ana Cristina Ferreira
WP participants: All the partners

Description of the WP: This work package is dedicated to the general coordination and management of the project including all administrative and financial issues. This will ensure the timely implementation of the tasks, the smooth running of the project and successful dissemination of outputs. Specific tasks will be dedicated to data and risk management. Three workshops will be will be used for coordination of work, exchanges of information and collaborative development of the toolkit. The coordinator will ensure successful management, integration and delivery of the project, and will coordinate and provide effective reporting and communications between project partners. A steering committee composed of one representative for each participating institute will be set up to discuss the advancement of the project through progress reports. The WP leaders report on their respective WP, whereas the coordinator is responsible for the final report.

Task **JRP19-WP7-T1**: Coordination and organisation
Task start month: 25
Task end month: 54
Task Leader: Claire Ponsart
Deputy Task Leader: Ana Cristina Ferreira
Task Participants: All the partners

Description of the task: WP leaders assisted by Deputy WP leaders will be in charge of coordination and management inside WPs and of the preparation of activity reports to be compiled by the project coordinator. An annual workshop will be organised in order to exchange information about the workplan and deliverables. Each meeting will include collaborative work between partners and stakeholders (projects, EU existing networks, ECDC, EFSA) to conceive the final toolkit throughout the project. Regular exchanges within the steering committee will be organised by video- or telephone conferences. ANSES will organize the first annual workshop.

The kick-off meeting including all project participants will be hosted by ANSES. The purpose of this meeting will be to discuss in details the workprogramme to be done in the period lying ahead, to present and plan the first defined milestones and deliverables and to exchange about specificities of atypical Brucella (culture, serology). A practical demonstration of different atypical *Brucella* will be organised during the workshop.

Task **JRP19-WP7-T2**: Data management
Task start month: 25
Task end month: 54
Task Leader: Guillaume Girault
Deputy Task Leader: Ana Pelerito
Task Participants: All the partners

Description of the task: As required, we will use the first five months of the project to develop a Data Management Plan (DMP). The DMP will be drafted to identify information generated by the project and define a common shared platform to store all data obtained during the entire project. The partners will be responsible for sharing and uploading data. This plan will include conditions of use associated with newly generated data. All data, derivative analyses, protocols and the toolkit will made available for the consortium and scientific community.

Task **JRP19-WP7-T3**: Risk management
Task start month: 25

Task end month: 54
Task Leader: Claire Ponsart
Deputy Task Leader: Ana Cristina Ferreira
Task Participants: All the partners

Description of the task: Changes necessary in order to deliver milestones on time as well as delays and problems in achieving the milestones will be reported in advance of workshops by work package leaders and deputy leaders. Modifications to the project made possible by newly available materials and techniques will be discussed, evaluated and, if considered appropriate, implemented in a coordinated manner, as will possible changes in emphasis based on developments in perceived threats and newly published work appearing in the literature.

Task **JRP19-WP7-T4**: Synthesis and dissemination of recommendations coming from the project outputs
Task start month: 25
Task end month: 54
Task Leader: Claire Ponsart
Deputy Task Leader: Ana Cristina Ferreira
Task Participants: All the partners

Description of the task: A notification system including emerging and non-regulated *Brucella* will be achieved to track and collect epidemiological and clinical data.

Deliverables

Ref	Title	Due month
D-JRP19-WP7.Del1	First draft of data management plan	30
D-JRP19-WP7.Del2	Creation of a data sharing common platform	32
D-JRP19-WP7.Del3	Final draft of data management plan	34
D-JRP19-WP7.Del4	Report of the first annual workshop including exchanges between partners and inputs from external stakeholders	36

Milestones

Ref	Title	Due month
M-JRP19-M2	Conference call of the steering committee regrouping WP leaders + deputy leaders on data management	28
M-JRP19-M3	Definition of type of data generated for each WP and structure of the data sharing platform	28
M-JRP19-M4	Conference call of the steering committee regrouping WP leaders and deputy leaders	28
M-JRP19-M10	Definition of the programme of the annual workshop	30
M-JRP19-M11	Organisation of accommodation and logistic aspects	32
M-JRP19-M12	Implementation of first annual workshop	34
M-JRP19-M13	Definition of criteria to be included in the notification system	34
M-JRP19-M14	Choice of the informatic system to implement the notification system	34

2.4.2.1.18 JRP18-R2-ET1.1-MEmE

Reference to the Stategic Research Agenda (please refer to D2.7)			**ET 1.1: Development and harmonization of NGS and non-NGS methods (e.g. pheno-genotypic and histochemical methods) for the detection of foodborne parasites**							
Research Project Title			**Multi-centre study on *Echinococcus multilocularis* and *Echinococcus granulosus s.l.* in Europe: development and harmonization of diagnostic methods in the food chain.**							
Research Project Acronym			**MEmE**							
Leading Organisation			**P27-ISS**			Deputy Leading Organisation		**N/A**		
Project Leader			**Adriano Casulli**			Deputy Project leader		**N/A**		
Project Start month			**M25**			Project End month		**M54**		
Participant	**1**	2	4	6	7	8	9	**10**	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	**FLI**	RKI	DTU
PM	**22**							**9**		
Participant	**13**	**14**	**15**	16	17	18	19	20	21	22
	SSI	**UT**	**VFL**	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM	**9.9**	**15.5**	**5.4**							
Participant	23	24	25	26	**27**	28	29	**30**	31	32
	UoS	OKI	NUIG	TEAGASC	**ISS**	IZSAM	IZSLER	**RIVM**	WbvR	FHI
PM					**11.6**			**2.46**		
Participant	**33**	**34**	**35**	**36**	37	38	39	40	**41**	
	NVI	**PIWET**	**INIAV**	**INSA**	IISPV	IC	SLV	FOHM	**SVA**	
PM	**7**	**11.7**	**9.16**	**2.7**					**8.5**	

Objectives

- Production of Standard Operating Procedures (WP1-T1) for the sampling of matrices that will be used to feed WP2, WP3 and WP4.
- **SAMPLING** of different matrices from naturally or experimentally infected definitive and intermediate hosts (WP1-T2,T3) to feed WP2, WP3 and WP4 on **1)** validation of parasitological/molecular assays; **2)** development and validation of new tools and production of data relevant for epidemiological assessments, and **3)** Proficiency Testing Schemes.
- Validation of established **PARASITOLOGICAL** (Segmental Sedimentation and Counting Technique, SSCT) and novel **MOLECULAR** diagnostic procedures (multiplex PCRs and Magnetic

Capture - Real Time PCR assay) to detect *E. multilocularis* (Em) and *E. granulosus s.l.* (Eg) in different matrices along the food chain (WP2-T1,T2,T3).

- Development and validation of **NEW TOOLS** such as: **1)** New molecular markers for *Echinococcus* species (from rapid diagnostics to source attribution); **2)** new multiplex qPCR for detection and discrimination of Em/Eg and Eg genotypes; **3)** sequencing using Region-Specific Extraction (RSE) and NGS for the detection of Em/Eg in complex samples; **4)** biomarker discovery in exosomes from sheep plasma for diagnosis of CE infection (WP3-T1,T2,T3,T4).
- Production of **DATA RELEVANT for EPIDEMIOLOGICAL ASSESSMENTS** on: **1)** contamination of vegetables for human consumption by eggs of Em/Eg; **2)** prevalence of Em/Eg in dog faeces; and **3)** identification of potential risk factors for human CE/AE infection through questionnaires (WP3-T6,T7,T8).
- **TRAINING** scientists of MEmE in parasitological and molecular identification of *Echinococcus* spp. by exchange of key staff members (WP4,T1).
- **DISSEMINATING PROJECT RESULTS** at different levels (general public, populations at risk, biologists, veterinarians, clinicians, health authorities, policy makers and media) (WP4,T2).

Description of work

WP: **1 (SAMPLIG STRATEGY)** (takes place over the 3rd and 4th year)
WP start month: **25**; WP end month: **48**; WP Leader: **PIWET**
WP participants: ISS, FLI, ANSES, RIVM, PIWET, SVA, NVI, SSI, INIAV, INSA, VFL, CVRL, BIOR, CMRIXJ, JLU, VRIXJ
Description of the WP: This WP is aiming to **collect (in the field/research facilities) and produce in the laboratory, the biological material** needed to implement the activities of MEmE. Samples will be used for validation of parasitological/molecular methods (WP2), to generate innovative tools and epidemiological data (WP3), and prepare Proficiency Testing Schemes (WP4).

WP1	M25-26	M27-28	M29-30	M31-32	M33-34	M35-36	M37-38	M39-40	M41-42	M43-44	M45-46	M47-48	M49-50	M51-52	M53-54
T1.		D1.1													
T2.						D1.2									
T3.															

Task: **JRP-WP1-T1. SOPs for sampling in matrices** (takes place over the 3rd year)
Task start month: 25; Task end month: 28; Task Leader: ISS
Task Participants: ISS, ANSES, PIWET, NVI, INIAV, INSA, CVRL, BIOR, CMRIXJ
Description of the task: This task will be used to produce Standard Operating Procedures (SOPs) for the sampling of matrices that will be used to feed WP2, WP3 and WP4. SOPs will be generated for: **1)** sampling of fecal material in dog feces collected from the environment and their processing for molecular analysis; **2)** sampling of the small and large intestine of foxes and their processing for parasitological and molecular analyses; **3)** sampling of feces of experimentally infected foxes and their processing for parasitological and molecular analyses; **4)** sampling of blood from experimentally infected sheep and sheep at abattoirs and its processing to obtain plasma suitable for proteomic analysis of exosomes; **5)** sampling of *E. granulosus* cysts from naturally infected sheep and pigs at abattoirs and experimentally infected sheep, and their processing for molecular analyses; **6)** sampling of vegetables for human consumption and their processing for molecular analysis.
Task: **JRP-WP1-T2. Matrices collection in the field from intermediate and definitive hosts** (takes place over the 3rd year)
Task start month: 27; Task end month: 36; Task Leader: PIWET

Task Participants: ANSES, RIVM, PIWET, FLI, SVA, NVI, SSI, INIAV, INSA, VFL, CVRL, BIOR, CMRIXJ, JLU, VRIXJ

Description of the task: Biological samples will be collected from:

1) intestines of red foxes (N=400) sampled in the frame of monitoring programmes in endemic areas (FLI, ANSES, RIVM, PIWET, SSI, INIAV, VFL, BIOR, JLU, VRIXJ). Samples will be examined by SSCT and *Echinococcus* **worms** will be isolated (ANSES, PIWET, FLI, INIAV, BIOR). **Faeces** from the distal part of the large intestine of red foxes will be also collected. *Echinococcus* **eggs** will be isolated from mature worms or faeces. Moreover, intestines (N=300) of red foxes from *E. multilocularis* free areas will be collected (CVRL) for use in the validation of diagnostic tests (WP2-T1,T2,T3; WP3-T1,T2,T3,T5; WP4-T3). **Other parasites** from existing sample collections will be retrieved and used as controls for specificity evaluation (*Mesocestoides* spp., *Taenia* spp, etc.) (FLI, ANSES, PIWET, SVA, SSI, INIAV, BIOR, JLU, VRIXJ);

2) *E. granulosus* cysts-derived material (protoscoleces, germinal layer, hydatid fluid, etc.) (N=400) for molecular analyses (WP2-T2,T3; WP3-T1,T2,T5; WP4-T3) and blood samples stored in DMSO (N=80) for proteomic analysis (WP3-T4) will be obtained from sheep and pigs slaughtered at abattoirs (ISS, ANSES, PIWET, INIAV, JLU, VRIXJ);

3) Dog faeces (N=2,400; see WP3-T7) will be collected for producing epidemiological data on prevalence of Em/Eg in selected endemic areas (ANSES, PIWET, INIAV, NVI, SSI, VFL, BIOR, JLU, VRIXJ, CMRIXJ) (WP3-T7). Faeces from *Echinococcus* spp. uninfected dogs will be also used as a matrix for spiking with eggs for validation of molecular assays (WP2-T2,T3; WP3-T1,T2,T3,T5; WP4-T3).

Task: JRP-WP1-T3. Matrices collection from experimental animal models

(takes place over the 3rd and 4h year)

Task start month: 27; Task end month: 48; Task Leader: ANSES

Task Participants: ANSES, INIAV, VRIXJ, JLU

Description of the task: All experiments will be conducted in compliance with international and national laws of the participants' states upon obtainment of ethical clearance.

ST1. Mouse/fox model of Em. A red fox model will be used to produce reference material to validate selected parasitological/molecular tests, NGS included (see WP3) and for production of reference intestines that will be use to train laboratory personnel on SSCT and/or to provide material for proficiency tests (see WP5). Red foxes will be infected using protoscoleces produced in mice (N=60) infected by the larval form (metacestodes) of E. *multilocularis* though intraperitoneal injection of protoscoleces. Experimentally infected mice will be kept in animal facilities until fully developed fertile metacestodes (i.e. containing protoscoleces) are produced. Foxes, the main definitive host for Em, will be maintained in the animal facilities of ANSES. Fox animal model will be also supported by JLU and VRIXJ in China.

Fox faeces will be collected daily to provide feces from pre-patent and patent periods from infected animals (see WP2-3). Foxes will be euthanized at 28-36 days post-infection to provide intestines with mature worms and faeces with parasite eggs. Intestines will be used to train laboratory staff on SSCT and/or to provide material for proficiency tests (see WP4). Worms produced will be used as reference material for molecular tests, NGS included (see WP3).

ST2. Sheep model of Eg. A sheep model will be used to produce material for infection biomarker discovery through proteomic analysis of exosomes from plasma. A previous proteomic study on exosomes isolated from plasma of patients infected with CE, carried out within the project HERACLES, has identified potential biomarkers of cyst viability (see WP3-T4). The analysis of the exosome proteomic profile obtained from plasma of experimentally infected sheep with CE and uninfected controls, maintained in a protected environment, will allow exploring the presence of these newly discovered potential biomarkers, as well as additional ones, in the natural intermediate host. Sheep (N=10) will be purchased at weaning age and N=5 will be infected orally with 1000 viable *E. granulosus* eggs, isolated from infected dogs faeces (see WP1). Sheep will be maintained for 1 year post-infection in an animal facility of INIAV, kept indoors and separated

from other animals. Plasma will be collected at different time points throughout infection, and cysts will be collected when sheep will be euthanized 1 year post-infection. CE cysts will provide reference material for molecular analyses (see WP3), training, and proficiency tests (see WP4).

Deliverables		
Ref	**Title**	**Due month**
D-JRP22-WP1.1	SOPs for sampling in matrices shared within the Consortium	M28
D-JRP22-WP1.2	Collection of samples in the field finalized	M36
Milestones		
Ref	**Title**	**Due month**
M-JRP22-03	Ethics approvals for the use of animal model	M28
M-JRP22-12	Interim evaluation of collection of samples in the filed	M32

WP: **2 (VALIDATION of PARASITOLOGICAL and MOLECULAR ASSAYS)**
(takes place over the 3rd and 4th year)
WP start month: **29**; WP end month: **44**; WP Leader: **ANSES**
WP participants: ISS, FLI, ANSES, RIVM, PIWET, SVA, NVI, SSI, INIAV, UT, VFL, CVRL, BIOR
Description of the WP: This WP will focus on the **harmonization and validation of selected parasitological and molecular procedures**. Validation will include estimates of the analytical and diagnostic performance characteristics of tests. This research will be conducted in line with OIE principles and methods of validation of diagnostic assays for infectious diseases. Development and validation of analytical methods will be performed, where possible, in a quality system according to **UNI CEI EN ISO/IEC 17025:2005** (*General requirements for the competence of testing and calibration laboratories*). In the absence of an acceptable gold standard, the performance of the diagnostic tests will be assessed by means of **Bayesian modelling** (Branscum et al., Prev Vet Med 2005).

WP2	M25-26	M27-28	M29-30	M31-32	M33-34	M35-36	M37-38	M39-40	M41-42	M43-44	M45-46	M47-48	M49-50	M51-52	M53-54
T1.						D2.1									
T2.															
T3.															

Task: **JRP-WP2-T1. Segmental Sedimentation and Counting Technique, SSCT**
(takes place over the 3rd year)
Task start month: 29; Task end month: 36; Task Leader: ANSES
Task Participants: FLI, ANSES, PIWET, NVI, SSI, INIAV, VFL, BIOR
Description of the task: The diagnosis of an echinococcal infection in the definitive host is important for epidemiological studies. Difficulties in the detection of the adult parasite in definitive hosts mainly derive from the irregular shedding of *Echinococcus* spp. eggs and the inability to distinguish them morphologically from those of *Taenia* spp. Since the available diagnostic methods differ among laboratories both methodologically and in their performances, there is a need to validate and harmonize the detection methods. This task will focus on the validation of the technique currently considered the reference technique for the detection of Em/Eg in definitive hosts: Segmental Sedimentation and Counting Technique, SSCT (Umhang et al., Exp Parasitol 2011). In this task, the SSCT method will be evaluated for its diagnostic and analytical sensitivity and specificity.

For this purpose, well-characterized specimens (intestinal mucosa and faecal samples with known infection status) will be obtained in WP1. For validation, specimens will be analyzed by SSCT method versus SCT (Sedimentation and Counting Technique) through an interlaboratorty evaluation within the participants.

Task: **JRP-WP2-T2. Comparison of multiplex PCRs** (takes place over the 3rd and 4th year)

Task start month: 29; Task end month: 44; Task Leader: ISS

Task Participants: ISS, FLI, ANSES, PIWET, NVI, SSI, INIAV, VFL, CVRL, BIOR

Description of the task: The molecular characterization of *E. granulosus* isolates is pivotal to understand the spatio-temporal epidemiology and infection evolution of *E. granulosus* species complex in many endemic areas. Also of paramount importance is the correct identification of cystic lesions caused by different cestodes (such as *E. multilocularis*, and closely related members of the genus *Taenia*) in the **intermediate hosts**, both in livestock and humans. Boubaker and colleagues (2013) published a single-tube multiplex PCR allowing discrimination of *Echinococcus* at the level of species/genotypes. Moreover, Trachsel and colleagues (2007) developed a single-tube multiplex PCR targeting the **definitive host** for the identification of eggs belonging to *E. granulosus*, *E. multilocularis* and *Taenia* genus. This task is dedicated to the validation of these two molecular assays widely used for the detection of *Echinococcus* in definitive and intermediate hosts, targeting mitochondrial and nuclear markers (Trachsel et al., Parasitology 2007; Boubaker et al., PLoS Negl Trop Dis 2013).

Task: **JRP-WP2-T3. Magnetic Capture - Real Time PCR assay** (takes place over the 3rd and 4th year)

Task start month: 29; Task end month: 44; Task Leader: SVA

Task Participants: ISS, FLI, ANSES, RIVM, PIWET, SVA, NVI, SSI, INIAV, UT, VFL, BIOR

Description of the task: This task is dedicated to the validation of a molecular assay widely used in surveillance programmes for Em, in the context of the European regulation (EU No. 2018/772): Magnetic Capture - Real Time PCR assay (Isaksson et al., Parasite & Vectors 2014). This semi-automated magnetic capture probe DNA extraction method and real time hydrolysis probe polymerase chain reaction assay (MC-PCR) is used for the detection of *E. multilocularis* DNA in faecal samples from red foxes. This assay proved to have a high sensitivity and a very high specificity.

Deliverables		
Ref	**Title**	**Due month**
D-JRP22-WP2.1	Validation of SSCT	M36
Milestones		
Ref	**Title**	**Due month**
M-JRP22-04	SOP for validation of SSCT	M30
M-JRP22-05	SOP for validation of multiplex PCRs	M30
M-JRP22-06	SOP for Validation of Magnetic Capture - Real Time PCR assay	M30

WP: **3 (DEVELOPMENT/VALIDATION of NEW TOOLS and PRODUCTION of DATA RELEVANT for EPIDEMIOLOGICAL ASSESSMENTS)** (takes place over the 3rd, 4th and 5th year)

WP start month: **27**; WP end month: **52**; WP Leader: **FLI**

WP participants: ISS, FLI, ANSES, PIWET, SVA, NVI, SSI, INIAV, INSA, UT, VRIXJ, BIOR, CMRIXJ, JLU

Description of the WP: This WP is aiming to **generate new innovative tools** for rapid detection, differential diagnosis, and tracking of infection, both at large and small-scale settings. The tools will include:

1) new molecular markers (new mitochondrial and microsatellite markers, and NGS-based method);

2) new multiplex TaqMan qPCR for detection and genotyping of Eg s.l. and Em;

3) new NGS approach instead of the current sequencing methods;

4) biomarker discovery through proteomics in sheep plasma exosomes.

This WP will also collect field samples and data to generate **data relevant for epidemiological assessments** on:

1) contamination of vegetables for human consumption by eggs of Em/Eg;
2) prevalence of Em/Eg in dogs;
3) potential risk factors for human CE/AE infection.

WP3	M25-26	M27-28	M29-30	M31-32	M33-34	M35-36	M37-38	M39-40	M41-42	M43-44	M45-46	M47-48	M49-50	M51-52	M53-54
T1.															
T2.															
T3.															
T4.															
T5.															
T6.															
T7.															
T8.															

Task: **JRP-WP3-T1. New molecular markers for Em and Eg s.l.: from rapid diagnostics to source attribution** (takes place over the 3rd and 4th year)
Task start month: 27; Task end month: 48; Task Leader: ANSES
Task Participants: ISS, FLI, ANSES, NVI, SSI, UT, JLU
Description of the task:
ST1. New mitochondrial markers. Recent studies have revealed an unexpectedly high genetic diversity at the level of the mitochondrial genome for *E. granulosus sensu stricto* and for genotypes G6-G7. However, these studies covered only part of the genetic diversity as isolates from many important regions were missing (e.g. China, Russia). Moreover, for several species, including *E. multilocularis*, the mitogenome data are scarce. In order to develop species and genotype specific diagnostic assays, additional mitogenome data are required for all *Echinococcus* species using a larger worldwide panel of different *E. granulosus s.l.* species and genotypes (N=300), and also for *E. multilocularis* (N=200). From this, the knowledge of differences in nucleotide composition of mitochondrial genome will be used to develop a Toehold Exchange Probes based diagnostic tool able to provide a reliable and rapid identification of species/genotypes of *E. granulosus s.l.* and *E. multilocularis*.
ST2. New microsatellite markers. *E. granulosus sensu stricto* is the dominant species (88% of infections identified at species level) responsible of human cases of cystic echinococcosis in the world. The recent sequencing of the nuclear genome opens the possibility to use bioinformatic tools to search for microsatellite sequences. A first bioinformatics screening based on microsatellites characteristics will be performed, before to realize a targets selection providing correct PCR amplification. Easily interpretable profiles and selection of polymorphic targets will be assessed by observation of electrophoretic profiles obtained by capillary electrophoresis. A collection of human/animal CE samples from different geographical and host origins will be analysed using the selected microsatellites. According to the polymorphism observed, a panel of microsatellites will be validated to obtain a final panel of microsatellites with high discrimination power allowing to perform source attribution.
ST3. NGS-based method. The very high variability of *E. granulosus s.s.* 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.

Task: **JRP-WP3-T2. New multiplex TaqMan qPCR for detection and genotyping of Eg s.l. and Em** (takes place over the 3rd and 4th year)

Task start month: 27; Task end month: 48; Task Leader: FLI

Task Participants: ISS, FLI, ANSES, SSI, INIAV, UT, CMRIXJ, JLU

Description of the task: The *Echinococcus* genus can be currently divided into nine species: *E. multilocularis*, *E. shiquicus*, *E. oligarthrus*, *E. vogeli* and the *E. granulosus* complex pecies [*E. granulosus sensu stricto* (G1,G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6–G10) and *E. felidis*]. Affordable and species-specific molecular assays are required to investigate epidemiologically the dynamics, pathogenicity, as well as the infection routes of the members of this genus. Several PCR protocols for genotyping of *E. granulosus sensu lato* and *E. multilocularis* were published in the past. However, most of them are based on conventional PCR and require an additional sequencing step, or their application is limited to certain *Echinococcus* species. The aim of the present task is to develop a TaqMan probe-based multiplex Real-Time PCR as a tool for a simultaneous, rapid diagnosis and typing of *E. granulosus sensu lato* and *E. multilocularis*. For the development of the TaqMan multiplex Real-Time PCR, samples collected in WP1-T1 will be used.

Task: **JRP-WP3-T3. Detection of Em/Eg in complex samples: sequencing using Regions Specific Extraction (RSE) and NGS** (takes place over the 3rd and 4th year)

Task start month: 27; Task end month: 48; Task Leader: NVI

Task Participants: ANSES, SVA, NVI, SSI, UT, CMRIXJ, JLU

Description of the task: This task will employ a novel approach for *Echinococcus* spp. detection and characterisation by combining DNA-enrichment methods, using parasite-specific DNA-capture probes and magnets, from complex biological samples (e.g. faeces or raw vegetables), and characterisation of the captured DNA using high throughput sequencing technologies (nanopore and ILLUMINA). Access to these technolologies may give partners different alternatives for parasite detection and characterisation. In this task, we seek to investigate whether detection of *E. granulosus* and *E. multilocularis* DNA in complex samples is possible and whether standard protocols using the nanopore platform could provide partners with a fast and reliable alternative method for parasite identification that does not require heavy investments in infrastructure or instruments for DNA sequencing. Analysis of the generated data will be carried out using common computing platforms such as e.g. Epi2me/metrichor or Galaxy, which are commercially or freely available. This will enable shared access of sequence-data and analysis pipelines between partners in the project.

Task: **JRP-WP3-T4. Proteomic study on biomarker discovery in exosomes from animal plasma** (takes place over the 3rd, 4th and 5th year)

Task start month: 27; Task end month: 52; Task Leader: ISS

Task Participants: ISS, ANSES, INIAV

Description of the task: A previous proteomic study on exosomes isolated from plasma of patients infected with CE, carried out within the project HERACLES, has identified potential biomarkers of cyst viability (Fratini et al., manuscript in preparation; see the section "**Relation to ongoing national and international projects/activities**"). The aim of this task, continuing the investigations carried out in the project HERACLES, is to analyze the exosome proteomic profile obtained from plasma of sheep infected with CE and uninfected controls (see WP1). Plasma from sheep obtained at **abattoirs** (WP1-T2) will be used in particular to explore the presence of the potential biomarkers newly discovered in CE infected humans also in the main natural intermediate host.

Plasma from sheep maintained in protected **research facilities** after controlled infection (WP1-T3) will be used in particular for new potential biomarkers discovery in the natural intermediate host.

Task: **JRP-WP3-T5. Assessment of newly developed molecular methods against the existing techniques** (takes place over the 4th and 5th year)

Task start month: 43; Task end month: 52; Task Leader: FLI, ANSES

Task Participants: ISS, FLI, ANSES, NVI, SSI, INIAV, UT, CMRIXJ, JLU

Description of the task: In this task, the diagnostic performances of new and existing molecular and parasitological techniques will be compared. This will be performed with two sets of comparisons:

1) comparison of multiplex PCRs techniques (WP2-T2), magnetic capture-Real Time PCR technique (WP2-T3), and the newly developed multiplex Taqman qPCR (WP3-T2) using reference DNA obtained from adult *E. multilocularis* worms and *E. granulosus* cysts (WP1-T2);

2) comparison of the parasitological Segmental Sedimentation and Counting Technique (SSCT) (WP2-T1) with the above-mentioned molecular techniques (WP2-T2,T3 and WP3-T2) using intestines and DNA extracted from the faeces of the same experimentally infected foxes (WP1-T3), respectively.

Task: **JRP-WP3-T6. Contamination of vegetables for human consumption by Em/Eg** (takes place over the 3rd and 4th year)

Task start month: 29; Task end month: 48; Task Leader: ANSES

Task Participants: ISS, ANSES, UT, BIOR, CMRIXJ, JLU

Description of the task: Human infection by Em/Eg is caused by oral ingestion of microscopic parasite eggs. While the exact route of infection of individual human cases is generally unknown, the foodborne transmission is plausible. As eggs are inactivated by heat (i.e. cooking) the consumption of raw vegetables contaminated with viable eggs could lead to human infection, but today very scanty data are available on the degree of such contamination. Development of a robust and reliable method, coupling visual detection of eggs to assess their integrity and molecular biology for species identification, is required and will be developed for detection of Em/Eg eggs, specifically in lettuce. This method will be validated using samples spiked with known eggs numbers to assess the limit of detection. Using this method, a survey on 100 lettuce samples from each of the participating countries of Italy, France, Latvia and China will be conducted to obtain an epidemiological evaluation of the importance of the contamination of lettuce with Em/Eg eggs.

Task: **JRP-WP3-T7. Prevalence of Em/Eg in dog (faecal samples) from selected geographical areas** (takes place over the 3rd and 4th year)

Task start month: 29; Task end month: 48; Task Leader: PIWET

Task Participants: ANSES, PIWET, NVI, SSI, INIAV, UT, BIOR, CMRIXJ, JLU, VRIXJ

Description of the task: Dogs are main final hosts for *E. granulosus s.l.* and potential final hosts for *E. multilocularis* in Europe. Faeces of infected dogs is an important source of infection for humans. Due to the close contact with people, even the low prevalence of *Echinococcus* spp. in these animals may cause a significant risk of infection. In this task, faecal samples will be collected in selected endemic regions of Poland (PIWET, N=400), France (ANSES, N=400), Portugal (INIAV, N=300), Denmark (SSI, N=200), Latvia (BIOR, N=200), Norway (NVI, N=400) and China (CMRIXJ, JLU, VRIXJ N=500 from Xinjiang, Tibet, Sichuan and Inner Mongolia provinces). The total copro-DNA will be isolated using kits dedicated to faeces and examined by multiplex PCR (Trachsel et al., Parasitology 2007) for *E. multilocularis* and *E. granulosus s.l.* This task will provide current data on prevalence of these tapeworms in dogs from selected areas of northern, eastern and western Europe, and, additionally, endemic region of China, with a total of 2,400 faecal samples analysed.

Task: **JRP-WP3-T8. Potential human risk factors by means of questionnaires** (takes place over the 3rd and 4th year)

Task start month: 29; Task end month: 48; Task Leader: ISS

Task Participants: ISS, ANSES, SSI, INSA, BIOR, CMRIXJ, JLU, VRIXJ

Description of the task: Human CE and AE are considered food/water borne infections, however risk factors/at-risk habits and source attribution of human infections are poorly known. Semi-structured questionnaires on potential risk factors for human infection with CE and AE will be designed , taking advantage also of available **ECDC repositories and toolkits** for food and waterborne disease outbreaks investigation (https://ecdc.europa.eu/en/publications-data/outbreak-investigation-questionnaire-repository-questions-tool-5a; https://ecdc.europa.eu/en/publications-data/toolkit-investigation-and-response-food-and-waterborne-disease-outbreaks-eu). Questionnaires will be submitted for ethical approval to the relevant committees of the participating hospitals. Upon written informed consent, questionnaires will be_administered to volunteers with CE/AE and matched (by age, sex and country of origin) uninfected controls accessing clinical centres belonging to the European Register of CE **network** (http://www.heracles-fp7.eu/erce.html; Rossi et al., 2016), the **French National Reference Centre for Alveolar Echinococcosis (CNR-EA)** (Charbonnier et al., 2014), **Riga East University Hospital** (Latvia) (Laivacuma et al., 2019), **Hospital do Litoral Alentejano** (http://www.ulsla.min-saude.pt/) (Portugal), **Hospital do Espírito Santo de Évora, EPE** (Portugal) (http://www.hevora.min-saude.pt/), and **Hospital Santa Luzia de Elvas** (Portugal) (http://www.ulsna.min-saude.pt), **The First Bethune Hospital of Jilin University** (China) (http://www.jdyy.cn/) and **The First Affiliated Hospital of Xinjiang Medical University** (China) (http://www.xydyfy.cn/).

Deliverables		
Ref	**Title**	**Due month**
Milestones		
Ref	**Title**	**Due month**
M-JRP22-07	Protocols for New molecular methods, NGS included	M30
M-JRP22-08	Protocol for proteomic analysis in animals plasma	M30
M-JRP22-09	Protocol for contamination of vegetables by Em/Eg	M30
M-JRP22-10	Protocol for prevalence of Em/Eg in dog	M30
M-JRP22-11	Questionnaire scheme for potential human risk factors	M30

WP: **4 (TRAINING, DISSEMINATION and PROFICIENCY TESTING SCHEMES)**
(takes place over the 3rd, 4th and 5th year)
WP start month: **27**; WP end month: **54**; WP Leader: **ISS**
WP participants: ISS, FLI, ANSES, RIVM, PIWET, SVA, NVI, SSI, INIAV, INSA, UT, VFL, CVRL, BIOR, CMRIXJ, JLU, VRIXJ
Description of the WP: This WP will focus on:
1) establishing the most effective methods for **disseminating project results** at different levels (general public, population at risk, biologists, veterinarians, clinicians, health authorities, policy makers and media);
2) training scientists from institutions participating to MEmE in the parasitological and molecular identification of *Echinococcus* spp. This WP will also focus on the dissemination and training within **International Networks** linked to MEmE such as the NRL for Parasites/*Echinococcus* (https://w3.iss.it/site/EURLPMaps/MapsInfo.asp);
3) organization and delivery of PTs for the harmonization and validation of selected parasitological and molecular procedures.

WP4	M25-26	M27-28	M29-30	M31-32	M33-34	M35-36	M37-38	M39-40	M41-42	M43-44	M45-46	M47-48	M49-50	M51-52	M53-54
T1.															
T2.															

T3.															

Task: **JRP-WP4-T1. Trainings (parasitological and molecular approaches, NGS included)** (takes place over the 3rd and 4th year)
Task start month: 27; Task end month: 48; Task Leader: ISS, ANSES, SVA, FLI, NVI
WP participants: ISS, FLI, ANSES, RIVM, PIWET, SVA, NVI, SSI, INIAV, INSA, UT, VFL, CVRL, BIOR, CMRIXJ, JLU, VRIXJ
Description of the task: Training activities will focus on **Exchange of key staff members** between MEmE labs (short-term fellowships) in order to enhance cohesion and improve implementation of project activities, to share skills, expertise and protocols, to standardize the diagnostic tests used in the different laboratories and to prepare for PT schemes. It is expected that at least one member of each laboratory will visit a different laboratory within the Consortium. The European Union Reference Laboratory for Parasites (EURLP) **annual meeting** will also train **veterinarians** (http://www.iss.it/crlp/index.php?lang=2&anno=2016&tipo=25) from the National Reference Laboratories for Parasites on diagnostic procedures during surveillance activities. Training activities will focus on the following techniques:
1) Segmental Sedimentation and Counting Technique, SSCT (organized by ANSES);
2) Multiplex PCRs (organized by FLI and ISS);
3) Magnetic Capture - Real Time PCR assays (organized by NVI, SVA and FLI);
4) Detection of Em/Eg in complex samples: sequencing using Region Specific Extraction and NGS (organized by NVI).
Task: **JRP-WP4-T2. Dissemination of project results** (takes place over the 3rd, 4th and 5th year)
Task start month: 27; Task end month: 54; Task Leader: ISS
WP participants: ISS, FLI, ANSES, RIVM, PIWET, SVA, NVI, SSI, INIAV, INSA, UT, VFL, CVRL, BIOR, CMRIXJ, JLU, VRIXJ
Description of the task: Dissemination will cover three levels: **1) EU citizens** (general public) especially the populations at risk of Em/Eg infection, living in endemic areas of countries participating in this project; **2) general practitioners, veterinarians, clinicians and biologists** working in diagnostic laboratories; **3) health authorities** (both medical and veterinary) and **policy makers** at regional, national and EU (EFSA, ECDC) levels. Dissemination activities will be planned and implemented in order to ensure the appropriate **visibility** of the project. The dissemination plan will identify target **stakeholders** and **media** for such activities. The **dissemination activities** and **exploitation of the results** will be conducted along multiple lines: **1) presentation of scientific papers** will be one of the main expected outcomes from the project partners; **2) participation in national and international meetings, conferences, workshops**, academic courses and seminars; **3) individual presentations to stakeholders**.

Deliverables		
Ref	Title	Due month
Milestones		
Ref	Title	Due month
M-JRP22-14	Organization of training periods	M36

WP: **5 (SCIENTIFIC and ADMINISTRATIVE MANAGEMENT)**
(takes place over the 3rd, 4th and 5th year)
WP start month: **25**; WP end month: **54**; WP Leader: **ISS**
WP participants: ISS, FLI, ANSES, RIVM, PIWET, SVA, NVI, SSI, INIAV, INSA, UT, VFL, CVRL, BIOR, CMRIXJ, JLU, VRIXJ
Description of the WP: **This WP will focus on the scientific and administrative coordination, which** are foreseen in the project work plan to ensure efficient and successful cooperation between the project partners. This requires the setting up of effective decision-making bodies, clear external

communication and operational internal communication, and effective administrative and technical control. As such, **coordination** will be based on the following major principles: **1)** effective quality management; **2)** compliance with all relevant EC regulations and implementation of sound monitoring and professional administration to prevent time and cost escalation; **3)** strong research commitment from the entire team to enable efficient execution of activities, timely delivery of results, and horizontal data dissemination across the Consortium and stakeholders to ensure the exploitation of the results; **4)** flexible and responsive management to accommodate the need for changes as the Consortium advances its scientific agenda; **5)** identification, protection and management of the Intellectual Property Rights (IPR) generated by the project; **6)** management of ethical issues. This WP will ensure also the good management of the research data through the development of a **Data Management Plan** (DMP).

WP5	M25-26	M27-28	M29-30	M31-32	M33-34	M35-36	M37-38	M39-40	M41-42	M43-44	M45-46	M47-48	M49-50	M51-52	M53-54
T1.		D5.2	D5.1												
T2.						D5.5									
T3.															

Task: **JRP-WP5-T1. Setup of the project** (takes place over the 3rd year)
Task start month: 25; Task end month: 30; Task Leader: ISS
WP participants: ISS
Description of the task: This task will focus on responsibilities allocation among Consortium members during the inception of MEmE.
A common baseline for tasks and responsibilities allocation will be established. The overall project management will take measures to guarantee control, verification of the project results, and to ensure that plans are fulfilled and necessary corrective actions are implemented. The project management structure will be settled with the **Steering Committee (SC)** and the **External Advisory Board (EAB)** to support risk mitigation and strengthen the project's scientific excellence and worldwide impact. A **Data Management Plan** will be prepared and submitted as a deliverable, and updated regularly, including specifications on: **1)** handling of research data during and after the end of the project; **2)** type of data collected, processed and/or generated; **3)** methodology and standards applied; **4)** strategies for data sharing/open access; **5)** data preservation.
Task: **JRP-WP5-T2. Administrative and scientific management**
(takes place over the 3rd, 4th and 5th year)
Task start month: 27; Task end month: 54; Task Leader: ISS
WP participants: ISS, FLI, ANSES, RIVM, PIWET, SVA, NVI, SSI, INIAV, INSA, UT, VFL, CVRL, BIOR, CMRIXJ, JLU, VRIXJ
Description of the task: The goal of this task is to ensure the timely issue of periodic management reports and cost statements, to handle the EJP/EC project reviews and payment issues. Specific activities will include: **1)** daily management of administrative consortium activities; **2)** following/supporting the preparation and signature of the Agreement with **external partners**; **3)** checking the progress of administrative work; **4)** coordinating the administrative bodies and work of the different participants' institutions; **5)** coordinating the preparation of financial reports; **6)** supporting the organization of meetings. **Annual project meetings** will be organized as follows: Kick off meeting: beginning of 2020 at ANSES; Interim meeting: beginning of 2021 at FLI; Final meeting: mid 2022 at ISS.
Task: **JRP-WP5-T3. Ethics management** (takes place over the 3rd, 4th and 5th year)
Task start month: 25; Task end month: 54; Task Leader: ISS
WP participants: ISS, FLI, ANSES, RIVM, PIWET, SVA, NVI, SSI, INIAV, INSA, UT, VFL, CVRL, BIOR, CMRIXJ, JLU, VRIXJ

Description of the task: The Intellectual Property Rights (IPR) on products generated by the project will be managed and protected. A **Consortium Agreement** with **external partners** will be prepared to define the Consortium members' rights and duties, and to handle IPRs issues arising during the project. To ensure appropriate protection of the project's interest, the participants intend to organize the exploitation and the management of IPRs in accordance with all EC rules and in accordance with the **Guide to IPR Rules in Horizon 2020**, "*background*" and "*foreground*". All research activities carried out for the project MEmE will be in compliance with fundamental ethical principles including those reflected in the **Charter of Fundamental Rights of the European Union** and will take into account opinions of the **European Group on Ethics in Science and New Technologies (EGE)**. Participants in MEmE fully conform to national legislation and applicable codes of conduct and will seek the approval of the relevant ethics committees prior to start of the RTD activities **(99/167/EC: Council Decision of 25/1/99)**.

Deliverables

Ref	Title	Due month
D-JRP22-WP5.1	Data Management Plan (DMP)	M30
D-JRP22-WP5.2	Periodic technical and financial reports to EJP/Commission	M36
D-JRP22-WP5.5	Kick-off annual meeting in Malzéville (France) by ANSES	M27

Milestones

Ref	Title	Due month
M-JRP22-01	Tasks and responsibility allocation	M25
M-JRP22-02	Organization of Kick-off annual meeting by ANSES	M26
M-JRP22-13	Preparation of technical and financial reports to EJP/Commission	M35

2.4.2.1.19 JRP19-R2-ET1.1-PARADISE

Reference to the Stategic Research Agenda (please refer to D2.7)			**ET 1.1: Development and harmonization of NGS and non-NGS methods (e.g. pheno-genotypic and histochemical methods) for the detection of foodborne parasites**							
Research Project Title			**PARADISE– PARAsite Detection, ISolation and Evaluation**							
Research Project Acronym			**PARADISE**							
Leading Organisation			**P27-ISS**			Deputy Leading Organisation			**P41-SVA**	
Project Leader			**Simone M. Cacciò**			Deputy Project leader			**Karin Troell**	
Project Start month			**M25**			Project End month			**M54**	
Participant	**1**	2	4	6	7	**8**	**9**	10	**11**	12
	Anses	Ages	Sciensano	NDRVMI	SZU	**VRI**	**BfR**	FLI	**RKI**	DTU
PM	**6.5**					**4**	**0.3**		**14**	
Participant	**13**	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM	**9.05**									
Participant	**23**	**24**	25	26	**27**	28	29	**30**	31	32
	UoS	**OKI**	NUIG	TEAGASC	**ISS**	IZSAM	IZSLER	**RIVM**	WbvR	FHI
PM	**5.65**	**2**			**25.6**			**3.34**		
Participant	**33**	**34**	**35**	36	37	38	**39**	**40**	**41**	
	NVI	**PIWET**	**INIAV**	INSA	IISPV	IC	**SLV**	**FOHM**	**SVA**	
PM	**0.3**	**3.9**	**2.51**				**0.4**	**1**	**20**	

Objectives

- To organize the kick-off meeting, allocate tasks and establish the management team, steering committee and advisory board (WP1)
- Generation of new *C. parvum* genomes (WP2)
- Generation of new *G. duodenalis* genomes (WP2)
- *In silico* analysis of new and unpublished genomes available within the consortium (WP2)
- Establishment of a referenced genome database for foodborne parasites (FBPs) using available data and data generated within PARADISE (WP2).
- Use referenced database for interrogation of available metagenomes and detection of FBPs (WP2)
- Develop optimized protocol for amplicon-based metagenomics (WP2)
- Detection of FBPs on spiked food by amplicon-based and shotgun metagenomics approaches (WP2)

- *In silico* selection of markers suitable for high-resolution typing of *C. parvum* and *G. duodenalis* (WP3)
- Testing of the selected markers by a team of expert laboratories for development of MLST schemes for *C. parvum* and *G. duodenalis* (WP3)
- Development of pre-DNA extraction enrichment strategies, based on nanobodies and aptamers, for *C. parvum* and *G. duodenalis* (oo)cysts (WP4)

Development of post-DNA extraction enrichment strategy, based on hybridization capture probes, to concentrate low abundance target DNA in various matrices (WP4)

Description of work

The work is organized into 4 work packages (WPs), with specific tasks (T). The 2.5-year project spans over three Annual Periods (Y3-Y5) and is described in three Work Plans.

WP JRP-PARADISE_WP1 Coordination and impact

WP start month M25; WP end month M54

WP Leader: **Simone M Cacciò, ISS**; WP Deputy: **Karin Troell, SVA**.

WP participants: **ANSES, BFR, FOHM, INIAV, ISS, NVI, OKI, PIWET, RIVM, RKI, SLV, SSI, SVA, UoS, VRI, BIOR, CRU, HZAU, JLU, NMBU, UoM**

Description of the WP:

- WP1 takes place over the first, the second and the third annual period of the project. WP1 works closely with all the WPs of the project.

WP1 will be responsible for all reporting to the OHEJP coordinating body and will coordinate three annual meetings during the 3-year project. Because of the large number of partners in the consortium, and the need for interaction between WPs, WP1 will play an important role in ensuring that milestones and deliverables of the consortium are successfully completed and timely communicated.

The Coordinator at ISS and the Co-coordinator at SVA will collaborate in keeping continuous contact between the WPs and ensure proper dissemination of results.

A PARADISE Management Team (PAMT) comprising the Coordinator, Co-coordinator and WP leaders will be established. The project will also have a Steering Committee, with one representative per partner Institute. The Committee will meet during the three annual assemblies planned within PARADISE.

In addition to these, an Advisory Board comprising relevant stakeholders, including a representative from CDC and from EFSA, will be included. Members for the Advisory Board will be recruited at the start of the project. The Board will meet with the coordinators through Skype-meetings, and will be asked for advice on project directions two times in the project period.

Task: JRP-PARADISE-WP1-T1 T-0.1 Management, coordination and communication

Task start month: **M25**; Task end month: **M54**

Task Leader: **Simone M Cacciò**, **ISS**; Deputy Task Leader: **Karin Troell**, **SVA**

Task Participants: **ANSES, BFR, FOHM, INIAV, ISS, NVI, OKI, PIWET, RIVM, RKI, SLV, SSI, SVA, UoS, VRI, BIOR, CRU, HZAU, JLU, NMBU, UoM**

Description of the task :

- WP1-T1 takes place over the first, second, and third annual period of the project.

This task will include communication to all partners about project progress, upcoming deadlines, follow-up of deliverable completion, and reporting of deliverables to the OHEJP.

Coordination and management of the project. A PARADISE Management Team (PAMT) comprising the Coordinator, Co-coordinator and WP leaders will be established. Throughout the project, the PAMT will have regular teleconferences (at least four times per year or more often, if needed) to

communicate across WPs and ensure that the work flow within and between WPs proceeds as expected. A common platform will be set up to facilitate sharing of information and documents. Adherence to H2020 rules regarding e.g. ethics, dissemination and publication, will be ensured. The Coordinator at ISS will support all partners in the project with any questions regarding financial or procurement of goods and services.
Coordination of project meetings organization. In the first year of the project, a Kick-Off meeting (KOM) will be organized by the project Coordinator at ISS (Rome, Italy), where the consortium participants meet face-to-face.

WP: JRP-PARADISE_WP2 NGS-based genomics and metagenomics
Task start month: **M25**; Task end month: **M52**
WP Leader: **Yannick Blanchard**, **ANSES**; WP Deputy: **Simone M Cacciò, ISS**
WP Participants; **ANSES, BFR, FOHM, INIAV, ISS, NVI, OKI, PIWET, RIVM, RKI, SLV, SSI, SVA, UoS, VRI**
Description of the WP:

- WP2 takes place over the first, the second and the third annual period of the project.

WP2 aims at 1) generating novel genome data from selected isolates of *Cryptosporidium parvum* and *Giardia duodenalis* in WP2-T1; 2) using in silico approaches and a referenced database to interrogate available metagenomes to detect foodborne parasites in WP2-T2; and 3) using amplicon-based and shotgun metagenomics approaches to detect target parasites using spiked food matrices in WP2-T3.

Task: JRP-PARADISE-WP2-T1 **NGS-based genome study of selected isolates of *C. parvum* and *G. duodenalis***
Task start month **M25**; Task end month **M36**
Task Leader: **Simone M Cacciò, ISS**; Deputy Task Leader[1]: **Yannick Blanchard, ANSES**
Task Participants: **ANSES, BFR, FOHM, INIAV, ISS, NVI, OKI, PIWET, RIVM, RKI, SLV, SSI, SVA, UoS, VRI**
Description of the task:

- WP2-T1 takes place over the first year of the project

This task will address major gaps in current knowledge of the genomic variability of *C. parvum* and *G. duodenalis*, and will achieve this by sequencing an estimated one hundred genomes (50 for each parasite) chosen to allow better geographic coverage and host representativeness. Completion of this task will benefit from harmonized protocols for wet laboratory procedures and from analytical pipelines to process NGS data. These originates from the Horizon 2020 project COMPARE, to which ISS has participated. ANSES will act as a centralized sequencing center, in view of the consolidated experience in the use of various sequencing platforms (Illumina, Ion Torrent, PacBio and MinIon). Data analysis will receive contribution from bioinformatics expertise available at ANSES, ISS, RKI and SVA. Genomes will be sequenced at a coverage of 50x for robust identification of mutations.

Task: **JRP-PARADISE-WP2-T2 *In silico* analyses of metagenomes for detection of foodborne parasites (protozoa and helminths)**
Task start month **M25**; Task end month **M52**
Task Leader: **Frits Franssen, RIVM**; Deputy Task Leader: **Yannick Blanchard, ANSES**.
Task Participants: **RIVM, ISS, SSI, VRI**
Description of the task:

- WP2-T2 takes place over the first, second and third annual period of the project

This task will establish a referenced database of genomes of relevant unicellular and multicellular foodborne parasites (FBPs). Shotgun-derived metagenomes from human and animal gut

microbiomes, environmental samples (soil, water, and wastewater/sludge), and food (plant-related) will be selected. Next, raw sequence data from these metagenomes will be mapped, using stringent parameters, to each reference genome. RIVM will provide dedicated servers for big data in a high performance grid, granting the computational power needed to query large metagenome data. As a result, new information regarding the distribution of FBPs in different environments will be gathered, in turn helping source attribution efforts. Metadata analysis will allow evaluation of geographical information.

Task: **JRP-PARADISE-WP2-T3 Experimental amplicon-based and shotgun metagenomics for detection of foodborne parasites**
Task start month **M25**; Task end month **M52**
Task Leader: **Rune C. Stensvold, SSI;** Deputy task Leader: **Petr Kralik, VRI**
Task Participants: **SSI, ISS, RIVM, VRI**
Description of the task:

- WP2-T3 takes place over the first, second and third annual period of the project

Amplicon-based sequencing targeting ribosomal genes is a well-established methodology to profile bacterial communities. It can be used for eukaryotic parasites (and fungi), but the necessity to limit amplification of the host's ribosomal DNA while ensuring that of a taxonomically large array of target organisms, complicates this approach. Shotgun metagenomics is an untargeted sequencing approach used to profile taxonomic composition and functional potential of communities.
This task will compare the relative performance of the two approaches for the detection of different target organisms (e.g., *Cryptosporidium*, *Giardia*, and *Toxoplasma*). Food matrices will be spiked with known numbers of (oo)cysts, and nucleic acids will be sequenced to explore the limit of detection. An amplicon-based platform developed and extensively tested on clinical, food and environmental samples at SSI, will be further optimized for this task.

Description of work
WP: JRP-PARADISE_WP3 Design, implementation and validation of multi-locus typing schemes
Task start month **M25**; Task end month **M54**
WP Leader: **Karin Troell, SVA**; Deputy WP Leader: **Christian Klotz, RKI**
WP participants: **ANSES, BFR, FOHM, INIAV, ISS, NVI, OKI, PIWET, RIVM, RKI, SLV, SSI, SVA, UoS, VRI**

Description of the WP :

- WP3 takes place over the first, the second and the third annual period of the project

Based on whole genome sequencing data generated in WP2-T1, unpublished data from within the consortium and data publicly available, this WP aims to identify new markers suitable for robust high-resolution genotyping (WP3-T1). Candidate markers will be tested by expert laboratories, and the markers and typing approach will be iteratively developed together with WP3-T1 until respective typing protocols are produced (WP3-T2). The protocols will be further tested by inter-laboratory comparison (WP3-T3).

Task: JRP-PARADISE-WP3-T1 In silico selection of informative loci from comparative genomics data.
Task start month **M25**, Task end month **M42**
Task Leader: **Christian Klotz, RKI**; Deputy Task Leader : **Karin Troell, SVA**
Task Participants: **RKI, SVA, ISS**

Description of the task :

- WP3-T1 takes place over the first and second annual period of the project

This task will use sequence data and information generated in WP2–T1 as well as data already generated by consortium members to provide a comparative analysis from which a suite of unlinked multi-locus sequence markers will be identified. This analysis will be performed separately for *C. parvum* (at SVA) and *G. duodenalis* (at RKI).

Loci will be selected based on: (i) sufficient physical distance to minimize genetic linkage; (ii) coverage of all chromosomes; (iii) high genetic variability across genomes; (iv) *in silico* specificity for *C. parvum* or *G. duodenalis*. Suitability of the selected markers for MLST approaches will be tested within WP3-T2, and iteratively improved until appropriate discrimination of parasite strains is possible.

For *G. duodenalis* a set of approximately 30 genomes from recent patient isolates representing the human pathogenic variants (AI, AII, B) are already available within the consortium, and will be used to set-up the pipeline for marker identification based on polymorphic sites. As *G. duodenalis* represents a highly diverse species complex, it may be necessary to develop two typing strategies for Assemblage A and Assemblage B. Recently developed markers allowing high-resolution typing for Assemblage A will be included in the evaluation. Similarly, approximately 40 genomes of *C. parvum* are available within the consortium. These represents parasite isolates differing in geographical origin and host, and representing different genetic variants.

Task : JRP-PARADISE-WP3-T2 Development of MLST schemes for *C. parvum* and *G. duodenalis*
Task start month **M31**; Task end month **M50**
Task Leader: **Martha Betson, UoS**; Deputy Task Leader: **Simone M. Cacciò, ISS**
Task Participants: **ANSES, BFR, FOHM, INIAV, ISS, NVI, OKI, PIWET, RIVM, RKI, SLV, SSI, SVA, UoS, VRI**

Description of the task :

- WP3-T2 takes place over the first, second and third annual period of the project

An iterative approach will be employed in collaboration with WP3-T1 to ensure selection of the most appropriate markers. PCR amplification and Sanger sequencing will be used for marker typing, as this technology is readily available across the EU, the cost is continually decreasing and it allows detection of all SNPs in the loci of interest. However, alternative methods such as restriction fragment length polymorphism or fragment length analysis will also be considered. A collection of isolates of human, animal, water and food origin, covering different EU regions and additional countries, is currently available within the project consortium, including about 900 *C. parvum* and 600 *G. duodenalis* samples. Markers will initially be tested on a smaller group of samples representing parasites of different genotypes and from different geographical regions. The marker panel will be refined to a smaller set of markers (approximately 6-10 markers expected for each parasite) based on ease of amplification and typing, and ability to discriminate between parasite strains. Subsequently, the refined marker set will be tested on the complete sample collection to validate typability and resolution. Of the four expert laboratories involved in WP3-T2, one will undertake validation of the *Cryptosporidium* isolates, one will cover the *Giardia* isolates and two will cover both parasites. After validation, a typing protocol will be developed for each parasite. It is envisaged that the protocols will be flexible depending on the resolution required, with the possibility of using a smaller marker set for particular applications. Of note, the data generated during the marker validation will also provide the opportunity to conduct an analysis of the parasite population structure across Europe.

Description of work

WP : JRP-PARADISE_WP4 Parasite enrichment strategies
WP start month **M25**; WP end month **M54**
WP Leader **Marco Lalle, ISS**; Deputy WP Leader: **Mats Isaksson , SVA**
WP participants **ISS, SVA, RKI, ANSES, VRI INIAV, FOHM, SLV, SSI, RIVM**

Description of the WP :

- WP4 takes place over the first, the second and the third annual period of the project.

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.

Task : JRP-PARADISE-PARADISE-WP4-T1 Development of pre-DNA extraction enrichment strategies
Task start month **M25**; Task end month **M54**
Task Leader: **Christian Klotz, RKI**; Deputy Task Leader: **Gregory Karadjian, ANSES**
Task Participants: **RKI, ANSES, ISS, SVA,VRI**

Description of the task:

- WP4-T1 takes place over the first, the second and the third year of the project.

The objectives of this task are the development and evaluation of pre-DNA extraction protocols based on new affinity reagents (nanobodies and aptamers) for magnetic capture of *C. parvum* and *G. duodenalis* (oo)cysts in different matrices. The developed strategies will be compared by inter-laboratory comparison to evaluate the more reliable and easy-to-use method.
In Y3, the task will start the screening, selection and characterization of the highly affine and specific nanobodies and aptamers, with the ability of binding d *C. parvum* or *G. duodenalis* (oo)cyst, and the verification of cross-reactivity between *Cryptosporidium* and *Giardia*, but also with other parasites. Selection of a set of nanobodies reactive against *C. parvum* IOWA oocysts and a mixture of cysts from different *G. duodenalis* Assemblages, available at ISS, will be done at RKI. To stimulate the production of high-affinity nanobodies, extracts of parasite (oo)cysts or intact (oo)cysts will be mixed and used to immunize a naïve single Alpaca/Llama. Once immune response is achieved, total mRNA will be extracted from the lymphocyte population and used to construct a cDNA phage-library of the variable nanobody region. High-affinity binding sequences will be enriched by standard Phage display approaches or (oo)cyst binding enrichment procedures. Subsequently, a subset of highly affine nanobodies will be produced in *E. coli* and tested for their binding capacity to parasite (oo)cysts by flow cytometry.
ANSES and ISS will select at least ten DNA-based aptamers sets specific for *C. parvum* and *G. duodenalis* (oo)cysts, using two libraries (DNA and RNA based) and the cell-SELEX method (Systematic Evolution of Ligands by Enrichment). To ensure comparable selection strategies, a study visit is planned at the beginning of the project. Intact *C. parvum* or *G. duodenalis* (oo)cysts will be incubated with DNA library for multiple rounds (>10) with a progressive decrease in (oo)cysts number and interaction time at each round. Negative selection will be performed to avoid cross-reactivity using (oo)cysts from related organisms (i.e., *Toxoplasma gondii* in case of *C. parvum*). Selected aptamers will be identified by cloning-sequencing and/or NGS.

Task: JRP-PARADISE-PARADISE-WP4-T2 Development of post-DNA extraction enrichment strategies
Task start month **M25**; Task end month **M54**

Task Leader: **Mats Isaksson, SVA**; Deputy Task Leader : **Marieke Opsteegh, RIVM**
Task Participants: **RKI, ANSES, VRI, SVA, NFA, PHA, INIAV, RIVM**

Description of the task:

- WP4-T2 takes place over the first, the second and the third year of the project.

The objectives of this task is the development and evaluation of a protocol for post-DNA extraction enrichment based on sequence-based capture of low abundance target DNA from feces, different types of water (drinking, recreational, waste water and washing water from fresh produce) and archived DNA material from suspected foodborne outbreaks. By the use of hybridization biotinylated, single-stranded DNA probes complementary to target DNA regions, targeted DNA can be physically pull out using streptavidin-coupled magnetic beads. This method is commonly referred to as DNA fishing. Therefore, the targeted DNA is concentrated and can be more easily detected, amplified, sequenced or screened using specific qPCR. The developed strategies will then be implemented and tested among WP4 participants by a ring test.
In Y3, a set of biotinylated hybridization capture probes and helper probes for each parasite will be designed. Probes will be designed based on the markers selected in WP2 and WP3, and at least one probe for each marker will be then used in WP4-T2. Additionally, capture systems will be developed for *Cryptosporidium* spp. 18S ribosomal DNA as well as for the *Giardia* specific gene b-giardin. Initial work will be performed at SVA in collaboration with RIVM. To assess their specificity, hybridization probes will be tested against reference DNAs from *Cryptosporidium* and *Giardia*, as well as against DNAs from other common FBPs.

Deliverables

Ref	Title	Due month
D-JRP-PARADISE-WP1.1	Report of the kickoff meeting	M26
D-JRP-PARADISE-WP2.1	Protocol for 18S rDNA-based amplicon sequencing for detection of relevant FBPs	M30
D-JRP-PARADISE-WP3.1	Report on the *in silico* selection of highly polymorphic sequences in *C. parvum* and *G. duodenalis* genomes	M30
D-JRP-PARADISE-WP4.1	Report on the cloning and sequencing of aptamers specific for *C. parvum* and *G. duodenalis*	M36
D-JRP-PARADISE-WP4.2	Report on the selection of highly affine nanobodies specific for *C. parvum* and *G. duodenalis*	M36

Milestones

Ref	Title	Due month
M-JRP-PARADISE-1	Kick-off Meeting (WP1)	M26
M-JRP-PARADISE-2	Study visit (ISS-ANSES) for optimizing aptamer selection strategy	M28
M-JRP-PARADISE-3	Key isolates of *C. parvum* collected	M30
M-JRP-PARADISE-4	Key isolates of *G. duodenalis* collected	M30

M-JRP-PARADISE-5	Referenced database of foodborne parasite genomes established	M30
M-JRP-PARADISE-6	Pipeline for metagenome data analysis for FBPs optimized	M30
M-JRP-PARADISE-7	First set of candidate markers for MLST development available	M30
M-JRP-PARADISE-8	Animal immunization and cDNA library of nanobody sequences for *C. parvum* and for *G. duodenalis* completed	M30
M-JRP-PARADISE-9	Submission of genome sequences of *C. parvum* and *G. dudenalis* to public repository	M36
M-JRP-PARADISE-10	Aptamers sequences selected	M36
M-JRP-PARADISE-11	Highly affine nanobodies selected	M36
M-JRP-PARADISE-12	Pre-evaluation of probes directed towards markers commonly used for *C. parvum*	M36
M-JRP-PARADISE-13	Pre-evaluation of probes directed towards markers commonly used for *G. duodenalis*.	M36

2.4.2.1.20 JRP06-R1-FBZ1-NOVA

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ 1: Improved surveillance system and harmonized data analyses**									
Research Project Title	**Novel approaches for design and evaluation of cost-effective surveillance across the food chain**									
Research Project Acronym	**NOVA**									
Leading Organisation	**P41-SVA**					Deputy Leading Organisation			**P13-SSI**	
Project Leader	**Jenny Frössling**					Deputy Project leader			**Steen Ethelberg**	
Project Start month	**M1**					Project End month			**M36**	
Participant	**1**	2	**4**	6	7	8	**9**	10	**11**	**12**
	Anses	Ages	**Sciensano**	NDRVMI	SZU	VRI	**BfR**	FLI	**RKI**	**DTU**
P.M	**13.37**		**16.9**				**7**	0	**36**	**16**
Participant	**13**	14	15	**16**	**17**	18	19	20	**21**	**22**
	SSI	UT	VFL	**INIA**	**UCM**	MVNA	INRA	IP	**APHA**	**PHE**
P.M	**15**			**7**	**8**				**6.65**	**5.3**
Participant	23	24	25	26	27	**28**	29	**30**	31	**32**
	UoS	OKI	NUIG	TEAGASC	ISS	**IZSAM**	IZSLER	**RIVM**	WbvR	**FHI**
P.M						**2.5**		**3.2**		**11.34**
Participant	**33**	34	35	36	37	38	39	**40**	**41**	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	**FOHM**	**SVA**	
P.M	**1.62**							**2.5**	**16.5**	

Objectives

- Integrative research:
  - Evaluation of the effect of different surveillance programs in the food production on human health through mathematical modelling
  - Assessing the effect of using metagenomics in surveillance of foodborne zoonoses.
  - Developing models to answer questions of cost-efficiency and prioritisation of surveillance

- Common framework of terms describing foodborne zoonosis surveillance across sectors
- Descriptive mapping of unused potential food-chain surveillance resources across countries and of impediments towards their use

- Use of electronic food purchase data for surveillance
  - Description by member state of food purchase data sources and their availability
  - Structured review of the field of the use of food purchase data for outbreak investigation.
  - Manual of how to use food purchase data for outbreak investigations.
  - Case control studies of food risk factors for sporadic infections of important pathogens using electronic datasets of food purchase data.
  - Analysis of food consumption patterns in hospitals in relation to hospitals outbreaks of foodborne zoonotic diseases.
  - Tool for food risk mapping and trace-back as a computer software
- Syndromic surveillance tools
  - Inventory of data availability, quality and suitability
  - Evaluation of statistical syndromic surveillance approaches to detect foodborne diseases outbreaks
  - Evaluation of multivariate syndromic surveillance methods for foodborne diseases surveillance
- Spatial analysis tools
  - Maps of livestock prevalence and geographical patterns of Salmonella transmission in in areas of varying infection intensity
  - Analyses of the spatio-temporal associations with human salmonella cases
  - Geographically aided evaluation of optimal surveillance strategies.
  - Assessment of the potential effect of relevant interventions
  - Cartographic map of hot spot areas for Salmonella transmission between wild boars and low biosecurity systems.

- Potential new environmental surveillance indicators identified.

WP1: Food chain surveillance mapping

WP start month: M1

WP end month: M36

WP Leader: Sarah Welby, CODA-CERVA

Deputy WP Leader: Solveig Jore, NIPH

WP participants: SVA, SSI, DTU, ANSES, INIA, NVI, NIPH, RIVM, APHA, BfR, UCM, RKI, FLI, SpFrance, ISS, IZS, PHAS, PHE

Description of the WP: WP1 continues based upon what was achieved during the second annual period of the EJP. Effective and cost-efficient surveillance demands an integrated approach across the food chain, harmonised practices and communication among the different stakeholders. For the surveillance of foodborne zoonoses, a common understanding of the surveillance systems in place, their potential gaps, and alternative approaches, is needed. In this context, a common language is one important baseline for surveillance communication; i.e. terminology with Med-Vet joint definitions of phrases used to describe, analyse, and evaluate surveillance.

Various methods and tools for collecting information about foodborne pathogens and human health exist or are being developed at national and regional levels. Different stakeholders at different levels of the food chain process reveal different perceptions, needs and priorities. Development and implementation of traditional and new surveillance tools relies on effective communication among the parts involved, an issue particularly important when communication cross-domains is needed.

Objective: This WP aims at aligning perceptions and needs along the food chain, and across the Med-Vet domains, in two main ways: by providing a common language for discussion, and by performing a full mapping of the surveillance opportunities and hurdles along these dimensions. The tasks outlined below aim at analysing the different levels of the food chain in order to obtain a comprehensive overview of the different stakeholders involved as well as their terminology perception, needs and priorities. In addition, this WP aims at identifying stakeholders' perceptions on critical gaps and hurdles reducing the cost effectiveness of surveillance (i.e. legal, ethical and technical barriers to data communication). This, in turn, will enable a comprehensive approach to foodborne zoonosis surveillance optimisation. The study may use one specific pathogen as a proof-of-concept for other foodborne pathogens. Parts of the feed-to-fork data chain analysis may be focused in a specific sector (such as poultry, or pork). The pathogen and sectors will be defined in the start of the task, among partners. This WP will mainly be descriptive and will involve all partners. This WP can be partly linked to and will have continuous contact with the other WPs in this project, as well as WPs in some other EJP projects.

T-1.2 Mapping of surveillance: data, regulatory framework, key stakeholders, opportunities and barriers
Task start month: M1
Task end month: M36
Task Leader: Sarah Welby, CODA-CERVA
Deputy Task Leader: Solveig Jore, NIPH
Task Participants: ALL
Description: T-1.2 continues based upon what was achieved during the second annual period of the EJP. Within this task the participating partners will make a detailed overview of available surveillance information sources in the different MS, including both databases and other type of sources. Traditional surveillance data, but also all kind of other potential usable data sources such as registers, surveys, and reports, will be included. Understanding the context and drivers to surveillance is critical for designing successful interventions actions and that these intervention actions will only be effective if key players in the system accept them. In this task, the different surveillance strategies implemented in each MS, and the involved stakeholders, will also be described. This will provide input and support to the other WPs, in particular the simulation of alternative surveillance strategies in WP5. This task will be performed in close collaboration with WP2, WP3, WP4, and WP5
Objective: To find and describe all currently used and unused sources that potentially could be used for improving surveillance, and to investigate stakeholders' perceptions on the obstacles and opportunities for foodborne integrated surveillance associated with these sources.
Method: Again, the work of previous European funded projects will be used as starting point, but here the focus will be on the potential for synergy between med and vet with the aim of foodborne surveillance. Group discussions about this topic will be held and documented, some in connection to assemblies where experts are already participating. After identification of stakeholders across Member States, potential novel data sources or surveillance gaps will be further investigated by targeted surveys and/or interviews. Perceived and documented opportunities and barriers for systematic use of each new data source will be investigated. This will cover availability, cost, technical, legal, economic, socio-political, educational and ethical issues for each involved Member State. When relevant, motivators or incitements for supplying data i.e. feed-back information of interest for the data supplier will be examined. Participatory methods will be used to evaluate the functionality and acceptability of surveillance strategies. Flow and relational diagrams as well as Outcome and Assessment Information Set (OASIS) tool methods will be used to determine the main drivers and hurdles for effective surveillance.
Outcome: A comprehensive map describing important components and stakeholders across the food chain representing potential sources for improved surveillance; and an increased knowledge

about differences in Member States regarding implementation and execution of surveillance programs concerning food borne zoonoses. A description of identified opportunities and obstacles of each of these sources.

Deliverables

Ref	**Title**	**Due month**
D-2.4	Mapping of food chain surveillance across countries	M35

Milestones

Ref	**Title**	**Due month**
-	-	-

WP2: Analysis of food purchase data
WP start month: M1
WP end month: M36
WP Leader: Steen Ethelberg, SSI
Deputy WP Leader: Katja Alt, BfR
WP participants: SSI, RKI, BfR, ISS, SpFrance (InVS), PHE, RIVM, CODA-CERVA, PHAS, NIPH, NVI, IZS.
Description of the WP: WP2 continues based upon what was achieved during the second annual period of the EJP. In order to understand the transmission of foodborne diseases it's important to understand food exposures. This is done by applying questionnaires for patients and non-ill control subjects, asking them to report food histories. Often, however, such data are heavily biased or incomplete and their collection costly and difficult to obtain in a timely manner. Therefore, ways to obtain data on everything a patient or household consumes during an outbreak period have been pursued for some years, progressing from manual collection of receipts to capture of electronic purchasing data. Also, purchase data from restaurants or caterers for hospitals or other institutions for vulnerable populations can be tracked. Additionally, information obtained from food sales data (big data covering affected regions) and tracing analyses potentially enhance evidence during outbreak investigations. This WP aims to use food consumption patterns as a new digital surveillance tool to aid our understanding of both outbreaks as well as determinants for sporadic foodborne illness.
Objective: This WP will perform research into how surveillance of food consumption patterns can be used to better understand foodborne zoonoses: to aid surveillance, to explore risk factors and for outbreak investigations.
Structure: The WP is divided into five separate, partly interdependent tasks, each containing subtasks.

<u>T-2.2 Food purchase data for outbreak investigations</u>
Task start month: M2
Task end month: M30
Task Leader: Steen Ethelberg, SSI.
Deputy Task Leader: TBD.
Task Participants: SSI, ISS, SpFrance, PHE, BfR, RIVM, CODA-CERVA, PHAS, NIPH
Description of the task: T-2.2 continues based upon what was achieved during the second annual period of the EJP. Patient supermarket data have been used successfully in several EU member states for specific outbreak investigations. There are approx 10 documented accounts hereof (published papers), performed by partners in this application (SpFrance, SSI, PHE, RKI, RIVM). So far however, this has occurred in an ad hoc manner and results and possibilities have never been collectively described nor have the methods been attempted to be standardised. We believe a more efficient and more general method could be described and propose to collect data and experiences from partner institutes and work towards a common description and methodology which should pave the way for increased use of this potentially powerful tool.

Objective: Subtask 1: Identify and review existing use of purchase data for outbreak investigations. Subtask 2: Develop Best Practice / Standard Operating Procedures for such studies. Subtask 3: Describe potential for use as an analytical tool (rather than simply a hypothesis-generating tool).

T-2.3 Big data analysis of risk factors for sporadic disease
Task start month: M1
Task end month: M30
Task Leader: Frederik Trier Møller, SSI.
Deputy Task Leader: Steen Ethelberg, SSI.
Task Participants: SSI, PHAS, PHE, RIVM, NIPH.
Description of the task: T-2.3 continues based upon what was achieved during the second annual period of the EJP. The level of digitalization of purchases in Denmark is high with more than 80% of transaction being digital. Recently, the SSI has contracted with a digital receipts provider covering at least 50% of daily consumables expected to rise to 80% before 2018 (consumers can see their purchases, current and historical, via a smartphone app). Aligned with several years of experience with epidemiological use of purchase data this makes Denmark the ideal country to pilot the use of consumer purchasing data. We propose to make a structured comparison between known cases and non-ill persons for whom we can assess electronic complete purchase data.
Objective: Build and maintain research infrastructure to achieve consent from sporadic cases of salmonella and campylobacter and healthy controls to use their purchasing data to conduct: Subtask 1: Case control study of foods posing a risk for sporadic salmonella infections. Subtask 2: Case control study of foods posing a risk for sporadic campylobacter infections.
Perspective: Depending on results from Denmark, studies can be made in selected other countries. Sweden and Norway, where the data accessibility is also maturing rapidly, are good candidates. Also, other pathogens and other study objectives can be envisioned and may be included in this phase.

T-2.5 Trace back and food risk mapping
Task start month: M1
Task end month: M36
Task Leader: Katja Alt, BfR.
Deputy Task Leader: Madelaine Norström, NVI.
Task Participants: BfR, NVI, NIPH, ISS.
Description of the task: T-2.5 continues based upon what was achieved during the second annual period of the EJP. Published likelihood-based tools have previously been developed at partner institutes (BfR, NVI) that potentially could accelerate the identification of contaminated food products using spatio-temporal distribution of food sales (food risk mapping). We suggest further development of the methods to include time and Global Trade Identification Number (GTIN) in the analysis, to evaluate various aggregation levels before analysis, and to increase robustness in case of inaccurate or missing information. Furthermore, these results will subsequently be integrated into the state of the art tracing tool FoodChain-Lab.
Objective: Subtask 1: Investigate the availability and usefulness of data from WP2, tasks 1-3. If new real data should not be available for the project, the partners will work on data provided for previous studies. Subtask 2: Develop the likelihood method so that it handles data on time together with GTIN. Work out guidance for selection of unit for aggregation of data before analysis, and decide on ways to handle inaccurate and missing information on case purchase patterns and distribution patterns, and others. Subtask 3: Import of available food sales and eventually food purchase data structures into FoodChain-Lab. Implementation of the likelihood method as cloud service, and integration of the results of the likelihood method into the analysis of FoodChain-Lab.
Outcome: Integration of improved tools for food risk mapping into the state-of-the-art tracing tool FoodChain-Lab will allow for open access, cloud based use in the scientific community. Additionally,

the structure of the collected sales data will be evaluated for tracing purposes (in alignment with EFSA working group report) and subsequently integrated as import routine into FoodChain-Lab.

Deliverables

Ref	Title	Due month
D-2.2.3	Assessment of the use of the method as an analytical tool, rather than merely hypothesis-generation.	M30
D-2.3.3	Case control study of food risk factors for sporadic campylobacter infections.	M34
D-2.5.4	Description and documentation of software, development of tutorials and training material.	M34

Milestones

Ref	Title	Due month
M-FBZ1.NOVA.22	Case control study of food risk factors for sporadic salmonella infections.	M30

WP3. Syndromic surveillance

WP start month: M1
WP end month: M36
WP Leader: Adeline Huneau, ANSES
Deputy WP Leader: Fernanda Dórea, SVA
WP participants: ANSES, NIPH, SVA, NVI, CODA-CERVA
Description of the WP: WP3 continues based upon what was achieved during the second annual period of the EJP. Syndromic surveillance (SyS) is a relatively recent approach of surveillance used since early 2000s in human health and more recently in animal health (http://www.syndromicsurveillance.eu/). It consists in the real-time collection, analysis, interpretation and diffusion of non-specific indicators in order to early detect any change in human or animal health. SyS systems, usually used in complement of traditional public health surveillance systems, are expected to be earlier and more cost effective than ongoing systems. Nevertheless, due to the use of nonspecific indicators, there is still doubt about the efficiency of the approach and performances of syndromic surveillance have to be evaluated for the monitoring of FBD.
Objective: Assess whether SyS in animal and food sectors could be a valuable add-on to the current or future surveillance of FBD in humans. The expected impacts are a better understanding of the interplay between animal and human health and an improvement of the prediction of human FBD outbreaks.
Structure: The WP has three tasks, where T-3.2 and T-3.3 are dependent from T-3.1.

<u>Task 3.2. Univariate syndromic surveillance development for FBD (and AMR)</u>

Task start month: M11
Task end month: M30
Task Leader: Carole Sala, ANSES
Deputy Task Leader: Petter Hopp, NVI
Task Participants: ANSES, NIPH, SVA, NVI, CODA-CERVA
Description: T-3.2 continues based upon what was achieved during the second annual period of the EJP.
Objective: evaluate statistical approaches and potential of the data sources identified in Task 1 to detect FBD outbreaks using data.
Method: A set of modules/components, representing different statistical methods will be implementing and applied to the relevant data sources identified in Task 1.SyS data will be analysed with a panel of commonly used time series models (GLM, GAM, ARIMA, Holt-Winters…) combined with algorithms for signal detection (use of CI, control charts…).Simulated dataset will be used to complete data sources when needed and, particularly to mimic various outbreak patterns helping

in the evaluation of the performance of the SyS in terms of sensitivity, specificity and timeliness. The scoring approach proposed by Dórea *and coll* (2013) on univariate SS data source will be implemented to assess the improvement of the SS performances by combining methods. This will lead to the identification of the relative timeliness/sensitivity/specificity of each of the methods, and whether some methods are more suited for certain outbreak types i.e. large vs small, spatially diffuse versus localised. Human outbreak data will be used to valid results and performances of the approach.
Result: Evaluation of the potential interest of the development of SS for FBD based on real examples

Task 3.3. Evaluation of multivariate syndromic surveillance for FBD
Task start month: M11
Task end month: M36
Task Leader: Gry Marysol Grøneng, NIPH
Deputy Task Leader: Fernanda Dórea, SVA
Task Participants: NIPH, SVA, NVI, ANSES, CODA-CERVA
Description: T-3.3 continues based upon what was achieved during the second annual period of the EJP.
Objective: Assess whether the combination of surveillance results from different SyS components could lead to an improvement of the surveillance system for the selected FDB cases study. That is, to investigate how results from different components, developed in Task 2, can support the evidence from the others, and lead to improved decision making. Integration across components is understood here as integration along the disease transmission chain for specific pathogens within humans, within animals, and across the med-vet domains.
Method: Considering the data sharing barrier, the goal will be to combine the evidence from results of independent monitoring of different epidemiological sources of data, with the overall goal of public health protection. By combining the evidence gathered through these punctual monitoring along the food production chain, we can monitor FBD risk from farm to fork, accumulating evidence at every step. A possible example of case study is the use of the results from veterinary and food monitoring system such as *Campylobacter* detection in broilers and meat, to support results from the human syndromic surveillance system based on primary health consultations for gastro-intestinal diseases (Sykdomspulsen) in Norway.
Result: Identify possible methods to improve the global surveillance by integrating and consolidating results from different sources across the med-vet domains. Barriers to achieving this goal will be identified and prioritised.

Deliverables

Ref	Title	Due month
D-3.4	Recommendations about the quality standardisation of data produced across the food chain for their use in SyS ()	M30
D-3.5	Contribution of multiple syndromic surveillance components in the FBD surveillance	M36

Milestones

Ref	Title	Due month
M-FBZ1.NOVA.27	Integration of multiple sources developed and tested	M34

WP4: Spatial risk mapping
WP start month: M1

WP end month: M36

WP Leader: Ana de la Torre, INIA

Deputy WP Leader: Stefan Widgren, SVA

WP participants: INIA, RIVM, UCM-VISAVET, SSI, SVA, FLI, CODA

Description of the WP: WP1 continues based upon what was achieved during the second annual period of the EJP. This WP deals with the development of modelling tools for spatial analysis of FBZ and the identification and assessment of new surveillance indicators/risk factors. Spatial epidemiological tools are increasingly being applied for the study of emerging zoonoses, partly because of improving analytical methods and technologies for data capture and management, and partly because the demand for more objective ways of allocating limited resources in the face of the emerging threat posed by these diseases is growing.

Objective: This WP will perform research into how spatial analysis can improve the knowledge on disease behaviour (spatio-temporal patterns) and identify risk factors/indicators for designing efficient risk-based surveillance strategies and favour timely interventions. Following the recommendation of the Special report on eradication, control and monitoring programmes to contain animal diseases (EU, 2016), new indicators related to the environment, including wildlife, will be explored to optimize the effectiveness of surveillance programs.

Scope: The practical approach of this WP will be done using *Salmonella* infection in swine as a proof of concept. *Salmonella* has been selected as example of FBD as it is one of the most common public health problems, causing significant human morbidity and even mortality and consequently high economic losses in both developing and developed countries. Foods of animal origin are still one of the major sources of infection for the general public, with eggs, broiler chickens and pigs being consistently identified among the top attributed food sources. Whereas control programmes for *Salmonella* in poultry have been applied in the whole UE with high success, only few European countries have implemented eradication or control programmes of *Salmonella* in swine, beef, or dairy production. Results from efforts made in Denmark, Sweden, Finland, Norway, Ireland, Germany, Great Britain and Holland, are somewhat inconsistent. So far, there are no national control programmes established in any Mediterranean country, where >40% of the pig farms were positive to *Salmonella* (EFSA baseline study, 2009). In consequence, further efforts to implement control programs for reduction of the prevalence of *Salmonella* infection in swine in the near future are envisioned. Prerequisites to the implementation of such an approach; scientific efforts directed to improve our preparedness and develop an effective risk-based surveillance system should be carried out. Results from this WP would also be relevant for surveillance of many other FBZ diseases, as the explored risk mapping techniques and developed tools will be generic.

Structure: The WP is divided into 3 tasks. Task 4.1 deals with disease behaviour to identify spatial relationships and patterns in *Salmonella* prevalence in livestock, slaughterhouses and its association with human cases; Task 4.2 and Task 4.3 deals with the identification of new risk factors, namely animal feed (Task 4.2) and wildlife and the environment (Task 4.3).

Links with other WP: The current *Salmonella* surveillance programmes compiled under WP1 will be reviewed to identify the main requirements for applying risk mapping techniques. Data on *Salmonella* prevalence in livestock and human will be explored through the source data explored in WP3. Usefulness of the risk mapping tools developed and new risk factors identified will be evaluated in close collaboration with WP5, where alternative surveillance approaches will be tested.
Results applicability: The spatio-temporal patterns of disease occurrence in livestock and human would allow finding an optimized strategy for each epidemiological scenario, e.g. by identifying high risk administrative areas to focus resources for prevention and control of diseases. The identification of new surveillance indicators will allow designing more cost-effective surveillance

and control strategies as recommended in the special report on eradication, control and monitoring programmes to contain animal diseases (EU, 2016). Finally, the cartographic map of hot spot areas for *Salmonella* will improve disease awareness in pig producers and hunters, and development of contingency plans and control strategies in EU member states with low biosecurity farms.
All the information generated will be available at the Internet as interactive high resolutions maps, graphs and visual information. As such it will improve the technology transference of on-going research effort and help educators, veterinarians, technicians and policy makers to better understand, evaluate and fight FBD.

Task 4.2. Risk of introduction of *Salmonella* in pig farms through animal feed.
Task start month: M1

Task end month: M36

Task Leader: UCM-VISAVET

Deputy Task Leader: INIA

Task Participants: INIA, UCM-VISAVET, CODA-CERVA

Description of the task: T-4.2 continues based upon what was achieved during the second annual period of the EJP. *Salmonella* present in animal feed is a significant source of infection in pigs. In order to develop systems for its inclusion in risk assessment/mapping, feeding flows for some of the largest swine systems in high prevalence regions will be described and mapped.

Objective: The aim of this task is to assess the risk of *Salmonella* introduction in pig farms through contaminated animal feed.

Methods: The spatial network structure (feed plants-swine farms) will be explored by spatial analysis techniques (e.g. spatial modelling, network analyses) [M1-M12]. Data on prevalence of *Salmonella* infection, and serotypes and antimicrobial resistance patterns in intensively managed pig farms will be explored in combination with information on animal feed data to analyse the importance of unsterilized animal feed in the introduction of *Salmonella* in the farms and to improve zoonotic hazard management in the pork food chain [M13-M24]. Using this model, the potential effect of changes in feed management (such as the withdrawal of the use of formaldehyde-based feed treatments, the most common alternatives to provide an effective antimicrobial control and whose use is controversial due to its risk for workers) will be explicitly considered and evaluated [M25-M36].

Outcome: Characterize the feeding flows for large swine systems in a high prevalence regions using sociograms. Assess the potential effect of changes in feed conservation treatments (i.e., withdrawal of formaldehyde-based feed treatments).

Task 4.3. Role of the environment in the occurrence and maintenance of *Salmonella* infection in extensive farming.
Task start month: M1

Task end month: M36

Task Leader: INIA

Deputy Task Leader: RIVM

Task Participants: INIA, UCM-VISAVET, RIVM, FLI, CODA-CERVA

Description of the task: T-4.3 continues based upon what was achieved during the second annual period of the EJP. Wildlife often serves as a reservoir for (re-)emerging zoonoses. Infection of wildlife or vectors is frequently linked to environmental and climatic conditions, which largely take place outside the field of view of medical and veterinary experts and affect transmission in a complex manner. This lack of insight in the mechanisms that drive the occurrence and spread of wildlife zoonoses hinders appropriate risk assessment and the development of interventions to reduce public health risks, particularly in a changing environment.

Objective: The aim of this task is to investigate the overlap between habitats of suitable wildlife, e.g. wild boar, with primary production and other environmental variables, which may influence transmission of pathogens from wildlife to livestock farming. *Salmonella* transmission between wild boars and low biosecurity systems such as extensive farming-oriented systems or backyard farms will be selected as a working example. It will be studied in selected Mediterranean countries, e.g. Spain, where this type of non-controlled housing is present and there is a high prevalence of infection in livestock.

Methods: First, available data on infection and serotype prevalence in extensively managed pig farms (WP3) will be mapped and analysed to characterize its spatial distribution and identify patterns, processes, and relationships (e.g. smoothing technique, cluster analyses) [M1-M12]. Second, hot spot areas with higher potential for *Salmonella* transmission between wild boars and low biosecurity systems will be identified by overlapping wild boar distribution maps (Bosch et al., 2016), extensive farming systems geodata and known serotype spatial distribution (spatial autocorrelation analyses) [M13-M24]. Finally, state-of-the art machine learning algorithms will be employed to correlate infection data with environmental drivers. They will be spatially explored and analysed through Bayesian classical and stochastic modelling approaches (correlating covariates, expert opinion, mapping techniques....) to assess their availability/competence as surveillance indicators [M25-M36]. Reliable data of paramount importance will be gathered from free sources such as 'worldclim' (climate), 'CORINE' (land-use) and 'MODIS' (satellite imagery). The model will be an extension of a previously developed model, which was successfully used for tick presence forecasting.

Outcome: A high-quality cartographic map of hot spot areas will be developed that would help to understand and visualize risk scenarios to make decisions based on the best available information. This tool could also be potentially used for implementing the surveillance systems of other FBZ diseases shared between wild boar and domestic pigs, as trichinellosis. Identification of potential new surveillance indicators will be shared with WP5, where alternative surveillance approaches will be tested.

Deliverables

Ref	Title	Due month
D-4.6.	Assessment of the potential effect of the withdrawal of the use of formaldehyde-based feed treatments done.	M36
D-4.9.	Potential new environmental surveillance indicators identified.	M36

Milestones

Ref	Title	Due month
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.	M36
M-FBZ1.NOVA.29	Result reporting on new environmental surveillance indicators.	M36

WP5: Evaluation of surveillance programs & cost efficiency

WP start month: M1
WP end month: M36
WP Leader: Håkan Vigre, DTU
Deputy WP-leader: Annemarie Pielaat, RIVM
WP Participants: DTU, SVA, APHA, RIVM, ANSES, CODA-CERVA, FLI
Description of the WP: WP5 continues based upon what was achieved during the second annual period of the EJP. To support the identification and prioritization of control measures in food production, mathematical models have become an important tool as they help a risk manager to predict the effect of different intervention strategies. Thus far, different mathematical frameworks have been developed for i) estimating the occurrence of pathogens in the food chain e.g QMRA), ii) develop probability theorems around sampling and detection of pathogens, iii) estimate the importance of different sources for human disease (source attribution) and iv) estimate the consequence of different diseases (burden of disease).
The overall objective of this WP is to develop modelling approaches to predict the impact of surveillance programs applied in the food production chain on human exposure and public health. Initially, all models will be developed using case-studies focusing on specific pathogen-food combinations that are of primary importance to the public health within the EU. Each case study will utilise nation and region specific data within the farm to fork approach (e.g. population structure in animal production systems, prevalence and concentration of pathogens in animals or specific food products, consumption patterns and food handling by consumers). These will then be used to develop a framework that can be applied to other pathogens and food items. The output will be a modelling-tool that will form the scientific basis for the design of surveillance programs across Europe, which will enhance the comparability of surveillance data between countries.
The novelty in this WP is that it will combine and further develop currently segregated mathematical frameworks to model the efficiency of different surveillance programs in a human health and cost efficiency context. This approach will advance the focus on human health impact when developing surveillance programs in food production, thereby optimizing decisions about how to distribute limited capacity for surveillance.

T-5.1: Adapt infectious disease models for assessing the effect of surveillance programs in primary animal production on consumer exposure to foodborne pathogens.
Task start month: M1
Task end month: M36
Task Leader: Mark Arnold APHA
Deputy Task Leader: Thomas Rosendal, SVA
Task Participants: APHA, DTU, SVA, CODA-CERVA
Description of the task: T-5.1 continues based upon what was achieved during the second annual period of the EJP. In this task we will adapt existing risk assessment models for foodborne zoonotic pathogens to include modules for simulating the effect of surveillance programs (detection and control) in the food-chain on human exposure to zoonotic pathogens.
sT-5.1.1
Sub-Task Leader: Mark Arnold, APHA
Sub-Task Participants: APHA, SVA, DTU
For several foodborne zoonoses, the difficulty of detection is compounded by the lack of clinical signs in the animals. Therefore, improved surveillance measures are required to detect infected animals, and thus limit the exposure of humans to contaminated products by prevention of further spread in the animal population, and/or withdrawal of products from the market. The objective of this task will be to adapt existing models for the transmission of Salmonella in pigs in GB (APHA), in cattle in SWE (SVA) and in eggs in DK (DTU) to (i) take into account the effect surveillance activities on consumer exposure to Salmonella and (ii) if applicable, adapt the models to represent additional pathogens.

sT-5.1.2
Sub-Task Leader: Thomas Rosendal, SVA
Sub-Task Participants: SVA, CODA-CERVAThe surveillance layer of existing models describing spread of a food-borne zoonosis in an animal population will be adapted to generate different global metrics of performance. These will include: time to detection, extent of an outbreak, spread at the time of detection and animal population strata affected. Other surveillance metrics will also be explored to find those that are most descriptive in situations of endemic disease, freedom from disease and low prevalence disease states. These metrics will be used to assess different surveillance strategies and their capacity to minimize the exposure of foodborne pathogens among the citizens in a country or region in case of an outbreak or change in prevalence of disease in the animal population.

T-5.3 Modelling the effect of surveillance programs in the food production on human health.
Task start month: M1
Task end month: M36
Task Leader: Annemarie Pielaat, RIVM
Deputy Task Leader: DBD
Task Participants: RIVM, APHA, DTU, SVA, CODA-CERVA, FLI, ANSES
Description of the task: T-5.3 continues based upon what was achieved during the second annual period of the EJP. We will broaden the assessment of surveillance from one specific pathogen-food combination at a specific stage in the food chain to surveillance of different pathogens in several food production chains simultaneously up until the moment of consumption. Disability Adjusted Life Years (DALYs) will be used as a consistent measure for the subsequent public health risk. Accounting for mortality, severity and duration of a disease in a single method is a powerful tool to compare the actual health impact of a given intervention in the surveillance of different zoonoses.
sT-5.3.1.
Sub-Task Leader: Håkan Vigre, DTU
Sub-Task Participants: DTU, ANSES, RIVM, FLI
In this subtask we will develop a model consisting of parallel parts for surveillance of a pathogen in several sources based on the cases studies in sT-5.1.1 (Salmonella in egg, pork, poultry and beef). The objective is to combine the effect on human health based on individual surveillance programs into an overall effect on human health when different programs are combined. Connecting surveillance in several sources allows the simulation of changing the distribution of surveillance capacity across sources. This modelling approach will consider countries having several sources for a zoonotic infection (e.g. *Salmonella* in pork from Denmark is one source and *Salmonella* in pork from France is another source, and so on). By parameterizing each country-specific model by country-specific data and weighting the contribution from each country by import/export data, this approach can support the prioritization of surveillance across European countries.
sT-5.3.2
Sub-Task Leader: Annemarie Pielaat, RIVM
Sub-Task Participants: RIVM, DTU, SVA, FLI, ANSES, CODA-CERVA, APHA
In this subtask we will develop a modelling approach for optimizing the distribution of sampling capacity of different food safety authorities in the EU over products in retail according to their contribution to the microbiological public health risk in a most cost-efficient way. DALYs will be used as a consistent measure for the public health risk of different food pathogens over Europe. Exposure assessment (including consumption data and food preparation at home) from St-5.3.1 will be combined with DALYs and a monitoring budget to define an optimizing criteria for risk based sampling. The method has already been implemented in The Netherlands for *Campylobacter* in pork and poultry, *Salmonella* and *Toxoplasma gondii* in pork, Shiga-toxin producing *E. coli* and ESBL *E.coli* in beef. In this subtask we will improve the current methodology through the contribution of other

data sources as identified in this project (e.g. retail purchase data following the EFSA zoonoses report) and other methods for surveillance.		
Deliverables		
Ref	**Title**	**Due month**
D-5.1.1	Report comparing performance of surveillance strategies	M30
D-5.1.2	Recommendations for metrics to evaluate surveillance performance	M36
D-5.3.1	Report assessing the quantitative effect on human health of changing surveillance capacity across different sources in an MS	M36
D-5.3.2	A practical risk based sampling approach that combines exposure to zoonoses with disease burden and costs for sampling at a European level.	M30
Milestones		
Ref	**Title**	**Due month**
M-FBZ1.NOVA.23	surveillance layer added to models	M30
M-FBZ1.NOVA.24	A dynamic modelling layer of surveillance is overlaid the submodels	M30
M-FBZ1.NOVA.25	initial set of results circulated to project team	M32

2.4.2.1.21 JRP07-R1-FBZ2-LISTADAPT

Reference to the Stategic Research Agenda (please refer to D2.7)			**FBZ 2: Development and harmonisation of NGS-based methods for detection and tracing of FBZ agents, emerging threats and AMR determinants**							
Research Project Title			**Adaptive traits of *Listeria monocytogenes* to its diverse ecological niches**							
Research Project Acronym			**LISTADAPT**							
Leading Organisation			**P1-ANSES**			Deputy Leading Organisation			**P33-NVI**	
Project Leader			**S. Roussel**			Deputy Project leader			**T. Skjerdal**	
Project Start month			**M1**			Project End month			**M30**	
Participant	**1**	**2**	4	6	7	**8**	9	10	11	12
	Anses	**Ages**	Sciensano	NDRVMI	SZU	**VRI**	BfR	FLI	RKI	DTU
P.M	**29.75**	**3.83**				**4.00**				
Participant	13	14	15	16	17	18	**19**	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	**INRA**	IP	APHA	PHE
P.M							**9.81**			
Participant	23	24	25	26	27	**28**	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	**IZSAM**	IZSLER	RIVM	WbvR	FHI
P.M						**11.56**				
Participant	**33**	34	35	36	37	38	**39**	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	**SLV**	FOHM	SVA	
P.M	**8.13**						**0.22**			

Objectives

WP0 (coordination), and some tasks of WP4 and WP5 are involved in year 3 of LISTADAPT project. The objectives related to each of these WPs are described below:

For Year 3, WP 4 aims to:

- Conduct biostatistics analysis of 4000 genomes: 2000 obtained in this project compared with additionally 2000 genomes presently available from food, food processing plants, and clinical cases (T4.3).
- Uncover new genes or new mechanisms related to virulence of clinical human and animal strains (T4.3).

- Determine which genes or lack of genes involved in the strain ecophysiology that results in some clonal complexes being successful in a given environment i.e. humans, food matrixes, storage and unsuccessful in another in terms of adaptation (T4.3, T4.4).
- Compare genotypical data with phenotypical data to identify new determinants or genetic markers needed for improving surveillance for prevention and control (T4.4).

For Year 3, WP5 aims to :

- Set up appropriate quality standards and WGS Proficiency Testing Trials, in close collaboration with Compare (WP 2) and the Global Microbial Identifier (GMI) (T5.3)
- Communicate and inform the scientific community about "Listadapt" progress and results obtained (T5.4).

Description of work

WP 0 : Coordination

WP0 takes place over the first and the second annual period of the EJP. WP0 continues based upon what was achieved during the first annual period of the EJP

WP start month : M1
WP end month : M30
WP Leader : ANSES (L Guillier)
WP participants: All : DTU, ANSES, AGES, IZSAM, NVI, VRI, NFA, INRA
Description of the WP :
The coordinator will invest a large amount of time in the Listadapt project. He will maintain regular contacts between the 8 different partners and will be responsible for the efficient progress of the project. He will make sure that all deliverables and milestones are reached in the timeframe envisioned for the project. He will organize interactions between groups, and meetings every six months (one face-to face meeting and one telephonic conference) to continuously monitor progress of the objectives and to discuss scientific advances and problems. The coordinator will take advantage of the venue of 6 NRLs involved in "Listadapt" at the annual EURL /NRL workshops dedicated to Listeria and located at ANSES Maisons-Alfort to organize the annual face-to face "Listadapt" meetings.
Each partner will provide the coordinator with scientific and financial statements, from which the coordinator will prepare the reports (cost details, research results and technical advancements). Any problem related to the financial management of the project will be discussed.

WP 4 : Identification of genetic traits in *Listeria monocytogenes* underlying adaptation to the ecological niches

WP4 takes place over the first, the second and the third annual period of the EJP. WP4 continues based upon what was achieved during the first and second annual period of the EJP

WP start month : M1
WP end month : M30
WP Leader : DTU (René Hendriksen)
WP deputy leader : NVI (Karin Lagesen)
WP participants : ANSES, DTU, NVI, AGES, IZSAM, VRI, NFA, INRA
Description of the WP:

Task4.3.: Biostatistic analysis of annotated genomes

Task 4.3 continues based upon what was achieved during the first annual period of the EJP

Task start month :M6
Task end month : M29
Task Leader: NVI
Deputy Task Leader: DTU
Task Participants: ANSES, AGES, IZSAM
Description of the task :
This task focuses on the differences between the 2000 genomes obtained in WP2 with the additional 2000 genomes presently available within the consortium. This could be *via* statistical approaches based on the bioinformatic analysis and also metadata associated with the samples
We would like (i) to determine which genes or lack of genes that make some clonal complexes successful in a given reservoir but unsuccessful in another (ii) uncover new genes or new mechanisms involved in the strain ecophysiology and in the virulence of clinical human and animal strains. Of particular interest are genetic factors relating to virulence, survival, colonization, persistence, adaptation. For practical considerations, this task is divided into two subtasks:
Sub-Task: 4.3.1 Identification of statistically relevant methods and development of analysis
Sub-Task start month: M6
Sub-Task end month: M22
Sub-Task Leader: NVI
Sub-Task description:
Existing software will be evaluated to find software suitable for this task, and an analysis procedure will be developed using a small subset of the listerias available as a test set.
Sub-Task: 4.3.2 Processing of all isolates
Sub-Task start month: M16
Sub-Task end month: M29
Sub-Task Leader: NVI
Description of the sub-task:
Once a good procedure has been laid out, all the isolates available will be analyzed.

Task 4.4 : Comparative analysis phenotypical data / genotypical data
Task 4.4 continues based upon what was achieved during the first annual period of the EJP

Task start month :M17
Task end month : M29
Task Leader: ANSES
Deputy Task Leader: IZSAM
Task Participants: All : DTU, ANSES, AGES, NVI, VRI, NFA, INRA
Description of the sub-task :
The aim of the task is to correlate the findings of phenotypic analysis obtained in WP3 (T3.2, T3.3, T3.4) with the results from the studies conducted in WP4 (T4.3.2)
The data correlation and association will be investigated by multivariate analysis; a particular emphasis will be devoted to the analysis of population genetics within the Listeria isolates into each biological niche by analysis of molecular variance for which specific software have been implemented (AMOVA, Arlequin)
We expect to clarify which genes are involved in adaptation of *Lm* to different ecological niches and discover strain-specific genes/markers which will strongly assist in diagnostic, phylogenetic and functional research. Strains would be assigned into well-defined clusters relevant to biological investigations.

WP 5 Trainings and dissemination

WP5 takes place over up to the end of the EJP. WP5 continues based upon what was achieved during the two first years of the EJP

WP start month : M1
WP end month : M30
WP Leader: Given as the strategic importance of this WP, the coordination will be done by the "Listadapt" coordinator and the leaders and the deputy-leaders of the WPs.
WP participants: All : DTU, ANSES, AGES, IZSAM, NVI, VRI, NFA, INRA
Description of the WP

Task 5.3.: Proficiency Testing Trials
Task start month : M22
Task end month : M26
Task Leader: DTU
Deputy Task Leader: IZSAM
Task Participants: DTU, ANSES, AGES, IZSAM, NVI, VRI, NFA
Description of the task :
In "Listadapt", we plan to set up appropriate quality standards and WGS Proficiency Testing Trials, in close collaboration with **Compare** (WP 2) and the **Global Microbial Identifier** (GMI) networks. The PT trial aims at evaluating the ability of NRLs to perform WGS according to Compare SOPs.
Quality management will be ensured and regularly tested using reference strains and reference datasets from humans, animals, environment and food. The SOPs and workflows from Compare will be evaluated here and validated by a PT trial on (1) sample preparation, (2) sequencing and (3) data analysis. René Hendriksen (DTU) chairs the working group for proficiency testing of the GMI initiative. Cesare Camma (IZSAM) is also involved in GMI. Their expertise will be then instrumental in this task.

Task 5.4.: Dissemination

Task 5.4 continues based upon what was achieved during the first annual period of the EJP

Task start month : M1
Task end month : M30
Task Leader: ANSES
Deputy Task Leader: DTU
Task Participants: All : DTU, ANSES, AGES, IZSAM, NVI, VRI, NFA, INRA
Description of the task:
We have to regularly communicate and inform the scientific community about "Listadapt" progress and results obtained.

Delivery of 2000 new genomes
One of the outcomes of the project (WP2, T2.3) is the delivery of 2000 new genomes of *Listeria monocytogenes.* The sequences will be made available to the scientific community. They will be deposited in public access internet databases. Whole Genome Sequence data will be described in relevant Genome Announcements (American Society for Microbiology) and also deposited at NCBI.

Participation in national and international conferences, seminars and symposia

Sharing information in national and international meetings and congresses will also be done, in particular during the annual EURL/NRLs workshop dedicated on Listeria. Our results will be presented to the International research community, in major conferences and congresses such as :

- International Symposium on Problems of Listeriosis (ISOPOL XX) organized by the European Molecular Biology Organization (EMBO) in 2019 ;
- 14th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases (MEEGID)
- 12th International Meeting on Microbial Epidemiological Markers (IMMEM XII)

Publishing in peer-reviewed scientific journals

Results obtained in the framework of this project will be aimed for publication in high impact journals (Plos One, Frontiers in Microbiology, Applied and Environmental Microbiology, International Journal of Food Microbiology…). Med –Vet EJP funding will be acknowledged in every publication and communication.

Promotion of Listadapt

We promote the project by using the EURL Listeria website (https://eurl-listeria.anses.fr) and also magazines on line such as Euroréférence to reinforce the visibility of Listadapt among all referencing stakeholders in Europe.

Our results will be communicated to the non-scientific community whenever possible, via press releases, with the help of Press services from each partner organization. We will also promote the fast circulation of the project results to a large public, by writing popular articles.

Deliverables

Ref	**Title**	**Due month**
D-JRP7-5.3	Publications and communications	M30
Milestones		
Ref	**Title**	**Due month**
M-JRP7-20	Bioinformatic analysis done for all the strains	28
M-JRP7-21	Bioinformatic analysis done for all the strains	28
M-JRP7-22	Ad hoc batch Lm genomes annotation completed	20
M-JRP7-23	Face-to face meeting -2020	25
M-JRP7-24	WGS Proficiency Testing trial (PTtrial) done	26

2.4.2.1.22 JRP08-R1-FBZ2-METASTAVA

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ 2: Development and harmonisation of NGS-based methods for detection and tracing of FBZ agents, emerging threats and AMR determinants**									
Research Project Title	**Standardisation and validation of metagenomics methods for the detection of foodborne zoonoses, antimicrobial resistance and emerging threats.**									
Research Project Acronym	**METASTAVA**									
Leading Organisation	**P4-Sciensano**					Deputy Leading Organisation		**P10-FLI**		
Project Leader	**Steven Van Borm**					Deputy Project leader		**Dirk Höper**		
Project Start month	**M1**					Project End month		**M30**		
Participant	**1**	2	**4**	6	7	8	9	**10**	11	12
	Anses	Ages	**Sciensano**	NDRVMI	SZU	VRI	BfR	**FLI**	RKI	DTU
P.M	**5.99**		**4.09**					**6.80**		
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
P.M										
Participant	23	24	25	26	27	28	29	30	**31**	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	**WbvR**	FHI
P.M									**12.39**	
Participant	33	34	35	36	37	38	39	40	**41**	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	**SVA**	
P.M									**2.00**	

Objectives

- Identifying knowledge and data gaps in concertation with ongoing initiatives (COMPARE, EFFORT, and GMI). Collecting reference data to allow informed design of metagenomics experiments fitting to a precisely defined diagnostic question
- Collecting validation data to understand and document the analytical test properties (sensitivity, specificity, robustness) of metagenomics workflows for model pathogens (in their most relevant sample matrices), including hepatitis E, norovirus, large DNA viruses, antibiotic resistant bacteria and Shigatoxigenic Escherichia coli (STEC).
- Proposing quality assurance metrics to guide the evaluation and interpretation of metagenomics datasets. To this purpose, available datasets will be collected and investigated in a meta-analysis. Sample type specific qualitative and quantitative metrics

for the evaluation of metagenomics datasets will be proposed. In addition, we aim to develop a system of relevant process controls to monitor the robustness of the complete assay including sample treatment, data generation, and analysis. • Provide dissemination of the outcome of other initiatives & METASTAVA.

- Ensure interlaboratory reproducibility of the delivered data and principles by organizing a proficiency test that is relevant for diagnostic laboratories and by cross analysing metagenomics data generated by the partners

Description of work

WP1: Collect reference data from other metagenomic projects select the metagenomic methods to be used for the project, and provide guidance data for informed metagenomic workflow design.
WP1 takes place over the first , the second and the third (6 month time extension) annual period of the EJP
WP start month M1
WP end month M30
WP Leader Dirk Höper
Deputy WP Leader Frank Vandenbussche
WP participants Steven Van Borm, Sander van Boheemen, Lihong Liu, Yannick Blanchard, Sigrid DeKeersmaecker, Isabelle Kempf, Kevin Vanneste, Frank Vandenbussche, Wim van der Poel, Alex Bossers.

Description of the WPi
WP1 collects reference data from literature, sequence databases, partner's datasets, and ongoing initiatives. Sequence data, metadata, and published data about error rates and biases in metagenomics data generation and analysis workflows will be included in a meta-analysis aiming to produce guidelines for informed metagenomics experiment design, and quality metrics for the validation and interpretation of metagenomic data.

Task T-1.4: propose a standardised framework for the description of the application scope and analytical properties of a metagenomics assay.
Task start month M18
Task end month M30
Task Leader: Steven Van Borm
Deputy Task Leader: Dirk Höper
Task Participants: all
Description of the task:
proposed on all delivered data (WP1-WP3), a workgroup will propose a
standardised framework for the description of scope & analytical properties of a metagenomics assay.

WP2: Quality assurance tools for the validation and interpretation of metagenomics.
WP2 takes place over the first , the second and the third (6 month time extension) annual period of the EJP
WP start month M10
WP end month M30
WP Leader Sander van Boheemen
Deputy WP Leader Yannick Blanchard
WP participants Steven Van Borm, Sander van Boheemen, Lihong Liu, Yannick Blanchard, Sigrid DeKeersmaecker, Isabelle Kempf, Kevin Vanneste, Frank Vandenbussche, Wim van der Poel, Alex Bossers

Description of the WP
WP2 develops quality assurance tools for metagenomics in a diagnostic context, including quality metrics, process controls, data about reproducibility and batch effects, and proficiency tests.

TaskT-2.1: develop quality metrics for metagenomics dataset evaluation.
T-2.1 takes place over the first and the second annual period of the EJP
Task start month M10
Task end month M30
Task Leader: Kevin Vanneste
Deputy Task Leader: Steven Van Borm
Task Participants: Van Borm, De Keersmaecker, van Boheemen, Höper, Blanchard, Vandenbussche, Bossers.

Description of the task
Based on the propositions of T-1.4, develop and validate both raw data QC metrics and endogenous (internal) control target sequences that can provide indications about the quality of a metagenomics dataset
TaskT-2.2: Development and evaluation of external controls for metagenomics.
T-2.2 takes place over the first and the second annual period of the EJP
Task start month M1
Task end month M24
Task Leader: Frank Vandenbussche
Deputy Task Leader: Yannick Blanchard
Task Participants: Vandenbussche, Blanchard, Van Borm, van Boheemen.

Description of the task
Develop and evaluate exogenous process controls that can be spiked in samples to allow a full control of the assay from sample treatment until data interpretation.

Task T-2.4: evaluate QC metrics on additional datasets.
Task start month M13
Task end month M30
Task Leader: Steven Van Borm
Task Participants: all

Description of the task
partners will analyse existing and generate additional metagenomics datasets to cross-validate the developed QA principles (QC metrics and/or process controls) on additional case studies and samples.

TaskT-2.5: METASTAVA metagenomics proficiency test.
Task start month M13
Task end month M30Task Leader: Lihong Liu
Deputy Task Leader
Task Participants: all

Description of the task
In concertation with ongoing initiatives, we will provide added value by providing a PT focused on diagnostic sample matrices (both at the sample and dataset level) and targeted to diagnostic

institutes. The collected reference samples and datasets will provide leverage for benchmarking and validation of future methodological developments in data generation and analysis.

WP3: evaluation of the analytical properties of metagenomics workflows.
WP3 takes place over the first , the second and the third (6 month time extension) annual period of the EJP
WP start month M1
WP end month M30
WP Leader Yannick Blanchard
Deputy WP Leader Lihong Liu
WP participants Steven Van Borm, Sander van Boheemen, Lihong Liu, Yannick Blanchard, Sigrid DeKeersmaecker, Isabelle Kempf, Kevin Vanneste, Frank Vandenbussche, Wim van der Poel, Alex Bossers

Description of the WP
WP3 experimentally determines the analytical properties (sensitivity, specificity) and robustness of metagenomics data generation and analysis protocols for the selected model pathogens in diagnostically relevant sample matrices.

Task T-3.1: analytical sensitivity, hepatitis E virus.
Task start month M1
Task end month M30
Task Leader: Nicole Pavio
Task Participants: Pavio, Martin-Latil, Liu, Van Gucht, Suin, Van Borm, Vandenbussche, Cotten, Koopmans, Mikhayil Hakhverdyan, van der Poel, Bossers.

Description of the task
Replicate metagenomics analysis of hepE dilutions in diagnostically relevant sample matrices for human, animal and food samples to estimate the analytical sensitivity and robustness.

TaskT-3.2: analytical sensitivity, norovirus.

Task start month M1
Task end month M30
Task Leader: Sandra Martin-Latil
Task Participants Martin-Latil, Liu, van Boheemen, Koopmans

Description of the task
Replicate metagenomics analysis of norovirus dilutions in diagnostically relevant sample matrices for human and food samples to estimate the analytical sensitivity and robustness. Higher uncertainty for deliverables on food items (very low NoV titers), high certainty for human (fecal or vomit) samples.

TaskT-3.3: analytical sensitivity, large DNA viruses.
Task start month M1
Task end month M30
Task Leader: Dirk Höper
Task Participants: Beer, Höper, Pohlmann, van Boheemen, Koopmans, De Clercq

Description of the task
Replicate metagenomics analysis of poxvirus dilutions in diagnostically relevant sample matrices.

TaskT-3.4: analytical sensitivity, STEC.
Task start month M1
Task end month M24
Task Leader: DeKeersmaecker
Task Participants: Kempf, Blanchard, Denayer

Description of the task
Replicate shotgun metagenomics of STEC dilutions in diagnostically relevant sample matrices for human and/or food samples to estimate the analytical sensitivity and robustness.

Task T-3.5: analytical sensitivity, detection of ABR genes.
start month M1
Task end month M30
Task Leader: Kempf
Task Participants: DeKeersmaecker, Blanchard, van der Poel, Bossers
Description of the task
Replicate shotgun metagenomics of antibiotic resistant bacteria in diagnostically relevant sample matrices to estimate the analytical sensitivity and robustness.

Task T-3.6: bioinformatics and statistical analysis of analytical performance experiments
T-3.6 takes place over the first and the second annual period of the EJP
Task start month M1
Task end month M30
Task Leader: Kevin Vanneste
Task Participants: Van Borm, Vandenbussche, Blanchard, Höper, van Boheemen, DeKeersmaecker, Bossers
Description of the task
Concertation to align the analysis of all data generated in T-3.1 - T-3.5.

WP4: Concertation with ongoing efforts and dissemination.
WP4 takes place over the first , the second and the third (6 month time extension) annual period of the EJP
WP start month M1
WP end month M30
WP Leader Steven Van Borm
WP participants all

Description of the WP
WP4 assures via direct concertation that all research overlap with ongoing initiatives is avoided. It also ensures formal PI issue arrangements in accession to data, knowledge, tools, and samples where needed. In addition, WP4 assures dissemination of the project findings.

TaskT-4.1: concertation with ongoing initiatives.
Task start month M1
Task end month M30
Task Leader: Steven Van Borm
Task Participants: Van Borm, Blanchard, Koopmans, Beer, Roosens, De Keersmaecker.

Description of the task

Coordination with the related projects of COMPARE, EFFORT, DECATHLON and GMI, to give input for WP1, 2, and 3. Identify gaps in the current technology relevant for applying metagenomics for diagnostics. In addition, existing ISO workgroups on QA of NGS based methods will be contacted. A coordination meeting will be organised with participants from these other projects, Intellectual property (IP) issues regarding access to their knowledge and technology will be addressed (as will any background IP at the start of the consortium), this information will be provided to WP1 and WP2, and an analysis of important gaps in the current technology for applying metagenomics for diagnostics will be identified. Continued coordination with related initiatives to exchange findings.

TaskT-4.2: Formal dissemination.
Task start month M1
Task end month M24
Task Leader: Steven Van Borm
Task Participants: all

Description of the task
SOPs, scientific papers, conference contributions, participation to working groups

Task T-4.3: dissemination of recommendations to stakeholders
T-4.3 takes place over the first and the second annual period of the EJP
Task start month M1
Task end month M30
Task Leader: Van Borm
Task Participants: all

Description of the task
Dissemination of recommendations from project findings to the various stakeholders (e.g. policy-makers, industry, citizens, research community in the different domains of 'One Health').

Task T-4.4: Organization of a scientific meeting
Task start month M24
Task end month M30
Task Leader: Van Borm
Task Participants: all

Description of the task
Organisation of a scientific meeting to disseminate the project findings.

WP5: Project management
WP5 takes place over the first , the second and the third (6 month time extension) annual period of the EJP P
WP start month M1
WP end month M30
WP Leader Steven Van Borm
Deputy WP Leader Dirk Höper
WP participants all
Description of the WP
Responsibilities include liaising with the EU, consolidating a consortium agreement, organising project meetings, reports, and internal coordination. The project coordinator will be assisted in these duties by a deputy coordinator, work package leaders and task leaders. A General Assembly (coordinator + WPLs) will be established to allow important decisions impacting on project

organisation and output. Yearly project meetings will be organised, supplemented with additional online meetings (either project-wide, or WP or task specific).

Task T-5.2: internal communication.
Task start month M1
Task end month M30
Task Leader: Steven Van Borm
Task Participants: all

Description of the task
Yearly progress meetings and intermediary communication

Task T-5.3: reporting and liaising with the EU
Task start month M24
Task end month M30
Task Leader: Van Borm
Task Participants: all

Description of the task
Half term (Y1) reporting and final report.

Deliverables

Ref	Title	Due month
D-1.4	SOP: guidelines for the description of scope and analytical properties of a metagenomic method in a diagnostic context	M30
D-1.5	Review paper	M30
D-2.1	SOP: use of quality metrics for metagenomics dataset evaluation	M30
D-2.2	Report and guidelines for the use of exogenous process controls in metagenomics	M30
D-2.5	Report on proficiency test	M24
D-3.3	Report: analytical sensitivity and robustness , hepE	M30
D-3.4	Report: analytical sensitivity and robustness , NoV	M30
D-3.5	Report: analytical sensitivity and robustness , pox	M30
D-3.6	Report: analytical sensitivity and robustness , STEC	M30
D-3.7	Report: analytical sensitivity and robustness , ABR genes detection	M30
D-4.2	SOP's , guidelines, scientific papers, presentations	M30
D-4.3	Minutes of scientific meeting	M30
D-5.2	Progress and final meeting minutes	M30
D-5.3	Half term report	M13
D-5.4	Final report	M30

Milestones

Ref	Title	Due month
M-FBZ2.metastava.3	Proficiency test panel ready for shipping	M20

M-FBZ2.metastava.6	Scientific meeting	M30
M-FBZ2.metastava.7	Dissemination to various stakeholders including joint communication with ongoing initiatives	M30
M-FBZ2.metastava.9	Final meeting	M30

2.4.2.1.23 JRP09-R1-FBZ3-AIR-SAMPLE

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ 3: Biosecurity and other interventions**									
Research Project Title	**Air-sampling: A Low-Cost Screening Tool in Biosecured Broiler Production.**									
Research Project Acronym	**AIR-SAMPLE**									
Leading Organisation	**P12-DTU**					Deputy Leading Organisation			**P33-NVI**	
Project Leader	**Jeffrey Hoorfar**					Deputy Project leader			**Mona Torp**	
Project Start month	**M1**					Project End month			**M30**	
Participant	1	2	4	6	7	**8**	9	10	11	**12**
	Anses	Ages	Sciensano	NDRVMI	SZU	**VRI**	BfR	FLI	RKI	**DTU**
PM						**2.60**				**20.61**
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	23	24	25	26	27	**28**	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	**IZSAM**	IZSLER	RIVM	WbvR	FHI
PM						**4.00**				
Participant	**33**	**34**	35	36	37	38	39	40	41	
	NVI	**PIWET**	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM	**0.70**	**3.10**								

Objectives:

- To prepare scientific manuscripts for publication.
- To prepare Standard Operating Procedures (SOP's) and propose standards.
- To disseminate the above protocols through hands-on workshops.

Description of work

WP2. Validation and standardization.
WP start month: 13.
WP end month: 30.
WP Leader: Gro Johannessen (NVI).
Deputy WP Leader: Ivana Koláčková (VRI).

WP participants: Ivana Kolackova (VRI), Julia Christensen (DTU), Mona Torp and Gro Johannessen (NVI), Kinga Wieczorek and Jacek Osek (NVRI), Giuliano Garofolo (IZSAM).

Description of the WP: WP2 departs from the achievements of the first annual period and takes place over the second annual period of the EJP. Validation is important to regulatory approvals before the methods can be used. Hence, the air sampling protocol from WP1 will be validated. Following statistical data analysis of the results obtained, the best performing protocol will be drafted as a Standard Operating Procedure (SOP). This will be disseminated among the EJP partner laboratories involved in testing of Campylobacter, and as a new working item to microbiology working groups of International Organization for Standardization (ISO) and Committee of European Normalization (CEN). A draft manuscript will be submitted to the EFSA Journal.

Task 2-2. Statistical analysis, Standardization and dissemination.
Task start month: M21
Task end month: M30
Task Leader: Renata Karpickova (VRI)
Deputy Task Leader: Jeffrey Hoorfar (DTU)
Task Participants: Gro Johannesen (NVI), Jacek Osek (PIWET), Mona Torp (NVI), Giuliano Garofolo (IZSAM).

Description of the task: The main aim of this task is to disseminate the outcome. Results of air samples used in qPCR and culturing will be statistically validated against sock samples, but also using latent class analysis where no gold standard is assumed.

Project results will be communicated through various relevant channels as follows:

1. Preparation of a draft manuscript to the official journal of European Food Safety Authority.
2. Preparation of an SOP to be submitted to the consortium and to ISO and CEN for consideration as a future guideline or standard
3. Communication of the project outcome to the national food safety authorities
4. Online video animation material for the education of broiler industry, and
5. Dissemination through a hands-on, wet-lab workshop.

Deliverables

Ref	Title	Due month
D-JRP9-2.3.	Draft manuscript for publication in EFSA Journal.	M24
D-JRP9-2.4.	Hands-on, wet-lab workshop for relevant EJP partners.	M24

Milestones

Ref	Title	Due month
M-JRP9-4	Statistical analysis completed.	M21

2.4.2.1.24 JRP10-R1-FBZ3-MoMIR-PPC

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ 3: Biosecurity and other interventions**									
Research Project Title	**Monitoring the gut microbiota and immune response to predict, prevent and control zoonoses in humans and livestock in order to minimize the use of antimicrobials.**									
Research Project Acronym	**MoMIR-PPC**									
Leading Organisation	**P19-INRA**					Deputy Leading Organisation			**P17-UCM**	
Project Leader	**Velge Philippe**					Deputy Project leader			**María Ugarte Ruiz**	
Project Start month	**M1**					Project End month			**M36**	
Participant	**1**	2	4	**6**	7	**8**	9	10	11	12
	Anses	Ages	Sciensano	**NDRVMI**	SZU	**VRI**	BfR	FLI	RKI	DTU
P.M	**2.5**			**12.43**		**13**				
Participant	13	14	15	16	**17**	18	**19**	20	21	22
	SSI	UT	VFL	INIA	**UCM**	MVNA	**INRA**	IP	APHA	PHE
P.M					**7,5**		**9,45**			
Participant	**23**	24	25	26	**27**	28	**29**	30	**31**	**32**
	UoS	OKI	NUIG	TEAGASC	**ISS**	IZSAM	**IZSLER**	RIVM	**WbvR**	**FHI**
P.M	**3**				**7,75**		**20,50**		**14,45**	**8,40**
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
P.M										

Objectives

- Development of tools to predict Super-shedder animals and human prolonged *Salmonella* carriers
- Development of pro- and pre-biotics to prevent the appearance of Super-shedders
- Development of new models of pathogen transmission
- Development of new intervention strategies to fight zoonotic pathogens
- Dissemination of the original information acquired during this project

Description of work

WP0, Task-0.3 Control and manage activity progresses, the timely delivery of project tasks and outputs
Task end month: M36
Task Leader: P18-Tours, Philippe Velge ; Task Participants: All partners
Description of the task:
The steering committee will have the responsibility for ensuring that milestones and deliverables will be accomplished on time. He will facilitate the day-to-day running of the project. These will include regular and effective communication, troubleshooting and the overseeing of general administrative duties.

WP0, Task-0.4 Control and manage the project closure and outputs
Task end month: M36
Task Leader: P18-Tours, Philippe Velge
Deputy Task Leader: P18-Tours, Beatrice Laroche
Task Participants: All partners
Description of the task:
Project closure plans will be developed in line with Horizon 2020 requirements. The detailed closure process comprising a template and related guidelines developed by the INRA Team will be used as a framework for this process. The closing meeting will be held at the University of Surrey. It will allow discussion about future initiatives.

WP1: Risk prediction for Super-shedder animals and human prolonged Salmonella carriers through the use of gut microbiota and immune status analyses.
WP Leader: P22, Roberto La Ragione
Deputy WP Leader: P29, Giovanni Loris Alborali
WP participants: P1, P8, P16, P18, P22, P27, P29, P32
Description of the WP:
The goal of this WP is to identify immunological and microbiota markers that are associated to high/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 will use already developed infection models (P1, 18 and 27) but also animal (Task 1 and 2) and human infections (Task 3) in “field condition” (P12, P29, P32). Taking into account the great adaptability of pathogens for their hosts, the virulence and immunogenicity of Salmonella strains of human and animal origins and from Super- and non Super-shedders will be compared (P18-Tours, P22). The gut microbiota composition (P8, P22), the cellular biomarkers and the immune response parameters (P18, P27, P29) will be studied with the same chickens and pigs. The second year will be devoted to the analysis of the data recovered the first year in order to define the predictive markers associated to high and low shedders .
This WP will be divided into 4 tasks:

Task-1.1: Predictive immunological markers associated to the high and low shedders in chickens and pigs
Task Leader: P27, Barbara Chirullo
Deputy Task Leader: P1, Martine Denis
Task Participants: P1, P16, P18-Tours, P27, P29
Description of the task:
The goal of the task will be 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 will be focused on the most prevalent serotypes: S. Typhimurium for pig, and S. Enteritidis for chicken. They will use global approaches but also already published markers (Knetter et al. Innate Immun,

2015; Chaussé et al. Infect Immun 2011). Moreover, we will study the predictive power of the IgM and IgA dynamics before and after infection to distinguish the super-shedders.

Task-1.2: Predictive microbiota markers associated to the high and low shedders in chickens and pigs
Task Leader: P8, Ivan Rychlick
Deputy Task Leader: P22, Roberto La Ragione
Task Participants: P1, P8, P18-Tours, P22, P27, P29, P32
Description of the task:
The goal of the task is to analyse the gut microbiota composition before and after Salmonella infection in pig and poultry to identify Predictive microbiota markers associated to the high and low shedders in chickens. Samples from representative pigs and poultry will be analyzed (P8 and P22 respectively) based on Illumina sequencing of V3/V4 variable region of 16S rRNA genes and the microbiomes related to shedding status for Salmonella. Several bacterial genera have been already correlated with susceptibility/ resistance to become a super-shedder. However, it is important to demonstrate numerous correlations in poultry and/ or pig before to purify and test these commensal bacteria in WP2.

Task-1.3: Risk factors associated with prolonged convalescent Salmonella shedding in humans
Task Leader: P32, Anke Corinna Stüken
Task Participants: P22, P32
Description of the task:
This task will encompass a Norwegian prospective cohort study as detailed for task 1.3 in the first annual period of the EJP. In the second annual period, the last recruitment of participants and sampling will take place (Feb-19). Furthermore, stool samples for microbiome analyses will be selected: To identify predictive markers for prolonged convalescent shedding in host microbiota composition, all stool samples received from cases with prolonged shedding (positive >2 month) and at least an equivalent number of stool samples from cases without prolonged shedding (positive ≤2 month) will be subjected to microbiome analyses. The data will be analyzed for differences in diversity between prolonged and short-term shedder microbiomes, as well as for associations between shedding status and the presence or absence of specific taxonomic groups. In total, a maximum of 90 stool samples will be subjected to microbiome analyses. In addition, the remaining initial Salmonella isolates submitted to NRL will be whole genome sequenced, serotyped and screened for antibiotic susceptibility to beta-lactams (ampicillin, ceftazidime, cefotaxime, meropenem), quinolone (pefloxacin), aminoglycoside (gentamicin) and trimethoprim-sulfamethoxazole by disk diffusion. In the second annual period of the EJP, all genome sequenced Salmonella isolates from participants will be screened for the presence of resistance genes to the different classes of antibiotics and virulence genes such as fimbrial operons (e.g. bcf, csg, stb, sth, sti), colonisation factors (e.g. misL, bapA, sinH) and invasins (pagN, Rck), T3SS-1 and T3SS-2 genes. Furthermore comparative genomic analyses will search for possible additional marker genes that may predict long term Salmonella excretion in humans.

Task-1.4: Virulence of *Salmonella* strains originated from high and low shedders
Task Leader: P18-Tours, Ignacio Caballero, Deputy Task Leader: P22, Roberto La Ragione
Task Participants: P18-Tours, P22, P32
Description of the task:
We will continue to compare the adhesion and invasion capabilities as well as the induced immune response between different Salmonella of animal origins and from super- and low-shedders using epithelial pig and chicken cell lines (P18, P22). Transcriptomic analysis will be set up to compare the immune response. For the Salmonella strains, which will be sequenced, within the project a

comparative study between the virulence and the genome will be performed. In addition, the isolates will be screened for their ability to attach to abiotic surfaces and for biofilms by partner 18.

WP2: Prevention of the appearance of Super-shedders by modifying feed and/or microbiota
WP Leader: P16 VISAVET-UCM. Antonio Rodríguez Bertos
Deputy WP Leader: P29, Giovanni Loris Alborali
WP participants: P6, P8, P16, P18-Tours, P22, P27, P29,
Description of the WP:
Production animals when infected by a foodborne pathogen such as Salmonella, Campylobacter or toxigenic E. coli, are in most cases mere asymptomatic carriers, which release the bacteria to the environment through their faeces. The removal of super-shedders from a population may significantly reduce transmission and contamination with these foodborne pathogens. However, far from being beneficial, prolonged antibiotic treatment may alter dramatically the intestinal flora composition, may increase the pathogen´s antimicrobial resistance and trigger the super-shedder phenotype (Croswell et al. 2009 Infect immun ; Sana & Monack 2016 Nature).

The aim of WP2 is to ascertain, through the use of –omics technology, whether pre-biotics, pro-biotics or nutraceutical formula would be able to considerably lower the numbers of Salmonella (and other foodborne pathogens such as *Campylobacter* and toxigenic *E. coli*) released by super-shedders. The impact of these food supplement formula on gut microbiota composition, immune response and susceptibility to pathogens like *Salmonella* will be investigated. We will also analyze their impact on the resistome of the microbiome and on animal´s parameters (P29, P6 respectively). Close cooperation with the other partners will allow us to better understand
super-shedding basis and to set up the best options to achieve a drastic reduction of Salmonella release to the environment. We will test the efficacy of a series of commensal bacteria (probiotics) and of natural diet additives which are expected to alter the intestinal microbiota by promoting the growth of “beneficial” microorganisms, whilst avoiding the use of antibiotics. The main goal would be to modify microbiota composition to improve immune response and the barrier effect procured by gut microbiota, with the ultimate goal of preventing super-shedder status in animals and carriage status in humans. Some approaches have been reported on this respect in human and animal (Drakoularakou et al. 2010 Eur J Clin Nutri; Yadav et al (2016) J Exp Biol Agri Sci).

Task-2.1: Use of probiotics in chicken and pig
Task Leader: P8, Ivan Rychlick
Deputy Task Leader: P29, Giovanni Loris Alborali
Task Participants: P6, P8, P12, P18-Tours, P22, P27, P29,
Description of the task:
The main objective of this task will be to improve immune response and barrier effect procured by gut microbiota by introducing already identified probiotics (first year) and other candidate probiotics, which will be identified in WP1 and isolated from healthy pigs and poultry. These commensal bacteria will be phenotypically and genotypically characterised using WGS, Biolog, NMR and in vitro organ culture studies (P8, P22). We will also test in vitro the competitive exclusion of these commensal bacteria by themselves and/or the metabolites generated for Salmonella. The effect on gut microbiota and pathogen infection will be tested in established experimental avian and porcine models described in WP1 (P18-Tours, P8, P27), but also on farms with presence of Salmonella (P6, P29). The pro and pre-biotics (task 2.2) will be tested in pig farms (P6, P29) and in broiler farms (P6). Blood count test - hemoglobin, hematocrit, white blood cells, total blood protein, blood albumin, glucose, serum bactericidal activity and immunoglobulin levels according ELISA method will be recorded as well as welfare, health status and animal performance parameters.

Task-2.2: Use of pre-biotics and nutraceutical already defined by the consortium partners in chicken and pig.
Task Leader: P16, María Ugarte Ruiz
Deputy Task Leader: P6, Hristo Daskalov:
Task Participants: P6, P8, P16, P22
Description of the task:
The goal of this Task is to measure the effect of several pre-biotics (pellet, meal, liquid, or feed with various fibers) (P1, P6) and nutraceuticals (P16) in farms and experimental conditions. We will analyse their effects on animal health status (P6, P16), blood parameters (P6), animal performances (P6, P16), gut microbiota composition (P8, P16, P22), immune response (P6, P27, P29) and welfare (P6, P29). Microbiota composition and immune status will be tested with the same approaches than in WP1. They will be mainly analysed the second year from the samples recovered the first year.

WP3: Modelling the transmission of zoonotic agents to improve intervention strategies on livestock farms
WP Leader: P30 Thomas Hagenaars
Deputy WP Leader: P18-Jouy, Beatrice Laroche
WP participants: P18-Tours, P30
Description of the WP:
WP3 will focus on the problem of Salmonella in poultry and pig in order to develop the building blocks and tools that are needed for design and assessment of intervention strategies. For this purpose we will develop 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 will be designed to allow assessment of the efficiency and/or cost-effectiveness of different intervention strategies. In particular, assessment of intervention strategies targeting super-shedders and based on probiotic and/or prebiotic treatments are of interest, as well as combinations of such strategies with biosecurity measures based on hygiene and movement restriction. This WP will be divided in two main tasks:

T-3.1: Transmission modelling at within-host and between-host scales
Task Leader: P18-Jouy, Beatrice Laroche Deputy Task Leader: P30, Thomas Hagenaars Task Participants: P18-Jouy, P30, P41
Description of the task:
In this task, mathematical models will be developed to integrate quantitative knowledge on the heterogenity of infection and the role of gut microbiota, linking up two scales. The models, build the first year, will be improved the second year after inclusion of data obtained in WP1 and WP2.

Sub-Task 3.1.1: Within-host scale: modelling individual responses and shedding
Sub-Task Leader: P18-Jouy, Béatrice Laroche
Sub-Task Participants: P18-Jouy,
Description of the Sub-task:
Our ambition is 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.This work will rely on the data generated by WP1 and WP2. The general idea would be to implement stochastic or deterministic dynamic interaction models between groups of gut bacteria (typical models are generalized Lotka-Volterra models, but other options are possible). Some bacterial genus will be explicitly represented, based on the knowledge generated in WP1. For other operational taxonomic units, aggregation prior to modelling is a key problem that we will address through clustering approaches. The links with the hosts immune response will be modelled, first in order to include possible feedback loop between the host and its microbiota and second in order to include super-

shedder detection strategies in the model. The final models will include as much as possible the mechanisms described in WP1 and WP2

Sub-Task 3.1.2: Between-host scale: modelling transmission, linked to within-host results
Sub-Task Leader: P41, Mart de Jong
Sub-Task Participants: P18-Jouy, P30, P41
Description of the Sub-task:
The modelling aims to integrate, the second year, data from the two other WPs and already available datasets (first year). Between-host transmission models will be formulated based on the results of the within-host modelling, relating host susceptibility and transmission parameters to intra-host characteristics (P18-Jouy, P30). Then, environmental dissemination of infectious material has to be incorporated, based on part on data from experiments. The coupling of between-animal heterogenities (of shedding) to environmental transmission is important to both distinguish and combine host-pathogen interactions and pathogen-enviroment interactions.We envisage including both transmission between stables (animal groups coinciding temporally but not spatially) and between rounds (animal groups coinciding spatially but not temporally), such that this model can in task 2 be further developed to mimic interventions: super-shedder detection/removal, between-stable biosecurity measures, between-round biosecurity measures.

T-3.2: Interventions strategies: Identification and evaluation tools
Task Leader: P30, Thomas Hagenaars
Deputy Task Leader: P18-Jouy, Béatrice Laroche
Task Participants: P18-Jouy, P30, P41
Description of the task:
The results of WP1 and WP2 will help designing intervention strategies targeting super-shedders and based on probiotic and/or prebiotic treatments. As these rely on a complex interplay between the ecological behaviour of the gut microbiota and the host immune response, the multi-scale modelling approach is essential to assess and improve the efficiency of these intervention strategies. Moreover, such strategies can be combined with other biosecurity measures based on hygiene and movement restriction.

Sub-Task 3.2.1: Systematic inventory of relevant intervention measures
Sub-Task Leader: P30, Thomas Hagenaars
Sub-Task Participants: P30, P41
Description of the Sub-task:
In order to identify specific husbandry and/or biosecurity actions that can form building blocks for intervention strategies in combination with those based on probiotic and/or prebiotic treatments, knowledge of the husbandry practices is important. Based on expert knowledge, an inventory of such practical building blocks will be developed in year 1. Based as much as possible on results from the models of task 3.2.2, these building blocks can be weighed using a HACCP methodology. A first such weighing will be attempted in year 3.

Sub-Task 3.2.2: Inclusion of potential interventions into the modelling
Sub-Task Leader: P18-Jouy, Béatrice Laroche
Sub-Task Participants: P18-Jouy, P30, P41
Description of the Sub-task:
In part based on WP2 results, mathematical representations of the mechanisms through which potential intervention measures act will be included in the models developed in task 3.1.2

Sub-Task 3.2.3: Development of economic analysis tools
Sub-Task Leader: P30, Ron Bergevoet

Sub-Task Participants: P30, P41
Description of the Sub-task:
Economic analysis tools will be developed that can be used in combination with the transmission-dynamical models to evaluate cost effectiveness of intervention strategies. In this context the socio-economic viability of intervention strategies, including consumer acceptance, needs to be taken into consideration, as well as effective implementation strategies of promising interventions. For this, behavioural insights will be applied to the agricultural domain to predict the adoption of policy measures and interventions. In year 3 the economic analysis tools will be developed from a first description of these tools completed in year 1.

WP4: Communication and Dissemination for Impact
WP Leader: P18-Tours, Philippe Velge
Deputy WP Leader: P32, Anke Corinna Stüken
WP participants: All partners
Description of the WP:
This WP will be embedded in the animal and human value chain through all work being carried out. It aims to raise awareness of the progress and benefits of the project by continually communicating with stakeholders through the targeted routes and media. The Steering committee will identify and set clear communication objectives that will ensure that all messages are tactically planned. Dissemination and communication strategies will acknowledge demand from beyond Europe for new knowledge that will afford effective infection-control measures. Individual partners' specific roles in communication activities will be set out and agreed during the initial stages of the project. The communication strategy will remain sufficiently flexible to respond positively to unforeseen opportunities and may evolve during the life of the project.

T-4.1: Dissemination of data within the project and management of data
Task Leader: P18-Tours, Philippe Velge
Deputy Task Leader: P32, Anke Corinna Stüken
Task Participants: All partners
Description of the task:
The main objectives of this task were to develop:
- The project website and the Data management policy and strategies

T-4.2: Dissemination of data outside the project and management of data
Task Leader: P32, Anke Corinna Stüken Deputy Task Leader: P1, Martine Denis
Task Participants: All partners
Description of the task:
The overarching objective of WP4 is to ensure that new knowledge, information and data from MoMIR -PPC are passed to relevant user communities and other recipients – including the general public – in a timely and accessible manner in order to ensure that maximum impact potential is achieved. The applicants have extensive experience of delivering both research outputs and more general information on food-borne pathogens through all branches of the popular media, and will play an active role in engaging with the public. They will be supported by the press offices in their respective organizations that will help draft and target press releases and arranging interviews and media visits. The consortium well understands that issues of food quality and hygiene, public health and farm-animal welfare offer excellent opportunities for engaging with a public that has high interest in such matters.
- We will disseminate our research findings through peer-reviewed publications and through presentations at national and international conferences.
- MoMIR-PPC partners will communicate with policy-makers and regulators to ensure, wherever relevant and practical, that significant ground-breaking results are encapsulated within formal best-

practice guidelines, national policies and/or statute. Dissemination activities will further extend to the scientific and lay-public communities		
Deliverables		
Ref	**Title**	**Due month**
D-JRP10-2.02	Description of the microbiome and resistome in farms	36
D-JRP10-1.11	Recovery of all human samples	32
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
D-JRP10-2.03	Characterization of protective commensal bacteria able to inhibit Salmonella colonization (second rounds)	34
D-JRP10-1.06	Definition of predictive immunological markers associated to the high and low shedders in chickens and in pigs.	34
D-JRP10-1.07	Definition of immunological markers associated to the high and low shedders in chickens and in pigs.	34
D-JRP10-1.09	Definition of predictive microbiota markers associated to the high and low shedders in chickens and in pigs.	32
D-JRP10-1.10	Definition of microbiota markers associated to the high and low shedders in chickens and in pigs.	32
D-JRP10-2.11	Evolution of the immune-signature in pig, chicken and/ or human according to the context (infection, treatment…)	34

2.4.2.1.25 JRP11-R1-FBZ4-MedVetKlebs

Reference to the Stategic Research Agenda (please refer to D2.7)			**FBZ 4: Source attribution and transmission routes**							
Research Project Title			**Klebsiella pneumoniae: from ecology to source attribution and transmission control**							
Research Project Acronym			**Med-Vet-Klebs**							
Leading Organisation			**P20-IP**			Deputy Leading Organisation			**N/A**	
Project Leader			**Sylvain Brisse**			Deputy Project leader			**N/A**	
Project Start month			**M1**			Project End month			**M30**	
Participant	**1**	**2**	4	6	7	8	9	10	11	**12**
	Anses	**Ages**	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	**DTU**
P.M	**4.86**	**2.25**								**0.55**
Participant	**13**	14	15	16	17	18	**19**	**20**	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	**INRA**	**IP**	APHA	PHE
P.M	**6.17**						**5.05**	**19.06**		
Participant	23	24	**25**	26	27	**28**	29	**30**	31	32
	UoS	OKI	**NUIG**	TEAGASC	ISS	**IZSAM**	IZSLER	**RIVM**	WbvR	FHI
P.M			**1.59**			**12.31**		**12.00**		
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
P.M										

Objectives

Objectives of Year 3 will be to pursue the broad sampling of Kp in multiple sources; Broad sampling will be supported by the design in previous year of a qPCR to detect Kp in complex matrices; To define the best sources where to perform deep sampling; and perform genomic sequencing analyses and modelling of transmission among sources

Description of work

WP1, Task 1.2
Real-time PCR assays will be evaluated for sensitivity and specificity from spiked matrices, first in simple matrices, then in more complex and relevant ones as defined in task 2.1. The limit of

detection will be determined. The qPCR developed at INRA will be tested at Pasteur and IZSAM and validated protocols will be sent to all partners.

WP1, Task 1.3
An important objective of the project is to diffuse protocols and harmonize their use. This has been achieved through our scientific meeting of Month 13 through a dedicated session, by e-mail exchanges, and by TCs between participating institutions. Five partners will contribute to this work, and have already implemented the method in their lab; and are testing chicken, salads and other samples for Kp contamination at the moment.

WP2 consists of sampling and evaluating of the prevalence and types of Kp in reservoirs and sources. It encompasses two complementary and temporally ordered approaches. First (T2.1), we will sample broadly in order to probe the presence of Kp in as many as possible relevant types of sources. This will provide comparative data on the occurrence of Kp in various types of samples and will lead to the identification of the most relevant potential sources in terms of transmission to animals and humans. In a second step (T2.2), we will focus our sampling on the most important sources of Kp in order to define precisely the prevalence of Kp in these sources, and quantify occurrence as a function of geography or other relevant parameters (processing, conservation). Finally (T2.3), selected Kp isolates from positive samples will be characterized for antimicrobial susceptibility and by genomic sequencing to define their subtype and harbored genes.

WP3 will deal with the analysis of genomic sequences, and the development of models of Kp transmission.
In T3.1, we will deliver clonal subtypes and gene distribution in the sources, which will be used for transmission models refinement and testing. In task 3.2, we will improve current modelling methods and develop specific extensions, using two different approaches: (1) source attribution methods and (2) dynamic modelling.

WP4 will deal with overall coordination, management and dissemination

Deliverables

Ref	Title	Due month
D-JRP11-2.2	Prevalence in selected sources	24
D-JRP11-2.3	Prevalence in selected sources	30
D-JRP11-2.4	Quantification of Kp in selected sources	24
D-JRP11-2.5	Quantification of Kp in selected sources	30
D-JRP11-2.6	Genome sequence data	24
D-JRP11-2.7	Genome sequence data	30
D-JRP11-3.1	Source distribution of clonal groups, plasmids and genes	24
D-JRP11-3.2	Source distribution of clonal groups, plasmids and genes	30
D-JRP11-3.3	Source attribution models by microbial subtyping and comparative exposure assessment	30
D-JRP11-3.4	Computer program for dynamic models simulation	24
D-JRP11-3.5	Estimates of the attribution proportion for different food, animal and environmental sources for the countries included.	30
D-JRP11-3.6	Estimates of the relative contribution of different transmission routes for exposure to Kp in the different countries included.	30
D-JRP11-4.2	Project Periodic Reports	24

D-JRP11-4.5	Consortium meetings – Review of work done/progress made and definition of priorities for next period	30
D-JRP11-4.7	Plan for the dissemination and exploitation of results	24
D-JRP11-4.8	Final Report	30
Milestones		
Ref	**Title**	**Due month**
M-JRP11-8	Initial prevalence, quantification and genomic data for model refining	24
M-JRP11-9	1st batch of clonal groups, plasmids and genes defined, for refinement of models	24
M-JRP11-10	Compilation and integration of the data produced in WP1 and WP2 to be used in the dynamic and source attribution models	24
M-JRP11-11	Identification of a list of scenarios for control measures to be assessed through model simulations	24
M-JRP11-12	Consolidated prevalence, quantification and genomic data for modeling of kp transmission	30
M-JRP11-13	Application of dynamic and source attribution models to data collected from different countries	30
M-JRP11-14	Reporting of transmission and source attribution estimates	30

2.4.2.1.26 JRP20-R2-FBZSH3-DISCoVeR

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ-SH 3: Source attribution of bacterial foodborne zoonoses and antimicrobial resistance considering also the environment and non-livestock reservoirs (e.g. pets and wildlife) as sources**									
Research Project Title	**Discovering the sources of Salmonella, Campylobacter, VTEC and antimicrobial Resistance**									
Research Project Acronym	**DiSCoVeR**									
Leading Organisation	**P12-DTU**					Deputy Leading Organisation			**P30-RIVM**	
Project Leader	**Tine Hald**					Deputy Project leader			**Eelco Franz**	
Project Start month	**M25**					Project End month			**M54**	
Participant	**1**	2	4	6	7	**8**	**9**	10	11	**12**
	Anses	Ages	Sciensano	NDRVMI	SZU	**VRI**	**BfR**	FLI	RKI	**DTU**
PM	**12.5**					**16**	**11.8**			**14.1**
Participant	**13**	14	15	16	17	18	19	20	**21**	**22**
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	**PHE**
PM	**7.4**				4				**8.57**	**1**
Participant	23	24	25	**26**	**27**	28	29	**30**	**31**	**32**
	UoS	OKI	NUIG	**TEAGASC**	**ISS**	IZSAM	IZSLER	**RIVM**	**WbvR**	**FHI**
PM				**2.6**	**12**			**7.73**	**4.96**	**10.28**
Participant	**33**	**34**	**35**	**36**	37	38	39	40	**41**	
	NVI	**PIWET**	**INIAV**	**INSA**	IISPV	IC	SLV	FOHM	**SVA**	
PM	**3.85**	**8**	**8.8**	**23.13**					**16.25**	

Objectives

DiSCoVeR's objectives are in alignment with objectives of priority topic FBZ 2.1. for three of the main zoonotic bacterial pathogens (Salmonella, Campylobacter, and STEC/VTEC) and antimicrobial resistance (AMR). We will

- Fill knowledge gaps regarding potential sources of foodborne zoonoses by including data on non-livestock reservoirs and non-food sources and by providing source attribution estimates at different points in the exposure chain (e.g. at reservoir, retail and exposure) level and across EU regions and countries.

- Critically assess and improve existing source attribution models including approaches based on microbial subtyping, case-control studies, outbreak investigations and exposure assessment.
- Quantify the contributions of animal reservoirs, including wildlife and companion animals, food and environmental sources and their transmission routes to the burden of foodborne zoonotic disease, considering geographical differences throughout Europe and consortium partners.
- Use both phenotypic and genomic typing techniques for pathogen and antimicrobial resistance characterization, as well as epidemiological data, for the purposes of source attribution.
- Improve existing and develop new models for source attribution that account for multi-directionality of transmission incl. transmission among reservoirs and within the human population.
- Providing a critical evaluation of the evidence and uncertainty obtained by the source attribution models to improve the characterisation of the sources and transmission pathways implicated in the epidemiological cycles of the hazards focused by the project.
- Provide recommendations on how to transfer results from source attribution models in to actions for prevention and control.
- Evaluate the transferability of the source attribution approach in terms of opportunity to strengthen the technical and institutional capacity building for the One-Health surveillance.

The focus of this project will be on three of the main bacterial foodborne zoonotic pathogens in the EU for which previous source attribution analyses have generally neglected the environment and non-livestock reservoirs: non-typhoid *Salmonella* spp., *Campylobacter jejuni/coli*, and Shiga toxin-producing *Escherichia coli* (STEC/VTEC), incl. their antimicrobial resistant strains. Because of the transfer of antimicrobial resistant determinants between bacterial species, e.g. between commensals and pathogens, we will also attempt to include attribution approaches for AMR using metagenomics data. When relevant for the models applied, we will attempt to include data from all parts of the food production chain including the retail level. Finally, it should be noted that not all source attribution models are relevant or applicable to all the focus pathogens, and that data availability and accessibility often influence the choice of methods.

Description of work

WP 1. Project coordination and administration

WP start month 25

WP end month 54

WP Leader: Tine Hald (DTU)

Deputy WP Leader: Eelco Franz (RIVM)

WP participants: All partners

Description of the WP: WP1 takes place over the first, second and third year of the project.

This WP will be responsible for all reporting to the One Health EJP coordinating body and will organise 3 face-to-face meetings and quarterly web-based meetings during the 2.5-year project. Due to the relatively large number of consortium partners and the need for interaction between WPs, WP1 will play an important role in ensuring that milestones and final deliverables of the consortium are successfully completed and communicated. The WP leader at DTU and deputy

leader at RIVM are also the overall project coordinator and project deputy leader, which creates integrity throughout the project and ensure a continuous communication between the WPs and the timely dissemination of results. A Project Management Team (PMT) will be formed consisting of all WP and Task Leaders and their deputies. The PMT will hold monthly webmeetings.

To ensure coordination of work between WPs and the different hazard-specific tasks, WP1 will start out by mapping the existing knowledge gaps and recommend new studies and/or methods that are needed to fill them. This will be done in close collaboration with WP2, which will be initiated by mapping the existing data available to the consortium members. This can be data generated directly by the partners or publicly available data. As several of the consortium partners plan to collect new data during the course of the project, the recommendations from this WP may include the collection of specific samples from the environment and non-livestock reservoirs, and non-animal derived food sources, different water sources and soil.

In close collaboration with WP5, WP1 also takes responsibility for communicating with stakeholders at the EU-level (e.g. ECDC, EFSA, EMA) and Member State-level authorities, farmers' organisations and representatives from the food industry, as well as other projects within and outside the One Health EJP. In Month (M) 30 and back-to-back with the final annual project meeting, we will organise a 1-day stakeholder workshop, where newly developed methods, results and recommendations for future actions will be presented and discussed. The stakeholder workshop is included as Milestone 5.2 in WP5.

Task: *JRPFBZ-1-WP1-T1 Project management*

Task start month 25; **Task end month** 54

Task Leader: Tine Hald (DTU); **Deputy Task Leader:** Eelco Franz (RIVM)

Task Participants: SVA, ANSES, ISS

Description of the task: This task will include communication to all partners about project progress, upcoming deadlines, follow up of deliverable completion, and reporting of progress, milestones and deliverables to the One Health EJP coordinating body. The PMT will be formed and will throughout the project hold monthly webmeetings to ensure communication across WPs and ensure that the work flow within and between WP proceed as expected. Staff for administrative support, including financials, will also be included in the PMT and attend meetings as required. The first webmeeting of the PMT will be held in M25 of the project (January 2020).

The first annual project meeting including all project participants will be hosted by DTU in M27 (March 2020). The purpose of this meeting will be to introduce consortium partners to one another and discuss in detail the work to be done in the period lying ahead and how to achieve the defined milestones and deliverables in the work packages most efficiently. The preliminary recommendations from WP1-T2 will also be presented and discussed.

Quarterly webmeetings with all consortium partners will also be organised. The quarterly webmeetings will replace the monthly PMT meetings in the months they take place. The first quarterly webmeeting for all consortium partners is scheduled for M31 of the project (June 2020), as all partners are meeting at the first annual meeting in M3 of the project.

Task: *JRPFBZ-1-WP1-T2 Mapping of existing knowledge gaps and recommendations on how to fill them*

Task start month 25; **Task end month** 28

Task Leader: Annemarie Kassbohrer (BfR); **Deputy Task Leader**: Tine Hald (DTU)

Task Participants: RIVM, ANSES, NVI

Description of the task: WP1 will take a systematic approach to map existing knowledge gaps based on a review of current data availability (as mapped by WP2), existing source attribution approaches, and published source attribution estimates. The review will be done per hazard group (i.e. for Salmonella, Campylobacter, VTEC, and AMR) and identify for which sources and hazards, SA estimates are lacking or very uncertain. This knowledge will form the basis for making recommendations for the other WPs for generating/collecting new data (WP2), developing new or modify existing models (WP3), and applying new or updated hazards' data in existing SA approaches with the overall purpose to generate new or improved SA estimates. Several of the consortium partners have indicated that they plan to generate (e.g. WGS of existing isolates) and/or collect new data during the course of the project. The recommendations from this task are therefore expected to include the collection of new samples from the environment and non-livestock reservoirs (e.g. pets and wildlife), and non-animal derived food sources such as vegetables, different water sources and soil.

Task: *JRPFBZ-1-WP1-T3 Data Management Plan (DMP)*

Task start month 25; **Task end month** 30

Task Leader: Tine Hald (DTU); **Deputy Task Leader**: Marianne Chemaly (ANSES)

Task Participants: All partners

Description of the task: As required by the OHEJP, we will use the first six months to develop a Data Management Plan (DMP). The DMP will explain how we plan to manage, describe, analyse, and store the data collected throughout the project, and how we will ensure that the data will be preserved and made available beyond the duration of the project.

WP 2. Data – Coordination of the collection of genomic data, other microbiological data and epidemiological data.

WP start month 25

WP end month 48

WP Leader: Marianne Chemaly (ANSES)

Deputy WP Leader: Robert Söderlund (SVA)

WP participants: All partners

Description of the WP: WP2 takes place over the first and second year of the project.

The purpose of this WP is to coordinate the identification and collection of relevant data between the projects partners considering the data requirements established by WP1, which identify data gaps, and WP3, which identify data for the specific methods. The work will start by mapping existing data and establishing a joint data-sharing platform. Data will be organised according to pathogen and type of attribution approach, although some data will be useful for more than one approach e.g. WGS data. Besides genomic and phenotypic data and their metadata, and bioinformatics data (e.g. SNP matrices, cg/wg MLST), the data to be collected includes epidemiological data (i.e. from case-control studies, outbreaks, prevalence and microbial count data), and demographic and population data (consumption patterns, production and trade, travels, etc.). We will also collect data from available metagenomics samples to analyse the resistomes from different sources including humans for the purpose of source attribution.

Task: *JRPFBZ-1-WP2-T1* Mapping of existing data and establishing a joint data-sharing platform

Task start month 25; **Task end month** 36

Task Leader: Tine Hald (DTU); **Deputy Task Leader**: Eelco Franz (RIVM)

Task Participants: All partners

Description of the task: A map of the relevant data sources from of existing or upcoming surveillance efforts and research projects in the partner countries that will support this project will be conducted. For example several partners are also involved in the EJP proposal 'Assessing Determinants Of the Non-decreasing Incidence of *Salmonella*, ADONIS' (JRP FBZ 2.4), in which genomic data will be collected from avian *Salmonella* and could be included in this project. Data will largely be organised according to pathogen and type of attribution approach, although some data will be useful for more than one approach e.g. WGS data. In this project, for WGS data we will rely on existing genomic platforms specialised to store such data and make easy accessible links from our platform to these archives through e.g. the EU H2020 project "COMPARE" (http://www.compare-europe.eu/). It would also be relevant to seek collaboration with the consortium that is going to address the integrative topic IA 2.1 on joint databases of reference material and data incl. metadata. Exactly how we end up collecting and sharing data will be described in detail in DMP (see WP1-T3).

Task: *JRPFBZ-1-WP2-T2* Data collection for *Salmonella*

Task start month 25; **Task end month** 48

Task Leader: Magdalena Zając (PIWET); **Deputy Task Leader**: Angela Pista (INSA)

Task Participants: ANSES, VISAVET-UCM, NIPH, APHA, Teagasc, WbvR, VRI, INIAV, PHE

Description of the task: This task, dedicated for *Salmonella*, will enable the collection of genomic data and their metadata (i.e. epidemiological information describing the sequences), bioinformatics data (e.g. SNP matrices, MLST, cg/wg MLST, AMR determinants, etc.), other epidemiological data (incl. data from case-control studies, outbreak investigations, prevalence and microbial count data), and a range of demographic and population data (consumption patterns, production and trade of food, geographical locations, travels, age and gender distribution).

The partners involved in this task will offer access to their datasets recovered from National Reference Laboratories, official monitoring programs and epidemiological investigations. We will determine the minimum required data and metadata. The data include strains from food, different animal sources (cattle, poultry, turkey, pigs, wildlife, pet animals, reptiles), environment, and fertilizers with organic component or sewage. Proposed typing data include PFGE, MLVA and WGS of different serovars. Data from travel and non-travel associated *Salmonella* cases and from joint outbreak investigations (veterinary and public health) are also available. Data from food business operators (FBO) will be included, if available and accessible and the quality is sufficient for source attribution. Sampling from new sources and sequencing of existing isolates will be performed to meet the objectives of the project following the data gaps identified by WP1.

Task: *JRPFBZ-1-WP2-T3* Data collection for *Campylobacter*

Task start month 25; **Task end month** 42

Task Leader: Eva M. Nielsen (SSI); **Deputy Task Leader**: Mónica Oleastro (INSA)

Task Participants: DTU, ANSES, VISAVET-UCM, Teagasc, PIWET, SVA, WbvR, INIAV

Description of the task: This task, dedicated for *Campylobacter*, will enable the collections of the same types of data as for Salmonella (WP2-T2): Genomic, epidemiological, bioinformatics and a range of demographic and population data. The partners involved in this task will provide access to large collections (several thousands) of *Campylobacter* isolates and data collected within NRLs context, monitoring programs and investigations from different animal sources (cattle, poultry, pigs, pets, veal calves, shellfishes, wildlife, water fowl), food (beef carcasses, chicken meat),

environment (sewage water) and humans. Data from FBO will be included when available/accessible and relevant. All the isolates are characterized (e.g. MLST, AMR profiles) and subsets are already sequenced in the context of other research projects (Discerning Environmental Pathways of *Campylobacter* Transmission (DEPi*C*T), Campysource), but more sampling and sequencing will be performed within DiSCoVer to enable greater comparisons between facilities and production cycles. For the collecting new samples during the project, the partners will agree on the types of reservoirs and sources to investigate to help filling in knowledge gaps identified in WP1.

Task: *JRPFBZ-1-WP2-T4* Data collection for Verocytotoxin-producing *E. coli* (VTEC)

Task start month 25; **Task end month** 48

Task Leader: Camilla Sekse (NVI); **Deputy Task Leader**: Rosangela Tozzoli (ISS)

Task Participants: VISAVET, NIPH, APHA, SVA, INSA, PIWET, Teagasc, VRI, INIAV

Description of the task: This task, dedicated for VTEC, will enable the collection of the same types of data as WP2-T2 and WP2-T3: genomic, epidemiological, bioinformatics and a range of demographic and population data. The partners involved in this task will give access to their collections of VTEC strains and data collected within NRLs context, networks coordination, monitoring programs and investigations from humans, food (beef meat and carcasses) and different animal sources (cattle, sheep, other livestock, healthy dogs, outdoor cats, wild reindeer, moose, roe deer, red deer, wild boar and Spanish ibex, cervids, corvid birds) and from environmental sources. Many isolates are well characterized, in terms of serogroup, *stx*-subtyping and presence of additional virulence genes. The data will be shared for the project's purposes, provided together with a set of anonymized metadata of the strains. New sampling (wildlife and companion animals) and WGS will be performed during the project's activities, and will be available for enlarging the WGS dataset of data to be exploited for the source attribution modelling exercise carried out in DISCoVeR. For the collecting new samples during the project, the partners will agree on the types of wildlife and domestic animals to investigate and which serotypes or other VTEC types to look for. This will help filling in knowledge gaps regarding potential sources of VTEC as identified in WP1.

Task: *JRPFBZ-1-WP2-T5* Data collection for AMR

Task start month 25; **Task end month** 48

Task Leader: Julio Alvarez (VISAVET-UCM); **Deputy Task Leader**: Kaye Burgess (Teagasc)

Task Participants: NIPH, ANSES, INSA, PIWET, WbvR, VRI, INIAV, SSI

Description of the task: Data types for AMR will be collected for each pathogen from the previous WP 2 tasks (T2, T3 and T4) with a special focus on AMR determinants.

All the partners involved in this task will give access to isolates collected from humans, food, wildlife, animal sources and the environment through the existing network of collaborations.

Phenotypic and genotypic AMR on *Salmonella*, *Campylobacter* and VTEC will support DiSCoVeR with raw-data to allow for a comprehensive approach for the development of pathogen specific source attribution models. Most of the data proposed for DiSCoVeR has already been generated from various research projects. New data proposals will include genotypic AMR data based on WGS of *Campylobacter*, *Salmonella* and sequencing of ESBL and fluoroquinolone resistant *Enterobacteriaceae* in addition to information already collected in tasks T2-T4.

As metagenomics data potentially can identify all AMR determinants present in a samples, we will attempt to collect such data to develop a more a more comprehensive attribution model for AMR. A challenge here may be to find appropriate, particular, human metagenomic data in EU. We will explore this in this task. However, for model development purposes, we will have access to very appropriate datasets from four African countries, where samples from human and their immediate

exposure environment (including pets, livestock, food, recreational and drinking water), will be collected during the next two years as part of another DTU project. These data will be included in DiSCoVeR as well.

All the collected data on AMR will be centralized and categorized depending on their nature (i.e., phenotypic vs. genotypic, qualitative vs. quantitative) and grouped by antimicrobial family/drug.

WP3. Methods - Critical assessment/improvement of existing and development of new source attribution models.

WP start month 25

WP end month 48

WP Leader: Thomas Rosendal (SVA)

Deputy WP Leader: Lapo Mughini-Gras (RIVM)

WP participants: SSI, ANSES, NIPH, NCOH-UU, DTU, BfR, WbvR, ISS, PHE, INIAV

WP description: WP3 takes place over first and second year of the project.

This WP will focus on the cataloguing, evaluation and development of existing methods for source attribution and will also develop new methods for the critical assessment of source attribution models and finally suggest novel approaches for source attribution. To fully utilise risk factor studies and risk/exposure assessments to subtyping and WGS information, this WP will identify and develop novel approaches of source attribution and will pass on these findings to WP4.

Task: JRPFBZ-1-WP3-T1 Assessing and developing source attribution methods based on microbial subtyping

Task start month 25; **Task end month** 48

Task Leader: Thomas Rosendal (SVA); **Deputy Task Leader**: Lapo Mughini-Gras (RIVM)

Task Participants: DTU, NIPH, ANSES, BfR, NCOH-UU, SSI, WbvR, INIAV, APHA, PHE

The principle of the subtyping approach is to compare the distribution of subtypes in various sources with the distribution in humans and from this estimate the relative contributions of each of the sources to human infections. The approach includes the Hald model, the Dutch model, as well as population genetic models including the asymmetric island model, STRUCTURE, and BEAST discrete trait analysis, which will be evaluated for their scalability and use with WGS data. Nascent, supervised machine-learning methods will also be evaluated for usability for source attribution. We will develop new models that include covariates such as information on the pathogen (phylogeny, plasmid and prophage occurrence and transfer rates, virulence factors, antimicrobial resistance determinants, etc.), the sources (exposure rate, prevalence, geographical distribution, import-export flows, etc.) and the human host (consumption, travel history, etc.).

Task: JRPFBZ-1-WP3-T2 Assessing and developing source attribution methods based on phylogenetic data

Task start month 25; **Task end month** 48

Task Leader: Eva Møller Nielsen (SSI); **Deputy Task Leader:** Umaer Naseer (NIPH)

Task Participants: SVA, DTU, APHA, PHE

This task will integrate the linking between patient isolates and sources using WGS to develop a new approach for source attribution where concrete information on links to specific sources will be taken into account. Here, the linking of isolates from human infections and possible sources - by

direct matching at the high level of resolution offered by WGS as it is done in an outbreak situation - will be used to classify sporadic cases. This will give the possibility of a more transparent output. Furthermore, it will be explored if this approach can be used in real-time surveillance.

Task: JRPFBZ-1-WP3-T3 Evaluation of microbial subtyping source attribution by infectious disease modelling

Task start month 25; **Task end month** 48

Task Leader: Thomas Rosendal (SVA); **Deputy Task Leader:** Laurent Guillier (ANSES)

Task Participants: RIVM, DTU

Methods for source attribution have been difficult to evaluate and often the conclusion of the validity of an estimate is justified based on prior knowledge the behaviour of the pathogen. In this task, an approach will be used to estimate the spread of an infectious agent with multiple subtypes from several sources to humans. Artificial data will be used to evaluate the validity of the estimates generated by the proposed methods in task WP3-T1. Results of this task will be a method for empirical evaluation of a microbial subtyping source attribution approach.

Task: JRPFBZ-1-WP3-T4 Assessing and developing approaches for source attribution of antimicrobial resistance based on metagenomics

Task start month: 31; **Task end month:** 48

Task Leader: Sofia Duarte (DTU); **Deputy Task Leader**: Lapo Mughini-Gras (RIVM)

Task Participants: INIAV

The transition from typing of single isolates to metagenomics data requires an adaptation of the modelling approach. We hypothesize, however, that the principles from the microbial subtyping approach applies also to species-level resistomes, i.e. that there is a strong association between the abundance of particular resistance determinants and specific animal reservoirs. We will explore this by advancing the work started in EFFORT using a supervised classification machine-learning model identifying host-specific clusters of resistance determinants that can subsequently be used to estimate the probability of a human case to originate from each of the included reservoirs.

Task: JRPFBZ-1-WP3-T5 Assessing and developing source attribution approaches based on case-control study results

Task start month: 30; **Task end month:** 36

Task Leader: Axel Bonacic Marinovic (RIVM); **Deputy Task Leader:** Sara Pires (DTU)

Task Participants:

Here we will update meta-analyses of existing case-control studies of sporadic *Campylobacter* and *Salmonella* infections. We will also include the results of two ongoing meta-analyses of case-control studies of sporadic VTEC cases. This will be done by means of Bayesian evidence synthesis analysis. In addition, we will apply methods combining WGS data with structured epidemiological data from case-control studies, leading to optimally informed source attribution.

Task: JRPFBZ-1-WP3-T6 Assessing and developing source attribution approaches based on data from reported outbreak investigations

Task start month: 28; **Task end month:** 36

Task Leader: Sara Pires (DTU); **Deputy Task Leader:** Gaia Scavia (ISS)

Task Participants: RIVM

We will update existing studies led by DTU on *Salmonella* and VTEC based on data reported by EU MS to EFSA. We will explore the possibility of segregating the analysis by pathogen subtype and severity, and will perform sub-regional source attribution analyses, if sufficient data are available.

Task: JRPFBZ-1-WP3-T7 Assessing and developing source attribution approaches based on Risk-assessment

Task start month: 30; **Task end month:** 38

Task Leader: Eric Evers (RIVM); **Deputy Task Leader:** Annemarie Käsbohrer (BfR)

Task Participants:

Comparative exposure assessment and approaches such as linking specific bacterial genetic markers with better survival of the pathogens in different environments leading to exposure of humans and/or with higher virulence, i.e. giving rise to proportional more human cases or more severe symptoms than strains without such markers.

WP4. Results – Quantifying the contribution of various sources of foodborne zoonoses and AMR.

WP start month: 30;

WP end month: 49

WP Leader: Lapo Mughini-Gras (RIVM);

Deputy WP Leader: Sara M. Pires (DTU)

WP participants: PIWET, NCOH-UU, BfR, TEAGASC, NVI, NIPH, ANSES, WbvR, VRI, SVA, INSA, VISAVET-UCM, SSI, ISS, APHA, PHE

WP description: WP4 takes place over first, the second and the third year of the project.

In WP4, data collected in WP2 and the methods assessed/developed in WP3 will be used to quantify the contributions of the main sources of the three focus pathogens and AMR. Results will be presented per pathogen, attribution method, data type and, when/if applicable, geographical region/country. While overall ('European or multi-country') analyses will be performed, an important goal will be to identify country differences for pathogen-method combinations, which may reflect differences in their epidemiology. Particular attention will be given to environmental and non-livestock (pets and wildlife) sources besides the 'traditional' livestock/food sources. Different methods will be used in parallel to identify the best source attribution method for the type/amount of data available and the pathogen in question. The results of applied methods for each pathogen will be compared in 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.

Task: JRPFBZ-1-WP4-T1 Salmonella source attribution and comparison of results from different approaches

Task start month: 30; **Task end month:** 40

Task Leader: Sara Pires (DTU); **Deputy Task Leader:** Dariusz Wasyl (PIWET)

Task Participants: RIVM, ANSES, NIPH, VRI, INSA, VISAVET-UCM, APHA, PHE

As *Salmonella* has long been the focus of most research/surveillance activities in the EU, we expect it to have the largest amount of typing, epidemiological and exposure data available for source attribution. Source attribution based on microbial subtyping (MS) will be done using both phenotyping (serotyping, phage typing, AMR) and genomic (WGS, MLVA, MLST) data, using both well-established (frequency-matching and population genetics) and novel (machine-learning,

BEAST, etc.) approaches. Based on previous experience in *Salmonella* source attribution, we expect pets like reptiles and the environment to be of particular interest. Moreover, as salmonellosis often occurs as part of outbreaks, source attribution based on the analysis of outbreak data is expected to be particularly informative. The wealth of case-control studies for *Salmonella* will enable source attribution based on a meta-analysis of case-control studies, as well as combined case-control and microbial subtyping-based source attribution analyses. Comparative exposure assessment will be useful for estimating the likely contributions of sources like wildlife or the environment, for which there are fewer typing and epidemiological data.

Task: JRPFBZ-1-WP4-T2 Campylobacter source attribution and comparison of results from different approaches

Task start month: 30; **Task end month:** 42

Task Leader: Lapo Mughini Gras (RIVM); **Deputy Task Leader:** Katel Rivoal (ANSES)

Task Participants: NCOH-UU, DTU, SVA, INSA, VISAVET-UCM, SSI, PHE

Campylobacter has received considerable attention for source attribution using genomic data (particularly MLST) given the recognized limitations of phenotyping methods for source attribution of this pathogen. *Campylobacter* source attribution based on MS is expected to be possible using either allele-based schemes like the traditional 7-locus MLST scheme, cgMLST, wgMLST or SNP address, using both existing and novel approaches. There is strong indication that the environment and wild birds play an important role for *Campylobacter*, and data from these sources are available to perform robust analyses at different geographical scales. While the paucity of documented campylobacteriosis outbreaks is expected to limit the applicability of analyses of outbreak data, the many case-control studies of sporadic campylobacteriosis and comparative exposure assessments will offer opportunities to perform source attribution at the point of exposure. The phylogenetic approach to be developed in WP3-T2 is also considered to provide new insights for Campylobacter source, at least in some countries. Alike *Salmonella*, combined source attribution and case-control analyses will be performed.

Task: JRPFBZ-1-WP4-T3 VTEC source attribution and comparison of results from different approaches

Task start month: 30; **Task end month:** 49

Task Leader: Eelco Franz (RIVM); **Deputy Task Leader:** Gaia Scavia (ISS)

Task Participants: TEAGASC, DTU, ANSES, NVI, NIPH,VRI, SVA, INSA, VISAVET-UCM

VTEC is the pathogen for which the least amount of data will be readily available to perform source attribution, so collection of new data (e.g. sequencing of existing isolates) may be needed. Like for *Salmonella*, source attribution of VTEC using microbial subtyping is expected to be possible using either phenotyping (serotyping) or genomic data, with existing and novel approaches. A regional analysis of data on VTEC outbreak investigations has been recently conducted in a study led ny DTU, and we will build on this study to complement with newly available data. A systematic review of case-control studies has also recently been conducted, and we will retrieve results to compare with the output of our project. Data for comparative exposure assessments will be collected from harmonized EFSA databases and complemented with other available studies. For VTEC, the environment and wildlife reservoirs are expected to play a crucial role, so special attention will be given to the quantification of these sources. Alike *Salmonella* and *Campylobacter*, combined source attribution and case-control analyses will also be performed.

Task: JRPFBZ-1-WP4-T4 AMR source attribution results presented regionally and by region/country, for each applied method and integrated

Task start month: 30; **Task end month:** 49

Task Leader: Sofia Duarte (DTU); **Deputy Task Leader:** Thomas Hagenaars (WbvR)

Task Participants: VRI, INSA, VISAVET-UCM, RIVM

For AMR, the microbial subtyping approach will be particularly useful. Using either the phenotyping or genomic data for the three focus pathogens, we will identify AMR determinants to be used to define "types" for source attribution. In the EFFORT project, the first steps were made to develop an approach for source attribution of AMR using metagenomic data. We will advance this model using a supervised classification machine-learning model identifying host-specific clusters of resistance determinants that can subsequently be used to estimate the probability of a human case to originate from each of the included reservoirs. As humans are also a reservoir for AMR genes, we will include samples from human in the 'source' samples.

WP5 - Conclusions, recommendations and policy translation.

WP start month: 33

WP end month: 54

WP Leader: Gaia Scavia (ISS)

Deputy WP Leader: Tine Hald (DTU)

WP participants: all partner institutions

Description of the WP: WP5 takes place over first, the second and the third year of the project.

WP5 aims at providing a critical evaluation of the evidence and uncertainty obtained by the source attribution models to improve the characterisation of the sources and transmission pathways implicated in the epidemiological cycles of the hazards in focus (*Salmonella*, *Campylobacter*, VTEC and AMR). In addition, the transferability of the source attribution approach will be evaluated in terms of opportunity to strengthen the technical and institutional capacity building for the One-Health surveillance. Technical evaluations will allow to describe strength, limitation and fit for purposes of methodologies for source attribution modelling being applied, in light of the current availability and gaps of data in the various sectors (clinical, food, animal, environment), and considering the limitations and barriers connected to the current operational field context (Task 5.1). Expert evaluation will allow to elucidate how project outcomes could be interpreted and used to improve hazard control policies at both EU and national level and to identify which resources and components of the surveillance programmes would be needed to optimise the applicability of source attribution approach in the field (Task 5.2). On this basis, conclusions and recommendations will be formulated with the support of the relevant stakeholders (in particular EFSA and ECDC) and taking into account the control policies in place at the EU and national level, in the various sectors. In particular, the existing hazard specific control programmes such as the National Control Programme (NCP) for *Salmonella,* at the primary production level and other intervention strategies will be focused. Final recommendations will also be delivered in close cooperation with the WP2 of the EJP focusing 'Science to policy translation' to ensure an optimal level of harmonization within the EJP (Task 5.3).

This WP includes all the participant institutes. Activities will be organised into three separate, interdependent tasks. These will be carried out in close cooperation with the WP2, WP3 and WP4 leaders for the purposes of integration of contributions and comprehensive interpretation of the methodologies and output. Since the critical evaluation of the outcomes will require specific a robust knowledge on the epidemiology and surveillance context for each specific hazards, single hazard specific experts will be identified among participants and involved in the WP activities (expert group). Alignment with other projects within and outside the EJP (NOVA, Cohesive, Orion) will also be established in order to guarantee full internal harmonization and avoid duplication of the activities.

Task: JRPFBZ-1-WP5-T2 Translating source attribution estimates into options for control policies

Task start month: 33; **Task end month:** 50

Task Leader: Gaia Scavia (ISS); **Deputy Task Leader**: Sara Pires (DTU)

Task Participants: SVA, SSI, INSA, NVI, VRI

Description of the task: Under this task, the expert evaluation of the relevance of the results and the associated uncertainty in terms of public health will be carried out, in order to identify possible options for hazard control. In the first step, the evidence obtained by the current project and the elements of novelty granted by this novel comprehensive One Health approach will be used to further characterise the role of the various components of the epidemiological cycle of each single hazard, in the complexity of transmission pathways between animals, food, environment, and humans. Possible options for innovative hazard control intervention in the complex of the transmission pathways will then be evaluated/revised, taking into account the current policies in place at EU and national level. For this purpose, an inventory of the current existing hazards' control programme and strategies in the various sectors and tracts of the animal-environment-food-human chain will be mapped, in cooperation with other relevant EJP projects.

Deliverables

Ref	Title	Due month
D-JRPFBZ-1-WP1.1	Mapping of knowledge gaps and recommendations for new data generation and method development	28
D-JRPFBZ-1-WP1.2	Data Management Plan	30
D-JRPFBZ-1 WP2.5	Database/sharing platform solution established	36

Milestones

Ref	Title	Due month
M-JRPFBZ-1-01	Identification of Project Management Team	25
M-JRPFBZ-1-02	First annual project meeting	27
M-JRPFBZ-1-03	Completion of mapping of knowledge gaps and recommendations for new data generation and method development	28
M-JRPFBZ-1-04	Identification of types of samples to investigate and for which species to include in the sampling	29
M-JRPFBZ-1-05	Framework for evaluation of Microbial subtyping methods	33
M-JRPFBZ-1-06	Methods for source attribution based on outbreak data evaluated	33
M-JRPFBZ-1-07	Format for results presentation (standard structure) for all pathogens and AMR	33
M-JRPFBZ-1-08	Mapping of data available for Salmonella	34
M-JRPFBZ-1-09	Mapping of data available for Campylobacter	34
M-JRPFBZ-1-10	Mapping of data available for VTEC	34
M-JRPFBZ-1-11	Mapping of data available for AMR	34
M-JRPFBZ-1-12	Protocol for presentation of results for Salmonella, Campylobacter, VTEC and AMR	34
M-JRPFBZ-1-13	Framework for using phylogenetic data for source attribution	36
M-JRPFBZ-1-14	Comparison of data and methods for each pathogen and AMR	36

2.4.2.1.27 JRP21-R2-FBZ3.1-BIOPIGEE

Reference to the Stategic Research Agenda (please refer to D2.7)			**FBZ 3.1: Benchmarking biosecurity practices for pig farming across Europe using national surveillance data and management standards for identifying best practice to prevent biological hazards, particularly Salmonella and hepatitis E virus, from entering the food supply chain**							
Research Project Title			**Biosecurity practices for pig farming across Europe**							
Research Project Acronym			**BIOPIGEE**							
Leading Organisation			**P9-BfR**			Deputy Leading Organisation			**N/A**	
Project Leader			**Chris Kollas**			Deputy Project leader			**N/A**	
Project Start month			**M25**			Project End month			**M54**	
Participant	**1**	**2**	4	**6**	7	**8**	**9**	10	**11**	12
	Anses	**Ages**	Sciensano	**NDRVMI**	SZU	**VRI**	**BfR**	FLI	**RKI**	DTU
PM	**15**	**11**		**27**		**11**	**24**		**13**	
Participant	13	14	**15**	16	17	18	19	20	**21**	22
	SSI	UT	**VFL**	INIA	UCM	MVNA	INRA	IP	**APHA**	PHE
PM			**7.9**						**9.5**	
Participant	23	24	25	26	27	28	29	**30**	**31**	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	**RIVM**	**WbvR**	FHI
PM					6	9		**0,6**	**7,5**	
Participant	**33**	**34**	35	36	37	38	39	40	**41**	
	NVI	**PIWET**	INIAV	INSA	IISPV	IC	SLV	FOHM	**SVA**	
PM	**6,5**	**12,25**							**8,5**	

Objectives:

WP1

- Coordination of the project BIOPIGEE

WP2

- Review current biosecurity protocols and briefly literature on biosecurity effectiveness to help select, amend or design protocol(s) to be used in this project.
- Collect biosecurity protocol data from farms within participant countries, categorised as either high or low risk for Salmonella and/or Hepatitis E.
- Begin biosecurity field studies to fill key data gaps, mainly related to Hepatitis E.

WP3

- Main objective: to obtain better knowledge on how to combat Salmonella and HEV in biofilms/surface microlayers by disinfection in pig farms, so that this knowledge can be used for further development of a common biosecurity protocol
- Sub-objective 1: to establish a standardized method for testing the effect of disinfectants on Salmonella in biofilm.
- Sub-objective 2: To obtain knowledge on the effect of relevant disinfectants on Salmonella in biofilm by using the standardized method and a panel of relevant Salmonella strains from herds and abattoirs
- Sub-objective 3: To use the evaluated models to test disinfectants on HEV in biofilms/surface microlayers
- Sub-objective 4: To develop a suitable HEV infectivity assay to test different kind of samples that can be contaminated with HEV.
- Sub-objective 5: To implement, optimize and adapt a HEV infectivity assay for pig and environmental samples.
- Sub-objective 6: To assess HEV stability in relation to disinfection approaches in pig farms.

WP4

- Modelling of the cost and effectiveness of biosecurity measures

WP5

- Development of a catalogue of biosecurity measures relevant to reduce/limit occurrence of Salmonella and Hepatitis E virus (HEV) at different pig production stages
- Integration of gained knowledge on effectiveness and costs of biosecurity measures studied in WP2-4 into the catalogue
- Continued systematic review/meta-analysis to further fill the catalogue with relevant effective measures
- Identification and recruition of relevant experts to build a panel for assessing biosecurity practice in pig production

WP6

- To produce dissemination material on best biosecurity practices to reduce the presence of Salmonella and Hepatitis E virus in pigs, at farm level and along the food chain.
- To develop a support tool for calculating cost effectiveness of biosecurity related interventions.
- To organise a workshop series to enable exchange between researchers and stakeholders on biosecurity in pig production

Description of work:

WP1

WP: 1 Project coordination and integration of results
WP start month: 25
WP end month: 54
WP Leader: BfR (Chris Kollas)
Deputy WP:: BfR (Elke Burow)
WP participants: All members of BIOPIGEE
Description of the WP: The first work package leads and coordinates the project. Exchange between work packages and integration of results, as well as active linking to external partners and projects (EU activities) will be organised. Three major project meetings will be organised: First, a kick-off meeting will be held with all participants in month M26. Next, a mid-term meeting is planned for all WP-leaders and finally, the final project meeting or conference will be conducted in M53. All parts for the project reports, deliverables and milestones will be collected from WP-leaders and delivered to OHEJP The contact to EJP coordinators will be maintained. A data

management plan will be evaluated and revised throughout the project lifetime. A database structure to store data among all WP will be provided.

Task: JRP#-WP1-T1 Project management and meeting organisation
Task start month: 25
Task end month: 54
Task Leader: BfR (Chris Kollas)
Deputy Task Leader: BfR (Elke Burow)
Task Participants:, all BIOPIGEE members
Description of the task: The project organisation will keep the information flow between the WPs. Problems of participants will be solved, a place for common data exchange will be established and a smooth working atmosphere will be supported. For this, emails, web-conferences and eventually newsletters will be used. During the whole project period, the coordinators will take part in at least two European conferences that deal with the proposed project's topic, in order to inform the scientific and non-scientific audienceabout the BIOPIGEE project and its results.
Furthermore, a smooth communication will be guaranteed between the WP3 of OHEJP and the BIOPIGEE consortium. Participant's inquiries will be collected, answered (if possible) and forwarded to the board (if necessary). The main contact person of the proposed project will be Chris Kollas.
All kind of exchange between WPs will be supported. The connections between WPs, as shown in the Pert chart, need to be kept vital. Not only data, but also ideas and criticism will be exchanged with the help of the project coordination. For this, inter alia surveys will be circulated periodically, asking for progress, cooperation and exchange.
The coordination team will actively search for further external partners and projects and funding that fit to the topic of BIOPIGEE.
A kick-off meeting will be organised and held with all participants of BIOPIGEE in month M26. A mid-term meeting is planned (together with APHA) to join all WP-leader and the final project meeting / conference will be conducted (together with IZSAM) for all BIOPIGEE participants and eventually also guests in M53 or M54.
The task will mainly be carried out by C. Kollas and E. Burow.

Task: JRP#-WP1-T2 Development of data management plan
Task start month: 25
Task end month: 30
Task Leader: BfR (Chris Kollas)
Deputy Task Leader: BfR (Chris Kollas)
Task Participants: all BIOPIGEE members
Description of the task: Following H2020 rules a comprehensive data management plan will be developed for the BIOPIGEE project. The plan will include the following points: data summary, making data findable, making data openly accessible, making data interoperable, increase data re-use, allocation of resources, data security and ethical aspects. This will be achieved by close cooperation with the EJP-OneHealth WP4-group, which proposed support in developing the data management plan. Of course, all BIOPIGEE members will work on developing the plan and asked for agreement of the final plan.
A database structure for the catalogue of biosecurity measures (WP5) will be implemented by WP1. This data base structure will be accessible by all WPs to insert and extract gained knowledge on effectiveness, confidence and costs of biosecurity measures and will thereby be collectively developed and shared between WPs.

Task: JRP#-WP1-T3 Provision of project deliverables and reports
Task start month: 35
Task end month: 36

Task Leader: BfR (Chris Kollas)
Deputy Task Leader: BfR (Elke Burow)
Task Participants: BfR, AGES, ANSES, APHA, BFR, ISS, IZSAM, NDRVI, NVI, PIWET, RKI, SVA, VFL, WbvR
Description of the task: Project deliverables of all WPs will be collected, checked and transferred to the board of EJP-OneHealth. Further, yearly project reports will be compiled with the help of WP-leaders and also delivered to the board.

WP2
WP: JRP#-WP2 Biosecurity effectiveness studies
WP start month 25
WP end month 50
WP Leader APHA (Richard Smith)
Deputy WP Leader BfR
WP participants APHA, SVA, WbvR (UU), BfR, VFL (EMU), PIWET, VRI, IZSAM, ISS, AGES (VMU), NDRVMI, RIVM
Description of the WP: A review of biosecurity audit protocols supplemented by a brief, focused review of relevant literature on the effectiveness of biosecurity practices will define the most suitable protocol(s) to be used on a selection of pig farms in each participating country, covering a representation of European climates and production types. The farms will be identified as high or low risk, focusing on Salmonella and/ or Hepatitis E (HEV), to allow for case-control analysis in order to quantify the benefits of each practice. A structured approach will be used to standardise farm selection to reduce known biases and to provide criteria for ranking high/ low risk. Information collected on slaughterhouse biosecurity best practice will also define the impact on carcase contamination. The results will benchmark standards in each country and define overall best practice. Field studies will be completed to provide evidence for key knowledge gaps on the effectiveness of controls, particularly focused on the effect of biosecurity on HEV. The work on HEV will be supported by the HEVnet project providing a facility to upload sequences and metadata to allow investigation of HEV subtype diversity. This integrative approach, drawing evidence from a wide range of sources, will inform quantification of the benefit of the biosecurity practices against Salmonella and HEV at farm and at slaughter, which will be vital to the assessments of the cost and effectiveness of biosecurity analyses (WP4) and benchmarking biosecurity practice (WP5).

Task: JRP#-WP2-T1 Development of biosecurity protocol
Task start month 25
Task end month 28
Task Leader: BfR
Deputy Task Leader: SVA
Task Participants: PIWET, SVA, APHA, BfR, AGES, NDRVMI, WbvR, IZSAM
Description of the task: Expert knowledge within the consortium of existing biosecurity protocols (e.g. Biocheck, Combat, PADRAP) will be used to indicate which are most relevant to pig production and Salmonella and HEV. The evaluation will specify how the protocols have been used previously and how appropriate they would be for defining European pig farm biosecurity standards in the context of this project. This work will attempt to draw on knowledge gathered from previous studies, such as MINAPIG, which applied the Biocheck system to 232 farrow-to-finish farms in four EU countries. This task will also include a rapid, focused update of previously completed reviews of published literature on the biological effectiveness of biosecurity practices for Salmonella and HEV on European pig farms (such as Andres and Davies, 2015; https://onlinelibrary.wiley.com/doi/full/10.1111/1541-4337.12137). The findings from the search of literature will be supplied to WP5 (incorporated in the database structure of the catalogue of biosecurity meausres) where it will be further built upon and developed into a full review/meta-analysis.

Depending on the findings from these evaluations, a biosecurity protocol will be adapted, or a new one developed, for use in this project to assess the application of biosecurity measures on pig farms. This may be a single protocol covering general biosecurity questions relevant to both pathogens, with additional sections for questions specific to each pathogen, or if there is not enough overlap in relevance, then separate protocols may be designed for Salmonella and HEV.
The protocol will be expected to cover biosecurity areas such as: pig mixing and movement on farm, cleaning and disinfection, knowledge of pathogen status of incoming pigs, number of sources of pigs used, the source and structure of feed, feed ingredients and additives (e.g. organic acid). The protocol will also collect economic information on farm performance from all recruited farms, and the cost of specific biosecurity practices from a subset of farms in each country (e.g. costs of rodent control), as requested by WP4. The economic data will be supplied to WP4 to be fed into the cost-effectiveness models.
The protocol(s) will be developed in English and then translated, as necessary, by project partners for each participating country. If and where necessary, professional support for translation will be used. The protocol's evaluation system will be applicable for both Salmonella and Hepatitis E.

Task: JRP#-WP2-T2 Application of the biosecurity protocol
Task start month 27
Task end month 42
Task Leader: PIWET
Deputy Task Leader: AGES
Task Participants: BfR, APHA, WbvR, SVA, VFL, PIWET, IZSAM, AGES, ISS, NDRVMI, VRI
Description of the task: The biosecurity protocol(s) developed in task 2.1 will be utilized in a diverse group of European partner countries (UK, DE, IT, NL, PL, SE, AT, EE, BG, CZ) to provide data from a representative cross-section of pig farms (breeding, weaning/finishing, farrow-to-finish) across Europe.
The protocol will be applied on between 30-90 pig farms in each of the participating European countries (see Table 1, sampling scheme below). The surveyed farms will be selected due to their production type, and prior knowledge of their expected Salmonella or HEV status, to hopefully ensure a good representation of both high and low risk farms. Specific farm or slaughterhouse sampling completed for the project, or where possible, relevant biosecurity data collected from previous studies or existing information from surveillance will be incorporated where the data can be accessed for the purpose of this project. The information on Salmonella and HEV status will be used to categorise the farms as high or low risk for these pathogens. Criteria for high and low risk will be produced that will be flexible enough to be applied to farms sampled through either harmonized EU surveillance, country specific surveillance schemes or sample results collected specifically for this or previous project(s).
During the farm visits, documentation of best biosecurity practice (such as annotated photographs) will be gathered and forwarded to WP6 (T6.1).

Table 1: Sampling scheme, T2

Country	Salmonella # of farms	Farm type: Breeding	Farm type: Wean/fat	Farm type: Farrow-to-finish	Type of sample	Type of analysis	BIOPIGEE sampling/ existing data
UK	30	10	10	10	faeces	culture	BIOPIGEE
DE	60	20	20	20	faeces	culture	Existing
NL	18	8	10	0	faeces	culture	Exist./BIOP.
SE	30	10	10	10	faec./lymph n.	culture/ELISA	Existing
EE	30	10	10	10	faeces	culture	Existing
PL	30	15	15	0	faeces	culture	BIOPIGEE
IT	30	10	10	10	faeces	culture	BIOPIGEE
AT	30	15	0	15	faeces	culture	BIOPIGEE
BG	32	0	0	32	faeces	culture	BIOPIGEE
ES	0	-	-	-	-	-	-
FR	90	30	30	30	faeces	culture	Existing
CZ	45	0	0	45	caecal	culture	BIOPIGEE

Country	HEV # of farms	Farm type: Breeding	Farm type: Wean/fat	Farm type: Farrow-to-finish	Type of sample	Type of analysis	BIOPIGEE sampling/ existing data
UK	30	10	10	10	faeces	PCR	BIOPIGEE
DE	30	10	10	10	faeces	PCR	BIOPIGEE
NL	18	8	10	0	faec./blood	PCR/ELISA	Exist./ BIOP.
SE	0	-	-	-	-	-	-
EE	30	10	10	10	faec./blood	PCR/ELISA	Exist./ BIOP.
PL	30	15	15	0	faeces	PCR	BIOPIGEE
IT	42	14	14	14	faeces	PCR	BIOPIGEE
AT	30	15	0	15	faeces	PCR	BIOPIGEE
BG	32	0	0	32	faec./liver	PCR	BIOPIGEE
ES	0	-	-	-	-	-	-
FR	90	30	30	30	faeces	PCR	Existing
CZ	45	0	0	45	caecal	PCR	BIOPIGEE

Task: JRP#-WP2-T4 Field studies
Task start month 25
Task end month 48
Task Leader: APHA
Deputy Task Leader: IZSAM
Task Participants: APHA, IZSAM, WbvR, ISS
Description of the task: A limited number of identified data gaps on biosecurity effectiveness to the two pathogens of interest will be filled using small, targeted field trials. The trials will produce evidence of the effect of improvements to biosecurity to inform WP4 and 5. These will include 1) assessment of the Hepatitis E risk of environmental and wildlife reservoirs; 2) studies of the effectiveness of management controls for Hepatitis E; and 3) studies of the effectiveness of cleaning and disinfection on Salmonella and Hepatitis E positive farms.

1) A longitudinal study will be completed to investigate infection dynamics of HEV on farms. For this study eight farms will be selected: four high-seroprevalence and four low-seroprevalence farms. Twenty sows and 10 pigs per litter from two cohorts per farm will be sampled (blood and faeces) on at least five moments. Two groups of four farms are expected to provide sufficient

power to identify differences, because each farm includes a repetition that adds power in the hierarchical modelling. Quantification of the transmission dynamics in combination with simulation modelling will identify relevant transmission and intervention points in time and place during the pig production cycle. A pilot intervention study on three high-risk farms will be performed to test the potentially effective interventions identified, like specific hygiene interventions to reduce within farm transmission or reduce the risk of introduction to the farm.

2) In addition, five pig farms, representing different management strategies for mixing pigs at the weaning, growing and finishing stages, will be followed over time. This study will compare these differences on the spread of HEV on farm and the prevalence of HEV in the finishers shortly before slaughter. A single batch of pigs will be followed on each farm, with sampling visits expected to occur directly after weaning, at the growing stage and then within the week before the finished pigs are sent to slaughter. The results will be analysed against comparable data already collected from two similar farm trials. The study will also collect samples from potential environmental sources of HEV (e.g. farm waste, wildlife) to provide information on potential risks on farm, which will help infer which biosecurity practices could be effective in limiting HEV spread on farm.
3) For the evaluation of the effectiveness of cleaning and disinfection procedures in the farms against *Salmonella* and Hepatitis E contaminations, a minimum of five pig farms for each different management system will be chosen on the basis of the existence of regular cleaning programmes. The farms will be visited in order to determine their cleaning schedules. Samples will be taken before and soon after the cleaning and disinfection actions regularly adopted by the farms. In particular, samples will be collected from the most relevant places such as floors (surface samples with swabs/bootsocks), feeders (surface samples with swabs) and drinkers (water samples). Microbiological analysis will include counts of Enterobacteriaceae, *Salmonella* isolation and HEV RNA detection. The differences in the microbiological results before and after cleaning and disinfection procedures will be statistically evaluated in order to assess the efficacy of those cleaning programmes in place.
4) In this reporting year, the study documents and protocols will be designed and field visits started.

WP3

WP3: Impact of disinfection on persistence of pathogens in biofilm
WP start month: 25
WP end month: 54
WP Leader: Wim van der Poel (WbvR)
Deputy WP Leader: Live L. Nesse (NVI)
WP participants: WbvR, NVI, RKI, APHA

Description of the WP: The aim of the present WP is to obtain better knowledge on how to avoid and combat the build-up of environmental reservoirs of Salmonella and HEV in pig farms by disinfection. This knowledge will be the basis for rating effectiveness of cleaning and disinfection included in the catalogue of biosecurity measures and the subsequent benchmarking (WP5).

This will also contribute information to WP4 as the cost of disinfections to remove biofilms will impact overall biosecurity cost and effectiveness, and also to WP6 to raise awareness of the risks of biofilms and the difficulty in removing them.

Salmonella may be introduced into herds by contaminated feed or breeding animals. However, once introduced, it is well known that Salmonella can persist in farm environments from several months to years. Cleaning and disinfection are therefore important biosecurity measures. Salmonella are in general good biofilm-formers, and when residing in biofilms in the environment they are extremely well protected against environmental stress, including various disinfectants. Consequently, it is imperative to use disinfectants able to eradicate Salmonella in biofilm. Today, however, disinfectants are chosen based on their ability to kill planktonic bacteria in standard

laboratory tests, and these tests cannot be used to predict the effect on the bacteria in biofilm. Standard Methods for testing the effectivity of disinfectants against salmonella do not include biofilm-associated forms of the pathogens, thus making it difficult to give advice on which disinfectants to use. This problem will be addressed in WP3.

The aim of the Salmonella part of WP3 is to establish a standardized test for assessing the effect of disinfectants on Salmonella in biofilm, and apply this test to evaluate disinfectants for use in swine production. The work will be organized into three subtasks.

HEV may be introduced in pig herd via different routes. The most important introduction routes in pig herds are not really known. Contaminated feed, breeding animals as well as workers coming to farms may all play a role. Once introduced, HEV may persist in herds and farm environments for years. Good biosecurity protocols for HEV have not been developed yet. Usefulness of cleaning and disinfection are not really known because research is hampered by the fact that there is not a method available to test for HEV infectivity. It is possible that HEV persists better in environments with good biofilm-forming bacteria. Consequently, it may be indicated to use disinfectants able to inactivate biofilm forming bacteria as well as Hepatitis E virus itself. This approach will be tested in WP3.

The overall aim for HEV in WP3 will be to establish a standardized assay for assessing the effect of disinfectants in environmental samples in pig farms, and apply this assay to evaluate disinfectants for use in swine production, particularly in the case that biofilm forming bacteria are present. The work will be organized into three subtasks.

Task JRP#-WP3-T1: Comparison of methods for testing the effect of disinfectants
Task start month: 25
Task end month: 40
Task Leader: RKI
Deputy Task Leader: NVI
Task Participants: RKI, NVI, APHA
Description of the task: The objective of this task is to select a method for testing the effect of disinfectants on Salmonella in biofilm. Known methods with a potential for testing the effect of disinfectants on Salmonella in biofilms will be validated and standardized, thanks to collaboration of institutes working for both human (RKI) and animal health (NVI, APHA) with high level expertise within this area. Representative strains of Salmonella in biofilm-associated form will be assessed in terms of efficacy of disinfectants. The results for the Salmonella strains will be evaluated by comparing them with the results for the *E. coli* reference test organism as listed in the European Standard Norm DIN EN 14885. Results obtained from different laboratories will be compared with each other and one biofilm model will be selected.

Task JRP#-WP3-T2: Effect of disinfectants on biofilm-associated wild type Salmonella
Task start month:25
Task end month: 54
Task Leader: NVI
Deputy Task Leader : APHA
Task Participants: RKI, NVI, APHA
Description of the task: The objective of this task is to test the effect of relevant disinfectants against biofilm residing wild type Salmonella collected from farm and abattoirs. The validated method established in T3.1 and the strain panel selected in T3.2 will be used to test the effect of disinfectants commonly used in swine production. Several disinfectant types will be included, e.g. quaternary ammonium compounds (QAC), glutaraldehyde and oxidizing agents. The recommended

user concentrations and exposure times for each disinfectant will be used in the testing. Depending on the results, other concentrations and exposure times may also be tested, and separate testing of different active substances may also be done. Through this testing, we will identify the disinfectants most effective against Salmonella in biofilm. The results on the effectiveness of disinfectants will be added to the catalogue of biosecurity measures (WP5).
Sub-Task JRP#-WP3-T2-ST1: Selection of wild type salmonella isolates
Sub-Task start month: 25
Sub-Task end month: 36
Sub-Task Leader: APHA
Deputy Sub-Task Leader: NVI
Sub-Task Participants: APHA, NVI, RKI
Description of the sub-task: The objective of this sub-task is to collect and select wild type Salmonella isolates to be used in the testing of the disinfectants. Salmonella samples collected i WP2 will be screened for their biofilm formation capacities using a micro-titer plate method. The results will be combined with the information collected in WP2 to select a panel of relevant Salmonella strains to be used in the testing of disinfectants.
To assess the efficacy of disinfectants for HEV in environmental samples containing biofilm forming bacteria this will be studied in environmental matter spiked with the selected biofilm forming Salmonella stains using the developed infectivity methods. The disinfectants which are most effective against salmonella in bioflm will also be tested for their effect on HEV inactivation (T3.1-3.2).

Task JRP#-WP3-T3: Study of HEV stability in relation to disinfection approaches
Task start month:25
Task end month: 54
Task Leader: WbvR
Deputy Task Leader: WbvR
Task Participants: WbvR
Description of the task: The objective of this subtask will be to develop and optimize a culture system for Hepatitis E virus using primary pig liver cells and ultrathin pig liver slices. Primary pig liver cells will be harvested by flushing fresh pig livers obtained from spf piglets. Ultrathin pig liver slices of one or two cell thick will be obtained from fresh pig livers (also from spf-piglets) using a dedicated microtome procedure. Primary cells will be kept in monolayer cultures and ultrathin pig liver slices will also be kept in cell culture media to keep the cells alive as long as possible. Both methods will be assessed for suitability to test for HEV infectivity in different kind of HEV-positive pig and environmental samples The most suitable method will be implemented to assess the effect of HEV biosecurity practices, e.g. disinfection, on surface microlayers/biofilms contaminated with infectious HEV.

Sub-Task JRP#-WP3-T3-ST1
Sub-Task start month: M25
Sub-Task end month: M36
Sub-Task Leader: WbvR
Deputy Sub-Task Leader: WbvR
Sub-Task Participants: WbvR
Description of the sub-task:
The objective of this subtask will be to further adapt and implement the developed HEV infectivity assay for testing of microlayer surfaces /biofilms for assessing the inactivation of infectious HEV. As soon as the most suitable infectivity assay has been selected, this assay will be used to test different kinds of HEV positive pig samples and pig farm environmental samples. For the different kinds of

samples, preparation and processing methods will be optimized to improve the sensitivity and the selected assay. As needed the method will be adapted for routine use and standardized us much as possible.

WP4
Modelling of the cost and effectiveness of biosecurity measures
WP start month: 25
WP end month: 50
WP Leader: Beate Pinior
Deputy WP Leader: Simon Robin
WP participants: AGES (VMU), APHA, ANSES, SVA
Description of the WP 4: This work package will take place over years 3, 4, and 5 of the EJP. The overall aim of the work package is to assess the effectiveness and efficiency of the implementation of standard and specific intervention measures on prevalence reduction of Salmonella and Hepatitis E along the pig supply chain by consideration of e.g. differences in the effectiveness of intervention measures, pig production systems across Europe (e.g. herd size), temporal aspects (i.e. dynamic pig cycle, disease prevalence dynamics), spatial aspects (i.e. individual MS and EU), supply structure, fluctuations of supply, demand and prices on the market (e.g. meat and feed price, which are necessary for the economic welfare analysis). The partners of the present work package will maintain close contact to partners of WP 1, 2, 3 and 5 for legal implications of data sharing and the BIOPIGEE database structure (catalogue of biosecurity measures, WP5) will be used for insertion and extraction of measure's effectiveness and costs. The goals of work package 4 will be delivered via 4 tasks.

Task 4.1: Development of questionnaire on biosecurity costs
Task start month: 25
Task end month: 26
Task Leader: Beate Pinior
Deputy Task Leader:
Task Participants: AGES, ANSES
Description of the task 4.1: Task 4.1 takes place in year 3 of OHEJP. In this task we will develop a detailed questionnaire with respect to health and performance data of pigs located in farms (see WP 2) and the costs of biosecurity measures. The questionnaire will be delivered to the partners of WP2.1, who prepare a protocol on biosecurity practice and who will collect these economic data during their empirical survey at the farm level (WP2.2). Missing or further economic data on the subsequent production stages and human sector, such as data on the cost of illness will be collected from the literature in WP 4.1. WP 4.1 will feed WP 4.4 and WP 2.1.

Task 4.2: Stochastic simulations on the effectiveness of biosecurity measures
Task start month: 33
Task end month: 49
Task Leader: Simon Robin
Deputy Task Leader:
Task Participants: APHA, ANSES, SVA
Description of the task 4.2: Task 4.2 takes place over years 3, 4, and 5 of EJP. In this task we will adapt currently available transmission models such as QMRA for Salmonella and SimInF model for HEV and/or Salmonella of the consortium partners in order to analyse the impact of biosecurity measures on prevalence reduction of the zoonotic pathogens. The stochastic transmission models will simulate the spread of Salmonella and HEV across the production stages at the farm level with the provided set of biosecurity measures from WP 2 and WP 3, as well as with evidence-based best-practice measures from the literature search in **WP5** (accessed via collective database structure,

catalogue of biosecurity measures). The models will be developed for three countries, including the UK, GER, and IT. The models will be adapted with the current knowledge from epidemiology, such as transmission parameters, available demographical data, herd management data, and the provided collected data on the effectiveness of biosecurity measures from WP 2, 3 and 5.
The outcome of **WP 4.2** will be the level of prevalence (i.e. number of infected pigs at the farm level) i) without such biosecurity measures, ii) with individual standard biosecurity measures, iii) with individual specific mitigation measures, and/or iv) combination of a set of biosecurity measures.

Sub-Task 4.2.1: Data collection for the transmission models
Sub-Task start month: 33
Sub-Task end month: 36
Sub-Task Participants: APHA, ANSES, SVA
Description of the Sub-Task: Collection of transmission data, meta-data on the animal population, consumption data etc.

Task 4.3: Merge of models into one QMRA
Task start month: 34
Task end month: 50
Task Leader: Simon Robin
Deputy Task Leader:
Task Participants: APHA, ANSES, SVA
Description of the task 4.3: Task 4.3 takes place over the years 3, 4 and 5 of EJP. In **WP 4.3** the different outcomes of the transmission models (i.e. estimated prevalence at the farm level from the SimInF and QMRA models) at the primary production stage will be matched to a single QMRA model on Salmonella and HEV, which covers the entire food supply chain (i.e. subsequent processing stages as slaughterhouses but also the human sector). Data on the effectiveness of biosecurity measures at the slaughterhouse level from **WP2 (T2.3, collected in the BIOPIGEE' catalogue of biosecurity measures, WP5)** and/or the temperature impact on contamination reduction on the subsequent processing stages, and consumption data for pig meat in different countries will be incorporated in the single QMRA model. The final outcome of the one QMRA model will be the number of human cases for both Salmonella and HEV. The same three countries (UK, GER, IT) will be used to cover the range of potentially heterogeneous country-specific circumstances, such as the effectiveness of biosecurity measures, herd demographical data, and consumption data. The consideration of different country-specific circumstances is necessary in order to identify the "best" combination of cost-effective biosecurity measures across European countries. The outcome of **WP 4.3**, i.e. Salmonella and HEV prevalences at animal level and human level will feed into **WP 4.4**.

Sub-Task 4.3.1: Different transmission models will be matched to one QMRA zoonotic pathogen model
Sub-Task start month: 34
Sub-Task end month: 47
Sub-Task Participants: APHA, ANSES, SVA
Description of the Sub-Task: The different simulation models i.e. SimInF and QMRA model will be matched to onesingle QMRA zoonotic pathogen model because the outcome of the models, such as the SimInF model, will be the number of pigs infected with Salmonella and/or HEV at the production stages. Therefore, it will be necessary to match the different modelling approaches and considered pathogens in the models to one simulation QMRA model to obtain also the number of human cases. In this context, data of subsequent processing stages such as effective measures at the slaughterhouses from **WP2** and/or the temperature impact, and consumption data for pig meat in different countries will also be incorporated in this single QMRA zoonotic pathogen model. The

outcome will be the number of human cases for each considered pathogen, when no (biosecurity) intervention measures are implemented along the supply chain, compared to the scenario when intervention measures were implemented.

WP5
WP: 5 Benchmark of biosecurity practice
WP start month: 27
WP end month: 52
WP Leader: BfR
Deputy WP: APHA
WP participants: BfR, APHA, IZSAM, NDRVMI, AGES, PIWET, SVA, WbvR, VRI, SVA
Description of the WP:

In **WP5,** first 1) a catalogue of biosecurity measures and their effectiveness, confidence and costs to reduce/limit occurrence of Salmonella and Hepatitis E virus (HEV) in pig production and abattoir will be compiled and filled with information from different sources (studies, literature, machine learning, panel) (see sample spreadsheet Table 2 in the full proposal document). In a second step, 2) biosecurity practices (measures, categories, overall) will be benchmarked for their relevance to limit/reduce Salmonella and HEV in pig production. Different production stages and European regions will be considered.

In T5.1, the gained knowledge on effectiveness, confidence and costs of biosecurity measures from WP2-4 (results from cross-sectional on-farm study, study on slaughterhouse biosecurity, specific field studies, disinfection and biofilm, modelling effectiveness and costs) will be integrated in the catalogue of biosecurity measures which will refine the initial list of potentially relevant effective measures (biosecurity protocol developed in T2.1) for Salmonella and HEV. Knowledge gaps in the effectiveness will be filled by a literature search on peer-reviewed articles (T5.2) which will continue the brief search in T2.1, expand to a meta-analysis and last until M50 in order to collect most current published knowledge. If necessary, information from machine learning (T5.3) and an expert panel (T5.4) will supplement. Finally (5.5), biosecurity practice will be benchmarked for its effectiveness/relevance in order to limit/ reduce Salmonella and HEV occurrence in pig production. The results of WP5 will feed into WP4 and will be incorporated into the development of a support tool offered to veterinarians/consultants/farmers (T6.2) and be disseminated to stakeholders at a final workshop in (T6.3).

Task: JRP#-WP5-T1 Data integration from WP2-4 in catalogue of biosecurity measures
Task start month: 27
Task end month: 50
Task Leader: BfR
Deputy Task Leader: NDRVMI
Task Participants: BfR, NDRVMI, PIWET, SVA, APHA, AGES, IZSAM, WbvR
Description of the task: The catalogue of biosecurity measures relevant to limit/reduce Salmonella and HEV occurrence will be developed and continuously filled with findings on biosecurity measures' effectiveness and costs from WP2-4 throughout the project according to each WP's and Task's milestones and deliverables (results from cross-sectional on-farm study, study on slaughterhouse biosecurity, specific field studies, disinfection and biofilm, effectiveness and costs). This task will run until month 50.
Additionally, information on effectiveness from the current literature/meta-analysis, machine learning and panel information, gained in WP5, will be incorporated in the catalogue throughout the project. The reference origin will be noted (e.g. review, T2.2, meta-analysis, panel, machine learning, …) in the catalogue of biosecurity measures. The catalogue will, thanks to the database structure implemented in WP1, be accessible for insertion and extraction to all partners and

thereby continuously inform all WPs on current knowledge. The catalogue will form the base of the final benchmarking and dissemination of most current knowledge.

Task: JRP#-WP5-T2 Literature review/meta-analysis
Task start month: 27
Task end month: 50
Task Leader: IZSAM
Deputy Task Leader: BfR
Task Participants: PIWET, SVA, APHA, BfR, AGES, NDRVMI, IZSAM, WbvR, VRI
Description of the task:
A literature review and meta-analysis on peer-reviewed articles will fill knowledge gaps identified during the preparatory work and the brief initial review for the common biosecurity protocol in WP2. This work will begin in month 27, continue until month 50 and include on-going incorporation of current knowledge on biosecurity effectiveness into the catalogue of biosecurity measures. In case of a lack of information from the literature search (using terms like "biosecurity, pig, Salmonella and HEV"), the search will be expanded and articles on pathogens similar to Salmonella, namely Enterobacteriaceae (pathogenic *E. coli* from intestine and all *E. coli* in the environment of pigs) will be used for extrapolation on Salmonella (Filippitzi et al. 2018 and expert opinion*). Correspondingly, possible consideration of literature regarding biosecurity effectiveness for Salmonella/HEV in production systems of alternative livestock species as e.g. chicken will be clarified in expert interviews. Here also the use of effectiveness-information from non-European regions will be discussed.
The literature review will reveal biosecurity measures relevant for both Salmonella and HEV, for Salmonella only and for HEV only. Additionally, there may be measures relevant for all production stages or for only a part of the production stages. The catalogue of biosecurity measures will thus be stratified by pathogen (Salmonella, HEV) and production stage (breeding, weaner/fattener, farrow-to-finish, abattoir). Moreover, the country of origin will be documented. For the analysis, partner countries will be grouped by meaningful European regions (e.g. north, east, south) (see sample spreadsheet Table 2 in the full proposal document).
In month 36, a first part of literature review/meta-analysis, with overall values per measure/category will be estimated and offered to WP4.
*Filippitzi et al. 2018. Review of transmission routes of 24 infectious diseases preventable by biosecurity measures and comparison of the implementation of these measures in pig herds in six European countries. Transboundary and Emerging Diseases. 65, 381-398.

Task: JRP#-WP1-T4 Expert panel to add estimations on effectiveness/ weights
Task start month: 33
Task end month: 51
Task Leader: SVA
Deputy Task Leader: BfR
Task Participants: BfR, SVA, APHA, AGES, PIWET, NDRVMI, IZSAM, WbvR
Description of the task:
An expert panel will support the completion of information on biosecurity effectiveness and finally assess biosecurity practices in pig production. In the first year of BIOPIGEE, relevant experts will be identified and recruited among project partners, their collaborations and known experts from the literature (e.g. researchers, veterinarians, production consultants, pig production associations).

WP6
WP: JRP#-WP6 Dissemination
WP start month 25
WP end month 54
WP Leader Marie Sjölund, SVA

Deputy WP Leader BfR
WP participants SVA, BfR, AGES (VMU), APHA, VFL
Description of the WP:
Project results will be made available to students, farmers, veterinarians and the food industry through a number of tools and activities: Results will be presented and supplied as education and information material as well as through user-friendly interfaces on relevant existing websites. A support tool (Excel) to calculate cost effectiveness of interventions will be developed. A workshop-series will be organised to connect researchers and stakeholders “from farm to fork” in order to extract, discuss, inform about and rate country-specific biosecurity practices.

Collaboration with stakeholders in BIOPIGEE will build on previous activities and collaborations in the field of biosecurity. Building on these collaborations, knowledge and experience available in the field of biosecurity on the prevalence of Salmonella and Hepatitis E virus will be gathered and shared. Furthermore, these established collaborations will be used to disseminate the outcomes of BIOPIGEE to the stakeholders and have impact on the policy level.

In Austria, collaborations of BIOPIGEE partners with stakeholders include an expert group on antimicrobial resistance, EIP project partners and participants of a workshop on knowledge transfer on biosecurity. Collaborations are established with the Animal Health Service Austria (Tiergesundheitsdienst, TGD), the association of Austrian pig farmers (Verband Österreichischer Schweinebauern, VÖS) and the association of agricultural finishing producers (Verband landwirtschaftlicher Veredelungsproduzenten, VLV)), the Austrian Chamber of Agriculture, the Austrian Chamber of veterinary surgeons, as well as both ministries dealing with primary production, the Austrian Federal Ministry of Labour, Social Affairs, Health and Consumer Protection (BMASGK) and the Austrian Federal Ministry for Sustainability and Tourism (BMNT), Austrian Agency for Health and Food Safety (AGES), the University of Veterinary Medicine, Vienna (Vetmeduni Vienna), veterinary practitioners as well as several pig producers. In a workshop in autumn 2019, organized by Vetmeduni Vienna, the current situation as regards knowledge of biosecurity in primary animal production, research needs as well as communication needs and tools will be discussed among major stakeholders. Participants include the Animal health service, several associations for the livestock sectors including the association of Austrian Pig Farmers, the Austrian Chamber of Agriculture, the Austrian Chamber of veterinary surgeons, as well as both ministries dealing with primary production, the Austrian Federal Ministry of Labour, Social Affairs, Health and Consumer Protection (BMASGK) and the Austrian Federal Ministry for Sustainability and Tourism (BMNT).
In the Czech Republic, the Veterinary Research Institute (Czech BIOPIGEE partner) closely collaborates with the Ministry of Agriculture and the Ministry of Public Health through different institutions and working groups, which coordinate problems related to animal health care (State veterinary administration - legal office with nationwide competence), lab-based monitoring and epidemiological surveillance of pathogens (State veterinary institutes, National Institute of Public Health - National reference laboratories, Regional Public Health Authorities) or food safety (Czech agriculture and food inspection authority, Food Safety Center, EFSA focal point, Public Health Institutes). In addition, there is a longitudinal collaboration with many universities in the field of veterinary research and pre- or post-graduate education (e.g. University of Veterinary andPharmaceutical Sciences Brno, Mendel University, University of South Bohemia) and with proficiency associations e.g. Pigs Breeders' Association. The Veterinary Research Institute regularly organises workshops for veterinary practitioners and farmers to disseminate current knowledge.

In Germany, BfR houses the National Reference Laboratory for Salmonella, that annually receives a multitude of Salmonella strains from pigs all along the food chain from breeding units to pigs at

slaughter. Through its involvement in the monitoring of zoonoses in the food chain, BfR has intensive contacts to the veterinary authorities of the federal states and regular access to isolates from pig premises. Regular meetings with Universität Berlin and Hanover Veterinary School Foundation on questions of animal and environmental hygiene, i.e. biosecurity aspects. Collaboration with the Ministry of Food and Agriculture is established in the field of Salmonella control in poultry and on antimicrobial resistance. Finally, staff at BfR has also contacts to Agricultural faculties in Universities including those for Applied Sciences (Kiel, Göttingen, Berlin, Bonn, Kassel, Eberswalde, Osnabrück) as well as to farming/training schools. Furthermore, collaborations are established with German Animal Health Services. In the German project ORRES, dealing with risk factors for antibiotic use/resistance, BfR worked together with the Bavarian Animal Health Service (Tiergesundheitsdienst Bayern e.V.) and the Pig Health Service of the Agricultural Chamber of North Rhine-Westphalia. The animal health services have declared their intent to cooperate also for BIOPIGEE in order to find effective measures to limit/reduce Salmonella/HEV occurrence in pig production. Through their network of clients and their teaching obligations in training of farmers, these institutions can both serve as sources of data and assist in knowledge dissemination to current and future pig producers. BfR also has institutional contacts to the German Livestock Association (BRS) from a query on mortality data in pig production, and to Verband der Fleischwirtschaft e.V. (VDF), Bundesverband praktizierender Tierärzte (BPT) and Agricultural and Veterinary Faculties due to various presentations at related conferences of this association.

Input from and dissemination of information to UK livestock industry groups will be facilitated by the APHA membership of pig health and welfare council and various sub-councils, along with the Agricultural, Horticultural and Development Board for Pork (AHDB-Pork), British Pig Association, National Pig Association and pig veterinary society. Data retrieval and expert opinion on the BIOPIGEE project and dissemination of project results will be further facilitated by APHA staff who are members of the pig veterinary society and the APHA pig expert group which brings together representatives from AHDB-Pork, British Pig Association, National Pig Association and pig veterinary society.

In Sweden there is a long tradition of collaboration between SVA and the farm and veterinary advisory company Farm & Animal Health. The MINAPIG project in which Farm & Animal Health veterinarians assisted SVA in the collection of data on biosecurity and antimicrobial use in pig herds is one example. The results were then disseminated directly to participating and interested farmers in regional meetings. Another example of collaboration between Farm & Animal Health and SVA is the Swedish Veterinary Antibiotic Resistance Monitoring Programme – animal pathogens and food, in which resistance in animal pathogens are investigated. This activity is financed by the Swedish Board of Agriculture. The results are published in the yearly Swedres-Svarm report, a joint publication with the Public Health Agency (FOHM). Annual networking meetings on antimicrobial use and resistance are also hosted by SVA where representatives from competent authorities, the Swedish University of Agricultural Sciences, veterinary advisory companies, the Swedish Veterinary Association, Consumer Associations, farm animal and companion animal associations meet to share and discuss recent development within their field of work. Furthermore, SVA actively participates in under- and post graduate teaching of veterinary students.

Building on these exemplarily shown, but incomplete list of collaborations, available data will be collected and new data retrieved on the prevalence of Salmonella and Hepatitis E Virus in pig production in several countries, as well as on the knowledge and experience available in the field of biosecurity on a general level, and more specifically for the two pathogens addressed.JRP#-WP6-T1: Assembly and development of biosecurity information

Task start month 25

Task end month 54

Task Leader: APHA

Deputy Task Leader: AGES (VMU)

Task Participants: SVA, BfR, AGES (VMU), APHA, VFL

Description of the task: Results from WP2-5 will be provided by the respective work packages to WP6 to be assembled and further adopted for publishing. The results will be made available for farming schools through engaging and catchy education material. Moreover, the results will also be made available in a user-friendly interface on already existing websites of the One Health EJP, BIOPIGEE partners and relevant stakeholders such as previously identified associations of pig producers and veterinarians, authorities, educational bodies and companies along the food chain.

Sub-Task JRP#-WP6-T1-ST1 Identification of appropriate websites or other online channels
Sub-Task start month 25
Sub-Task end month 54
Sub-Task Leader: SVA
Deputy Sub-Task Leader: APHA
Sub-Task Participants: APHA, SVA, BfR, AGES (VMU)
Description of the sub-task: Already existing websites of partner organisations and interested relevant stakeholders such as previously described associations of pig producers and veterinarians, authorities, educational bodies and companies along the food chain in partner countries will be used to publish project results to reach out most efficiently. Both passive and active observations will be conducted throughout the project period to identify the at the time most appropriate organisations including their websites.

Sub-Task JRP#-WP6-T1-ST2a Provision of information for farming schools and websites
Sub-Task start month 31
Sub-Task end month 52
Sub-Task Leader: BfR
Deputy Sub-Task Leader APHA
Sub-Task Participants: APHA, SVA, BfR, AGES (VMU), VFL
Description of the sub-task: Relevant information material such as pictures of examples of good biosecurity practices will continuously be collected in WP2 (T2.2), forwarded to WP6 (sT6.1.2) and here be supplemented by existing and available material gathered from BIOPIGEE participants and cooperation partners. Information material (e.g. pictures) will be assembled, discussed with animal health services, training institutions and farming schools, and complemented with short messages by sT6.1.2 for dissemination towards e.g. farming schools at the end of the project. The material will also be available for the use on websites and be offered to relevant collaboration partners of BIOPIGEE participants.

Sub-Task JRP#-WP6-T1-ST2b Provision of slaughterhouse protocol to slaughter industry/related associations
Sub-Task start month 31
Sub-Task end month 52
Sub-Task Leader: BfR
Deputy Sub-Task Leader: APHA
Sub-Task Participants: APHA, BfR, AGES (VMU); VFL, IZSAM, ISS, WbvR, RIVM
Description of the sub-task:

A slaughterhouse biosecurity protocol applied and evaluated in WP2 T2.3 will be disseminated to inform slaughterhouses about effective biosecurity measures in participating countries.

Task JRP#-WP6-T3: Organisation of a workshop-series
Task start month 25
Task end month54
Task Leader: BfR

Deputy Task Leader: SVA
Task Participants: APHA, SVA, BfR, AGES (VMU)
Description of the task 6.3: Workshops will be organised to connect researchers and stakeholders in a farm-to-fork fashion to discuss and rate specific biosecurity measures and inform about obtained results. Three one-day-workshops will be co-organised by SVA and BfR in conjunction to relevant conferences such as the One Health EJP ASM, SafePork and the European Symposium of Porcine Health Management. Researchers and stakeholders representing all regions in Europe will have the opportunity to participate in discussions on biosecurity practices and to share their views on appropriate practices. Care will be taken to encompass participants from the different stages of the food chain from farm to fork. We seek to:

- extract and discuss country-specific biosecurity practices
- conduct an expert rating of biosecurity measures
- evaluate on the actors' responsibility of Salmonella-infection regarding the stages of the food chain
- inform stakeholders (results of WP2-5)

Sub-Task JRP#-WP6-T3-ST1 Identification of relevant stakeholders
Sub-Task start month 25
Sub-Task end month 52
Sub-Task Leader: BfR
Deputy Sub-Task Leader: SVA
Sub-Task Participants: APHA, SVA, BfR, AGES (VMU)
Description of the sub-task: The identification process will be carried out continuously to reach out to the at the time most relevant stakeholders of pig producers and veterinarians, authorities, educational bodies and companies along the food chain. This process will shift from being an active process at times to a more passive process of observations performed during everyday work and contact with stakeholders.

Sub-Task JRP#-WP6-T3-ST2 Identification of relevant conferences
Sub-Task start month 25
Sub-Task end month 26
Sub-Task Leader: BfR
Deputy Sub-Task Leader: SVA
Sub-Task Participants: APHA, SVA, BfR, AGES (VMU)
Description of the sub-task: The most appropriate conferences regarding content and audience as well as location and the time point they are to be held will be identified for organising workshops with stakeholders of pig producers and veterinarians, authorities, educational bodies and companies along the food chain.

Sub-Task JRP#-WP6-T3-ST3 Organisation of Workshop 1
Sub-Task start month 25
Sub-Task end month 30
Sub-Task Leader: BfR
Deputy Sub-Task Leader: SVA
Sub-Task Participants: APHA, SVA, BfR, AGES (VMU)
Description of the sub-task: The first workshop will be organised to gather input from stakeholders and inform about the project.

Deliverables

Ref	Title	Due month

D-JRP#-WP2.1	Biosecurity protocol (addressing Salmonella and HEV) designed for data collection in the field	28
D-JRP#-WP1.2	First draft of data management plan finished	30
D-JRP#-WP6.3	Workshop 1 completed	30
D-JRP#-WP1.4	Project report 1st year submitted	36
D-JRP#-WP3.5	Method for testing persistence of infectious HEV in surface microlayers	36
D-JRP#-WP3.6	HEV infectivity assay available	36
Milestones		
Ref	**Title**	**Due month**
M-JRP#-01	Kick-off meeting successfully organized	26
M-JRP#-02	Questionnaire on biosecurity costs	26
M-JRP#-03	Relevant conferences for workshops to be held at identified	26
M-JRP#-04	Biosecurity protocol designed for Salmonella and HEV	28
M-JRP#-05	Relevant stakeholders identified	28
M-JRP#-06	Appropriate websites or other online channels for dissemination identified	30
M-JRP#-07	Workshop 1 completed	30
M-JRP#-08	Design of field study protocols	32
M-JRP#-09	First part of meta-analysis finished	36
M-JRP#-10	Salmonella strains for testing are collected	36
M-JRP#-11	HEV infectivity assay available	36

2.4.2.1.28 JRP22-R2-FBZ4.1-TOXOSOURCES

Reference to the Stategic Research Agenda (please refer to D2.7)		**FBZ 4.1: Source attribution and transmission routes of foodborne pathogens other than bacteria, with emphasis on Toxoplasma gondii**								
Research Project Title		***Toxoplasma gondii* sources quantified**								
Research Project Acronym		**TOXOSOURCES**								
Leading Organisation		**P13-SSI**			Deputy Leading Organisation			**P30-RIVM**		
Project Leader		**Pikka Jokelainen**			Deputy Project leader			**Joke van der Giessen**		
Project Start month		**M25**			Project End month			**M54**		
Participant	**1**	2	4	6	**7**	**8**	**9**	**10**	**11**	**12**
	Anses	Ages	Sciensano	NDRVMI	**SZU**	**VRI**	**BfR**	**FLI**	**RKI**	**DTU**
PM	**13.5**				**3.7**	**13.5**	**1**	**9**	**6.1**	**1.1**
Participant	**13**	14	15	16	**17**	18	19	20	21	22
	SSI	UT	VFL	INIA	**UCM**	MVNA	INRA	IP	APHA	PHE
PM	**12.1**				**9.1**					
Participant	**23**	24	25	26	**27**	28	29	**30**	**31**	**32**
	UoS	OKI	NUIG	TEAGASC	**ISS**	IZSAM	IZSLER	**RIVM**	**WbvR**	**FHI**
PM	**3.75**				**15.3**			**9.39**	**1**	**3.1**
Participant	**33**	**34**	**35**	**36**	37	38	**39**	40	**41**	
	NVI	**PIWET**	**INIAV**	**INSA**	IISPV	IC	**SLV**	FOHM	**SVA**	
PM	**1.94**	**6.5**	**7.3**	**1.5**			**1**		**1.1**	

Objectives

- ✓ TOXOSOURCES evaluates existing source attribution models for their suitability to a parasitic foodborne pathogen, improves them, and applies them (WP2). Quantitative microbiological risk assessment (QMRA) existing at national basis for the meatborne pathway (tissue cysts) will be extended to a European level. Moreover, QMRA for the environmental pathway (oocysts) will be developed. Innovative integration of available data is emphasized, and existing major data gaps are identified and filled by targeted multicentre studies (WP2, WP3, WP4, WP5).

- ✓ TOXOSOURCES yields the first quantitative and comparable estimates of contribution of the different sources for the four regions within Europe (WP2). Moreover, TOXOSOURCES yields an overview of consumption habits that are also relevant for other foodborne pathogens (WP2, WP3). TOXOSOURCES addresses geographical differences in the epidemiology of T. gondii, in animals, humans and the environment (WP2, WP3, WP4, WP5). The consortium has excellent geographical balance within Europe for multicentre studies, and external partners from China add to the global trade aspect. The work includes collaboration with the food production and retail sectors (WP2, WP3, Interest Group).

- ✓ TOXOSOURCES will develop a novel serological method that specifically explores detection of infections caused by the oocysts (WP4) in Europe, and a novel NGS-MLST typing method that can detect within-genotype patterns that are important for understanding transmission routes (WP5). The latter will be a tool for source tracing, and improves preparedness to identify outbreaks and imported emerging atypical strains. The development processes will make optimal use of existing sample collections from suitable epidemiological frameworks.

- ✓ TOXOSOURCES consortium is very well aware of research that has been done previously in this field. Emphasis is put on complementarity, avoiding redundancy and overlap, and on impact, measurably advancing the knowledge on this major zoonotic pathogen. The results from the different approaches are integrated and compared to yield robust new information that is of importance to the Key EU Stakeholders and will contribute to the development of innovative and effective interventions at national, regional, European and global levels (WP1).

Description of work

The work is organized into 5 work packages (WPs) with tasks (T) and some subtasks (sT). The 2.5-year project spans over three Annual Periods (Y3-Y5) and is described in three Work Plans.

JRP-TOXOSOURCES-WP1 Coordination and impact

WP start month M25 - WP end month M54
WP Leader Pikka Jokelainen, SSI; Deputy WP Leader Joke van der Giessen, RIVM
WP participants SSI, RIVM, ISS, FLI, BfR, RKI, UCM, ANSES, PIWET, DTU, FHI, INIAV, INSA, NVI, SLV, SVA, SZÚ, UoS, VRI, WbvR, BIOR, CVRL, NMBU, BRCT/NRCT, AUTh, UASVMCN, HZAU, JLU
WP1 takes place over the first, the second and the third year of the project. WP1 works closely with all the WPs of the project. Objectives of WP1:

- To manage and coordinate the project.
- To draft and implement the Data Management Plan for TOXOSOURCES.
- To integrate all the results to contribute to developing interventions.
- To ensure the results will be useful (dissemination).

WP1 manages the project and is responsible for its progress, and integrates all the results of the project to make them useful, in particular to contribute to developing interventions.
WP1 ensures the project adheres to H2020 rules regarding e.g. ethics, IRP, dissemination and publication. Moreover, WP1 is responsible for the Data Management Plan of the project. WP1 coordinates the compiling of deliverables and reports and their timely submission to the Support Team, 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. During Y3, the main focus is ensuring the project starts efficiently, organizing the Kick-off Meeting, and drafting the Data Management Plan.

JRP-TOXOSOURCES-WP1-T1 Management, coordination and communication
Task start month M25 - Task end month M54
Task Leader: Pikka Jokelainen, SSI; Deputy Task Leader: Joke van der Giessen, RIVM
Task Participants: SSI, RIVM, ISS, FLI, BfR, RKI, UCM, ANSES, PIWET, DTU, FHI, INIAV, INSA, NVI, SLV, SVA, SZÚ, UoS, VRI, WbvR, BIOR, CVRL, NMBU, BRCT/NRCT, AUTh, UASVMCN , HZAU, JLU
WP1-T1 takes place over the first, the second and the third year of the project.
Coordination and management of the project. Coordination of compiling of deliverables and reports and their timely submission to the Support Team. Ensuring that the project adheres to H2020 rules regarding e.g. ethics, dissemination and publication. Drafting and implementing Data Management plan; SSI is the partner that is responsible of this. Coordination of organizing project meetings: during the first year of the project: Kick-off Meeting (KOM), where the consortium participants meet face-to-face and the work in the WPs is launched. Communication within the consortium: teleconference of the WP Leaders four times a year, email correspondence with the WP Leaders and the whole Consortium. Dialogue with the Interest Group is activated.

JRP-TOXOSOURCES-WP2 Multicentre quantitative microbiological risk assessment for *T. gondii* infections
WP start month M25 - WP end month M54
WP Leader: Marieke Opsteegh, RIVM; Deputy WP Leader: Sara Monteiro Pires, DTU
WP participants: SSI, RIVM, ISS, FLI, BfR, RKI, UCM, ANSES, PIWET, DTU, FHI, INIAV, INSA, NVI, SLV, SVA, SZÚ, UoS, VRI, WbvR, UASVMCN
WP2 takes place over the first (Y3), the second (Y4) and the third year (Y5). WP2 works closely with WP1 and WP3. Objectives of WP2:

- To estimate the relative contribution of food and environmental transmission routes (T1)
- To provide an overview of the prevalence in food animals and cats (T2)
- To quantify human exposure to possible sources of infection (T3)
- To provide an overview of the processing parameters for relevant meat products (T4)
- To provide an overview of prevalence and risk factors of human infection (T5)

WP2 estimates the relative contribution of different sources of *T. gondii* infection by quantitative microbiological risk assessment (QMRA). QMRA models for infection via tissue cysts (meat) and oocysts (fruits, vegetables and environmental pathways) will be developed and applied for nine countries (DK, NO, CZ, PL, PT, ES, FR, DE, NL) representing all four regions of Europe. During Y3, the main focus is building the QMRA model and collecting input data.

JRP-TOXOSOURCES-WP2-T1 QMRA modelling for human *T. gondii* infections
Task start month M25 - Task end month M54
Task Leader: Eric Evers, RIVM; Deputy Task Leader: Jakob Ottoson, SLV
Task Participants: SSI, RIVM, FLI, BfR, RKI, UCM, ANSES, PIWET, DTU, FHI, INIAV, INSA, NVI, SLV, SZÚ, VRI
WP2-T1 takes place over the first, the second and the third year of the project. During Y3, the main focus is building the QMRA model. The existing meatborne QMRA model will be adapted to include multiple countries and fit to consumption data that is specifically collected for this purpose (WP2-T3). Input from OHEJP JIP COHESIVE is included. A QMRA model for oocyst infections will be developed to estimate exposure via the environment and consumption of fresh produce.

JRP-TOXOSOURCES-WP2-T2 Review of prevalence of *T. gondii* infection in animals
Task start month M25 - Task end month M40
Task Leader: Radu Blaga, ANSES; Deputy Task Leader: Helga Waap, INIAV

Task Participants: SSI, RIVM, ISS, FLI, UCM, ANSES, PIWET, DTU, INIAV, NVI, SVA, UoS, VRI, WbvR, BIOR, UASVMCN
WP2-T2 takes place over the first and the second year of the project. Prevalence data from cats and animals raised or hunted for human consumption in Europe will be collated, building on the systematic reviews performed for EFSA and the Baltic and Nordic countries. Data will be analysed by Bayesian hierarchical modelling to estimate the prevalence by country and animal species, and delivered to WP2-T1.

JRP-TOXOSOURCES-WP2-T3 Quantitative exposure survey
Task start month M25 - Task end month M48
Task Leader: Arno Swart, RIVM; Deputy Task Leader: Jurgen Chardon, RIVM
Task Participants: SSI, RIVM, BfR, UCM, ANSES, PIWET, DTU, FHI, INIAV, VRI
WP2-T3 takes place over the first and second year of the project. A questionnaire designed to collect food consumption and handling data for QMRA will be expanded to capture exposure to oocysts and adapted to the QMRA-countries. Main focus in Y3 is to develop the generic questionnaire (considering e.g. FoodEx2, and databases of FAO and EFSA) and adapt it to capture region/country-specific products.

JRP-TOXOSOURCES-WP2-T4 Overview of processing parameters for relevant meat products
Task start month M25 - Task end month M45
Task Leader: Bretislav Koudela, VRI; Deputy Task Leader: Jacek Sroka, PIWET
Task Participants: SSI, RIVM, BfR, UCM, ANSES, PIWET, DTU, INIAV, NVI, SVA, VRI
WP2-T4 takes place over the first and second year of the project. Freezing, heating and salting of meat does not necessarily eliminate the risk of infection. Therefore, processed meat products may still be relevant for the QMRA. During Y3, partners from the selected countries will identify which processed meat products should be included in the survey (WP2-T3).

JRP-TOXOSOURCES-WP2-T5 Review of prevalence and risk factors for human *T. gondii* infection
Task start month M25 - Task end month M54
Task Leader: Agnetha Hofhuis, RIVM; Deputy Task Leader: Lasse S. Vestergaard, SSI
Task Participants: SSI, RIVM, RKI, UCM, ANSES, PIWET, FHI, INSA, SVA, SZÚ, UoS
WP2-T5 takes place over the first, the second and the third year of the project. WP2-T5 will provide an overview of seroprevalence/incidence and risk factors for human *T. gondii* infection in Europe. We will update and extend the review performed under Euro COST-FBP. During Y3, main focus is developing the systematic review protocol, perform searches, and initiate screening and data extraction.

JRP-TOXOSOURCES-WP3 Multicentre survey to fill the key existing gap: role of fresh produce (i.e. Ready-to-Eat salads)
WP start month M25 - WP end month M54
WP Leader: Marco Lalle, ISS; Deputy WP Leader: Anne Mayer-Scholl, BfR
WP participants: ISS, BfR, UCM, UoS, VRI, RIVM, NVI, PIWET, INIAV, ANSES, SSI, UCM, ISS, BIOR, AUTH, NMBU
WP3 takes place over the first, the second and the third year of the project. WP3 works closely with WP1, WP2 and WP5. Objectives of TOXOSOURCES WP3:

- To identify and assess the most appropriate procedure to detect *T. gondii* oocysts in fresh produce.
- To provide an overview of *T. gondii* oocysts in fresh produce and the environment.
- To conduct a risk-based pilot study based on available prevalence data (literature review), data on food production chains, EU trade patterns of selected fresh produce and available consumption data (WP2).

- To evaluate *T. gondii* oocyst contamination in selected fresh produce commodities by a multicentre pilot survey in representative EU regions.

WP3 aims to fill the knowledge gap concerning the relevance of fresh produce contamination by *T. gondii* oocysts as an infection source for humans. WP3 selects the most reliable methods for the molecular detection of *T. gondii* oocysts in fresh produce (i.e. vegetables, fruits and fruit juice) using a literature review and experimental evaluation through inter-laboratory comparison. Harmonised detection method(s) are implemented among the partners of WP3 by providing a standard operating procedure (SOP) and organizing a technical workshop. In Y3, the focus is on collection of existing data on prevalence of *T. gondii* oocysts in fresh produce and environment, as well as of information on fresh produce (e.g. RTE production trading and consumption) within EU. These data are provided to WP2. In WP3, these data and the selected, harmonised method are to design a risk-based sampling strategy for a multicentre pilot survey on *T. gondii* in fresh produce.

JRP-TOXOSOURCES-WP3-T1 Selection, evaluation and implementation of detection procedure for *T. gondii* oocysts in fresh produce
Task start month M25 - Task end month M39
Task Leader: Marco Lalle, ISS; Deputy Task Leader: Iva Slana, VRI
Task Participants: ISS, BfR, UCM, UoS, VRI, NVI, PIWET, BIOR, INIAV, ANSES, SSI, AUTH, NMBU
JRP-TOXOSOURCES-WP3-T1 takes place over the first and the second year of the project. ISS and VRI perform literature review on molecular detection methods for *T. gondii* oocysts in fresh produce (discussion by E-meetings will facilitate the decision process) to select for each analytical step (oocyst recovery, DNA extraction, parasite identification including target and primer selection and PCR vs. non-PCR based methods) those showing the most promising analytical characteristics, feasibility and potential for standardisation. Comparative experimental work at ISS and VRI are conducted on analytical steps to evaluate characteristics such as sensitivity, reproducibility, repeatability, ease of handling, availability and costs of reagents and equipment, hands on and turn-around times. Experiment are performed using representative fresh produce matrix (identified in WP3-T2) artificially spiked with *T. gondii* oocysts. An analytical procedure is defined and a preliminary SOP prepared. To ensure reproducibility and comparability of test results among the laboratories involved in the multicenter survey (WP3-T3), two technical trainings, based on the SOP are performed at ISS. For cost effectiveness, “satellite” partners (UoS, ANSES, SSI, INIAV, BIOR, AUTH, NMBU) expected to contribute only in the oocysts recovery step, will be trained only on this part of the procedure, whereas “main” partners (BfR, UCM; NVI, PIWET), performing the lab testing, will be trained in the full procedure.

JRP-TOXOSOURCES-WP3-T2 Design of a risk-based sampling strategy
Task start month M26 - Task end month M48
Task Leader: Rafael Calero, UCM; Deputy Task Leader: Martha Betson, UoS
Task Participants: ISS, BfR, UCM, UoS, VRI, RIVM, NVI, PIWET, BIOR, INIAV, ANSES, SSI, AUTH, NMBU
WP3-T2 takes place over the first and the second year of the project. The overall goal is to design the sampling plan for WP3-T3. Risk-based selection of fresh produce samples is done using existing data on *T. gondii* oocysts prevalence in fresh produce, and environment, and information on fresh produce (e.g. ready-to-eat (RTE) salads) production chains, trade patterns and consumption within EU. Data on *T. gondii* prevalence in fresh food (including vegetables, fruits and fruit juices) and environmental samples (including soil and water) in Europe are collected by extensive literature review following PRISMA recommendations. Peer-reviewed as well as “grey” literature are included, and advantage will be taken of national partners able to access information in different languages. Priority is given to data collection on prevalence in fresh produce to provide them to WP2 as input data for QMRA. Data on production chains (from field to table, including processing steps in different production types such as organic vs non-organic), trade patterns and consumption

within EU of fresh produce commodities (e.g. RTE) will be collected using publicly available information or data from European producer and retail associations. Information will be treated as Food Related Research Equivalent to Medical Research according to the Guidance Note-Ethics and Food-Related Research of the EC. This information will be combined with prevalence data to device the sampling strategy for WP3-T3 to include which products will be sampled, from which production system, as well as at which step in the food production chain. Sampling is adjusted taking into account sample size, feasibility and costs, as well as the experience gained and potentially samples collected during OHEJP JRP MedVetKlebs.

JRP-TOXOSOURCES-WP4 Serology method based on novel antigens to discriminate *T. gondii* infections acquired from oocysts
WP start month M25 - WP end month M54
WP Leader: Furio Spano, ISS; Deputy WP Leader: Frank Seeber, RKI
WP participants: ISS, RKI, UCM, NVI, RIVM, SSI, ANSES, FLI, PIWET, INIAV, WbvR, INSA, VRI, BIOR, HZAU, JLU
WP4 takes place over the first, the second and the third year of the project. Objectives of WP4:

- To identify *T. gondii* antigens with source-attributing potential (proteins of interest, POIs)
- To explore serological methods that discriminate oocyst- from tissue cyst-driven infections
- To estimate the prevalence of oocyst-driven infections in humans and animals

WP4 identifies novel antigens of *T. gondii* that have source-attributing potential and explores serological methods able to discriminate between oocyst- vs. tissue cyst-driven infections. During Y3, the main focus of WP4 is the bioinformatic selection and recombinant expression of a large set of sporozoite-specific antigens

JRP-TOXOSOURCES-WP4-T1 Identification and production of *T. gondii* stage-specific antigens for source attribution
Task start month M25 - Task end month M48
Task Leader: Furio Spano, ISS; Deputy Task Leader: Frank Seeber, RKI
Task Participants: ISS, RKI, FLI
WP4-T1 takes place over the first and the second year of the project. Applying a matrix of >40 variables (experimentally determined or predicted in silico) to the more than 2,500 proteins of the oocyst/sporozoite proteome stage-specific proteins will be identified (proteins of interest; POIs) and further prioritized based on features like abundance, antigenicity, solubility, etc. Genes or parts thereof encoding POIs will be PCR-amplified and cloned high-throughput into a validated bacterial expression vector, allowing C-terminal epitope-tagging for both, detection and affinity purification. Parallel small-scale purification and direct elution into plates will allow fast testing of multiple POIs in WP4-T2. The whole process is iterative, incorporating required experimental improvements or further POIs. In parallel a small set of known stage-specific proteins is tested to develop an antibody profiling method that aims to diagnose cyst-derived infections. For this the method will combine data regarding antibody isotype, IgG avidity and titer of the humoral response against bradyzoite-, tachyzoite- or sporozoite-specific antigens to allow the conclusion that freshly encysted bradyzoites (rather than oocysts) were responsible for infection. All POIs will be validated in WP4-T2 and up to mg quantities will be produced as required.

JRP-TOXOSOURCES-WP4-T2 Development of a novel stage-specific antigen-based ELISA to diagnose oocyst- and bradyzoite-driven *T. gondii* infections
Task start month M29 - Task end month M48
Task Leader: Gema Álvarez-Garcia, UCM; Deputy Task Leader: Luis Ortega Mora, UCM
Task Participants: UCM, ANSES, VRI, PIWET, RIVM

WP4-T2 takes place over the first and the second year of the project. POIs from WP4-T1 will be assessed with a panel of available pre-selected and validated reference sera from: i) animals experimentally infected with either *T. gondii* tachyzoites, bradyzoites or oocysts and ii) humans infected congenitally (tachyzoite-derived infection) or upon waterborne/environmental outbreaks (oocyst-derived infections), using a step-by-step validation process.

JRP-TOXOSOURCES-WP4-T2-sT1 Standardization of a POI-based ELISA to diagnose oocyst- and/or bradyzoite driven *T. gondii* infections using reference pig sera

Sub-Task Leader: Gema Álvarez-Garcia, UCM

Sub-Task Participants: ANSES, VRI, PIWET

Sera from experimentally infected pigs will be screened by two possible source attribution assays (sporozoite antigen-based ELISA or bradyzoite antibody profiling) in parallel. Sequential pig sera will come from animals infected with either oocysts or cysts. Sporozoite antigen based ELISA will measure specific IgG levels to diagnose oocyst driven infections. The bradyzoite antibody profiling will include the monitoring of IgM and IgG avidity kinetics. Next, the analytical specificity of the selected sporozoite or bradyzoite antigens showing the highest discriminatory ability among the different experimental groups will be checked by Western blot with sera from different host species infected with closely-related parasites.

JRP-TOXOSOURCES-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
Sub-Task Leader: Luis Ortega Mora, UCM
Sub-Task Participants: ANSES, RIVM, SSI
Differences linked to distinct transmission stages will be corroborated with selected and specific sporozoite or bradyzoite antigens and with reference sera from other animal species infected with: i) cysts (e.g. cat and mice sera); ii) oocysts (e.g. sheep sera and human sera from waterborne/environmental outbreaks); iii) tachyzoites (mice sera and sera from congenitally infected humans). All sera will be also analyzed by commercially available and routinely used serological tests to evaluate the additional diagnostic value of the new antigens tested.

JRP-TOXOSOURCES-WP5 Novel *T. gondii* typing method to detect within-genotype variation
WP start month M25 - WP end month M54
WP Leader: Gereon Schares, FLI; Deputy WP Leader: Simone Caccio, ISS
WP participants ANSES, BIOR, FLI, ISS, NVI, PIWET, RIVM, SSI, UCM, VRI, SZÚ/NIPH, HZAU, JLU
WP5 takes place over the first, second and the third year of the project. Objectives of WP5:

- To develop a novel *T. gondii* typing method with the discriminatory power needed in Europe
- To apply the novel method in pilot studies

WP5 aims to identify highly polymorphic regions in genomes of very closely related *T. gondii* strains across Europe. Preliminary NGS data on European Type II *T. gondii*, obtained in EURLP/ISS-FLI collaboration revealed substantial variation between isolates and relative to reference strains. Using a panel of representative strains, regions in the genome with an optimal SNP density are identified und used to establish a novel high-throughput targeted NGS-MLST-typing method.

JRP-TOXOSOURCES-WP5-T1 Retrieval of relevant *T. gondii* isolates or NGS-quality DNAs for NGS and NGS-MST
Task start month M25 - Task end month M44
Task Leader: Gereon Schares, FLI; Deputy Task Leader: Pikka Jokelainen, SSI
Task Participants: ANSES, BIOR, FLI, ISS, NVI, PIWET, RIVM, SSI, UCM, VRI, SZÚ/NIPH, HZAU, JLU

WP5-T1 takes place over the first and second year of the project. *T. gondii* isolates or WGS-quality DNAs from across Europe including also few isolates, referred to as atypical, recombinant or from other parts of world (e.g. China or South America). Isolates are going to be used either for whole genome sequencing (WGS) and validation of the novel NGS-MLST method. The focus is on *T. gondii* Type II as the vast majority (>80%) of European strains belong to this type. Type III isolates (a rare European Type) and atypical or recombinant isolates are collected, too. To ensure representativeness, the collection of 3 to 5 isolates or WGS-quality DNAs per region, i.e. northern, eastern, western and southern Europe: altogether 12-24 DNAs. After this, the task continues collection of further isolates or DNAs to establish a panel for the pilot studies (WP5-T3). For particular regions, we aim at a larger number of further isolates (about n=10) to later show that resolution of typing is high enough to trace differences of local isolates. All DNAs in the panel are also characterised based on polymorphism of fewer markers using existing standard techniques (PCR-RFLP and microsatellite (MS) typing).

JRP-TOXOSOURCES-WP5-T2 Novel, standardized high-throughput direct NGS-MLST *T. gondii* genotyping method
Task start month M25 - Task end month M48
Task Leader: Simone Caccio, ISS; Deputy Task Leader: Pavlo Maksimov, FLI
Task Participants: ANSES, BIOR, FLI, ISS, NVI, PIWET, RIVM, SSI, UCM, VRI, SZÚ/NIPH, HZAU, JLU
WP5-T2 takes place over the first and second year of the project. Whole Genome Sequencing (WGS) of key *T. gondii* isolates or WGS-quality DNAs (provided by WP5-T1) from the four European regions is performed. Genomic sequences on a representative number of *T. gondii* isolates covering entire Europe will be made available by entering them into existing public databases. Based on these sequences a novel, standardized high-throughput, targeted NGS-MLST genotyping method (using the Ion Torrent platform or other suitable platforms) is establishment to separate closely related *T. gondii* strains.

JRP-TOXOSOURCES-WP5-T2-sT1 Whole Genome Sequencing (WGS) of key *T. gondii* isolates and WGS-quality DNAs
Sub-Task start month M25 - Sub-Task end month M36
Sub-Task Leader: Simone Caccio, ISS; Deputy Sub-Task Leader: Pavlo Maksimov, FLI
Sub-Task Participants: FLI, ISS, UCM
Whole genomic sequences are generated on a representative number (n=12-24, provided by WP5-T1) of *T. gondii* isolates covering entire Europe. The new set of sequences is going to be completed with further sequences already available in public databases and as yet unpublished genomic sequences on European *T. gondii* isolates (e.g. unpublished sequences from an ongoing EURLP-FLI and UCM-CVI (Craig-Venter-Institute) cooperation. This includes also sequences of a few Type III and recombinant isolates already available at FLI and UCM.

JRP-TOXOSOURCES-WP5-T2-sT2 Establishment, validation and refinement of a novel, standardized high-throughput targeted NGS-MLST *T. gondii* genotyping method
Sub-Task start month M31 - Sub-Task end month M44
Sub-Task Leader: Pavlo Maksimov, FLI; Deputy Sub-Task Leader: Simone Caccio, ISS
Sub-Task Participants: FLI, ISS
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) are going to be selected for a new typing method. A novel, standardized, high-throughput targeted NGS-MLST genotyping method (using the Ion Torrent platform or other suitable platforms) is established to separate closely related *T. gondii* strains.

Deliverables

Ref	Title	Due month
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
D-JRP-TOXOSOURCES-WP1.1	Data Management Plan	M30
D-JRP-TOXOSOURCES-WP3.2	SOP on detection of *T. gondii* in selected fresh produce matrix	M36
Milestones		
Ref	Title	Due month
M-JRP-TOXOSOURCES-01	Kick-off Meeting held by WP1	M26
M-JRP-TOXOSOURCES-02	Bioinformatic selection of oocyst/sporozoite-specific antigens completed in WP4	M28
M-JRP-TOXOXOURCES-03	Key isolates summarized in WP5	M30
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
M-JRP-TOXOSOURCES-05	Experimental selection of the appropriate methods for samples analysis in WP3	M36
M-JRP-TOXOSOURCES-06	Delivery of data summary on literature-based prevalence of *T. gondii* oocysts in fresh produce from WP3 to WP2	M36
M-JRP-TOXOSOURCES-07	Production of the first sets of purified soluble recombinant proteins (up to 100) for WP4-T2 serological assays	M36
M-JRP-TOXOSOURCES-08	Evaluation of the source attributing ability of the first sets of stage-specific antigens produced in WP4-T1	M36

2.4.2.1.29 JRP23-R2-FBZSH5-ADONIS

Reference to the Stategic Research Agenda (please refer to D2.7)			**FBZ-SH 5: Determinants of the reversal of the decreasing trend in Salmonella incidence in humans and poultry in the EU**							
Research Project Title			**Assessing Determinants Of the Non decreasing Incidence of *Salmonella***							
Research Project Acronym			**ADONIS**							
Leading Organisation			**P30-RIVM**			Deputy Leading Organisation			**P34-PIWET**	
Project Leader			**Eelco FRANZ**			Deputy Project leader			**Dariusz WASYL**	
Project Start month			**M25**			Project End month			**M54**	
Participant	**1**	**2**	**4**	6	7	8	9	10	11	12
	Anses	**Ages**	**Sciensano**	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM	**21.7**	**4.5**	**7.04**							
Participant	**13**	14	15	16	**17**	18	19	**20**	**21**	**22**
	SSI	UT	VFL	INIA	**UCM**	MVNA	INRA	**IP**	**APHA**	**PHE**
PM	**15**				**16**			**14.4**	**7.9**	**1.25**
Participant	23	24	25	26	**27**	28	29	**30**	**31**	32
	UoS	OKI	NUIG	TEAGASC	**ISS**	IZSAM	IZSLER	**RIVM**	**WbvR**	FHI
PM					**4.2**			**10.08**	**5.5**	
Participant	33	**34**	**35**	**36**	37	38	39	40	41	
	NVI	**PIWET**	**INIAV**	**INSA**	IISPV	IC	SLV	FOHM	SVA	
PM		**9.5**	**10.45**	**21.46**						

Objectives

- to facilitate partners' cooperation and WP integration
- to guarantee timely delivery of periodic reports
- to accumulate, communicate and disseminate project outcomes

Description of work
JPR26-WP1 Project management
WP start month: 25
WP end month: 54
WP Leader: Eelco FRANZ (RIVM)
Deputy WP Leader: Dariusz WASYL (PIWET)

WP participants: ANSES, Sciensano, SSI, UCM, IP, RIVM, PIWET

Description of the WP:
WP1 takes place over the first, the second and the third year of the project. It is led by projects leaders supported by WPs leaders and deputies. It facilitates and cumulates all the efforts taken within other WPs, summarises and reports the outcomes to OHEJP management team, aligns and communicates to other OHEJP research and integrative activities, and disseminates the conclusions beyond OHEJP.
WP1 consists of two tasks ongoing throughout the project period. Since task 1 governs internal issues of the ADONIS consortium, task 2 is orientated to external partners and stakeholders.

JPR26-WP1-T1 Coordination
Task Leader: Eelco FRANZ (RIVM)
For fluent communication, planning and results sharing between partners and WPs three physical meetings and regular teleconferences are organised for the entire consortium, WPs leaders/deputies or given WP partners, depending on the issue.
Y3 Kick-off meeting, followed by teleconferences are organised in start-up phase to address planning and cooperation within the project.

JPR26-WP1-T2 Aligning and communication
Task Leader: Eelco FRANZ (RIVM)
T2 consists of two subtasks

JPR26-WP1-T2-ST1 Reporting
Subtask Leader: Eelco FRANZ (RIVM)
ST1 starts from Y4.

JPR26-WP1-T2-ST2 Alignment and communication
Subtask Leader: Dariusz WASYL (PIWET)
ST2 is responsible for aligment and communication with other OHEJP activities and stakeholders.
Y3 In start-up phase related OHEJP activities and relevant stakeholders is mapped and addressed to start aligning and communication process. The process is facilitated over operational phase.

JPR26-WP2 *Salmonella* controls at the primary production level
WP start month 25
WP end month 54
WP Leader Michel-Yves MISTOU (ANSES)
Deputy WP Leader Julio ALVAREZ SANCHEZ (VISAVET-UCM)
WP participants ANSES, APHA, PIWET, VISAVET-UCM, WVBR
Description of the WP[1]:
WP2 focuses on characterizing the increasing trend in *S.* Enteritidis prevalence in laying hens at the primary production level recently described along with that of other serotypes relevant for public health. Despite a common regulation, the application of National *Salmonella* Control Programs (NSCPs) in the various European countries is not harmonised, with varying levels of requirements. Variation in programme applications among countries may contribute to the difference in *Salmonella* prevalence trends observed among EU Member States. This WP is based on the comprehensive evaluation and comparative analysis of data coming from NSCP, official reports and available scientific literature. The ultimate objectives are to identify measures to improve *Salmonella* surveillance and control and flock management at country level (Task 1).

Additional missing information on the effectiveness of control measures implemented in laying hen farms will be gathered by both field sampling and through the use of questionnaires sent to relevant entities (Task 2). The outcomes of T1 and T2 will be used in Task 3 focused on identification of changes in primary production and weakness areas in *Salmonella* control programs that might lead to the increase in *Salmonella* incidents. Information generated in WP2 will be used to inform tasks carried out in WPs 3, 4 and 5. Data from NCSP in the poultry reservoir will be compared with those obtained in humans in the analyses planned in WP3. Whole genome sequencing of isolates retrieved from samples collected on farms in this WP will be part of the molecular genomic investigation performed in WP4. Finally, the comparative analysis of NSCP and sensitivity of surveillance activities among MSs will be exchanged with WP5 for informing the Multi-Criteria Decision Analysis (MCDA) modelling approach that will be carried out in WP5.

JPR26-WP2-T2.1 Comparative analysis of *Salmonella* controls and management measures at MSs level
Task start month 25
Task end month 48
Task Leader: Julio Alvarez Sanchez (VISAVET-UCM)
Deputy Task Leader[1]: Jowita S. Niczyporuk (PIWET)
Task Participants: ANSES, APHA, PIWET, VISAVET-UCM, WVBR
Description of the task[1]:
This task aims at the characterization and analysis of data from NSCPs, which can help to understand the trends in prevalence of certain serotypes in certain hosts and the differences between MSs. We will perform a comparative analysis of the *Salmonella* control plans in the poultry sector in the various participating countries with a particular focus on sampling plans and management measures. Analysis will be focused on serotypes target of control plans (Enteritidis, Typhimurium (including the monophasic variant), Infantis, Virchow and Hadar but also on other serotypes with an increasing relevance (such as *S*. Kentucky). Historical data on controls performed by competent authorities (official control) versus those carried out by food business operators (own checks) will be compared at the member state and host (i.e., breeding flocks, layers, broilers, turkeys) to assess the sensitivity of surveillance activities. The analysis of epidemiological investigations in outbreaks will be carried out when information is available to verify the application of prevention and management measures. We will trace the vaccination programs in the different MSs taking into account the type of live vaccines used.

Furthermore, and given that eggs and egg products are major factor in the epidemiology of S. Enteritidis, data from monitoring programs in eggs will be analyzed for participating countries with available information. This will allow the evaluation of the correlation between the prevalence and diversity of *Salmonella* serotypes in eggs and that observed in poultry based on official data.

JPR26-WP2-T2 Comparative analysis of *Salmonella* controls and management measures at farm level
Task start month 25
Task end month 48
Task Leader: Dariusz WASYL (PIWET)
Deputy Leader: Becky GOSLING (APHA)
Task Participants: ANSES, APHA, PIWET, VISAVET-UCM, WVBR
Description of the task[1]: Interrupting the Salmonella transmission cycle in infected farms after a *Salmonella* positive flock has been identified and removed is a critical point in the control and

prevention in poultry. This task aims to characterize the control and prevention procedures that are currently implemented on farms among the MSs.
ANSES and APHA will perform in Farms sampling campaign (ST2.1). ANSES, APHA, PIWET, VISAVET-UCM, WVBR will contribute to the elaboration and collection of questionnaires to evaluate flock management and control measures (ST2.2).

JRP26-WP2-T2-ST1 Field studies during outbreaks and sensitivity testing

SubTask Leader: Sophie LEBOUQUIN-LENEVEU (ANSES)
SubTask Participants: ANSES, APHA, VWBR
This sub-task focuses on epidemiological field surveys that will be carried out during outbreaks. Outbreak isolates will be typed and sequenced to help tracing *Salmonella* in the environment of the investigated flocks, including potential sources as other animal productions in the vicinity of the outbreaks (Anses, cf. WP4). .
APHA has a sample sensitivity model developed for laying flocks, the one referenced in the Arnold paper. APHA and Anses propose to update the outputs using information provided by sampling in infected flocks.

JRP26#-WP2-T2-ST2 On farm surveys

SubTask Leader: Jowita S NICZYPORUK (PIWET)
SubTask Participants: PIWET, VISAVET-UCM, VWBR, ANSES, APHA
A harmonised questionnaire on farm management, biosecurity, C&D, health status, and *Salmonella* testing result history will be prepared by all involved partners and then submitted to the stakeholders involved through different channels (such as poultry producer associations, veterinary associations, etc.). We will evaluate flock management and possible insufficiencies in control measures implemented to date in laying hen farms, in particular related to the implementation of vaccination programmes as well as the application of strict farm hygiene controls and sensitivity of the statutory sampling implemented in commercial laying hen flocks.

JPR26-WP3 Surveillance, epidemiology and source attribution
WP start month: 25
WP end month: 48
WP Leader: Roan PIJNACKER (RIVM)
Deputy WP Leader: Nina VAN GOETHEM (Sciensano)
WP participants: RIVM, Sciensano, AGES, INIAV, INSA, ISS, Pasteur, VISAVET

Description of the WP:
This WP will take place during the first and second year of the project and consists of three tasks. Because the recent plateau in human *S.* Enteritidis cases could be related to changes in human surveillance systems, this WP aims to evaluate MS national surveillance systems for *S.* Enteritidis in humans using an adapted version of the 2014 ECDC evaluation framework of public health surveillance systems. We will also assess changes over time regarding epidemiological characteristics, including demographics, severity of symptoms, food consumption patterns, sources of infection (using source attribution models), and fraction AMR among clinical isolates. Assessment of the power of the current monitoring program to capture the underlying variability in AMR phenotypes in humans and animals will be assessed by rarefaction analysis to estimate the richness of resistotypes. Furthermore, an exposure assessment of S. Enteritidis in table eggs will be performed to calculate the total reservoir output load before and after the levelling of the decreasing trend. We will strongly liaise with a possible funded project on FBZ2.1 where source attribution models are refined for Salmonella and a meta-analysis of performed case-control studies is planned which will give insights in possible changes in transmission routes.

JPR26-WP3-T1 Evaluation of surveillance systems in humans

WP start month: 26

WP end month: 48
Task Leader: Nina van GOETHEM (Sciensano)
Deputy Leader: Roan PIJNACKER (RIVM)
WP participants: RIVM, Sciensano, INSA, Pasteur, VISAVET-UCM, AGES

Description of the task: National surveillance systems for S. Enteritidis of three countries will be evaluated (three scenarios); one country where a decrease in the number of human salmonellosis cases has been observed, one country where it remained stable, and one country where it increased since 2014 as compared with before 2014. The surveillance systems will be described and evaluated in the 2014-2019 period and the 2008-2013 period using an adapted version of the 2014 ECDC evaluation framework of public health surveillance systems, focusing only on those surveillance attributes that could potentially affect the number of captured human S. Enteritidis cases. In the first year, surveillance systems will be described regarding several key elements, including surveillance objectives, surveillance category (e.g. event surveillance, monitoring), case definition(s), diagnostic tests used, data sources/flow, surveillance network (i.e. participants), population under surveillance, geographic coverage, period and frequency data collection, and type of surveillance (e.g. passive/active, compulsory/voluntary).

JPR26-WP3-T2 Assessment of changes in the epidemiology of human *S.* Enteritidis cases and other relevant serovars
WP start month: 29
WP end month: 48
Task Leader: Roan PIJNACKER (RIVM)
Deputy Leader: Angela PISTA (INSA)
WP participants: RIVM, INSA, INIAV, VISAVET-UCM

Description of the task: In the first and second year of the project, we will make an assessment of changes in the epidemiological characteristics and transmission routes of *S.* Enteritidis cases and other serovars relevant for public health. For the period 2008–2013 and 2014–2019, we will describe demographics (age, gender, etc.), severity of symptoms (defined by hospitalization or death), and the fraction of antimicrobial resistant isolates. For this purpose, we will use the ECDC TESSY dataset on human salmonellosis cases, complemented with national surveillance data if deemed necessary. Assessment of the power of the current monitoring program to capture the underlying variability in AMR phenotypes in humans and animals will be assessed by rarefaction analysis to estimate the richness of resistotypes. Moreover, we will compare food consumption patterns between these two periods based on production data and import/export data of food products. Although this has recently been done by EFSA (report “Salmonella control in poultry flocks and its public health impact”, published on 26 January 2019), these results were not stratified for different time periods, and hence, cannot be used to assess differences in consumption patterns over time. Lastly, source attribution will be performed using the modified Dutch model to identify the most likely sources of human *Salmonella* infection, likewise stratified by 2008 – 2013 and 2014 – 2019. For this purpose, we will use WGS data on *S.* Enteritidis in reservoirs, humans and food products. We will strongly liaise with a possible funded project on FBZ2.1 where source attribution models are refined for *Salmonella* and a meta-analysis of performed case-control studies is planned which will give insights in possible changes in transmission routes.

JPR26-WP3-T3 Assessment of human exposure to *S.* Enteritidis
WP start month: 32
WP end month: 48
Task Leader: Eric EVERS (RIVM)
WP participants: RIVM, WbvR

Description of the task: An exposure assessment of S. Enteritidis in table eggs will be performed for the three countries identified in WP3-T1 to calculate the total S. Enteritidis output load before and after the levelling of the decreasing trend. We will apply a simple exposure assessment approach such as sQMRA, deterministic or stochastic depending on data availability and applicability. The exposure assessment will start after the laying of the egg, or at the Salmonella status of the laying hen (flocks), and will end at the moment of consumption of the egg. It will include storage at the egg packing stations, transport to retail, and storage and preparation at the consumers' home. Data availability again might limit the calculations, however pre-calculation sensitivity analysis will indicate which data are relevant to calculate the human S. enteritidis exposure. Several aspects that play a role in the human exposure might have changed, such as the egg consumption rate, the way eggs are prepared, and storage times. Also, egg production rates in countries with different S. enteritidis egg prevalences might have changed, and import/export of eggs between EU countries or from/to outside the EU. The final output will be, per country, the S. Enteritidis prevalence and numbers in table eggs, and the total S. enteritidis load, at the moment of egg consumption.

JRP26-WP4 *Salmonella* Genomics
WP start month: 25
WP end month: 54
WP Leader: Eva Litrup (SSI)
Deputy WP Leader: Maria Pardos de La Gandara (IP)
WP participants: SSI, IP, RIVM, Piwet, Sciensano, WbvR, PHE, APHA, INSA, INIAV, ANSES, VISAVET, AGES, ISS
Description of the WP:
WP4 takes place over the first, the second and the third year of the project.
WP4 will investigate if one of the drivers of the recent increase in salmonellosis incidence could be related to the genetic variation of the bacterium. Emergence or clonal expansion of specific bacterial strains with increased fitness in the form of increased bacterial division, increased virulence, or antibiotic resistance can all impact the success of a clone. WP4 will provide an overview of the population structure of *S*. Enteritidis and perform comparative genomics regarding presence of virulence genes, (pro)phages, plasmids, replicons and AMR profiles in isolates representing the investigated time period. In addition, WP4 will elucidate the long and short term evolution and emergence of different clones and specific traits over time, as well as a potential increase in the bacterial effective population size. Finally, an investigation into possible differences in phenotype will be carried out and genomes will be analysed to elucidate any genomic differences that can be related to these phenotypes
SSI is WP lead and IP is deputy WP lead. All partners will supply Salmonella sequences from recent years and sequence historic strains from their respective strain collections and most partners will participate in bioinformatics analysis. Furthermore, some partners will take part in the phenotypic testing and mutant creation.
JRP26-WP4-T1 Collection overview
Task start month: 25
Task end month: 30
Task Leader: Christian Kornschober (Ages)

Deputy Task Leader: Michel -Yves Mistou (ANSES)
Task Participants: All institutes

Description of the task:
Task 1 takes place over the first year of the project.
Create an overview of WGS data available at all partners and in public repositories and determine gaps in data and possible strains to sequence at different partner institutes. The change in Salmonella incidence has varied over time and has been slightly varying in countries, these differences will be taken into account when sampling strains to sequence.

JRP26-WP4-T2 Population structure and comparative genomics
Task start month M25
Task end month M48
Task Leader: Eva Litrup (SSI)
Deputy Task Leader: Maria Pardos de La Gandara (IP)
Task Participants: SSI, IP, RIVM, Piwet, Sciensano, PHE, APHA, INSA, INIAV, ANSES, VISAVET, AGES, ISS

Description of the task:
Task 2 takes place over the first and the second year of the project.
Based on the large number of genome sequences available in public repositories and sequenced genomes collected or sequenced in Task JRP26-WP4-T1 we will analyze the population structure of S. Enteritidis through WGS, both at European level and at global level, before and after the decrease of *Salmonella* in Europe. Using established methods, we will additionally perform comparative genomics to search for the emergence and the prevalence of virulence genes, prophages, plasmids, replicons and AMR mechanisms.

JRP26-WP4-T3 Phylodynamics and Phylogeography
Task start month: 31
Task end month: 51
Task Leader: Eelco Franz (RIVM)
Deputy Task Leader:
Task Participants: SSI, IP, RIVM, Piwet, Sciensano, PHE, APHA, INSA, INIAV, ANSES, VISAVET

Description of the task:
Task 3 takes place over the first, the second and the third year of the project.
A combination of Bayesian and least-square methods will be used to elucidate the long and short term evolution and emergence of different clones over time, as well as a potential increase in the bacterial effective population size. The reconstruction of the ancestral state of specific biological discrete traits, e.g. AMR, virulence factors, or factors identified in Task JRP26-WP4-T2 will pinpoint the time of emergence of these traits.

JRP26-WP4-T4 Mutant creation and testing including GWAS studies
Task start month: 31
Task end month: 54
Task Leader: Eva Litrup (SSI)
Deputy Task Leader Michel-Yves Mistou (ANSES)
Task Participants: SSI, IP, Piwet, Sciensano, APHA, INSA, INIAV, ANSES, ISS

Description of the task:
Task 4 takes place over the first, the second and the third year of the project.

Following the bioinformatics studies in task JRP26-WP4-T2 and JRP26-WP4-T3, phenotypic testing will be performed on a range of strains identified during phylogenetic analysis of the population. We will identify possible targets responsible for fitness change in collaboration with JRP26-WP4-T2. The targets will be knocked-out and mutants will be tested in different phenotypic test, stress responses and fitness tests.
Furthermore GWAS studies will be performed to identify the possible genetic elements responsible for phenotype changes, and furthermore studies will be performed to possibly predict the genetic elements associated with the increased incidence of SE, based on the year of isolation.
Sub-Tas GWAS studies
Sub-Task start month: 37
Sub-Task end month: 54
Sub-Task Leader: Nicolas Radomski (ANSES)
Sub-Task Participants: SSI, IP, Piwet, Sciensano, APHA, INSA, INIAV, ANSES, ISS

WP5
WP: JRP26-WP5 MCDA model to support priority setting
WP start month 28
WP end month 52
WP Leader: Lapo Mughini-Gras (RIVM)
Deputy WP Leader: Nina van Goethem (Sciensano)
WP participants: RIVM, VBVR, Sciensano
Description of the WP: WP5 takes place over the first, the second and the third year of the project, and it consists of 2 tasks. It is possible that no single factor will explain the observed changes in S. Enteritidis incidence in humans and poultry, but that the reasons for these changes will turn out to be multifactorial and interconnected in nature. For priority setting of interventions, it is important to provide clear indications on the relative contributions of these factors and their interrelations. To do so, we plan to use a Multi-Criteria Decision Analysis (MCDA) modelling approach to operationalize an assessment framework for the identified determinants. Indeed, these determinants cannot be weighted (as such) against one another, as they have different units and measurement scales. Yet, their values can be converted into comparable dimensions using MCDA methods. MCDA provides stepping-stones and techniques for finding a compromise solution to complex issues and processes where multiple conflicting criteria are to be considered when assessing different options. In our case, we will use MCDA to assess how determinants are related to one another and which ones are more likely to have played a significant role in the in reversal of the decreasing trend of S. Enteritidis, i.e. to identify a trade-off among the possible effects of control measures at primary production, changes in surveillance, epidemiological and exposure patterns, and pathogen evolution.

Task: JRP26FBZ-4-WP5-T1 Framework building
Task start month 28
Task end month 48
Task Leader: Lapo Mughini-Gras (RIVM)
Deputy Task Leader: Nina van Goethem (Sciensano)
Task Participants: RIVM, Sciensano, WbvR
Description of the task: This task takes place during the first 2 years of the project. For the MCDA to be performed, an assessment framework for the different possible determinants will be built. This will consist of building a (hierarchical) structure of the transmission chain and all possible interrelations among primary production, exposure, and pathogen characteristics. After identifying all relevant nodes and their interconnections, as well as selecting the criteria (e.g. vaccine efficiency/coverage, efficiency surveillance systems, food consumption patterns, genetic variability

of S. Enteritidis strains, etc.) for these nodes, importance weights will be assigned by involving experts on the topic using a using the swing-weighting method.

Task: JRP26FBZ-4-WP5-T2 MCDA modelling
Task start month 36
Task end month 52
Task Leader: Lapo Mughini-Gras (RIVM)
Deputy Task Leader: Roan Pijnacker (RIVM)
Task Participants: RIVM, Sciensano, WbvR
Description of the task: This task takes place during the 3 years of the project. Here we will implement a hierarchical weighting through a method called Analytic Hierarchy Process (AHP). The AHP method requires the major criteria to be split into sub-criteria, possibly in a recursive manner. Criteria (leaves) in the framework structure (tree) can be further split into finer ones. Different scenarios for possible public health interventions to be evaluated (e.g. increasing/modifying sampling schemes at primary production, increasing vaccination, changing reporting procedures, reduce exposure to specific food products, etc.) need to be scored on all criteria that are not further subdivided. Saaty's weighting method based on pairwise evaluations will then be used. The advantage of AHP pairwise comparisons is that there is no need to convert all valuations into one unit, e.g. costs, rates, etc. One just needs to specify what is judged to be more important (pairwise) at the level of alternative criteria inside the criterion structure. There are many software available to perform this analysis (including R, among others), and input/output procedures can be implemented in a web-based interface and summarized in graphics and/or (Excel or HTML) tables. Extensions of the method are fuzzy set implementations (also in R) that would allow us to cope with uncertainty into the pairwise comparisons.

Deliverables

Ref	Title	Due month
D-JRP26-WP1.1	Detailed work plan developed (Kick-off meeting minutes)	28
D-JRP26-WP2.1	Report on the evaluation of the questionnaires	30
D-JRP26-WP3.1	Description of surveillance systems regarding their key elements	36
D-JRP26-WP3.2	Description of basic epidemiological characteristics of human *S.* Enteritidis cases and other relevant serovars	36
D-JRP26-WP4.1	Sequence inventory report	31

Milestones

Ref	Title	Due month
M-JRP26-1	Kick-off meeting organised	26
M-JRP26-2	Communication partners mapped	28
M-JRP26-3	Inventory of documents available for the comparative analyses	30
M-JRP26-4	Farm survey planed and questionnaires developed	30
M-JRP26-5	Selection of three countries of which their national *S.* Enteritidis surveillance systems will be evaluated	27
M-JRP26-6	Obtained national surveillance data on *S.* Enteritidis and other relevant serovars	30
M-JRP26-7	Sequence collection to be shared amongst partners	31

M-JRP26-8	Phylogenetic trees describing the sequence collection	32
M-JRP26-9	Inventory of MCDA methods suited to the data being collected.	30

2.4.2.1.30 JRP24-R2-FBZSH9-BeONE

Reference to the Stategic Research Agenda (please refer to D2.7)			**FBZ-SH 9: Better tools for detection and investigation of foodborne outbreaks, including antimicrobial resistant pathogens, as well as economic assessments of potentially increased cluster detection through whole genome sequencing**							
Research Project Title			**Building integrative tools for OneHealth Surveillance**							
Research Project Acronym			**BeONE**							
Leading Organisation			**P13-SSI**			Deputy Leading Organisation			**P30-RIVM**	
Project Leader			**Kristoffer Kiil**			Deputy Project leader			**Claudia Coipan**	
Project Start month			**M25**			Project End month			**M54**	
Participant	1	2	4	6	7	8	**9**	**10**	**11**	**12**
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	**BfR**	**FLI**	**RKI**	**DTU**
PM							**11**	**0.6**	**0.6**	**4**
Participant	**13**	14	15	16	17	18	19	20	**21**	**22**
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	**PHE**
PM	**26**								**0.2**	**1**
Participant	23	24	25	26	27	**28**	29	**30**	31	**32**
	UoS	OKI	NUIG	TEAGASC	ISS	**IZSAM**	IZSLER	**RIVM**	WbvR	**FHI**
PM						**4.5**		**10.67**		**0.58**
Participant	**33**	**34**	35	**36**	37	38	39	40	41	
	NVI	**PIWET**	INIAV	**INSA**	IISPV	IC	SLV	FOHM	SVA	
PM	**1.5**	**6**		**23.01**						

Objectives

- Establish state of the art M6
- Create datasets M8
- Establish method comparability M24
- Create a conceptual model for outbreaks M10
- Integrated model for outbreak detection/delineation using genomic and epidemiological data M22
- Guideline for cluster investigation using integrated data M24
- Database layout for national system M10
- Ontology based metadata standardization system M24
- Ad-hoc data sharing system M24

- Pipeline for strain characterization M12
- Dashboard M18
- Dashboard collaboration and analysis tool M24
- Framework for testing and feedback M4
- Successfully completed simulated outbreak trial M28

Description of work

WP1: Typing comparability and nomenclature

WP start month: 1
WP end month: 24
WP Leader: Vítor Borges, INSA(36)
Deputy WP Leader: Claudia Coipan, RIVM (30)
WP participants: SSI (13), RKI (11), IZSAM (28), RIVM (30), Piwet (34), FLI (10), APHA (21), DTU (12), FHI (32), PHE (22), NVI (33), BfR (09), INSA (36)
WP1 takes place over the first and second year of the project
Description of the WP:
WP1 lays a foundation for the work carried out in WPs 2, 3 and 4. It establishes state of the art within the areas covered by the BeONE project, building on findings from the ORION and COHESIVE projects, and doing additional research as necessary. It compiles the necessary datasets for both software testing in WPs 3 and 4, and model training, testing, and validation in WP 2. Finally, it explores the comparability of the clustering methods for various purposes by comparing cluster congruence at different scales.

Task: BeONE-WP1-T1 Establishment of state of the art
Task start month: 1
Task end month: 6
Task Leader: Vítor Borges, INSA (36)
Deputy Task Leader: Karin Lagesen, NVI (33)
Task Participants: INSA (36), RIVM (30), NVI (33), BfR (09)
T1.1 takes place over the first year of the project.
Description of the task:
The objective is to provide an overview of the genomics methods for WGS-based typing (core SNV, gene-by-gene, etc.) and existing solutions for each method, and provide a state of the art on the current need to:
i) ensure comparability between distinct methodologies to analyse, interpret and communicate WGS-derived data;
ii) establish thresholds at several levels (for different “epidemiological” goals) that can be the basis for efficient nomenclature systems;
iii) use relative cut-offs (i.e., ADs divided by the number of shared/analysed loci) instead of absolute cut-offs (ADs) in order to promote not only a better comparison across distinct datasets or between data collected with different cgMLST schemas for the same species, but also to facilitate the comparison across distinct species since each organism has rather different number of loci in gene-by-gene schemas;

Task: BeONE-WP1-T2 Dataset selection and curation
Task start month: 1
Task end month: 8
Task Leader: Vítor Borges, INSA (36)
Deputy Task Leader: Eva Litrup, SSI (13)
Task Participants: SSI (13), RKI (11), IZSAM (28), RIVM (30), Piwet (34), FLI (10), APHA (21), DTU (12), FHI (32), PHE (22), NVI (33), BfR (09), INSA (36)

T1.2 takes place over the first year of the project
Description of the task:
The objective is to compile and curate datasets for the different research and development activities within the project. Datasets consist of sequence reads and assemblies from strains and a wide array of associated metadata, depending on the sample source. Strains are collected with two aims in mind: 1) establishing a background dataset by capturing the genomic diversity within samples from all "one health" sectors, and 2) selecting sets of epidemiologically verified outbreak isolates and their immediate background, to train, test and validate models for outbreak delineation. Both a full dataset and a reduced test dataset will be compiled. All data will be obtained in anonymized form to protect the privacy of the entities from which the samples were isolated.

Sub-Task: BeONE-WP1-T2-ST1 Dataset selection and collection
Sub-Task: BeONE-WP1-T2-ST2 Quality check and assurance, and genome assembly

Task: BeONE-WP1-T3 Clustering congruence and thresholds
Task start month: 1
Task end month: 24
Task Leader: Vítor Borges, INSA (36)
Deputy Task Leader: Claudia Coipan, RIVM (30)
Task Participants: SSI (13), RKI (11), IZSAM (28), RIVM (30), Piwet (34), FLI (10), APHA (21), DTU (12), FHI (32), PHE (22), NVI (33), BfR (09), INSA (36)
T1.3 takes place over the first year and the second year of the project.
Description of the task:
The objective is to assess the cluster agreement at different threshold levels between different WGS-based typing approaches (e.g., cg/wgMLST; SNP-based), and to establish levels of resolution (i.e. threshold ranges) for different approaches that may correlate with distinct epidemiological settings, with focus on the resolution levels fitting the needs for long-term routine surveillance. The first step is to submit datasets of whole genomes sequences to typing based on various methods (SNP/allele matrices, etc.). Cluster generation will be then performed at different threshold levels with the same clustering methodology (e.g., goeBURST) in order to assess cluster stability individually for each selected WGS-based typing method (e.g., identify Phase I and II in nAWC graphs; Llarena et al, 2017;). This will allow defining, for each method, ranges of thresholds of similarity fitting the needs of the two settings of surveillance (Phase I: outbreak investigation; Phase II: long-term surveillance) and that may constitute the basis for robust hierarchical nomenclature systems. This task culminates with a measure of clustering concordance (e.g., Adjusted Rand and Wallace coefficients) between distinct typing methods at the defined levels and its correlation with existing hierarchical nomenclature.

Sub-Task: BeONE-WP1-T3-ST1 Selection of WGS-based typing methods to be evaluated
Sub-Task: BeONE-WP1-T3-ST2 Assessing clustering congruence between different methods at different hierarchical levels
Sub-Task: BeONE-WP1-T3-ST3 Correlating clustering congruence with existing nomenclature schemes

WP2: Joining molecular and epidemiological methods
WP start month: 1
WP end month: 24
WP Leader: Claudia Coipan, RIVM (30)
Deputy WP Leader: Vítor Borges, INSA (36)
WP participants: RIVM (30), INSA (36), SSI (13), PHE (22), NVI (33),RKI (11), APHA (21), PIWET (34)

WP2 takes place over the first and the second year of the project.
Description of the WP:
The objective is to generate an algorithm for outbreak detection that would incorporate both genomic (molecular) and epidemiologic data of the bacterial isolates. This algorithm would benefit public health, as it would allow for a comprehensive, multiple evidence-based, infectious disease outbreak investigation in a reproducible manner. At genetic/genomic level, detection of infectious disease outbreaks is based on quantifying the relatedness of the microbial isolates either at allele or at SNP level. The smaller the distance between any two isolates, the higher the chance that they share a recent common ancestor. At epidemiologic level, a disease cluster is traditionally defined as high incidence of patients with symptoms triggered by the same etiologic agent, and occurring at small intervals both spatially and temporally. The use of questionnaires further facilitates identification of exposure to same/similar risk factors. It is possible, however, to encounter genetically related microorganisms that do not come from the same source (convergent evolution, recombination, etc), as it is also possible to encounter isolates with larger genetic distances that still share a common source (variable within host mutation rate, quasispecies, etc). It is also possible to have a collection of patients with similar symptoms, etiologic agents, location, all within a short time interval, that are still not related epidemiologically (various microbial types, various sources, etc). The complementary use of epidemiological and genetic approaches in WP2 will take into account these aspects with the aim to provide new integrative algorithms for accurate identification of the related disease cases that might constitute an outbreak.

Task BeONE-WP2-T1: Conceptualization of epidemiological and biological factors impacting on fine resolution clustering and outbreak detection
Task start month 1
Task end month 10
Task Leader: Claudia Coipan, RIVM (30)
Deputy Task Leader: Vítor Borges, INSA (36)
Task Participants: RIVM (30), INSA (36), SSI (13), PHE (22), RKI (11)
T2.1 takes place over the first year of the project
Description of the task:
The objective of this task is to make an inventory of the factors, and the relations hereof, affecting the outbreak detection and the consequences for choosing/or not a threshold for outbreak delineation. This task will be primed at the project's kick-off meeting by an all partners' brainstorming session on the factors affecting outbreak detection. Based on literature survey and prior knowledge of the consortium partners, we aim to make a conceptual model of all the biological and epidemiological factors that impact on the ability to detect relatedness of microbial isolates. By the end of month 8 in the project we expect that the most relevant factors will be known. The expected deliverable of this task is drafting the manuscript of an opinion paper on the conceptual model. The factors found to be critical for outbreak detection will be explored in T2.2 by studying examples of bacteria with various evolutionary patterns.

Task BeONE-WP2-T2: Integrating genomics with epidemiology
Task start month 9
Task end month 22
Task Leader : Claudia Coipan, RIVM(30)
Deputy Task Leader: Vítor Borges, INSA (36)
Task Participants: RIVM(30), INSA(36), SSI(13), PHE(22), NVI(33), PIWET(34), APHA (21)
T2.2 takes place over the first and second year of the project.
Description of the task:
The objective of this task is to generate an algorithm that would allow outbreak detection by integrative use of genomic and epidemiologic data. The main activities of the task will be testing

the clustering methods designed in WP1 on datasets of four pathogens and testing the congruence with the associated epidemiological data. We envision this task as best performed if divided in four subtasks which will be performed in parallel and will each correspond to one of the selected gastrointestinal pathogens: *Campylobacter jejuni*, Shiga-toxin producing *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. The rationale for this is that although we aim for an optimum approach that would suit as many pathogens as possible it will still probably be challenging to find a one size fits all algorithm, given the variability of the envisaged pathogens. By fast tracking T2.2 we also ensure an optimal division of the human resources among the project partners, and therefore a schedule compression that will allow the completion of the task in due time.
The datasets compiled in WP1 will be used to compare the epidemiologic clusters with the genomic clusters of the microbial isolates, which will take place over the second year of the project. While the scientific literature describes a plethora of approaches for clustering microbial isolates at high resolution genomic level, e.g. established thresholds, internal validity indices, Dirichlet processes, Bayesian Gamma/Poisson models of substitution rate, as well as for identifying epidemiologic clusters, e.g. spatial autocorrelation , space-time scan statistics, a systematic comparison of the approaches and of their integrated results has not been yet attempted and validated against a large collection of bacterial isolates. For each of the four pathogens various genomic and epidemiologic clustering methods will be tested. The integration hereof might be done either by finding an optimum level of concordance between the two, or by incorporating them as variables in a multivariate analysis or evidence synthesis. The robustness of the outbreak detection algorithm will be tested by K-fold cross-validation within this WP, but also in independent analyses performed by each partner on own dataset, within WP5.
Since the generation of the outbreak detection algorithm is important for harmonization of epidemiological research among participating countries and it will feed T2.3 and T5.2, this algorithm will be a milestone in the project. The results of this task will be used to generate guidelines (T2.3) for outbreak detection. Both, the algorithm designed here and the guidelines will make the object of an original research paper to be submitted for publication in a peer-reviewed journal of specialty by the end of the project. To ensure the algorithm and the guidelines are functioning as desired we will try to have a provisional version of them by month 18. Following feed-back from the partners using the algorithm and the guidelines on their own datasets we will produce a final version hereof.

Sub-Task: BeONE-WP2-T2-ST1 Comparison of the epidemiologic clusters with the phylogenetic tree of the Campylobacter jejuni isolates
Sub-Task: BeONE-WP2-T2-ST2 Comparison of the epidemiologic clusters with the phylogenetic tree of the Shiga toxin producing Escherichia coli (STEC) isolates
Sub-Task: BeONE-WP2-T2-ST3 Comparison of the epidemiologic clusters with the phylogenetic tree of the Salmonella enterica isolates
Sub-Task: BeONE-WP2-T2-ST4 Comparison of the epidemiologic clusters with the phylogenetic tree of the Listeria monocytogenes isolates

WP3: Storage, management, and sharing for meta- and molecular data
WP start month: 1
WP end month: 30
WP Leader: Carlus Deneke, BfR (09)
Deputy WP Leader: Kim Ng, SSI (13)
WP participants: SSI (13), NVI (33), IZSAM (28), FLI (10), RKI (11), BfR (09), DTU (12)
WP3 takes place over the first, the second and the third year of the project.
Description of the WP:
In WP3, the results and experiences from existing projects in this field will be discussed and evaluated to generate a basis for further work. Based on this, a data sharing and management

concept will be designed and implemented. The main aim is to develop means to share content on decentralized instances of the same database system, rather than to build a central pan-national database. The database contains high-level molecular analysis data (as determined in WP 1) and relevant epidemiological metadata. For the latter, standardization and the use of ontologies is key and will be evaluated and integrated.
Automatic programming interfaces (APIs) for the communication with the database and thereby integration with existing national systems will be developed. In particular, a fully integrated data acquisition and sharing system will be implemented for an example molecular analysis workflow. Additionally, the data sharing model will be extensively tested and evaluated in a national pilot.

Task: BeOne-WP3-T1: Evaluate national level data sharing
Task start month 1
Task end month 10
Task Leader: Armando Giovannini, IZSAM (28)
Deputy Task Leader: Carlus Deneke, BfR (09)
Task Participants: NVI (33), IZSAM (28), INSA (36), BfR (09)
T3.1 takes place over the first year of the project.
Description of the task:
Discuss national experiences from different member states to get an overview over the national requirements and expectations for data sharing. In particular, results, obstacles, and opportunities from projects such as Innuendo or the EJP project COHESIVE will be taken into account.

Task: BeOne-WP3-T2: Metadata acquisition and standardisation
Task start month 1
Task end month 24
Task Leader: Simon Tausch, BfR (09)
Deputy Task Leader: Jörg Linde, FLI (10)
Task Participants: NVI (33), FLI (10)
T3.2 takes place over the first and second year of the project.
Description of the task:
The aim is the standardization of all steps of data processing (from data acquisition to result interpretation). Where different sectors (i.e. human, animal, food, epidemiology) intersect, finding a common language is often difficult. Standardized and comparable metadata will assist collaborative outbreak investigation. Within this task, BeOne will integrate and expand existing ontologies (coordinating with e.g. ORION and EFSA terminology) and develop modules and infrastructure to use it in BeONE.

Sub-Task: BeONE-WP3-T2-ST1 Metadata acquisition
Agreement between stakeholders on what is minimal and optimal set of metadata for outbreak analysis. This set of metadata will be collected for the shared outbreak data set in WP2.
Define a minimal set of necessary metadata that are relevant for outbreak investigation.
Wherever possible, controlled data entry (e.g. drop-down lists with ontologies) will be used to prevent ambiguities.
Sub-Task: BeONE-WP3-T2-ST2 Standardisation using ontologies
As this is a multi-language consortium with users and data from different fields and countries, it will use existing approaches for metadata ontologies (e.g. GenEpiO, FoodOn, ORION WP3).
Here, we evaluate and expand state of the art in 2020 of metadata ontology systems (EFSA scientific terminology, Orion ontology, GenEpio, FoodOn, Cohesive DoUtDes). Next, we apply of the selected ontology system to encode consortium's metadata.

Task: BeOne-WP3-T3: Database design and implementation

Task start month 1
Task end month 30
Task Leader: Kim Ng, SSI (13)
Deputy Task Leader: Simon Tausch, BfR (09)
Task Participants: SSI (13), BfR (09), IZSAM (28), NVI (33), DTU (12)
T3.3 takes place over the first, the second and the third year of the project.
Description of the task:
To have a database structure to store molecular data and epidata in a decentralized manner while structuring the data for sharing via the dashboard (WP4). Goal: Creation of a flexible decentralized database design which can be implemented on both a private and shared database system with expected structure generated from the analysis workflow WP2 and structured for the dashboard WP. The private database will be used inside each institute and may contain metadata and molecular data which is not (yet) shared with the consortium/public. By using the same database structure and ontologies, data can later be easily shared with selected partners.

Sub-Task: BeONE-WP3-T3-ST1 Determine and implement data structure for database
Determine data structure for database (with both molecular and epi data informed by WP1, WP2 and considerations from other projects including (COHESIVE, other existing national solutions). A potential database implementation which can solve this would be a shared NoSQL mongoDB.
Sub-Task: BeONE-WP3-T3-ST2 Implement API for import of data
Sub-Task: BeONE-WP3-T3-ST3 Data porting from an existing pipeline
Sub-Task: BeONE-WP3-T3-ST4 Data porting from further pipelines
Sub-Task: BeONE-WP3-T3-ST5 Expansion of the API for referencing/exporting of entries in other reference databases
Expansion of the API for import/export of data from one database to another. This will include considerations for anonymizing metadata (e.g. coarse graining location data) to factor in various sharing policies, and will be agreed on by all parties involved.
Sub-Task: BeONE-WP3-T3-ST6 Expansion of the API for queries from the dashboard WP4

WP 4: Development of a user-oriented interface for analysis and sharing of epi and molecular data
WP start month: 1
WP end month: 30
WP Leader: Kim Ng, SSI (13)
Deputy WP Leader: Rolf Sommer Kaas, DTU (12)
WP participants: BfR (09), SSI (13), DTU (12)
WP4 takes place over the first, the second and the third year of the project.
Description of the WP:
The aim of WP4 is to make a dashboard that interacts with the database so that members can utilize the tool for comparing isolates from different institutions against molecular and epidata in order to assist in surveillance. The goal is the implementation of a dashboard that can be applied against either private databases or shared databases for visualizing samples on various metrics including Geography, Phylogeny, Time, Metadata, etc. With the underlying database design from WP3 the dashboard can be run on all databases it is allowed to connect to. That means that the sharing of samples will be handled on the database level. However, the dashboard can inform users on where to request information from. The program will be open source and designed utilizing existing tools and platforms where reasonable (e.g. Dash/Plotly) with a plan to be web-based to leverage existing tools and interactivity. The dashboard should allow working spaces for members and request analysis to different servers. Centralized analytical components will also be done in this work package alongside the dashboard. Depending on technical challenges inspiration and aspects of existing packages including MicroReact, Signale, and NextStrain will be used.

Task: BeONE-WP4-T1 Dashboard
Task start month: 1
Task end month:18
Task Leader: Kim Ng, SSI (13)
Deputy Task Leader: Simon Tausch, BfR (09)
Task Participants: BfR (09), SSI (13), DTU (12), RKI (11)
T4.1 takes place over the first and second year of the project.
Description of the task:
Basic dashboard design and implementation. Results in an interactive read only view of the data in the database. Provides elements for selection and viewing of data based on Time, Geography, Phylogeny/clusters, Sample metadata, and predicted isolate phenotypes (ie AMR profile).

Sub-Task: BeONE-WP4-T1-ST1 Core display components
Sub-Task: BeONE-WP4-T1-ST2 Component integration

Task: BeONE-WP4-T2 Back end analysis implementation
Task start month: 1
Task end month:12
Task Leader: Rolf Sommer Kaas, DTU (12)
Deputy Task Leader: Kim Ng, SSI (13)
Task Participants: DTU (12), BfR (09), SSI (13)
T4.2 takes place over the first year of the project.
Description of the task:
The dashboard and database is independent of analysis pipelines, thereby making it possible for labs to employ the analysis tools that fit their needs. However, for the tools employed to be compatible with the database they must use an agreed upon format and report information required to do the necessary QC.
It will be the aim of this task to create a standardized format for input data to the database, based on the JSON format. Along with the format will also follow some minimum technical parameters that must be reported, so that versions of databases and software can be tracked, with the intention of making all reported analysis reproducible. In order to load the database with the results from the analysis pipelines, software will be written to read the specified format, enforce minimum parameters, and load the database. The developed software will be released as open source under the Apache version 2.0 license (https://apache.org/licenses/LICENSE-2.0.html).

Task: BeONE-WP4-T4 Data sharing front end
Task start month: 5
Task end month: 30
Task Leader: Kim Ng, SSI (13)
Deputy Task Leader: Simon Tausch, BfR (09)
Task Participants: BfR (09), SSI (13), DTU (12)
T4.4 takes place over the first, the second and the third year of the project.
Description of the task:
Build a front end for data sharing APIs developed in WP3-T3**.**

Sub-Task: BeONE-WP4-T4-ST1 Web-based input system
Sub-Task: BeONE-WP4-T4-ST2 Import/Export front end for reference databases
Sub-Task: BeONE-WP4-T4-ST3 BeONE data exchange system

WP5: Dissemination, Testing, Evaluation and Sustainability

WP start month: 1
WP end month: 30
WP Leader: Katrine Joensen, SSI (13)
Deputy WP Leader: Claudia Coipan, RIVM (30)
WP participants: SSI (13), RKI (11), IZSAM (28), RIVM (30), Piwet (34), FLI (10), APHA (21), DTU (12), FHI (32), PHE (22), NVI (33), BfR (09), INSA (36)
WP5 takes place over the first, the second and the third year of the project.
Description of the WP:
The aim of WP5 is to ensure that the dashboard will provide actual value to the participants by enabling straightforward and timely interpretation of relevant molecular and epidemiological data. The dashboard will be made useful across sectors (human, vet, food) to enable private data analysis within each institution as well as sharing of analysis results across institutions, sectors and countries when relevant. Extensive testing, feedback and evaluation during the whole project period will make sure that the dashboard can be employed by everyone. Thus, data output will cover specific features relevant for interpretation of public health aspects as well as for the food/veterinary side. The dashboard development will be guided towards a successful tool that can meet the various user requirements (analytical/molecular output, relevant epidemiological data, privacy/sharing) and facilitate user-friendly and timely interpretation for surveillance and outbreak investigations across sectors.

Task: BeONE-WP5-T1 Dissemination
Task start month: 1
Task end month: 30
Task Leader: Katrine Joensen, SSI (13)
Deputy Task Leader: Claudia Coipan, RIVM (30)
Task Participants: SSI (13), IZSAM (28), RIVM (30), Piwet (34), FLI (10), APHA (21), DTU (12), FHI (32), PHE (22), NVI (33), BfR (09), INSA (36)
T5.1 takes place over the first, the second and the third year of the project.
Description of the task:
The dissemination activities organized in WP5 will ensure a proper setup and use of the dashboard by participants. Specifically, the local installation of the dashboard by the participants will be described in a detailed instruction manual as well as a user instruction for specific input and output will be provided and continuously updated during the development steps during the project. The experts' decisions on analytical approaches for interpretation and sharing etc. will be handled in the respective WPs (WP1, WP2, WP3) while presented and discussed among partners at several points in T5.2, including at the two planned workshops.

Task: BeONE-WP5-T2 Continuous Testing and Feedback
Task start month: 1
Task end month: 30
Task Leader: Claudia Coipan, RIVM (30)
Deputy Task Leader: Katrine Joensen, SSI (13)
Task Participants: SSI (13), RKI (11), IZSAM (28), RIVM (30), Piwet (34), FLI (10), APHA (21), DTU (12), FHI (32), PHE (22), NVI (33), BfR (09), INSA (36)
T5.2 takes place over the first, the second and the third year of the project.
Description of the task:
The dashboard as well as specific WP outcome will be tested by the participants at several stages during the project to ensure a focused development. The participants will provide feedback to guide the development of the dashboard as well as for the specific WPs. Every 6 months a test is carried out targeting the aspects of the different WPs. These tests will be designed by the specific WPs (together with WP5). The tests may be designed to target specific sectors separately, if necessary.

For each of the WP tests, the participant feedback will be summarized and provided to the specific target WP as well as to WP4, which is in charge of the dashboard development.
This continuous testing will involve only a few partners at a time, while an overall evaluation of the dashboard with the participation of all partners is performed at the two workshops (sT5.2.2. and T5.3). The workshops will also be employed to present and discuss new features, developments, input/output, instructions, and interpretation.
The first workshop for general testing of the dashboard will take place during the second annual meeting, and the second workshop will be the final evaluation of the dashboard (T5.3.).
Sub-Task: BeOne-WP5-T2-ST1 Development of a detailed test framework
Sub-Task: BeOne-WP5-T2-ST2 Continuous testing of the platform

Task: BeONE-WP5-T4 Sustainability
Task start month: 1
Task end month: 30
Task Leader: Claudia Coipan, RIVM (30)
Deputy Task Leader: Katrine Joensen, SSI (13)
Task Participants: SSI (13), RKI (11), IZSAM (28), RIVM (30), Piwet (34), FLI (10), APHA (21), DTU (12), FHI (32), PHE (22), NVI (33), BfR (09), INSA (36)
T5.4 takes place over the first, the second and the third year of the project.
Description of the task:
Sustainability issues will arise in all WPs throughout the project period. The issues of sustainability will be identified and documented yearly by each WP leader. A final summary of the sustainability issues will be generated including recommendations for how to continue the work in the future.

WP6: Management
WP start month: 1
WP end month: 30
WP Leader: Kristoffer, Kiil, SSI (13)
Deputy WP Leader: Vítor Borges, INSA (36)
WP participants: SSI (13), RKI (11), IZSAM (28), RIVM (30), Piwet (34), FLI (10), APHA (21), DTU (12), FHI (32), PHE (22), NVI (33), BfR (09), INSA (36)
WP6 takes place over the first, the second and the third year of the project.
Description of the WP:
This WP will manage the project and its data, ensure timely delivery and reporting, internal communication as well as communication with stakeholders.
The WP will be responsible for the reporting to the One Health EJP coordinating body and will organise three general meetings and regular web-based meetings during the project period. These meetings are considered pivotal for the cohesion, internal communication and progress of this rather complex project.
In addition, WP6 will play an important role in ensuring that milestones and final deliverables of the consortium are successfully completed and communicated.
The project leader together with the WP leaders and deputy leaders (the project coordination team) coordinate the project. This team will ensure the overall progress and coordinate the work of all WPs towards their common goal: the development of a One Health surveillance tool for real-time analysis and sharing of molecular and epidemiological data. The team will ensure the continuous communication between the WPs and the timely dissemination of results. Communication to external stakeholders is also mainly the responsibility of the management team. The WP leaders/co-leaders will coordinate the tasks and the internal communication within each WP.

Task: BeONE-WP6-T1 Management
Task start month: 1

Task end month: 30
Task Leader: Kristoffer Kiil, SSI (13)
Deputy Task Leader: Vítor Borges, INSA (36)
Task Participants: SSI (13), INSA (36), RIVM (30), BfR (09), IZSAM (28), DTU (12), FLI (10), NVI (33)
T6.1 takes place over the first, the second and the third year of the project.
Description of the task:
At the beginning of the project, the project coordination team consisting of the project leader, the WP leaders and deputy leaders will be formed and regular web-meetings will be scheduled. The task includes internal communication to all partners about project progress, upcoming deadlines, milestones, deliverables, etc. In addition, this task is responsible for the cross-WP communication and coordination to ensure that the workflow within and between WPs proceed as expected. Staff for administrative support, including financials, will also take part in this task and participate in web-meetings when required.

Task: BeONE-WP6-T2 Communication
Task start month: 1
Task end month: 30
Task Leader: Kristoffer Kiil, SSI (13)
Deputy Task Leader: Vítor Borges, INSA (36)
Task Participants: SSI (13), RKI (11), IZSAM (28), RIVM (30), Piwet (34), FLI (10), APHA (21), DTU (12), FHI (32), PHE (22), NVI (33), BfR (09), INSA (36)
T6.2 takes place over the first, the second and the third year of the project.
Description of the task:
This task is responsible for the internal and external communication and the organisation of annual meetings for all participants as well as a hackathon for the developers. The coordination team will ensure the continuous communication between the WPs and the timely dissemination of results. The WP leaders/co-leaders will coordinate the tasks and the internal communication within each WP, including the organisation of specific web-meetings to ensure the optimal interaction of tasks. Communication to external stakeholders, e.g. ECDC, EFSA, and other projects within and outside the One Health EJP, is also coordinated in this task.

Sub-Task: BeONE-WP6-T2-ST1 Kickoff meeting
A kick-off meeting will be scheduled to take place within the first two months of the project. All involved scientists will meet at RIVM for a 2-day meeting, where participants will be introduced to each other and make the detailed plans for the project.
Sub-Task: BeONE-WP6-T2-ST2 2nd Annual Meeting
A general meeting for all participants will be organized and take place in April 2021 at INSA. Here, the progress will be presented and the plans for the final year will be discussed and planned.
Sub-Task: BeONE-WP6-T2-ST3 3rd Annual Meeting
The final meeting for all participants will take place at SSI close to the end of the project period, April 2022. Here, the project results will be presented and discussed and the work to finalise the remaining tasks will be planned in detail. The execution of the remaining communication and sustainability plans will be planned.
Sub-Task: BeONE-WP6-T2-ST4 Development hackathon
The hackathon for developers will be organized in the second half of the first project year and hosted by BfR.. This will be a 2-day working meeting for the developers in the project. The hackathon will ensure the united approach and crucial alignment of the development to be done in WP3 and WP4, as well as accommodate the smooth collaboration between developers at different partner institutes throughout the project.

Task: BeONE-WP6-T3 Data Management

Task start month: 1
Task end month: 30
Task Leader: Kristoffer Kiil, SSI (13)
Deputy Task Leader: Carlus Deneke, BfR (09)
Task Participants: SSI (13), BfR (09)
T6.3 takes place over the first, the second and the third year of the project.
Description of the task:
A data management plan will be created (deliverable) and this task will continuously track, monitor, analyse, and store data from the project. The initial project management plan will continuously be adjusted as needed. This task will ensure data availability for all purposes of the project and beyond, contributing to the sustainability of the project.

Deliverables

Ref	Title	Due month
D-BeONE.1.1	Report on the state-of-art	6
D-BeONE.6.1	Initial data management plan	6
D-BeONE.1.2	Finalized BeONE dataset	8
D-BeONE.2.1	Draft manuscript on the conceptual model	10
D-BeONE.4.1	Back-end analysis pipeline	12
D-BeONE.4.2	Web input system	12

Milestones

Ref	Title	Due month
M-BeONE.4.1	Initial requirement list from workshop at kick-off meeting	2
M-BeONE.3.1	Agreement on minimal and optimal set of metadata	4
M-BeONE.4.2	Implementation plan	4
M-BeONE.5.3	Initial test plan	4
M-BeONE.1.1	State-of-the-art completed	6
M-BeONE.1.2	Collected dataset	6
M-BeONE.1.4	Agreement on the methods/solutions to evaluate	6
M-BeONE.1.3	Curated WGS dataset completed	8
M-BeONE.2.1	The critical factors for outbreak detection have been identified	8
M-BeONE.4.3	Prototype dashboard	8
M-BeONE.3.2	Finalized evaluation of data sharing experiences	10
M-BeONE.3.3	Implementation of data structure	10
M-BeONE.4.4	Plan for data sharing system	10
M-BeONE.3.4	Finished implementation of controlled data entry	12

M-BeONE.3.5	Plan for ontology implementation	12
M-BeONE.3.6	Implementation of API for import of data	12
M-BeONE.4.5	Basic system for display component integration	12
M-BeONE.5.2	Yearly sustainability document	12

2.4.2.1.31 JIP01-R1-IA1.1-ORION

Reference to the Stategic Research Agenda (please refer to D2.7)			**IA 1.1: Interpretation of surveillance data - Standardised data formats and ontologies, common tools and procedures for data analyses, including interpretation of sequence data**							
Integrative Project Title			**One health surRveillance Initiative on harmOnization of data collection and interpretatioN**							
Integrative Project Acronym			**ORION**							
Leading Organisation			**P9-BfR**			Deputy Leading Organisation			**P41-SVA**	
Project Leader			**Matthias Filter**			Deputy Project leader			**Fernanda Dórea**	
Project Start month			**M1**			Project End month			**M30**	
Participant	1	2	**4**	6	7	8	**9**	**10**	11	**12**
	Anses	Ages	**Sciensano**	NDRVMI	SZU	VRI	**BfR**	**FLI**	RKI	**DTU**
P.M			**8**				**39**	**21**		**16**
Participant	**13**	14	15	16	17	18	19	20	**21**	**22**
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	**PHE**
P.M	**23.67**								**7.34**	**6.80**
Participant	23	24	25	26	27	28	29	**30**	**31**	**32**
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	**RIVM**	**WbvR**	**FHI**
P.M								**9.33**	**3.63**	**10.5**
Participant	**33**	34	35	36	37	38	39	**40**	**41**	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	**FOHM**	**SVA**	
P.M	**12**							**10**	**35**	

Objectives

- Finalize OH pilot studies
- Revise “OH Surveillance Codex”, including lessons learned from the OH pilots
- Revise “OH Knowledge Base – Epi”, including lessons learned from the OH pilots
- Revise “OH Knowledge Base –NGS”, including lessons learned from the OH pilots
- Revise “OH Knowledge Base – Integration”, including lessons learned from the OH pilots
- Revise “OH Harmonisation Infrastructure Hub”, including lessons learned from the OH pilots
- Revise “Sustainability Roadmap”

- Create “OHS Knowledge Hub” populated with resources from WP1, WP2 and WP3
- Perform dissemination and training activities

WP: **WP1 - “OH Surveillance Codex”**

WP1 takes place over the first, second, and third annual periods of the EJP

WP start month:	M1
WP end month:	M36
WP Leader:	9-BfR
Deputy WP Leader:	38-SVA
WP participants:	4 - Sciensano - 9-BfR, 10-FLI, 12-DTU, 21-PHE, 32-NIPH, 33-NVI, 37-FOHM, 38-SVA

Description of the WP:

This WP aims at the development of an integrated approach for harmonized description and categorisation of surveillance data covering all surveillance phases. The primary focus will be on the development of the first “OH Surveillance Codex”, a document with harmonized descriptive guidelines. WP1 will also define requirements for data standards and software tools that should be developed to facilitate broad and easy adoption of the framework. Some of these new resources will be implemented by WP3. The OH pilots carried out by WP1 have the goal of demonstrating that the “OH Surveillance Codex” guidelines and the proposed solutions supporting it’s adoption are practically applicable and generate the expected added value. Specifically the pilot performed in collaboration with WP3, EFSA & ECDC (so-called “supra-national pilot”) will also test and evaluate the supporting resources and solutions developed by WP1 and WP3. Based on the lessons learned from the OH pilot studies this WP will make necessary adjustments to the “OH Surveillance Codex” document and provide feedback to developers of OHS Codex solutions WP1 and 3. Throughout the duration of the project WP1 will use workshops organized by WP4 to train other EJP projects and stakeholders and disseminate the “OH Surveillance Codex” and the underlying conceptual framework. WP1 will further support the development of the “Sustainability Roadmap” (Task 4.3) to secure future development and adoption of the “OH Surveillance Codex” by EJP partners and European veterinary and public health agencies.

Task T-1.3: **One Health pilot**

Task 1.3 takes place over the first, second and third annual periods of the EJP.

Task start month:	M7
Task end month:	M30
Task Leader:	9-BfR
Deputy Task Leader:	38-SVA
Task Participants:	3 -CODA-CERVA/WIV-ISP, 9-BfR, 10-FLI, 21-PHE, 33-NVI, 37-FOHM, 38-SVA

Description of the task:

The OH pilot aims at illustrating the practical applicability of the “OH Surveillance Codex” when applied to real world surveillance data.

Subtask1 (M7 - M12):	Selection of the OH pilot study topic
Subtask2 (M13 – M18):	Planning and performing work necessary to prepare the execution of the OH pilot. This includes: specification of targets, definition of performance indicators, identification and making available required data sources and software tools etc.
Subtask3 (M19 – M30):	Execution of the OH pilot - applying the “OH Surveillance Codex” guide.

Task T-1.4: **Evaluation and Recommendations**

Task 1.4 takes place over the third annual period of the EJP.

Task start month:	M31

Task end month:	M36
Task Leader:	9-BfR
Deputy Task Leader:	38-SVA
Task Participants:	4 - Sciensano, 9-BfR, 10-FLI, 12-DTU, 21-PHE, 32-NIPH, 33-NVI, 37-FOHM, 38-SVA

Description of the task:

The aim of this task is the generation of lessons-learned from the OH pilot project. Specifically, it will analyse:

1. where the proposed "OH Surveillance Codex" has to be improved or adapted
2. what improvements and recommendations should be given regarding software provided by WP1 & 3
3. which implications the OH pilots have for the overall "ORION OH surveillance data harmonization framework".

In order to facilitate this WP4 will host a joint ORION Evaluation workshop in M33. There the results from all ORION OH pilots will be jointly discussed, analysed and critically evaluated. In addition this task will generate presentations, documents and training material that can be integrated into the "OHS Knowledge Hub" (operated by WP4).

WP:	**WP2-Epi**

WP2-Epi takes place over the first, second, and third annual periods of the EJP

WP start month:	M1
WP end month:	M36
WP Leader:	10-FLI
Deputy WP Leader:	9-BfR
WP participants:	All ORION partner

Description of the WP:

Within this work package we will focus on general surveillance data. In the first task we will carry out a requirement analysis to specify the state of the art of surveillance systems. We will then create an inventory of existing components of surveillance systems and identify gaps. Based upon the results, we will contribute to the development of a "OH Surveillance Codex" (WP1) and an application tool for surveillance systems. These tools will be tested in OH pilot studies and the result will be presented to the OH surveillance community.

Based on a literature review and a questionnaire survey, we create inventories on both data sources and data analysis; The inventory generated in this WP2 (OH Knowledge Base Epi) also covers current best practice guidelines and recommendations on using available resources. We will identify gaps and missing resources for reaching the ORION OH integration and harmonization objective. Appropriate diseases to be included in the OH Knowledge Base Epi might be Salmonella, Toxoplasma, Listeria, Campylobacter, VTEC/EHEC, Cryptosporidium, as well as antimicrobial resistance (AMR).

In a joint ORION prioritization workshop (M13) an "OH surveillance resource development plan" will be defined. This plan will detail and synchronizes the work carried out in all WP2s during task 2. New or improved resources will be integrated into the OH Knowledge Base Epi. In the second task, data from EJP partners of selected diseases or AMR will be collected, collated and prepared in order to use them for the OH pilot studies. In the third task WP2 -Epi will carry out OH pilot studies to prove whether (i) the OH surveillance framework previously developed by WP1 is suitable for the available data and (ii) if the data meet the requirements of the framework. The results of the pilot studies will be used to identify gaps in data availability and to improve the framework. Where appropriate these OH pilots will support the evaluation of resources developed by other WPs, e.g. WP1 and WP3.

WP2-Epi will integrate generated resources into OH Knowledge Base Epi which then becomes part of the "OHS Knowledge Hub" operated by WP4. Throughout the duration of the project WP2-Epi

will use workshops organized by WP4 to train other EJP projects and stakeholders and disseminate their knowledge.

Task T-2Epi.3: **Epi - OH pilot studies**

Task 2.3 takes place over the first, second and third annual periods of the EJP.

Task start month: M7
Task end month: M30
Task Leader: 9-BfR
Deputy Task Leader: 10-FLI
Task Participants: 9-BfR, 10-FLI, 12-DTU, 20-APHA, 21-PHE, 30-WbvR/CVI, 31-RIVM, 32-NIPH, 33-NVI, 38-SVA,

Description of the task:

The OH pilots will validate and exemplify the improved knowledge database generated in this WP. Topics for OH pilots will be selected along the overarching OH surveillance harmonization objective considering recommendations from EJP partners and other EJP sub-projects. Each of the OH pilots will be performed in a timely synchronized fashion:

Phase 1 (M7 - M12): Selection of the OH pilot study topic
Phase2 (M13 – M18): Planning and performing work necessary to prepare the execution of the OH pilot. This includes: specification of targets, definition of performance indicators, identification and collection of required data and software tools etc.
Phase3 (M19 – M30): Execution of the OH pilot - applying resources from "OH Knowledge Base Epi"
Sub-Task sT-2Epi.3.1 (M7-30) One Health Pilot 1: topic to be determined
Sub-Task sT-2Epi.3.2 (M7-30) One Health Pilot 2: Salmonella

A pilot study will be carried out by PHE and APHA on Salmonella surveillance using the OH surveillance framework. Within the study both partners will harmonise data transfer including data format, frequency, resolution and time. The partners will provide data to WP1 to be able to share data on early detection systems (EDS), methodology and application to salmonella datasets within the OH surveillance framework.

Sub-Task sT-2Epi.3.3 (M7-30) One Health Pilot 3: topic to be determined

Within this subtask 30-WbvR/CVI and 31-RIVM will carry out a common pilot study.

Task T-2Epi.4: **Evaluation and Recommendations Epi**

Task 2Epi.4 takes place over the third annual period of the EJP.

Task start month: M25
Task end month: M36
Task Leader: 30-WbvR/CVI
Deputy Task Leader: 38-SVA AND 13-SSI
Task Participants: all ORION partners

Description of the task:

This task aims at the generation of lessons-learned from the OH pilot project. Specifically, it will be analysed

1. where the OH Knowledge Base - Epi has to be improved or adapted
2. what improvements and recommendations can be given on the software provided by WP3
3. which implications this OH pilot has to the overall "ORION OH surveillance harmonization framework".

In order to facilitate this WP4 will host a joint ORION Evaluation workshop in M33. There the results from all ORION OH pilots will be jointly discussed, analysed and critically evaluated. In addition this

task will generate presentations, documents and training material that can be integrated into the "OHS Knowledge Hub" (operated by WP4).

WP: **WP2 – NGS**

WP2-NGS takes place over the first, second, and third annual periods of the EJP

WP start month:	M1
WP end month:	M36
WP Leader:	33-NVI
Deputy WP Leader:	32- FHI/NIPH
WP participants:	9-BfR, 10-FLI, 12-DTU, 13-SSI, 21-PHE, 30-WbvR/CVI, 31-RIVM, 32-FHI/NIPH, 33-NVI, 37-FOHM/PHA

Description of the WP:

There are several methods available today for identifying bacterial species and strains. Sequencing based strategies are rapidly gaining popularity, but that is not the only novel technique available (examples are NMR and MALDI-TOF). However, these methods may not be appropriate or applicable to a surveillance setting. Within this work package we will first do a review of existing and novel methods and systems for bacterial identification to find which methods/systems that are applicable for surveillance purposes. Based on the result we will set up an analysis pipeline, which may be within an existing system, which will be tested out in One Health pilots. The results will then be reviewed, and recommendations regarding analysis methods will be made. The work within this WP will primarily focus on NGS based methods, to better align with the work being done in other EJP projects.

Specifically, the aim of this' WP is the creation of an "OH Knowledge base NGS" - a cross-domain inventory of currently available data/methods/algorithms/tools/systems linked to existing and novel NextGen bacterial identification (primarily NGS) analysis methods and provide best practice solutions that support integrated OH surveillance data generation, data analysis, modelling and decision support. To accomplish that WP2-NGS will as described in previous WPs conduct expert interviews and SWOT analysis to identify gaps and missing resources.

The generated OH Knowledge Base NGS will for several relevant zoonotic agents contain meta-information on:

1. existing and novel analysis data sources
2. methods and algorithms for data management and integration
3. methods and approaches for data analysis

The analysis will also consider current experiences on practical issues such as data storage capacity, data exchange capacity, bioinformatics expertise, data exchange platforms, harmonised terminology etc.

WP2-NGS will participate in a joint ORION prioritization workshop (M13) as described in earlier WPs. WP2-NGS will also carry out OH pilots in a similar fashion as described in WP2-Epi, and will based on the results make necessary adjustments to the "OH Knowledge base NGS" and update the "OH surveillance resource development plan".

Task T-2Ngs.3: **NGS OH pilot studies**

Task 2Ngs.3 takes place over the first, second and third annual periods of the EJP.

Task start month:	M7
Task end month:	M30
Task Leader:	33-NVI
Deputy Task Leader:	32- FHI/NIPH
Task Participants:	31-RIVM, 32-FHI, 33-NVI

Description of the task:

Based on the results of 2Ngs.2.3 we will design an NGS based One Health pilot study. Two pathogens will be chosen for the pilot, in order to distinguish between pathogen specific issues and methodological issues. The OH pilot will be performed in the same fashion as previously described for WP-EPI timely synchronized with other ORION OH studies:

Phase1 (M7 - M12):	Selection of the OH pilot study topic
Phase2 (M13 – M18):	Planning and performing work necessary to prepare the execution of the OH pilot. This includes: specification of targets, definition of performance indicators, identification and making available required data sources and software tools etc.
Phase3 (M19 – M30):	Execution of the OH pilot - applying resources from "OH Knowledge Base – NGS"

Task T-2Ngs.4: Evaluation and Recommendations NGS

Task 2Ngs.4 takes place over the third annual period of the EJP.

Task start month:	M25
Task end month:	M36
Task Leader:	31-RIVM
Deputy Task Leader:	13-SSI, 33-NVI, 32- FHI/NIPH
Task Participants:	9-BfR, 10-FLI, 12-DTU, 13-SSI, 21-PHE, 30-WbvR/CVI, 31-RIVM, 32-FHI/NIPH, 33-NVI, 37-FOHM/PHA

Description of the task:

This task aims at the generation of lessons-learned from the OH pilot project. The task will be performed as previously described for WP-Epi, but with a focus on the areas examined in this WP. Specifically, it will be analysed

1. where the OH Knowledge Base - NGS has to be improved or adapted
2. what improvements and recommendations can be given on the software provided by WP3
3. which implications this OH pilot has to the overall "ORION OH surveillance harmonization framework".

In order to facilitate this WP4 will host a joint ORION Evaluation workshop in M33. There the results from all ORION OH pilots will be jointly discussed, analysed and critically evaluated. In addition this task will generate presentations, documents and training material that can be integrated into the "OHS Knowledge Hub" (operated by WP4).

WP: WP2 – Integration

WP2-Integration takes place over the first, second, and third annual periods of the EJP

WP start month:	M1
WP end month:	M36
WP Leader:	12-DTU
Deputy WP Leader:	13-SSI
WP participants:	9-BfR, 12-DTU, 13-SSI, 32-FHI/NIPH, 33-NVI, 37-FOHM/PHA, 38-SVA

Description of the WP:

This WP2 has two main aims:

1) to ensure integration of the knowledge and methods generated by the two subWP2s – Epi / NGS to avoid duplications and maximise benefits of the project;

2) a pilot study to demonstrate integration of all disciplines.

Specifically, during the first year we will create an inventory on WP2-Integration on current integrated OH surveillance approaches in use in the EU. The inventory generated in this WP2 (OH Knowledge Base Integration) also cover current best practice guidelines and recommendations. To accomplish that this WP2 will carry out jointly with the other ORION WPs expert interviews. Finally this WP2 will carry out a GAP analyses to identify gaps and missing resources for reaching the OH integration and harmonization objective.

In a joint ORION prioritization workshop (M13) a "OH surveillance resource development plan" will be defined. This plan detail and synchronize the improvement work carried out in all WP2s. New or improved resources developed by this WP2 will be integrated into the OH Knowledge Base Integration. In the third task this WP2 will carry out a pilot study that enhances OH surveillance on a specific topic, e.g. AMR. The pilot will not only integrate human, veterinary and food surveillance data, but also integrate the traditional disciplines with NGS from the work in the other WP2s. A specific objective of this OH pilot study is to ensure a robust, novel, future surveillance framework without losing the value of the historic data. The pilot study will try to use relevant outputs from all ORION WPs. WP2-Integration will further support the development of the "Sustainability Roadmap" (Task 4.3) in order to secure continued implementation and adoption of the "OH surveillance resource development plan" created by WP2 (M13).

Task T-2Int.3: **Integration OH pilot studies**

Task 2Int.3 takes place over the first, second and third annual periods of the EJP.

Task start month: M7
Task end month: M30
Task Leader: 12-DTU
Deputy Task Leader: 13-SSI
Task Participants: 12-DTU, 13-SSI, 33-NVI
Description of the task:

A multi-domain topic will be chosen, such as AMR, Listeria or VTEC/EHEC. This pilot will not only integrate human, veterinary and food surveillance, but also integrate the disciplines in WP2 – epidemiology, microbiology and novel genomic sequencing methods, to ensure a robust future surveillance system without losing the value of the historic data.

Phase1 (M7 - M12): Selection of the OH pilot study topic
Phase2 (M13 – M18): Planning and performing work necessary to prepare the execution of the OH pilot. This includes: specification of targets, definition of performance indicators, identification and making available required data sources and software tools etc.
Phase3 (M19 – M30): Execution of the OH pilot - applying the resources from "OH Knowledge Base Integration"

Task T-2Int.4: **Evaluation and Recommendations - Integration**

Task 2Int.4 takes place over the third annual period of the EJP.

Task start month: M25
Task end month: M36
Task Leader: 12-DTU
Deputy Task Leader: 13-SSI
Task Participants: 9-BfR, 12-DTU, 13-SSI, 32-FHI/NIPH, 33-NVI, 37-FOHM/PHA, 38-SVA
Description of the task:

This task aims at the generation of lessons-learned from the OH pilot project. Specifically, it will be analysed

1. where the "OH Knowledge Base Integration" has to be improved or adapted
2. which implications this OH pilot has to the overall "ORION OH surveillance harmonization framework".

In order to facilitate this WP4 will host a joint ORION Evaluation workshop in M33. There the results from all ORION OH pilots will be jointly discussed, analysed and critically evaluated. In addition this task will generate presentations, documents and training material that can be integrated into the "OHS Knowledge Hub" (operated by WP4).

WP: **WP3 - OH Surveillance Harmonisation Infrastructure**

WP3 takes place over the first, second, and third annual periods of the EJP

WP start month:	M1
WP end month:	M36
WP Leader:	38-SVA
Deputy WP Leader:	37-FOHM/PHA
WP participants:	9-BfR; 10-FLI, 12-DTU; 13-SSI; 20-APHA; 21-PHE; 31-RIVM; 32-FHI/NIPH, 33-NVI; 37-FOHM/PHA; 38-SVA

Description of the WP:

WP3 will focus on identifying infrastructural resources which can support the harmonization and integration of existing methods to collect and analyse surveillance data. These infrastructural resources include - among others - data standards, software libraries, ontologies, terminology mappings, semantic networks, software tools. The specific development tasks to be carried out by WP3 will be identified by requirements expressed from WP1 (in relation to the "OH Surveillance Codex"), WP2 (supporting their developments) and through interviews of domain experts from other EJP partners and projects. Through the close interaction with WP1 and WP2 in the requirement analysis phase, WP3 will identify existing resources along the full surveillance data lifecycle. WP3 will inventory and where possible / required improve these tools and resources. The decision on the specific WP3 developments to be carried out in task 3.2 will be taken together with WP1 and WP2 during the joint ORION prioritization workshop (M13). In contrast to the other ORION WPs, this WP will focus on specifications for necessary metadata along the full surveillance data lifecycle – from annotation of analytical data, documentation of analytical methods, applied data processing and modelling steps to visualization of results. These proposed solutionswill exploit the "One-Health Surveillance Ontology" also generated by this WP.

One national (Sweden) and one supra-national (WP1+WP3 +EFSA+ECDC) pilot will allow for testing how the improved infrastructural resources, and in particular the proposed approach to provide data and metadata as Linked-Open-Data, support the goal of surveillance data harmonisation. Based on the lessons-learned from these OH pilot studies WP3 will make necessary adjustments to their developed resources and update the inventory of OH surveillance infrastructural resources that then becomes part of the "OHS Knowledge Hub" operated by WP4. Throughout the duration of the project WP3 will use workshops organized by WP4 to train other EJP projects and stakeholders and disseminate its knowledge. WP3 will further support the development of the "Sustainability Roadmap" (Task 4.3) in order to secure continued development of OH surveillance infrastructural resources.

Task T-3.3: One Health pilot

Task 3.3 takes place over the first, second and third annual periods of the EJP.

Task start month:	M7
Task end month:	M30
Task Leader:	38-SVA
Deputy Task Leader:	37-FOHM/PHA
Task Participants:	33-NVI, 37-FOHM/PHA; 38-SVA

Description of the task:

The OH pilot aims at illustrating the usefulness and the added value of the infrastructural resources collected and improved within this WP. The pilot will be carried out by a veterinary health partner (National Veterinary Institute (SVA)) and a public health partner (National Public Health Agency (FoHM) of Sweden). Other participating institutions will be encouraged to join the OH pilot. The pilot will also assess the sustainability value of newly developed infrastructural resources when applied across domains.

Phase (M7 - M12):	Selection of the OH pilot study topic which will be a zoonotic agent from the following four candidates that have already been pre-selected by the WP

	partners: Campylobacter, Salmonella, VTEC/EHEC, and Listeria.
Phase (M13 – M18):	Planning and performing work necessary to prepare the execution of the OH pilot. This includes: specification of targets, definition of performance indicators, identification and making available required data sources and software tools etc.
Phase (M19 – M30):	Execution of the OH pilot - applying resources from the OH Harmonisation Infrastructure Hub

Task T-3.4: Evaluation and Recommendations

Task 3.4 takes place over the third annual period of the EJP.

Task start month:	M25
Task end month:	M36
Task Leader:	38-SVA
Deputy Task Leader:	37-FOHM/PHA
Task Participants:	9-BfR; 10-FLI, 12-DTU; 13-SSI; 20-APHA; 21-PHE; 31-RIVM; 32-FHI/NIPH, 33-NVI; 37-FOHM/PHA; 38-SVA

Description of the task:

This task aims at the generation of lessons-learned from the OH pilot project. Specifically, it will be analysed

1. where the improved infrastructural resources have to be further improved or adapted
2. which implications this OH pilot has to the overall "ORION OH surveillance data harmonization framework".

In order to facilitate this WP4 will host a joint ORION Evaluation workshop in M33. There the results from all ORION OH pilots will be jointly discussed, analysed and critically evaluated. In addition this task will generate presentations, documents and training material that can be integrated into the "OHS Knowledge Hub" (operated by WP4).

WP: WP4-Coordination, Communication, Training and Sustainability

WP4 takes place over the first, second, and third annual periods of the EJP

WP start month:	M1
WP end month:	M36
WP Leader:	9-BfR
Deputy WP Leader:	38-SVA
WP participants:	all ORION partners

Description of the WP:

This WP integrates all horizontal project activities including the coordination of internal and external communication, the set-up of shared technical resources (Wiki's, web-based OHS Knowledge Hub, software code repositories etc.), and the development of a "Sustainability Roadmap" paving the way to broad adoption of project results in future European surveillance practice. This WP will also organize all cross-WP workshops (M4, M13, M33) and promote wider group assessment / validation of findings at critical points in the project. The training and dissemination activities organized by this WP will promote knowledge exchange between project partners as well as other EJP partners and projects. Special focus will be given to the synchronization with the other(s) EJP Integrative action project(s), funded under IA-2. All external communication and knowledge transfer activities will be integrated into the overarching activities carried out by EJP WP5 "Science to Policy Translation". This includes promotion of knowledge transfer to responsible entities (e.g. EU reference laboratories, EFSA, ECDC) and international statutory bodies.

Task T-4.1: Internal project coordination

Task 4.1 takes place over the first, second and third annual periods of the EJP.

Task start month:	M1
Task end month:	M36
Task Leader:	9-BfR
Deputy Task Leader:	38-SVA
Task Participants:	all ORION partners

Description of the task:
This task takes responsibility for and effective project coordination. This will be facilitated by hosting monthly full consortia conference calls / WebEx meetings, one full consortia project meeting and dedicated cross-WP workshops in month 4, 13, and 33 to promote synchronized decision making and development within the ORION project. This task also disseminates duties to corresponding partners and keeps track of timely provisioning of partner's deliverables. Further it will manage the creation of yearly project reports.
WP4 will host a joint ORION Evaluation workshop in M33, where the results from all ORION OH pilots will be jointly discussed, analysed and critically evaluated. In addition this task will generate presentations, documents and training material that can be integrated into the "OHS Knowledge Hub" (operated by WP4).

Task T-4.2: External project integration (synchronized with EJP WP5)

Task 4.2 takes place over the first, second and third annual periods of the EJP.

Task start month:	M1
Task end month:	M36
Task Leader:	38-SVA
Deputy Task Leader:	9-BfR
Task Participants:	all ORION partners

Description of the task:
This task will deal with the coordination of *collaboration* with other EJP initiatives, and also *support* to other projects. We will *support* other funded EJP initiatives in two main ways: by including these projects in the phase of requirement analysis, for all WPs, so that they have a chance to contribute their views on current gaps and opportunities for OH integration; and by providing training (see specific training tasks below). The requirement analysis is also a point of close collaboration in the work to be carried out. We foresee that many projects may plan to conduct tasks similar to ORION's requirement analysis, and partner interviews. We suggest that integration with other projects will be needed to maximize resources, and avoid reducing the quality of survey responses due to an excessive number of questionnaires being mailed to EJP partners. Further, the results of this project should be closely tied to the Integrative Area 2, so that the accomplishments in integration of data collection and analysis can be in line with the issues of knowledge integration and decision making.

Task T-4.3: Sustainability roadmap

Task 4.3 takes place over the first, second and third annual periods of the EJP.

Task start month:	M7
Task end month:	M36
Task Leader:	9-BfR
Deputy Task Leader:	38-SVA
Task Participants:	all ORION partners

Description of the task:
Sustainability issues will arise throughout the project, in all WPs, and in all tasks. For this reason, it has been assigned to the coordination to follow and document raised issues of sustainability, whether legal or technical. Budget has been planned for legal and technical consultation, where needed, in order to inform potential solutions to sustainability issues, and trace a road map of sustainability for the integration of data and methods in a OH surveillance context. The final "Sustainability Roadmap" document will be created based on the results of the joint ORION Evaluation workshop hold in M33.

This document will give recommendations on how the "ORION OH surveillance data harmonization framework" with all its components should be consolidated by EJP partners and European veterinary and public health agencies in the future.

Task T-4.4: Training and Dissemination

Task 4.4 takes place over the first, second and third annual periods of the EJP.

Task start month: M1
Task end month: M36
Task Leader: 38-SVA
Deputy Task Leader: 9-BfR
Task Participants: all ORION partners
Description of the task:
This task will be concerned with planning and conducting, throughout the project, training that aims at sharing the knowledge already available within the consortium among all partners, training partners in the use of new tools produced/adopted, and disseminating results and training external partners in the adoption of tools and guidelines published by ORION. All partners have assigned a minimum of 2PM to training, so that they can take part in the activities listed below – receiving training, training others, or helping with dissemination activities.

Sub-Task sT-4.4.1 (M1- M12): Internal training (sharing knowledge on currently available national solutions)
Sub-Task Leader: 9-BfR
Sub-Task Participants: all ORION partners
Description of the task:
During the first year 2 workshops are planned in order to integrate knowledge available within the partner institutions. As the requirement analysis progresses, we will identify capacity already available in some partners, and carry out workshops to allow partners to train each other on their tools of expertise.

Sub-Task: T-4.4.2 (M7 – M36): Knowledge integration (web portal, Wiki, curricula, tutorials, videos, sample data)
Sub-Task Leader: 9-BfR
Sub-Task Participants: all ORION partners
Description of the task:
All WPs have tangible outcomes that are meant to benefit the surveillance community - e.g. the OH Surveillance Codex from WP1, the OH surveillance data inventories with validated tools in WP2, the ontology generated in WP3. This task will oversee the production of dissemination means, while training will be provided in the following Sub-Task. Workshops and webinars to the overall public are planned to the last year of project, while permanent dissemination means – such as videos and a wiki – will guarantee access to the project outcomes after the conclusion of the project. Particular emphasis is given to the "OHS Knowledge Hub", which will be a repository of all the tools and guidelines produced in the project.
Through continuous liaison with EFSA and ECDC it will be guaranteed that with respect to the information and results generated in the ORION project there will be no 'duplication' with similar efforts provided by stakeholders and that generated information remains freely available after the end of the project.

Sub-Task T-.4.4.3 (M7 - M36): Training and support for other EJP projects & partners
Sub-Task start month: M7
Sub-Task end month: M36

Sub-Task Leader:	38-SVA
Sub-Task Participants:	all ORION partners

Description of the task:
Training on all the guidelines, framework, and tools produced as part of ORION will be provided to other interested EJP partners. Two dedicated training workshops for external EJP project members are planned for the third year. These trainings as well as the ORION pilot projects will continouosly be used to attract additional partners to join the ORION project.

Deliverables		
Ref	**Title**	**Due month**
D-1.3	Revised OH Surveillance Codex, including lessons learned from the OH pilots	M36
D-2.7	Revised OH Knowledge Base - Epi, including lessons learned from the OH pilots	M36
D-2.8	Revised OH Knowledge Base -NGS, including lessons learned from the OH pilots	M36
D-2.9	Revised OH Knowledge Base - Integration, including lessons learned from the OH pilots	M36
D-3.3	Revised OH Harmonisation Infrastructure Hub, including lessons learned from the OH pilots	M36
D-4.4	Revised Sustainability Roadmap	M36
D-4.5	OHS Knowledge Hub populated with resources from WP1, WP2 and WP3	M36
D-4.6	Two additional training workshops and two webinars	M36
Milestones		
Ref	**Title**	**Due month**
M-IA1.ORION.3	ORION evaluation workshop	M33

2.4.2.1.32 JIP02-R1-IA1.2-COHESIVE

Reference to the Stategic Research Agenda (please refer to D2.7)	**IA 1.2: Common reporting and signalling procedures, joint platform for sharing surveillance data and their interpretation, incl. risk assessments**									
Integrative Project Title	**One Health Structure In Europe**									
Integrative Project Acronym	**COHESIVE**									
Leading Organisation	**P30-RIVM**				Deputy Leading Organisation			**P31-WbvR**		
Project Leader	**Kitty Maassen**				Deputy Project leader			**Wim van der Poel**		
Project Start month	**M1**				Project End month			**M30**		
Participant	1	**2**	**4**	6	7	8	**9**	**10**	11	**12**
	Anses	**Ages**	**Sciensano**	NDRVMI	SZU	VRI	**BfR**	**FLI**	RKI	**DTU**
P.M		**7.67**	**2.70**				**22.10**	**1.50**		**1.25**
Participant	13	14	15	16	17	18	19	20	**21**	**22**
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	**PHE**
P.M									**6.86**	**1.08**
Participant	23	24	25	26	27	**28**	**29**	**30**	**31**	**32**
	UoS	OKI	NUIG	TEAGASC	ISS	**IZSAM**	**IZSLER**	**RIVM**	**WbvR**	**FHI**
P.M						**49**	**9**	**14**	**3.06**	**8.36**
Participant	**33**	34	**35**	36	37	38	**39**	**40**	**41**	
	NVI	PIWET	**INIAV**	INSA	IISPV	IC	**SLV**	**FOHM**	**SVA**	
P.M	**4.05**						**3**	**1.60**	**8.70**	

Objectives

- Stimulating sustainable One Health approaches at the national level within EU countries, focussing on strengthening human-veterinary collaboration with respect to early signalling and assessing zoonotic threats.
- Roadmap towards an EU zoonoses risk-assessment or risk-analysis structure
- Design and test a common platform for the collection and analysis of surveillance and outbreak data on foodborne zoonoses.
- Capacity building within and between EU countries at several levels within the area of zoonotic diseases

Description of work

WP1 Coordination, communication and sustainability

WP start month: 1 WP end month: 36

WP Leader: Kitty Maassen, RIVM

Deputy WP Leader: Wim van der Poel, WbvR

WP participants: Bfr, APHA, IZS AM, WbvR, RIVM, SVA: All actively involved in this WP. All other project partners will have a reactive role in this WP, such as attending annual meetings.

Description of the WP: WP1 continues based upon what was achieved during the first and second annual period of the EJP. The objective of this WP is to coordinate the activities of the partners as described in the various WPs and ensure the smooth running of the project. This also includes overseeing the links between the WPs and assist with linking to other projects within the EJP and outside. The project is aiming at sustainable products (risk-analysis structures, collaborations, data platform). The steering group will stimulate partners and associate partners to reach for those goals. Within this WP also internal and external communication is coordinated.

Task: T-1.1 Coordination

Task start month: 1

Task end month: 36

Task Leader: Kitty Maassen, RIVM

Deputy Task Leader: Wim van der Poel, WbvR

Task Participants: WP (deputy)leaders: Bfr, APHA, IZS AM, WbvR, RIVM,SVA

Description of the task: Task 1.1 continues based upon what was achieved during the first and second annual period of the EJP. The RIVM is project coordinator and responsible for the project management and leading the steering group. WbvR will be deputy task-leader to ensure a good follow-up and management of the task. WbvR will be also the first sparring partner of the coordinator to discuss upcoming problems with. A steering group is formed consisting of the WP-leaders and deputy WP-leaders. To ensure efficient progress within the WPs, regular communications moments will be organized. They will have at least one meeting per year, most likely coupled to the annual meeting. The steering group will ensure the completion of deliverables in time and reaching the milestones (in time). Complete and timely reporting to the EJP management team is a joined responsibility of the steering group with final responsibility lying with the project coordinator. The project coordinator will liaise with the overarching EJP management. Important is also to oversee possibilities to connect activities between different WPs, look for opportunities to involve external organisations and link to other (EU) projects. The steering group can stimulate and facilitate to act upon such opportunities. Also contacts with EFSA and ECDC have been established and will be maintained in order to inform them on our progress and forfill the needs from EFSA and ECDC where possible and build upon already exisiting databases, models etc. In the summer of 2019, two new partners were added to the consortium (INIAV and VRI). Currently in the process of including more partners who already indicated their wish to become a partner, which includes the counterparts of the new members.

Task: T1.2 Communication/dissemination

Task start month: 1

Task end month: 36

Task Leader: Kitty Maassen, RIVM

Deputy Task Leader: Wim van der poel, WbvR

Task Participants: WP (deputy)leaders: Bfr, APHA, IZS AM, WbvR, RIVM, SVA
Description of the task Task 1.2 continues based upon what was achieved during the first and second annual period of the EJP. Within this task internal project communication is coordinated in close collaboration with task T-1.1. Annual meetings will be organized for all partners. In the meeting general project issues will be discussed and progression in the WPs is shared and discussed. In view of efficiency, the steering group meetings most likely will be coupled to the annual meetings. In addition, there is also the possibility to combine it with workshops or other meetings organized within other WPs.

At the end of the project a closing symposium will be organized. It is one way to disseminate the various products obtained in the project and transfer knowlegde. This symposium is open to all EJP partners and preferably to all EU countries, also those not involved in the EJP consortium.

A general EJP One Health website has been developed. This website does have the possibilities for private groups. Three groups are set up. One group for (secure) exchange of information between members of the steering group, one to inform all Cohesive members and one to inform all interested EJP members. We will develop opportunities to disseminate information to the public, most likely via the public area of the website. Targeted dissemination of information will be also defined, to address specific stakeholders with project progress and main results/deliverables.

Concerning sustainability, basic activities such website maintenance, networking with partners will be planned to continue after the end of the project. The main tools and procedures developed will be disseminated not only among the project partners but also to other national/international institutions/organizations potentially interested in the activities, favouring the further development of the tools and the extended networking.

WP2 Integrated risk-analysis at the national level

WP start month: M1 WP end month:M36

WP Leader: Kitty Maassen, RIVM

Deputy WP Leader: Charlotte Cook, APHA

WP participants: BfR, APHA, FHI/NIPH, ISZ LER, ISZ AM, FLI, SLV/NFA, PHE, ISS, NVI, CODA-CERVA, WIV-ISP, WbvR, RIVM, SVA, FOHM/PHA, INIAV.

Description of the WP: WP2 continues based upon what was achieved during the first and second annual period of the EJP. The objective of this WP is to provide common procedures and tools that can be used to improve the human-veterinary collaboration in the area of (emerging) zoonosis or even implement a sustainable integrated One Health structure at the national level. Examples of best practise methods will be used to design common procedures and tools, but also differences between countries and barriers for efficient collaboration will be taken into account as well. Importantly, the project will facilitate in the first step of creating support for a One Health structure by organizing local workshops with national stakeholders. In addition, a systematic way to select tools to help assess zoonotic threats will be developed to be used within the One Health structures.

Task: T-2.1 Development of guidelines for national One Health structures

Task start month: M1

Task end month: M26

Task Leader: Paul Bijkerk, RIVM Deputy Task Leader:

Task Participants: BfR, APHA, FHI/NIPH, ISZ LER, ISZ AM, FLI, SLV/NFA, PHE, ISS, NVI, CODA-CERVA, WIV-ISP, WbvR, RIVM, SVA, FOHM/PHA, INIAV

Description of the Task: Task 2.1 continues based upon what was achieved during the first and second annual period of the EJP. First, an inventory was made of current practice of One Health risk analysis (structures) at the national level. For this purpose, a workshop was organized accessible to all project partners, EJP partners and EU countries outside the consortium. This workshop was focused on current practice on human-veterinary collaboration, risk analysis and good practice, but will also reveal barriers to good practise, such as technical, political barriers, legal issues, logistics, difference in interests (economic versus health), difference in terminology and privacy. In this workshop also national goals and ambitions to improve the integration of human-veterinary risk-analysis were inventoried. As in the second year, also in the third year dedicated workshops will be organized to discuss and progress towards possible solutions for the most important barriers as identified within the first workshop. This WP will be in close collaboration with WP3, because of similar issues arise on European level. All information gathered will be used to develop common procedures and guidelines for setting-up/improving a national One Health structure. The guidelines will also contain suggestions on how to incorporate and use the national information system and tracing framework to be developed in WP4. The products developed will be publically available and will be used as input for task 3 of this WP and in WP3.

Task: T-2.3 Knowledge transfer and dissemination

Task start month: M25

Task end month: M35

Task Leader: Kitty Maassen, RIVM Deputy Task Leader:

Task Participants: BfR, APHA, FHI/NIPH, ISZ LER, ISZ AM, FLI, SLV/NFA, PHE, ISS, NVI, CODA-CERVA, WIV-ISP, WbvR, RIVM, SVA, FOHM/PHA, INIAV

Description of the Task: Task T-2.3 takes place over third annual period of the EJP. The common procedures and tools developed in Task T-2.1 and T-2.2 of this work package will be pilot-tested in partner and interested EJP-countries (upon request) via short-term missions by the consortium. The main goal is to create support in an interested country. It is known that it is difficult to get support for a structured collaboration when there is no crisis to show its importance. Therefore, we would like to organize short-term missions in order to locally provide information to all national stakeholders using experiences from others countries. Leading up to the meeting and during the meeting the developed common tools and procedures are tailored to the needs of the country and can be used for further implementation.

WP3 Towards an EU zoonoses structure

WP start month 1 WP end month 36

WP Leader: Elina Lathi, SVA

Deputy WP Leader: Charlotte Cook, APHA

WP participants: BfR, APHA, FHI/NIPH, FLI, SLV/NFA, PHE, ISS, NVI, CODA-CERVA, WIV-ISP, WbvR, RIVM, SVA, AGES, INIAV.

Description of the WP: WP3 continues based upon what was achieved during the first and second annual period of the EJP. The overall aim of the workpackage is to bring together existing risk analysis and epidemiological tools, skills and facilities that are required for a ‘one-health’ approach with respect to zoonotic diseases in Europe. One of the main tasks will be in assessing the barriers

encountered by risk analysis experts including surveillance experts to collaborate across country borders and recommending changes to be made to overcome them and ensure that surveillance experts have access to exactly the tools and data (see WP4) that are needed to provide a comprehensive risk analysis and surveillance service. As much progress in this area has already been made by some partners, a literature search will be undertaken to include white papers and informal reports and experiences by partners and other institutes. Identifying good practice throughout this area will require maintaining a knowledge base that is constantly curated by experts and subject to scrutiny both within and outside the project. The aims of workpackage 3 will be delivered via 5 tasks:

Task: 3.3 Retrospective systems analysis of detection of outbreaks

Task start month: 6

Task end month: 30

Task Leader: Charlotte Cook, APHA

Deputy Task Leader:

Task Participants: APHA, FHI/NIPH, FLI, SLV/NFA, PHE, ISS, NVI, CODA-CERVA, WIV-ISP, WbvR, RIVM, SVA, INIAV

Description of the task: Task 3.3 continues based upon what was achieved during the first and second annual period of the EJP. Task T-3.2 takes place over the first, second and third annual period of the EJP. Using a workshop approach, with relevant participants identified in the inventory workshop of the project as well as the listed partners, a systems analysis of selected surveillance systems will be used to identify certain key areas that are strong in identifying zoonotic pathogens of concern. This may include selecting previous outbreaks of a pathogen and mapping systematically the processes that are involved in detecting that an outbreak is occurring. Such analysis will aim to identify strengths and weaknesses in current surveillance systems whilst building relationships and cross –country/-department knowledge of surveillance systems and institutional knowledge. The extension of this task is the development of a pilot into task 3.4.

Task: 3.4 Pilot One-Health Early Warning System

Task start month: 25

Task end month: 36

Task Leader: Charlotte Cook, APHA

Deputy Task Leader:

Task Participants: APHA, FHI/NIPH, FLI, SLV/NFA, PHE, ISS, NVI, CODA-CERVA, WIV-ISP, WbvR, RIVM, SVA

Description of the task: Task T-3.4 takes place over third annual period of the EJP. Taking place in the last year of EJP this pilot system should include the relevant partners involved in both tasks 3.1 and 3.3 to build on and extend the initial findings. EU commission, ECDC and EFSA will be invited to participate in this task. This task includes how the exchange of signals can take place, for example in a formal meeting setting or an informal teleconference/video link. This pilot will also be an opportunity to trial some of the solutions to overcome barriers experienced in task 3.1. This task will also identify routes to escalate possible issues or recommend further data collection that should be undertaken. There will also be a need to keep the activities of the group open and transparent to allow countries outside the task to learn from the project. The outcome of this task will be in the form of expertise that ECDC and EFSA can draw on for development of a formal signal exchange system. This would include recommended requirements for the system as well as weaknesses and

potential problems that have been encountered during the pilot. The pilot study will include tools an platform that have been developed in work packages 2, 3 and 4 as well as relevant tools and networks that have been developed in other areas of the EJP, such as IA-1.

Task: 3.5 Development of tool for systematic cost-benefit analysis

Task start month: 25

Task end month: 36

Task Leader: Charlotte Cook, APHA Deputy Task Leader:

Task Participants: APHA, BfR, AGES, CODA-CERVA

Task T-3.4 takes place over third annual period of the EJP. In this task we will refine a tool for conducting cost- benefit analysis throughout the farm-to-consumption food chain using data collected on control measures. WP4 will be developing a platform for collating data related to risk assessment that this task will work with closely. The tool will developed in harmony with task 4.3 to ensure that all the tools developed in the project are produced in a similar framework to reduce the complexity for users. This will also minimise the training required and ensure that dissemination activities can be unified across the work packages where possible. The data used for validating and verifying the tools developed will be collected from research or surveillance projects both within and outside this EJP project.

Working from tools that have been developed previously for specific projects (http://www.efsa.europa.eu/en/supporting/pub/46e), further refinements will be made to enable the tool to be used on a wider variety of data and also to incorporate an economic component. This will enable control and surveillance measures to be evaluated against incidence reduction, both human and animal, and also to provide an estimate of whether these measures are likely to be economically viable. Such information will be necessary when evaluating the long-term OH surveillance and risk analysis strategies. Other member states will be encouraged to be part of the task for knowledge transfer and parameterisation of the tool at an individual MS level. Options for expanding the analysis to multiple member state blocks will be evaluated

WP4 Data platform to facilitate risk-analysis and outbreak control

WP start month: M1

WP end month: M36

WP Leader: Armando Giovannini, IZS-AM Deputy WP Leader: Armin Weiser, BfR

WP participants: BfR, FLI, IZS, FHI/NIPH, NVI, NFA, PHE, IZSLER, ISS, WbvR, FOHM/PHA, CODA-CERVA, WIV-ISP, AGES

Description of the WP: WP4 takes place over the first, second and third annual period of the EJP. The aim of this platform is to provide IT tools and data that are quickly available, usable and helpful in outbreak situations and for risk assessments. Especially tools for tracing, WGS and standardized risk assessments are established, harmonized and improved. All tools are planned to be developed into one software platform which will be available in the cloud to be evaluated during the project. These tools will be tested in a subset of the country involved in the project, willing to participate in the experimentation and to provide test data. The countries in this subset will be called "participating countries" thereafter.

WP4 as a whole is going to spread the usage of this set of tools (genetics, analysis, tracing and risk assessment tools) via workshops, interactive online courses and also bilateral courses and historical data applications with all partners including EFSA and ECDC. The aim is that it is usable by everyone – for local, but also for international outbreaks.

WP4 will take care of synchronization with other projects, especially IA-1: ORION and the other work packages WP2 and WP3. The development of other tools/data structures will be supported.
The ownership and privacy of data is a major important point when providing central software platforms. The data protection procedures will be evaluated case by case, on the basis of the contents of the national databases of participating countries.
WP4 will be very aware of this fact and will take care of integrated solutions for this in collaboration with WP3.

The COHESIVE project will provide a reasoned list of the available Information Systems for WGS data (NGS raw data and output of additional bioinformatics analysis) and associated meta data. This reasoned list will include the specifications of the available databases (data structures, etc.) and pros and cons of each information system. This reasoned list can be used by Member States to build up their own official system. Thus, it is anticipated the possibility of different implementations of the Information System for WGS data and associated meta data at Member States level. Such different implementations, however, should be harmonized and able to exchange their information at the European level. Specific task of the COHESIVE project will be to foster the harmonization and to implement the exchange tools for the various information systems implemented (or experimented) at the national level. No new databases will be produced by COHESIVE, but support is given to combine available databases into practical Information Systems, within Member States.

Task: T-4.1 Molecular typing data and metadata – database creation

Task start month: M1

Task end month: M32

Task Leader: Cesare Cammà, IZS-AM Deputy Task Leader:

Task Participants: BfR, FHI/NIPH, FLI, IZS-AM, IZS-LER, ISS, NVI, WbvR
Description of the task: Task T-4.1 takes place over the first, second and third annual period of the EJP. The adoption of WGS is becoming more and more widespread and it is progressively replacing older techniques (e.g. PFGE) for the definition of the links between cases within an outbreak and to link cases with their putative sources. What is missing is a link to be promptly used to connect results of WGS with other information on the various components of an outbreak: human cases, contaminated foods, pathway of contamination along the food processing chain, environmental conditions, location, etc.
The activities of this task will be based on the results of a set of activities and projects now ongoing at the EU level (ORION, COMPARE, EFSA and ECDC activities), which will give the outlines for the standardization of the databases to be used in European countries. Based on the specifications given by EFSA and ECDC, national databases will be experimentally implemented in this WP, able to feed data into a common European database and useful for the experimentation of epidemiological analysis of WGS data at the national level. In the lack of usable databases, for the sake of the fulfilling of the objectives of this project, prototype databases will be implemented, completely in compliance with the European standards set by EFSA and ECDC. EFSA/ECDC WGS mandate will be monitored in order to ensure that the final data models proposed by COHESIVE will be compliant with EFSA/ECDC WGS system.
For a more detailed description of the prototype system to be experimented, we will make reference to the architecture of the information systems relevant for our tasks already implemented in Italy (as a paradigmatic example of a member state), and with which we need to interface.
A set of databases are already operative in Italy (see diagram A1, buckets colored in blue for genetic data and yellow for metadata and other data useful for health purposes):

1. The database of the strains of pathogens isolated from human beings and clinical cases, including the genetic typing of these strains when available. This database is at the Italian national health institute (ISS), one of the partners of COHESIVE.
2. The databases of the strains of pathogens isolated from animals, food and the environment, including the genetic typing of these strains when available. These databases are mainly at the Italian national laboratories for the pathogens. The IZSAM is NRL for Campylobacter and has been recently appointed as the National Reference Center for Whole Genome Sequencing of microbial pathogens: database and bioinformatic analysis.
   The databases in (1) and (2) also include metadata directly related to each isolate (date, place, name of the patient or holding code, etc.)
3. The databases of the animal holdings and of animal movements, which contain the complete register of active animal holdings and the Italian animal trade networks. These databases are hosted in the servers at IZSAM.
4. The database of the food companies and plants, which is incomplete, contains only the main companies, especially those involved in the intra-community trade and in export to third countries. No data are readily available of the food trade networks. To be useful in outbreak investigations, therefore, this database needs to be integrated with ad hoc investigations in the case of an outbreak.

Another very important database with which the COHESIVE information system has to interface is the EU Joint DB EFSA/ECDC (colored in blue and with an EU flag in the diagrams).

The core of the prototype we are proposing to develop is the Prototype COHESIVE Information System (the bucket colored in red in the diagrams). The Prototype COHESIVE Information System is not a database with data of its own, rather it is a collection of procedures, queries, indexes, pipelines, needed to link all the other relevant and already existing databases each other. In some cases, to speed up tracing activities and analyses, part of the data stored in the national databases or in the EU joint DB, could be temporarily downloaded to the Prototype COHESIVE Information System.

One of the tasks of the Prototype COHESIVE Information System will be to harmonize and feed the genetic typing data produced at the national level into the EU Joint DB EFSA/ECDC. It is, therefore, interfaced with the national databases and the EU Joint DB.

It also has a number of other important tasks:

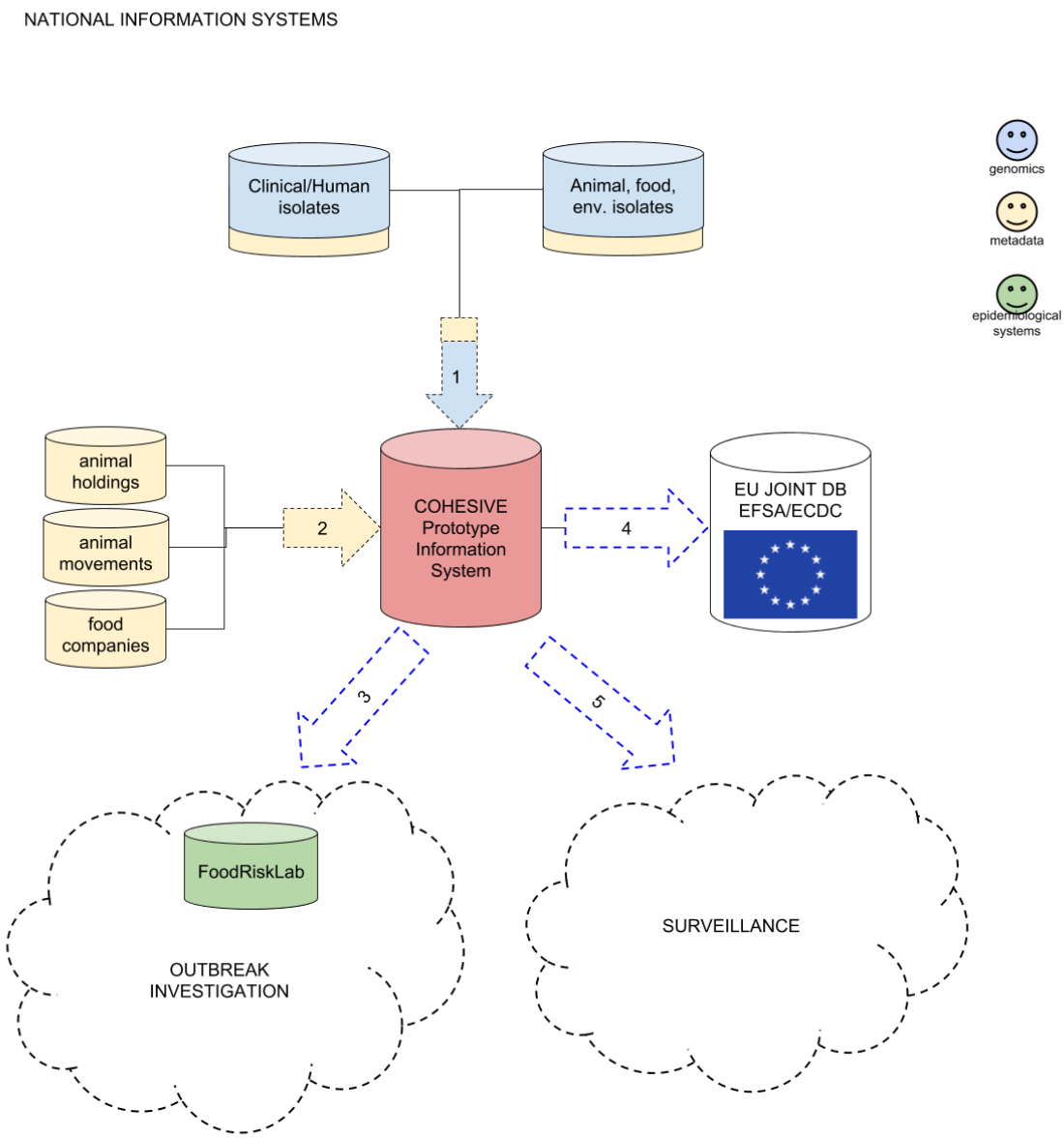
1. It will perform bioinformatic analysis on the data stored in the national Clinical/Human isolates DB and in the Animal/Food/Environment isolates DBs. Through these analyses, it may produce (see diagram A2, outputs) periodic reports on
   a. AMR profiles and trends in AMR,
   b. virulence profiles,
   c. phyloGIS (geographic distribution of genetic profiles)
   d. movements of genetic profiles in the trade network
   e. clustering (phylogenetic trees)
2. In the case of an outbreak without involvement of other European countries (diagram A2, outputs), the Prototype COHESIVE Information System will feed the systems for epidemiological analysis and tracing developed by the Task 4.2 (colored in green in the diagrams) with the relevant data stored in the National databases. The aim of this activity is to trace the origin of the outbreak (and possibly foresee its potential spread through the trade network).

3. In the case of an outbreak involving other EU member countries (diagram A3, EU outbreak), the linking of the Prototype COHESIVE Information System with the EU Joint DB EFSA/ECDC and with systems like TRACES may facilitate the transboundary outbreak investigation (not included as a possible output of COHESIVE).

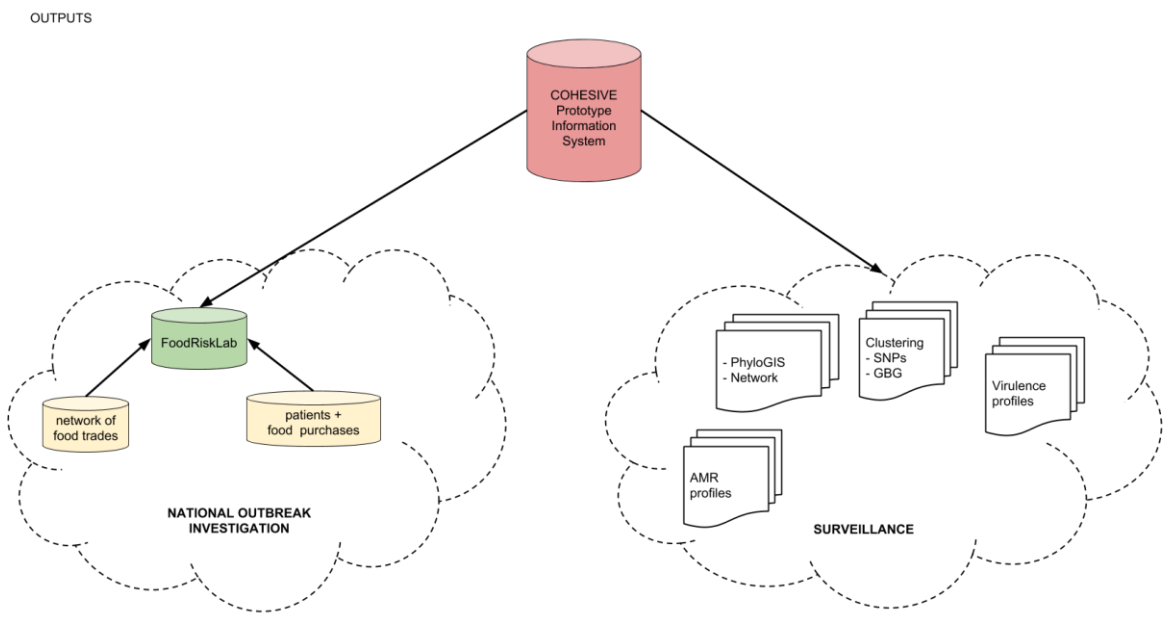
In conclusion, it is important to underline that no official EU wide system will be implemented. On the contrary, a study on the best possible integration of different information systems at member state level will be provided: for both WGS information, and associated metadata.

The final output of such study will be some Information system specifications. A member state could then realize an official national information system based on such specifications. The advantage will be that different systems among member states will be harmonized.

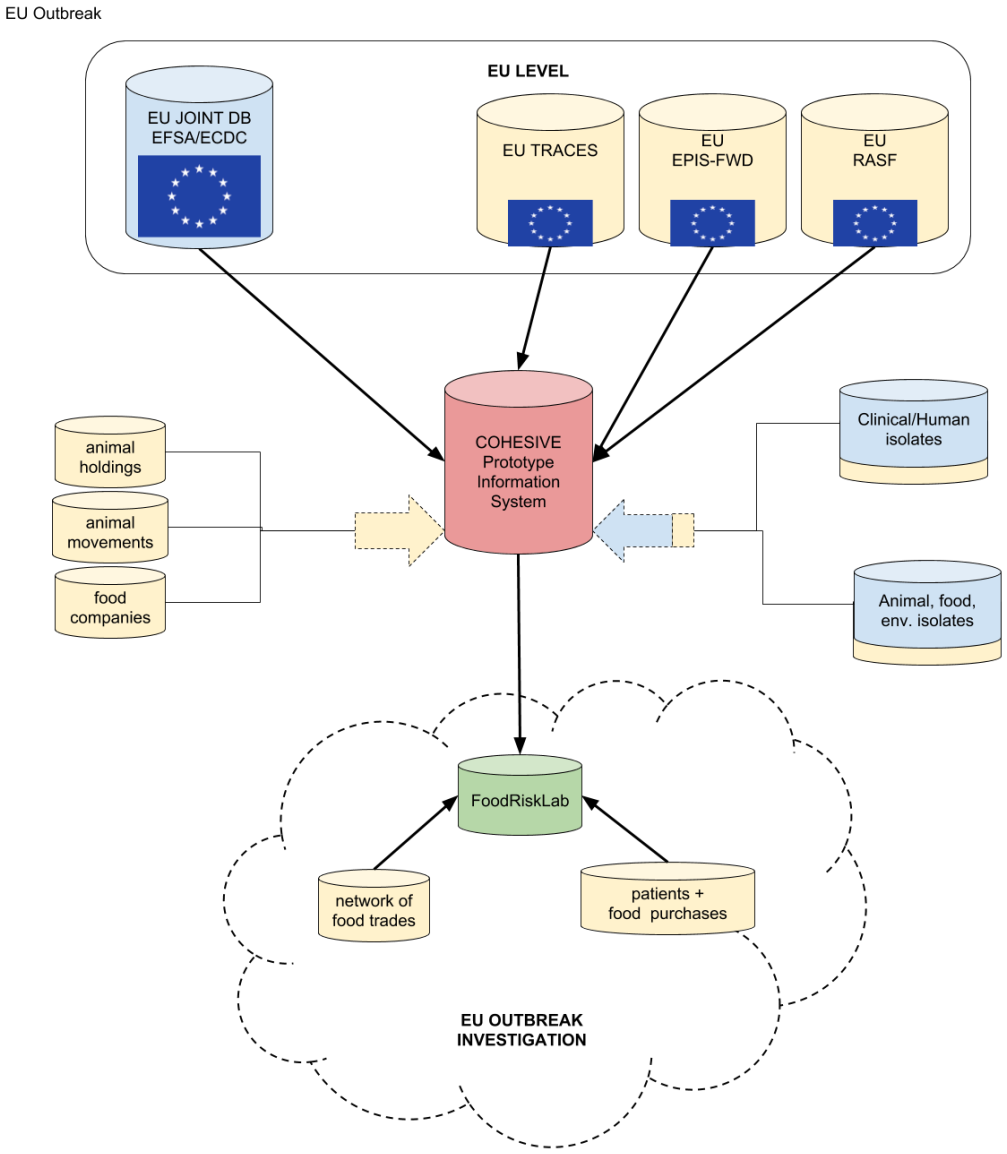
[Diagram A1: National information systems]



[Diagram A2: Outputs]



[Diagram A3: EU Outbreaks]



This task is subdivided into 6 sub-tasks, which will be realized at the national level by the Member countries and institutions involved in this WP:

Sub-Task sT4.1.5: Filling of DBs

Sub-Task start month M15

Sub-Task end month M26

Sub-Task Participants: IZS-AM, IZS-LER, FLI, ISS, NVI
Task T-4.1.5 takes place over the second and third annual period of the EJP. In this sub-task, based on the analysis performed in sub-task 4.1.4, we will implement the procedures necessary to link the national DBs relevant for this project (WGS, metadata and classical epidemiology) with the COHESIVE prototype information system and the tools for the epidemiological analysis of the data contained in the DBs. As far as possible, and depending on the priorities of each participating country, the data used to test the system will refer to the paradigmatic pathogens chosen during

the initial workshop. The amount of time allocated for this activity is because of possible unexpected difficulties faced during the linking of data, which are very likely to occur every time data are translated and migrated between two different systems.
This sub-task will produce the third deliverable of the task 4.1 (D-4.1.3): Databases containing the country data linked to the epidemiological analysis tools realized by tasks 4.2 and 4.3. The conclusion of this sub-task will be monitored using the Milestone M-A2.COHESIVE.4

Sub-Task sT4.1.6: Design and implementation of pipelines

Sub-Task start month M21

Sub-Task end month M32

Sub-Task Participants: IZS-AM, Bfr, FLI, NVI
Sub-task T-4.1.5 takes place over the second and third annual period of the EJP. The final sub-task of task 4.1 is the Design and implementation of the pipelines to feed the tools for epidemiological analysis from the relevant DBs by those Member States and institutions willing to experiment on the use of genetic data for the outbreak investigations.
The first step will be a survey of available bioinformatics analysis (pipelines) and, in particular, the output format. Consequently, the output format will follow a flexible data model, able to accept the results of pipelines used by different laboratories around Europe. No design of additional pipelines is foreseen in the project. This sub-task will be performed in close cooperation with the units at BfR responsible for tasks 4.2, 4.3, and 4.4. This sub-task will produce the fourth deliverable of task 4.1 (D-4.1.4), consisting in the description of the pipelines implemented to feed the analysis tools with the data stored in the three databases of this project.
The conclusion of this sub-task will be monitored using the Milestone M-A2.COHESIVE.5

Task: T-4.2 Development of a platform-independent tracing framework

Task start month: M1

Task end month: M32

Task Leader: Marion Gottschald, BfR Deputy Task Leader: Birgit Lewicki, Bfr

Task Participants: BfR, IZS-AM, FHI/NIPH, IZSLER, FLI, NVI, ISS, FOHM/PHA

Description of the task: Task 4.2 continues based upon what was achieved during the second annual period of the EJP. This task is going to combine and improve available tracing algorithms with analysis and visualization of WGS data into modern software integrating surveillance and outbreak data. Within this task a server system will be provided freely offering all developed tools browser-ready and open source.

The server will run on a Linux machine and the framework used is a Node.js / Angular framework providing secure areas for sensitive data. The server will be available worldwide for modern browsers, which mainly mean that only very old versions of common browsers are excluded, but the Internet Explorer is completely excluded.

Sub-Task sT4.2.2

Sub-Task start month M1

Sub-Task end month 32

Sub-task Participants: BfR, IZS-AM, FHI/NIPH, IZSLER, FLI, FOHM/PHA, NVI

Sub-task T4.2.2 continues based upon what was achieved during the first and second annual period of the EJP. This subtask is mainly a software programming and algorithm developing task. It seems

as if FoodChain-Lab (https://foodrisklabs.bfr.bund.de) is the state of the art tracing tool in Europe supporting quick supply chain data gathering and fastening in solving outbreak investigations. FoodChain-Lab has some room for improvements, e.g. eased availability and implementation of additional algorithms like traditional network analysis or modelling on the spread of germs along the collected supply chains. Therefore, a cloud-based browser-ready and user-friendly tracing tool based on FoodChain-Lab will be developed.

The deliverable of this subtask is the ready-to-use tool with integrated network analysis module.

Sub-Task sT4.2.3

Sub-Task start month M13

Sub-Task end month M32

Sub-task Participants: BfR, IZS-AM, FHI/NIPH, IZSLER, FLI, FOHM/PHA, ISS

Sub-task T4.2.3 continues based upon what was achieved during the second annual period of the EJP. This subtask takes care of the integration of even more meaningful surveillance and outbreak data into the software platform for analysis. Especially for 4.2.2, further data sources from MED- and VET-side, like cases, sampling results and also analysis results based on WGS-data from WP4.1 are going to be implemented resulting in a new algorithm based on probabilities for the scoring of common sources of an outbreak.

This subtask does also take care of the synchronization with IA-1: ORION and WPs 2 and 3. Selected promising tools will be taken into account for integration into the software platform of WP4. Especially, Task5 from WP3 seems promising, and it may come out that Task3 also develops into a candidate for the platform.

The deliverable of this subtask is the ready-to-use tool with extended WGS-Epi module and a report on additionally needed tools.

Task: T-4.3 Development of a platform-independent risk modeling framework

Task start month: M1

Task end month: M30

Task Leader: Christin Wallstab, BfR

Deputy Task Leader: Christine Müller-Graf, BfR

Task Participants: BfR, FHI/NIPH, FLI, FOHM/PHA, NVI

Description of the task: Task 4.3 continues based upon what was achieved during the first and second annual period of the EJP. The risk modelling framework will provide a flexible support to conduct high-end statistical modelling and simulation (including two-dimensional Monte Carlo and Markov chain Monte Carlo techniques, uncertainty and sensitivity analysis) with automatic reporting and model documentation, modular structure and options for using pre-defining templates for parts of the model. Risk scenarios defined in other task groups are considered as use cases.

The graphical user interface for risk modelling will be developed and implemented with shiny R package to build an interactive web app. The app will hosted on BfR webpage and server. Moreover, all scientific code will be made available under open source license model.

Sub-Task sT4.3.2 Implementation

Sub-Task start month M10

Sub-Task end month M30

Sub-task Participants: BfR, IZS-AM, FHI/NIPH, FLI, FOHM/PHA, NVI

Sub-task T4.3.2 continues based upon what was achieved during the first and second annual period of the EJP. a. implementation of standards and specific tasks of the risk modelling framework, including automated thorough documentation of the risk model,

b. GUI implementation,

c. technical documentation and user guidance.

Sub-Task sT4.3.3 Validation of the risk modelling framework

Sub-Task start month M19

Sub-Task end month M33

Sub-task T4.3.3 continues based upon what was achieved during the second annual period of the EJP.

a. Defining testing scenarios for verification and validation for each programme module,

b. Testing using testing scenarios and benchmarking (i.e. runtime),

c. Comparison with external programmes when available.

Sub-Task sT4.3.4 Deployment of the risk modelling framework

Sub-Task start month M34

Sub-Task end month M36

Sub-task Participants: BfR, FLI, IZS, FHI/NIPH, NFA, PHE, IZSLER, ISS, WbvR, FOHM/PHA, CODA-CERVA, WIV- ISP, AGES

Sub-task T-4.3.4 takes place over the third annual period of the EJP.

Task: T-4.4 Dissemination Task start month: M25

Task end month: M36

Task Leader: Marion Gottschald, Bfr Deputy Task Leader:

Task Participants: BfR, IZS-AM, NVI

Description of the task: Task T-4.4 takes place over the third annual period of the EJP. Dissemination via workshops and supporting of real/historical outbreak investigations (bilateral possible). All tools developed in WP4 will have enduring support and interactive live online courses will be offered. The tools will also be used in pilots in WP2 and WP3.

Deliverables

Ref	Title	Due month
D-1.5	Annual meeting	M26
D-1.6	Annual report	M28
D-1.7	Closing symposium	M36
D-1.8	Annual report	M36
D-2.5	Development of common procedures and tools	M26
D-2.6	Knowledge transfer and dissemination	M34
D-3.6	Knowledge transfer and dissemination	M34
D-3.7	Tool for systematic cost-benefit analysis	M36
D-4.1.3	Databases containing the country data	M26
D-4.1.4	description of the pipelines implemented to feed the analysis tools with the data stored in the three databases of this project	M32
D-4.2.2	FoodChain-Lab with integrated network analysis module as a web-service browser-ready, open source and implemented in the BfR cloud	M32
D-4.2.3	Integration and analysis of epi-data from 4.1 → WGS-Epi module and selected features from IA-1, WP2, WP3	M32
D-4.3.2	Populating a model project model repository Harmonised graphical user Interface prototypes Technical documentation and user guidance	M30
D-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)	M36
D-4.4.1	each subtask conducts workshops, provides documentation and tutorials	M36
Milestones		
Ref	**Title**	**Due month**
M-AI2.COHESIVE.1.3	Annual report	M28
M-AI2.COHESIVE.1.4	Closing symposium	M36
M-AI2.COHESIVE.1.5	Annual report	M36
M-AI2.COHESIVE.2.2	Development of common procedures and tools	M26
M-AI2.COHESIVE.2.3	Knowledge transfer and dissemination	M34
M-AI2.COHESIVE.3.1	Development of common procedures and tools for horizon scanning	M26
M-AI2.COHESIVE.3.3	Local dissemination	M34
M-A2.COHESIVE.4.7	Databases containing the country data	M26
M-A2. COHESIVE.4.8	Prototype for risk modeling framework ready	M30
M-A2.COHESIVE.4.9	Availability of the pipelines from the databases to the analysis tools	M32
M-A2. COHESIVE.4.10	Analysis of epi-data into tracing framework enclosed	M34
M-A2. COHESIVE.4.11	Final release of risk modeling framework	M34

2.4.2.1.33 JIP03-R2-IA2.3-CARE

Reference to the Stategic Research Agenda (please refer to D2.7)			**IA 2.3: Joint databases of reference materials and data, incl. metadata.**							
Integrative Project Title			**Cross-sectoral framework for quality Assurance Resources for countries in the European Union**							
Integrative Project Acronym			**CARE**							
Leading Organisation			**P12-DTU**			Deputy Leading Organisation			**P13-SSI**	
Project Leader			**Rene Hendriksen**			Deputy Project leader			**Mia Torpdahl**	
Project Start month			**M25**			Project End month			**M54**	
Participant	**1**	2	4	6	7	8	9	10	11	**12**
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	**DTU**
PM	**23.5**									**17.5**
Participant	**13**	14	15	16	**17**	18	**19**	**20**	**21**	22
	SSI	UT	VFL	INIA	**UCM**	MVNA	**INRA**	**IP**	**APHA**	PHE
PM	**19**				**13**		**19.6**	**3**	**1.62**	
Participant	23	24	25	26	**27**	**28**	**29**	**30**	**31**	32
	UoS	OKI	NUIG	TEAGASC	**ISS**	**IZSAM**	**IZSLER**	**RIVM**	**WbvR**	FHI
PM					**6**	**10**	**19**	**6.5**	**4.4**	
Participant	33	**34**	35	36	37	38	**39**	**40**	**41**	
	NVI	**PIWET**	INIAV	INSA	IISPV	IC	**SLV**	**FOHM**	**SVA**	
PM		**36**					**2.9**	**3.5**	**4.95**	

Objectives

- To coordinate collaboration between the public health, food and animal health sectors participating in CARE

Description of work
WP: WP0 Project coordination
WP start month: 25
WP end month: 54
WP Leader: Rene Hendriksen, DTU
Deputy WP Leader: Mia Torpdahl, SSI

WP participants: ANSES, DTU, SSI, UCM, INRA, IP, APHA, ISS, IZSAM, IZSLER, RIVM, WbvR, PIWET, SLV, FOHM, SVA.
Description of the WP: JIP IA 2.1-WP0
The coordinator and coordinating institute, DTU will invest a large amount of time in the CARE project. The coordinator will maintain regular contacts between the 16 different partners and will be responsible for the efficient progress of the project. The coordinator will make sure that all deliverables and milestones are reached in the timeframe envisioned for the project. The coordinator will organize interactions between groups, and meetings (one annually meeting and quarterly telephonic conferences) to continuously monitor progress of the objectives and to discuss scientific advances and problems. The kick off meeting will take place at DTU, Kgs. Lyngby, Denmark, the interim meeting at ANSES, Paris, France and the closing meeting at SSI, Copenhagen, Denmark.
All partners are aware that strict management and clear lines of communication are essential to the successful completion of the proposed work programme. All partners have previously been involved in EU, ECDC, EFSA or other large consortia and are familiar with working in multi-centre, multinational projects. The partners have collaborated in different constellations with each other before and have been chosen both for their scientific credentials and proven ability to deliver in such projects. Excellent lines of communication and working relationships between all the partners have already been established. Efficient communication between partners will be achieved using electronic mail, and frequent telephone and web meetings.
Each partner will provide the coordinator with scientific and financial statements, from which the coordinator will prepare the reports (cost details, research results and technical advancements). Any problem related to the financial management of the project will be discussed.

Task: JIP IA 2.1-WP0-T1 Over-all project coordination
Task start month: M25
Task end month: M54
Task Leader: Rene Hendriksen; DTU
Deputy Task Leader: Mia Torpdahl, SSI
Task Participants: ANSES, DTU, SSI, UCM, INRA, IP, APHA, ISS, IZSAM, IZSLER, RIVM, WbvR, PIWET, SLV, FOHM, SVA.
Description of the task: Maintain regular contacts between the 16 different partners and monitor progress of the project through tele-conferences every 2nd month. Thus, to ensure that all deliverables and milestones are reached in the timeframe envisioned for the project.

Sub-Task JIP IA 2.1-WP0-T1-ST1: Organize kick off meeting.
Sub-Task start month: M25
Sub-Task end month: M25
Sub-Task Leader: Rene Hendriksen, DTU
Deputy Sub-Task Leader: Mia Torpdahl, SSI
Sub-Task Participants: ANSES, DTU, SSI, UCM, INRA, IP, APHA, ISS, IZSAM, IZSLER, RIVM, WbvR, PIWET, SLV, FOHM, SVA.

Deliverables

Ref	Title	Due month
D.0.1	Consortium agreement	M25
D.0.2	Internal progress reporting templates	M26
D.0.3	Dissemination plan	M28
D.0.4	Minutes of the kick off meeting - 2020	M26

Milestones		
Ref	Title	Due month
M.0.1	Kick off meeting - 2020	M25

WP1

Objectives

To develop guidance for cross-sectoral proficiency testing, aimed at trialling the collaborative systems' ability to solve food-borne outbreaks and other trans-sectoral challenges.

Description of work

Task: **IA 2.1-WP1:** Develop of cross-sectional PT´s
WP start month: 25
WP end month: 54
WP Leader: 13 SSI; Jeppe Boel
Deputy WP Leader: 12 DTU, Rene Hendriksen

WP participants: DTU, SSI, UCM, PIWET, APHA, ISS, RIVM, WbvR, SLV, FOHM, SVA, ANSES.
Proficiency testing- (PT) or external quality assurance (EQA) schemes is an integrated part of quality assurance management for laboratories within the field of human and animal medical microbiology. The aim of this WP is to develop guidance for proficiency testing schemes that can be used cross-sectorial. This will be done by mapping the currently available PT´s and assess the usefulness of these schemes in a cross-sectorial context and by developing proposals for new PT schemes that can accommodate unmet and new needs.
The rapid emergence of whole genome DNA sequencing (WGS) methodologies for characterization of pathogens including analytical approaches that can predict virulence factors, antimicrobial resistance, clonal relationship, and identify food- and water borne outbreaks is an area where there is a need for new cross-sectorial PT schemes. Likewise, in the area of molecular based methodologies, including WGS, for culture-free detection/identification of pathogens in complex sample material new PT schemes are needed.
In order to facilitate the development of the new PT proposals the WP will include a number of well-designed pilot PT´s that will be performed among the WP participants.
The WP will be concluded by developing a final guidance document giving specific recommendations in relation to which PT´s that should be prioritized and containing specific guidance on how to perform the proposed cross-sectorial PTs.
The WP will be divided into three parts (tasks):

- Mapping of existing PT's and proposals for new PT schemes (6 months, M25-30)
- Pilot trials (organized as sub-tasks) and documentation of outcome (18 months, M31-48).
- Development of guidance document and proposals for SOPs where appropriate with suggestions for design of future cross-sectorial PT schemes (6 months, 49-54).

Task: **IA 2.1-WP1-T1 Mapping of existing and proposals for new PT schemes (6 months)**
Task start month: 25
Task end month: 30
Task Leader: RIVM/ Kirsten Mooijman
Deputy Task Leader: Open
Task Participants: DTU, SSI, UCM, APHA, ISS, RIVM, WbvR, SLV, FOHM, SVA, PIWET, ANSES.

Description of the task:
The purpose is to map the currently available PT schemes and to identify existing and propose new PT schemes that can be used in a cross sectorial context.

The mapping will be conducted by performing a systematical review of the currently available relevant PT schemes, including a review of the database of PT´s that are maintained by "Bundesanstalt für Materialforschung und -prüfung (BAM)", see www.eptis.org/index.htm in which >4000 international PTs are listed.
The WP will address the need for development of new PT's that are relevant from a one-health perspective. The focus will be on new molecular based methodologies, e.g. WGS that are being used cross-sectorial. The areas covered will include suggestions for proficiency testing of both wet and dry laboratory procedures, and include detection of pathogens in primary materials and methods used for characterization of isolates. A further, and novel component, is to develop a PT scheme where the proficiency in the speed of response to an outbreak is assessed.
The outcome of the work will be a short report (D.1.1) summarizing the usefulness of the currently available PT´s and to give a roadmap for the work in IA 2.1-W1-T2 which eventually will support the development on the final guidance document, IA 2.1-W1-T3

Task: **IA 2.1-WP1-T2 Pilot trials and documentation of outcome (18 months)**
Task start month: 31
Task end month: 48
Task Leader: SSI, Jeppe Boel
Task Participants: WP participants: DTU, SSI, UCM, PIWET, APHA, ISS, RIVM, WbvR, SLV, FOHM, SVA, ANSES.
Description of the task:
Based on the outcome of **IA 2.1-WP1-T1** a number of well-designed pilot PT´s will be organized to support the design of new cross-sectorial PT´s. The PT´s will be performed in accordance with the criteria stipulated in ISO17043:2010, "Conformity assessment - General requirements for proficiency testing"
The pilot PT´s will include both wet and dry laboratory exercises, and include different approaches, including culture and non-culture based detection methods and characterization methods with special focus on WGS based methodologies. A novel component is to perform pilot PT schemes where the proficiency in the speed of response to an outbreak is assessed. The participating laboratories will both provide analytical results as well as information about the applied methods.
The activities in this task will be coordinated with JRP/FBC 2.5 "Better tools for detection and investigation of foodborne outbreaks, including antimicrobial resistant pathogens, as well as economic assessments of potentially increased cluster detection through whole genome sequencing".
The precise activities within each sub-task will be determined and coordinated at the first meeting at the initiation of the CARE project.
All learnings from the pilot PTs will be used directly in development of the design of the future PT schemes
The pilot PT's will be performed as three subtasks and documented in reports that will be used in **IA 2.1-W1-T3.**

Sub-Task: **IA 2.1-W1-T2-ST1 Pilot PT on isolation/detection and characterization of pathogens**
Sub-Task start month: M31
Sub-Task end month: M48
Sub-Task Leader: SVA, Elina Lahti
Deputy Sub-Task Leader: FOHM, Cecilia Jernberg
Sub-Task Participants: DTU, SSI, UCM, APHA, ISS, RIVM, WbvR, SLV, FOHM, SVA, ANSES
Description of the sub-task:
The work will be focused on the laboratories ability to detect one or several different pathogens in different matrices; food and caeca/fecal samples. The pilot PT will target pathogens that are likely

to be present in foods and known to cause illness in humans. The pilot PT will include the detection/isolation methods that are used in the different sectors, including the possibility to use culture, molecular and metagenomics based methods. The pilot PT will be performed as a cross-sectoral exercise where different strains with a matrix will be distributed to veterinary and food laboratories and simultaneously a set of strains will be distributed to public health laboratories. This will be followed by comparison of the reported results on detection, isolation and level of characterization from the sectors.
The work will be finalized with a report (D.1.2.1) describing the outcome of the pilot PT and recommendations for a design of cross-sectoral PTs within this area.

Sub-Task: **IA 2.1-W1-T2-ST2 Pilot PT on typing/characterization including WGS**
Sub-Task start month: M26
Sub-Task end month: M32
Sub-Task Leader: DTU, Rene Hendriksen
Deputy Sub-Task Leader: WBWR, Kees Veldman
Sub-Task Participants: DTU, SSI, UCM, APHA, PIWET, ISS, RIVM, WbvR, SLV, FOHM, SVA, ANSES.

Description of the sub-task:
The pilot PT will include pure cultures as well as DNA samples. The proficiency will be assessed by evaluating the laboratories ability to predict geno- and phenotypic traits in bacteria from WGS data (e.g. serotype, MLST, SNP types, virulence factors, prediction of antimicrobial resistance patterns (wild type or non-wild type and possibly MIC values). The quality of the derived sequences will be addressed by considering generic elements such as DNA quality, library preparation, coverage, depth, sequencing platforms, and bioinformatics tools for data analysis.
The work will be finalized with a report (D.1.2.2) describing the outcome of the pilot PT and recommendations for a design of future cross-sectoral PTs within this area.

Sub-Task: **IA 2.1-W1-T2-ST3 Pilot PT on outbreak surveillance based on WGS data**
Sub-Task start month: M31
Sub-Task end month: M48
Sub-Task Leader: SSI, Jeppe Boel
Deputy Sub-Task Leader: APHA, Nick Coldham
Sub-Task Participants: DTU, SSI, UCM, APHA, PIWET, ISS, RIVM, WbvR, SLV, FOHM, SVA, ANSES.
Description of the sub-task:
Typically, for proficiency testing a turnaround time of up to three weeks would be expected. However, this might be too slow where a rapid response is required in an outbreak situation and member states need to introduce control measures.
In silico pilot PT trials will be launched to assess the laboratories ability to identify the source of foodborne outbreaks. The pilot PTs will include a scenario exercise, where laboratories shall identify an outbreak based on simulated data that will be provided to the laboratories in real-time. The exercise will test the laboratories ability to analyse, interpret and communicate data in real time, and test overall collaborative performance of the systems in place for outbreak detection. Additionally, an important element of the pilot PT will be to evaluate the speed of the response.
Standard techniques for phylogenetic comparison of bacterial strains will be evaluated, as this information may be essential for discovering new emerging pathogenic strains, tracing outbreaks, and introducing appropriate control measures across countries. Common target species such as *Salmonella* and *E. coli* will be included and the pilot PT might also include testing for AMR genes as this is a generic end point for many laboratories.
The work will be finalized with a report (D.1.2.3) describing the outcome of the pilot PT and recommendations for a design of future cross-sectoral PTs within this area.

Deliverables		
Ref	Title	Due month
D.1.1	WP1-T1: Report - Mapping of existing and proposals for new PT schemes	M30
Milestones		
Ref	Title	Due month
M.1.1	Kick off meeting for WP1	M25
M.1.2	Review of task 1 and description of relevant pilot PTs	M32

WP2 Creation of EUROpanelOH

Description of work

WP: 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
WP start month: 25
WP end month: 52
WP Leader: Olivier Chesneau, IP
Deputy WP Leader: Stefano Morabito, ISS
WP participants: ANSES, DTU, UCM, INRA, IP, ISS, IZSAM, IZSLER, WbvR, SLV, FOHM, PIWET, SVA, SSI.

Description of the WP: Bacterial reference materials (RM) are dispersed in Europe. Control strains are regularly distributed for EURLs PTs, PulseNet surveillance programs and EQAs organized by ECDC, but completeness and harmonization in providing RM for the application development and validation of analytical methods in a One Health perspective is still scanty. The need of certified RM is crucial in detail for the validation of novel methods, which is currently an active area of development, especially in the field of methods capable to identify and characterize bacterial strains on the basis of whole genome sequence (WGS) analyses. Valuable on-line resources to infer virulence and identify genetic features associated to antimicrobial resistance from WGS data are already available through several bioinformatics suites of programs, either commercially available or distributed through open web portals, such as services provided by the Center for Genomic Epidemiology at DTU (http://www.genomicepidemiology.org) or ARIES webserver hosted by EURL VTEC (https://www.iss.it/site/aries). Nevertheless, novel tools are being developed continuously for bioinformatics analysis of WGS data and there is a necessity of certified RM for their benchmarking and validation in laboratories.

The first objective of WP2 will consist of making an inventory of current use and existence of RM among CARE partners and other voluntary OH-EJP partners with a balance of leading sources according to the sector(s) of the partner: Animal, Food, Human (JIP IA 2.1-WP2-T1). Type specimens will be listed from pathotypes to phylotypes among prioritised pathogens, with a special effort made in the field of antimicrobial resistance to cover all mechanisms and track major allelic variants. Attention will be made to associate reference isolates with the corresponding genomic sequences. Redundancy will be avoided, phenotypes and genotypes will be reported, and there will be an assortment of clinically important resistance mechanisms outside the zoonotic foodborne potential, contributing to the One-Health perspective. A systematic process of gathering information that is appropriate and sufficient to develop an effective database (strains and genomes) will address the groups' needs and wants (JIP IA 2.1-WP2-T2). The database with epidemiological, phenotypic and genomic information will be organized for each selected bacterial species. A sub-database will be dedicated specifically to antimicrobial resistance mechanisms. Each CARE partner will be asked to contribute for enriching the databases by fill in the gaps through better characterization of selected isolates that will be shown of interest (JIP IA 2.1-WP2-T3). At the end of CARE and for next future developments, the EUROpanelOH will be a curated repository of

certified RM that will serve as a centralized source of challenge sets for surveillance and advancing basic science research.

Task: JIP IA 2.1-WP2-T1 **Inventory of current use and existence of reference materials** across CARE / OHEJP partner institutes, for selected, prioritized pathogens and antimicrobial resistances
Task start month: 26 (duration 10 months)
Task end month: 35
Task Leader: ANSES, Anne Brisabois
Deputy Task Leader: ISS, Stefano Morabito
Task Participants: IZSLER, IZSAM, SSI, IP, DTU, WbvR, SVA, UCM, INRA, Anses, PIWET
Description of the task: The availability of RM for bacterial pathogens, including either reference isolates or genomic sequences, will be investigated among partners of CARE and OH-EJP by launching a dedicated survey. The survey will include the possibility to point at reference collections accessed by the partners through repositories made available by other organizations (e.g. online repositories, PulseNET, ECCO, WFCC, FAO ..). Besides, the aim will consist of identifying the approach currently used by experts in the field to select appropriate RM, the localization where RM are stored, and the sector Animal / Food / Human from which RM have been isolated. This information will be used to make a list of routinely used certified RM for the bacterial pathogens considered of main interest in the field of OH-EJP. Attention will be paid to associate the reference isolates with a minimal mandatory set of metadata (e.g. source, year and country of isolation) and to include all kinds of analyses and characterization data obtained through validated standard methods. For antimicrobial resistance genes, a separate list including isolates of diverse bacteria resistant to specific compounds or associations will be appended to the list of the targeted pathogens, along with the susceptibility testing data. There exists a need to have a single standardized challenge database that contains all validated antimicrobial resistance genes (ARG) classified by mechanisms. This will be used as a gold reference data set to analyze RM genomes sequences. For all the listed isolates, a link to the genomic entries eventually available in public repositories (NCBI, ENA or DDBJ) will be added. Similarly, other molecular characteristics (e.g. MALDI-TOF mass-spectra or toxin profiling) will be also considered when available. Finally, the location where the bacterial cultures are currently stored will be included in the document, in view of facilitating the work of WP3 which aims to make RM more easily accessible. A subset of well-characterized RM will constitute the first inputs for feeding WP3.

Task: JIP IA 2.1-WP2-T2 **Gap analysis** with respect to accessibility, quality and usefulness of existing and potential new reference materials from a One-Health perspective.
Task start month: 36 (duration 7 months)
Task end month: 42
Task Leader: IP, Olivier Chesneau
Deputy Task Leader: INRA, Sophie Roussel
Task Participants: SSI, DTU, ISS, SSI, WbvR, SVA, UCM, ANSES, IP, PIWET, INRA.
Description of the task: Following the results of the survey conducted in JIP IA 2.1-WP2-T1 and the construction of the database listing the available RM (i.e. what we have), a need assessment will be conducted in JIP IA 2.1-WP2-T2 and will require consultation with partners in CARE, in OH-EJP and among a broader scientific audience to identify gaps related to the desirable RM (i.e. what we should have). These could include emerging threats or reference strains for additional features of already represented species, including reference isolates with unknown or elusive mechanisms of resistance, due to novel horizontally acquired genes or novel mutations arisen in the genomes. In addition, this gap analysis will be a key insight to stress the need of reference genomic sequences for many of the identified RM which will be identified and listed in this task. Such a need assessment will contribute to feed the JIP IA 2.1-WP2-T3. The mass-spectrometry (MS) data of at least some representative RM for the pathogens included in the list could be also desirable in order to compile

spectra which could serve the needs of laboratories working in food safety. The whole process of JIP IA 2.1-WP2-T2 will aim to determine what should be done in the enrichment and characterization of RM for the benefit of the laboratories that develop or validate methods for pathogen detection and antimicrobial resistance prediction.

Deliverables	
Title	Due month
Shared database gathering all information	M34
Sub-database for antimicrobial resistances	M35
Milestones	
Title	Due month
Identification and localization of available RM among CARE and OH-EJP partners	M30

WP3

Objectives

- To identify existing systems (software, databases) in place to record and query collections of biological materials held by OHEJP partners, for routine diagnostic and research needs.
- To ensure the long-term availability and sustainability of the reference material collection.
- To develop an approach for making such collections more widely accessible, within defined limits.

Description of work

WP: WP3 - Access and sustainability of well-defined microbial reference materials (RM)
WP start month: **25**
WP end month: **54**
WP Leader: Sophie Roussel (INRA)
Deputy WP Leader: Anne Brisabois (ANSES)
WP participants: ANSES, INRA, IP, PIWET , IZSAM, SVA, SSI, WbvR, IZSLER, SLV

Description of the WP:
This WP seeks to make visible, accessible and sustainable the reference material well-identified and characterized in WP2 with the complete data panel associated. The second objective of this WP is to make visible the microbial collections of CARE partners. Firstly, from existing portal data infrastructures, an information system will be developed including a web site and a searchable electronic collection catalogue (JIP IA 2.1-WP3-T1). This information system will facilitate the open access to reference strains and data for the scientific community use. Reference strains identified in WP2 will be maintained and stored in microbial collections considered as “sustainable” (JIP IA 2.1-WP3-T2). This work should enable us to ensure the long-term availability of RM in order to contribute the needs of the European research. Moreover, we plan a training session dedicated to the management of microbial collections (JIP IA 2.1-WP3-T2). This training should enable CARE partners to update and manage their microbial collections. In a third task (JIP IA 2.1-WP3-T3), we will ensure wide dissemination and accessibility of the RM. We aim to connect the CARE information system with data portals of existing international and solid infrastructures, to ensure the long-term availability of the CARE information system.
Task: JIP IA 2.1-WP3-T1 Development of an information system for making RM more widely accessible and visible
Task start month: M25 (Duration : 11 months)
Task end month: M35
Task Leader: INRA, Sophie Roussel
Deputy Task Leader:, IZSAM, Giuseppe Aprea

Task Participants: INRA, IP, DTU, PIWET, IZSLER, SSI, IZSAM, SLV, SVA, WbvR.
Description of the task: The Task JIP IA 2.1-WP3-T1 relies on an informatic approach. Beforehand, existing systems to record and query collections held by OHEJP partners will be listed. An information system (IS) will be developed to enable users evidence, query, and access of reference material. It will consist a minima of (i) a searchable electronic catalogue containing a maximum of information and data on the reference strains preserved and where they are stored (ii) a website. In addition, the IS will contain IT tools to support the curators and to provide users information in a structured way. A plan management data will also be established. Data will be of different types including mandatory- and -not mandatory data: "passport" data, sequencing and phenotyping data, and "regulation" data for easy access in compliance with applicable legislation (Nagoya Protocol).
The CARE catalogue will be connected to a broad range of European and National databases of microbial collection (including databases of CARE partners). We will follow recommendations established by the scientific community such as for instance the US AgBiodata consortium (Harper et al., 2018). The CARE IS will embrace the Findable, Accessible, Interoperable and Reusable (FAIR) principles established by stakeholders from the scientific, publishing and library communities (Wilkinson et al., 2016, 2019). The IS must be regularly updated, upgraded and curated. The CARE IS will link established databases and bridge the gaps between biodiversity repositories, sequence databases and characterization data obtained in WP2 (outputs WP2D2.1 ; WP2D2.6). The data portal infrastructure will be comparable and compatible to (i) this already existing of the Global Genome Biodiversity Network (GGBN) (Droege et al., 2013) and (ii) the future one that will be developed within the European Research Infrastructure MIRRI (Microbial Resource Research Infrastructure) (Casaregola et al., 2016).
This portal will make reference strains available for the European research, be it public or private. Data associated with strains will be freely available through IS. This portal will be a key tool as it will allow users to access strains and relevant information *via* one entry point, avoiding time-consuming searches for necessary information on specific strains. Through CARE, a new level of interoperability between strains, metadata and databases will be obtained and should boost the European research.

Glossary

- ECCO: European Culture Collections Organisation
- ELIXIR: brings life science resource from across Europe. These resources include databases, software tools, training material, cloud storage and super computers.
- EMBRC: European Marine Biological Resource Centre
- EOSC: European Open Science Cloud
- ESFRI: European Strategy Forum on Research Infrastructures
- MIRRI: Microbial Resource Research Infrastructure
- OECD: Organisation for Economic Cooperation and Development
- OIE: World Organization for Animal Health
- WFCC: Word Federation for Culture Collections

References

- Casaregola S, Vasilenko A, Romano P, et al (2016) An Information System for European culture collections: the wayforward. Springerplus. 5:772.
- Droege G, Barker K, Astrin JJ et al (2013). The global genome Biodiversity Network (GGBN) Data Portal. Nucleic Acid Research, 42607-612.
- Harper L, Campbell J, Cannon EKS et al., (2018). AgBioData consortium recommendations for sustainable genomics and genetics databases for agriculture. Database 1-32
- Janssens D, Arahal DR, Bizet C et al (2010). The role of public biological resources centers in providing a basic infrastructure for microbial research. Research in Microbiology 161, 422-429

- Wilkinson MD et al., 2016. The FAIR Guiding Principles for scientific data management and stewardship. Scientific data.
- Wilkinson MD et al., 2019. Addendum. The FAIR Guiding Principles for scientific data management and stewardship. Scientific data.

Deliverables

Ref	Title	Due month
D.3.1	Establish a searchable catalogue containing RM connected to a broad range of external databases including databases of CARE partners	**M35**

Milestones

Ref	Title	Due month
M.3.1	List of existing software and databases in place to record and query collections	**M30**

WP4

Objectives

- The aim of WP4 is to investigate and benchmark the availability and quality of the existing (meta)-data (in close collaboration with WP2) among the OHJPE members relevant for risk assessment.
- WP4 also aims, for higher comparability, to harmonise data and to increase the accessibility of the data by all OHEJP members.
- To avoid duplication and limited comparability with existing EU data collections and initiatives the CARE consortium aims to collaborate with ECDC and EFSA.
- To promote national participation in initiatives from EU authorities that serve to make high quality (meta)-data more readily available for risk assessments.

Description of work

WP: WP4
WP start month: 25
WP end month: 54
WP Leader: Moez Sanaa, IP
Deputy WP Leader: Henk Aarts, RIVM
WP participants: ANSES, RIVM, IZSAM, DTU.

WP4 will result in an assessment and benchmarking of the availability, relevance and quality of the existing (meta-)data, that is currently used among the OHJPE members for risk assessment. WP4 will also result in a high level of harmonised datasets and an increased accessibility of the data by all OHEJP members. Furthermore, in order to avoid duplication and limited comparability with existing EU data collections and initiatives the CARE consortium aims to collaborate with ECDC and EFSA. WP4 will not create its own database but will utilize an existing web platform to make the surveillance data accessible for simple visualizations, comparisons with other open sources of data, and download in a single place.

The first activity of WP4 is to conduct a survey among the OHJPE members regarding the available data for risk assessment. Based on the outcome of this survey a roadmap will be developed in how to collaborate with (reporting) initiatives (like the SIGMA project, RAKIP etc.) and existing databases maintained by EU authorities to prevent limited comparability and duplication. Furthermore, a dissemination plan for encouraging the national authorities in charge to put emphasis on the collection and reporting of the above identified data to enable readily availability and an increasing amount of data for risk-assessments.

Haberbeck, L. U., Plaza-Rodríguez, C., Desvignes, V., Dalgaard, P., Sanaa, M., Guillier, L., Nauta, M. & Filter, M. (2018). Harmonized terms, concepts and metadata for microbiological risk assessment models: the basis for knowledge integration and exchange. Microbial Risk Analysis, 10, 3-12.

Task: JIP IA 2.1-WP4-T1
Task start month: 25
Task end month: 40
Task Leader: Henk Aarts, RIVM
Deputy Task Leader: Moez SANAA, ANSES
Task Participants: ANSES, RIVM, IZSAM.
Description of the task:
This task will develop and launch a survey among OHEJP members to identify which data/databases (and associated metadata) is currently used in risk assessment. A survey template will be developed and sent out to all OHEJP members. WP4 members will analyze the outcome of the survey and, to avoid duplication and limited comparability with the already reported data, develop a roadmap for collaboration with (reporting) initiatives (like the SIGMA project, RAKIP etc.) and existing databases maintained by EU authorities (ECDC and EFSA). The CARE Consortium will benefit from JIP ORION, which aims at establishing and strengthening inter-institutional collaboration and transdisciplinary knowledge transfer in the area of health data integration. The CARE consortium will use the controlled vocabularies and the harmonized model annotation for risk assessments models proposed in ANSES/BFR/DTU RAKIP model (Haberbeck et al. 2018).

Task: JIP IA 2.1-WP4-T2 Metadata web platform
Task start month: 34
Task end month: 52
Task Leader: Jolene Lee Masters Pedersen, DTU
Deputy Task Leader: Rene Hendriksen, DTU
Task Participants: ANSES, RIVM, IZSAM, DTU
Description of the task:
Based on the survey a user guide will be developed describing how to access the available data and furthermore to develop a strategy to raise awareness by EU authorities of collecting relevant and high quality data for risk-assessment and implement this strategy. WP4 will incorporate the surveillance data into a web platform that aims to facilitate the exploration of antimicrobial resistance and infectious disease data together with publically available predictors of these factors. It will be possible to get a visual overview of available surveillance materials, test simple correlations with other important factors and download the data directly. Antimicrobial resistance and infectious disease data together with publically available predictors of these factors will be used as a case study of data connection, analysis and visualization.

Deliverables

Ref	Title	Due month
D.4.1	A survey on (meta-)data relevant for risk assessment The establishment of connection of this CARE activity with the databases and initiatives already in place	30 35
D.4.3	An executed dissemination plan	54

Milestones

Ref	Title	Due month
M.4.1	Survey Design Survey administration Survey analysis	28 30 36

2.4.2.1.34 JIP04-R2-IA2.2-OH-HARMONY CAP

Reference to the Stategic Research Agenda (please refer to D2.7)	**IA 2.2: Harmonised protocols and common best practice**									
Integrative Project Title	**One Health Harmonisation of Protocols for the Detection of Foodborne Pathogens and AMR Determinants**									
Integrative Project Acronym	**OH-Harmony Cap**									
Leading Organisation	**P13-SSI**					Deputy Leading Organisation			**P13-SSI**	
Project Leader	**Flemming Scheutz**					Deputy Project leader			**Nadia Boisen**	
Project Start month	**M25**					Project End month			**M54**	
Participant	**1**	2	4	6	7	8	**9**	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	**BfR**	FLI	RKI	DTU
PM	**6.2**						**6**			
Participant	**13**	14	15	16	17	18	19	20	**21**	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	PHE
PM	**29.5**								**15.3**	
Participant	23	24	25	**26**	**27**	28	29	**30**	31	**32**
	UoS	OKI	NUIG	**TEAGASC**	**ISS**	IZSAM	IZSLER	**RIVM**	WbvR	**FHI**
PM				**2.4**	**13.5**			**3.8**		**5.07**
Participant	**33**	**34**	**35**	**36**	37	38	**39**	**40**	**41**	
	NVI	**PIWET**	**INIAV**	**INSA**	IISPV	IC	**SLV**	**FOHM**	**SVA**	
PM	**2**	**4.83**	**5.45**	**14**			**0.8**	**3.5**	**14**	

Objectives (for WP1-5)

- Development and test of a pilot OHLabCap survey for NRLs
- Current and best sampling practise (PH) and methods for monitoring programs (F and AH)
- Collection of laboratory protocols for model organisms

WP1: Project Coordination

This WP takes place over the first, second, and third annual period of the project. All the tasks run concurrently

WP start month: M25

WP end month: M54

WP Leader: 13-SSI

WP participants: 1-ANSES, 9-BfR, 13-SSI, 30-RIVM, 26-Teagasc, 41-SVA

Description of WP1: Description of WP1: This WP will focus on effective project management subdivided between internal coordination, external engagement, and knowledge building. The purpose is to enabling collective activities, and ensure cohesiveness, efficacy, and sustainability. The intent is not only to enable seamless coordination between 5 WPs, 11 countries, and 15 institutions but also to be flexible and address unforeseen problems as they arise. Further this WP ensures that roles and responsibilities are clearly defined.

Task: WP1-T1: **Internal coordination**

Task start month: M25

Task end month: M54

Task Leader: 13-SSI

Task Participants: 1-ANSES, 9-BfR, 13-SSI, 30-RIVM, 26-Teagasc, 41-SVA

Description of the task: This task is centred on SSI's long track record of coordinating complex health activities across multiple EU countries. With 15 participating institutions, the aim is to move the project forward, keeping staff on budget, meet deadlines, ensure quality deliverables, and realise the full impact of the project. Intra-project exchanges will be focused on effective communication strategy, i.e. conference calls, five face-to-face meetings, and automated data collection such as surveys. This task also ensures that the "model organism" tracks will be carried out into the WP-specific work.

Task: WP1-T2: **External coordination, outreach and engagement**

Task start month: M25

Task end month: M54

Task Leader: 13-SSI

Task Participants: 1-ANSES, 9-BfR, 13-SSI, 30-RIVM, 26-Teagasc, 41-SVA

Description of the task: External engagement will be important to find synergies with other projects, obtain important and timely feedback from external partners, and effectively disseminate our own results. The aim is then to stay connected with external stakeholders. Particular attention will be paid to collaboration with other EJP initiatives, particular MATRIX and CARE, so they have a chance to contribute their views on current gaps, which will, benefit OH integration. Practically, this will be achieved by workshops described in WP5.

Task WP1-T3: **Data management**

Task start month: M25

Task end month: M54

Task Leader: 13-SSI

Task Participants: 1-ANSES, 9-BfR, 13-SSI, 30-RIVM, 26-Teagasc, 41-SVA

Description of task: This task will continuously track, monitor, analyse, and store data in order to ensure progress throughout the project. The outcome then is a data asset that will be readily available for all our purposes, as well as those that may need this data in the future, also

contributing to the sustainability of the project. The specific deliverable will be a preliminary data management plan, which can be adjusted as the project progresses.

Task WP1-T4: **Sustainability**

Task start month: M25

Task end month: M54

Task Leader: 13-SSI

Task Participants: 1-ANSES, 9-BfR, 13-SSI, 30-RIVM, 26-Teagasc, 41-SVA

Description of task: Sustainability is an important cross-theme to all WPs, with considerations before, during, and after the project. The intent is that others find value in this work and build upon it once the project has concluded. Mechanisms include, but are not limited to, dissemination in peer reviewed publications, participations in external events, data sharing, and future collaborations across the OneHealth interface.

WP2: Development of the OHLabCap

WP2 takes place over the first, second and third year of the project

WP start month: M25

WP end month: M54

WP Leader: 13-SSI

Deputy WP Leader: 30-RIVM

WP2 participants: All OH-Harmony-Cap participants

Description of WP2: The aim of WP2 is to develop a benchmarking instrument "OHLabCap", by surveying OneHealth laboratory interoperability, capacity and performance 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. An efficient tool *must* support harmonised data collection and validation, and encourage cross-sectoral communication and assist competent authorities in establish and strengthen National networks. This requires identifying and characterising OneHealth laboratory interoperability, capacity and performance in each step within the system (e.g. coordination, data collection, analysis, interpretation, and dissemination of data). The tool will produce country-scale profiles of laboratory interoperability and identify gaps in capacity. This tool will make use of the existing EULabCap without repeating existing surveys and incorporate AH and FS. The surveys will define targets across three dimensions: 1. primary diagnostic testing (e.g. regulation, guidelines, use, and AST), 2. NRL services (e.g. regulation, reference material, typing, and AMR markers), and 3. laboratory-based interoperability and communication (e.g. national networks, participation in EQAs, communication on OH levels). To ensure this, two surveys will be developed, a pilot survey at the NRL level and an adjusted survey in collaboration with the NRLs of the primary sector, and four tasks will be undertaken. The four tasks will run consecutively.

Task WP2-T1: **Development and testing of a pilot survey (NRL)**

Task WP2-T1 takes place in the first year of the project

Task start month: M25

Task end month: M30

Task Leader: 13-SSI

Deputy Task Leader: 30-RIVM

Task Participants: All OH-Harmony-Cap participants

Description of the task: Development and execution of a pilot survey of the project across OH interface on the National reference laboratory level. The pilot survey will define targets across the three dimensions (primary diagnostic, NRL services, and laboratory-based interoperability and communication) which will be assigned to three subtasks. The subtasks will establish indicators on structure, processes, capability, capacity, interoperability and communication for all the mentioned organisms. The survey population will be the contact points for each specific organism as defined by EFSA and ECDC and the tools will be electronic questionnaires.
Subtask WP2-T1-S1 (M25-M27): Compilation of indicators across three dimensions from the primary sector and national microbiology reference laboratory services

Subtask WP2-T1-S2 (M27-29): Choice of analytical software, e.g. EUSurvey, to be used for the surveys. Creation of a beta version questionnaire in the chosen software. Usability test of beta version among a smaller group of WP2 OH-Harmony-Cap participants

Subtask WP2-T1-S3 (M30): Finalise the first questionnaire and circulate to the survey population (D-2.1)

Task WP2-T2: **Scoring of collected data and chosen indicators (NRL)**

Task WP2-T2 takes place in the first year of the project

Task start month: M31

Task end month: M36

Task Leader: 32-FHI

Deputy Task Leader: 30-RIVM

Task Participants: All OH-Harmony-Cap participants

Description of the task: The aim of this task is to evaluate, assess, and score the collected data and chosen indicators. This will be achieved using scoring options similar to those used in the EULabCAP survey (WP2-T1). Three subtasks will be undertaken

Subtask WP2-T2-S1 (M31-M33): Collection and analyses of the survey results. Identification of gaps and prioritization of targets to be considered in the second, adjusted primary sector version of “OHLabCap”.

Subtask WP2-T2-S2 (M33-34): Determine which pathogens should be included or excluded from the adjusted survey

Subtask WP2-T2-S3 (M34-M36): Prepare the One Health instrument of the levels of system capability/capacity/interoperability for each of the EU/EEA countries (D-2.3).

WP3: One Health Laboratory Interoperability Guidance

WP3 takes place over the first, second and third year of the project

WP start month: M25

WP end month: M54

WP Leader: 26-Teagasc

Deputy WP 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

Description of WP3: 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 will be guidance (in a series of summary reports), for the chosen model organism(s), for systematic methodological improvement with respect to One Health laboratory interoperability. This includes developing a modern harmonised system for food, feed, animal and human sampling and testing in Europe. Three tasks will be undertaken; Sampling and Testing, Characterisation, and Data management. This WP does *not* include analysis of specific the protocols. Shiga Toxin producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), *Cryptosporidium*, and AMR for *Salmonella* and *Campylobacter* have been pre-selected for the project's purposes.

Task WP3-T1: **Sampling and Testing**

Task WP3-T1 takes place in the first year of the project

Task start month: M25

Task end month: M36

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, 39-SLV, 40-FOHM, 41-SVA

Description of the task: 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 approach for food, feed, animal and human testing, based on the most effective methods currently available, including the opportunities offered by new technologies such as WGS.

A working group (WG) of 6-8 relevant experts will be formed from within the project, including all required expertise and experience to undertake Task WP3-T1.

Sub-task WP3-T1-ST1(M25-M30): Establishing current practice (questionnaire)
A questionnaire based survey of all relevant laboratories in the EU (EURL network of NRLs, EFSA Zoonoses network, ECDC Food and Waterborne Disease network, etc.) will be used to establish; [1] what sampling plans, sampling and testing methods are currently used; [2] what strain characterisation (typing, AMR testing, etc.) is routinely performed; [3] how this is undertaken (methods used); [4] why these methods are used, including any limitations to applying new technologies such as WGS and [5] how data is managed (collated and stored within MSs and communicated to EFSA and ECDC). The data obtained will inform all tasks in WP3 and not just T-1.

Sub-task WP3-T1-ST2 (M31-M36): Best practice
Information from a systematic review of the scientific and other relevant literature will form the basis for a review of currently available sampling and testing methods. This will include limitations and highlight knowledge/methodology gaps which should be addressed through future research and development (R&D).

The outcome will be a summary report including; [1] the current and best practice in designing statistically based sampling plans; [2] the most appropriate sampling methods; [3] all currently available testing (detection) methods; [4] how these are applied and [5] the latest technologies/technological developments.

WP4: Harmonisation of Protocols

WP4 takes place over the first and second year of the project

WP start month: M25

WP end month: M48

WP Leader: 27-ISS

Deputy WP Leader: 1-ANSES

WP4 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

Description of WP4: The aim of WP4 is to produce recommendations and protocols to harmonise the methodology for the detection and typing of the model pathogens. These pathogens will be selected as example for the project's purposes, and identified candidates include Shiga Toxin producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), Cryptosporidium, and AMR for *Salmonella* and *Campylobacter*. This would allow us to take advantage of the experience of the EURL for *E. coli* and the EURL for Parasites, hosted by ISS and involved in the WP, who are continuously making efforts in the harmonisation of methods applied by Veterinary Laboratories through the coordination of the National Reference Laboratories (NRLs) networks. The strategy and the approaches adopted in the framework of WP4 activities can be, if needed, transferred to other pathogens, enabling harmonisation of methodologies in use in the EU/EEA Laboratories. During the first year of the project, information on the methodologies and protocols in place for the detection of the selected pathogens in Laboratories working in the different sectors will be collected.

Task WP4-T1: **Collection of the laboratory protocols for model organisms**

Task WP4-T1 takes place in the first year of the project

Task start month: M25

Task end month: M33

Task Leader: 27-ISS

Deputy Task Leader: I-ANSES

Task 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

Description of the task: Collection 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 EU/EEA laboratories. The collection will include; 1) Acquire an overview of the methods adopted for the detection, characterisation and typing of the selected pathogens. 2) Compilation of information about the methodologies applied for the detection of the selected pathogens and for AMR. Notably, the laboratories enrolled, in this collection, will be the NRLs for *each* specific organism as defined by EFSA and ECDC. The collection will be administered by the OHEJP partner institutes. Information on the methodologies applied in the networks of National Reference Laboratories (NRLs) for *E. coli* and Parasites, nominated according to EU Regulation 625/2017 in the Veterinary field, will also be shared. Proprietary methods, certified by a third party (such as Nordval: https://www.nmkl.org/index.php/nb/nordval, Microval: https://microval.org/, AFNOR: the French standardization organization) in accordance with the protocol set out in EN/ ISO standard 16140 or other internationally accepted similar protocols will also be considered. Additional Laboratories working in Public Health sector participating in the ECDC Proficiency Testing schemes may also be enrolled.

Task WP4-T2: **Evaluation of the collected laboratory protocols**

Task WP4-T2 takes place in the first and second year of the project

Task start month: M31

Task end month: M39

Task Leader: 41-SVA

Deputy Task Leader: I-ANSES

Task 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

Description of the task: 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. Three subtask will be undertaken and they will run concurrently. Three working groups (WG) of 4-6 relevant participants, with required expertise and experience in STEC/ETEC, *Cryptosporidium*, and AMR for *Salmonella* and *Campylobacter*, will undertake Task WP4-T2.

WP4-T2-S1(M31-M39): Evaluation of the laboratory protocols for the detection and typing STEC/ETEC

WP4-T2-S2 (M31-M39): Evaluation of the laboratory protocols for the detection and typing for AMR *Salmonella* and *Campylobacter*

WP4-T2-S3(M31-M39): Evaluation of the laboratory protocols for the detection and typing of *Cryptosporidium*

Deliverables

Ref	Title	Due month
D-IA.2.2.OH-Harmony-Cap.1.1	Preliminary data management plan	M30
D-IA.2.2.OH-Harmony-Cap.2.1	Completed pilot survey	M30
D-IA.2.2.OH-Harmony-Cap.2.2	Technical report of the survey results	M36
D-IA.2.2.OH-Harmony-Cap.3.1	Technical report on the current and best practice in sampling	M36

Milestones

Ref[1]	Title	Due month[1]
M-IA2.2.OH-Harmony-Cap.1	Kick-off meeting completed	M25
M-IA2.2.OH-Harmony-Cap.2	WP3-T1-ST1 completed	M30
M-IA2.2.OH-Harmony-Cap.3	Preparing a list of methods for the detection, Characterisation and typing of the selected pathogens in the EU/EEA NRLs	M30
M-IA2.2.OH-Harmony-Cap.4	WP3-T1-ST2 completed	M36

2.4.2.1.35 JIP05-R2-IA2.1-MATRIX

Reference to the Stategic Research Agenda (please refer to D2.7)			**IA 2.1: Common frameworks for design and methods to assess equivalence between surveillance and control activities**							
Integrative Project Title			**MATRIX: Connecting dimensions in One-Health surveillance**							
Integrative Project Acronym			**MATRIX**							
Leading Organisation			**P13-SSI**			Deputy Leading Organisation			**P41-SVA**	
Project Leader			**Katrin Gaardbo Kuhn**			Deputy Project leader			**Fernanda Dórea**	
Project Start month			**M25**			Project End month			**M54**	
Participant	**1**	2	4	6	7	8	**9**	**10**	11	**12**
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	**BfR**	**FLI**	RKI	**DTU**
PM	**3.15**						**19.85**	**8.4**		**2.5**
Participant	**13**	14	15	**16**	**17**	18	19	20	**21**	22
	SSI	UT	VFL	**INIA**	**UCM**	MVNA	INRA	IP	**APHA**	PHE
PM	**12**			**3**	**8**				**3.39**	
Participant	**23**	24	25	26	**27**	**28**	29	**30**	**31**	**32**
	UoS	OKI	NUIG	TEAGASC	**ISS**	**IZSAM**	IZSLER	**RIVM**	**WbvR**	**FHI**
PM	**8.4**				**4.5**	**27**		**8.5**	**3**	**10.94**
Participant	**33**	**34**	35	**36**	37	38	39	**40**	**41**	
	NVI	**PIWET**	INIAV	**INSA**	IISPV	IC	SLV	**FOHM**	**SVA**	
PM	**3.89**	**9.65**		**17.4**				**10**	**21**	

Objectives

- Generate a sector based (e.g. animal health (AH), public health (PH), & food safety (FS)) inventory of existing frameworks for surveillance of inter-sectorial hazards, and review existing methods for evaluating these frameworks
- Bring forward a map of the surveillance chain considering all sectors for each of the hazard-tracks
- Identify cross-sectorial surveillance chain linkages, focusing on outputs and sharing
- Propose best-practice guidelines and effective strategies for data collection, analysis, and dissemination aimed at multi-sectorial One-Health (OH) OH collaboration, for each specific hazard-track
- Inventory of past work and current practice

- Identification of operational partners and stakeholders
- Perform a requirement analysis for national One-Health surveillance (OHS) roadmaps
- Create a Knowledge Integration Platform
- Training and dissemination
- Country-based Identification of existing cross-sectorial OHS activities
- Define the content needs for cross-sectorial decision and collaboration dashboards

WP0: Coordination
This WP takes place over the third, fourth, and fifth annual period of the EJP
WP start month: M25
WP end month: M54
WP Leader: 13-SSI
Deputy WP Leader: 41-SVA
WP participants: 1-ANSES, 9-BfR, 10-FLI, 12-DTU, 13-SSI, UCPH, 16-INIA, 17-UCM, 21-APHA, 23-UoS, 27-ISS, 28-IZSAM, 30-RIVM, 31-WbvR, 32-FHI, 33-NVI, 34-PIWET, 36-INSA, 40-FoHM, 41-SVA
Description of the WP: This is a "housekeeping" collection of activities, to ensure project cohesiveness, efficacy, and sustainability.

Task: WP0-T1: Internal coordination
Task start month: M25
Task end month: M54
Task Leader: 13-SSI
Deputy Task Leader: 41-SVA
Task Participants: 1-ANSES, 9-BfR, 10-FLI, 12-DTU, 13-SSI, UCPH, 16-INIA, 17- -UCM, 21-APHA, 23-UoS, 27-ISS, 28-IZSAM, 30-RIVM, 31-WbvR, 32-FHI, 33-NVI, 34-PIWET, 36-INSA, 40-FoHM, 41-SVA
Description of the task: Regular meetings to coordinate the work across WPs and weave the work of tracks into the WP-specific work.

Task: WP0-T2: External coordination and communication
Task start month: M25
Task end month: M54
Task Leader: 13-SSI
Deputy Task Leader: 41-SVA
Task Participants: 1-ANSES, 9-BfR, 10-FLI, 12-DTU, 13-SSI, UCPH, 16-INIA, 17- UCM, 21-APHA, 23-UoS, 27-ISS, 28-IZSAM, 30-RIVM, 31-WbvR, 32-FHI, 33-NVI, 34-PIWET, 36-INSA, 40-FoHM, 41-SVA
Description of the task: continuous work to publish project progress updates and stay connected with other OHEJP projects (from the first and second phase), as well as external projects of relevance. Continued communication with stakeholders.

Task: WP0-T3: Data management
Task start month: M25
Task end month: M54
Task Leader: 13-SSI
Deputy Task Leader: 41-SVA
Task Participants: 1-Anses, 9-BfR, 10-FLI, 13-SSI, 21-APHA, 28-IZSAM, 41-SVA
Description of the task: Development and continuous update of a data management plan throughout the project.

Task: WP0-T4: Sustainability
Task start month: M25
Task end month: M54
Task Leader: 13-SSI
Deputy Task Leader: 41-SVA
Task Participants: 9-BfR, 13-SSI, 28-IZSAM, 33-NVI, 41-SVA
Description of the task: Continuous identification and documentation of sustainability challenges and opportunities to address them. Sustainability here refers specifically to the maintenance of project outputs after its conclusion.

WP1: Existing frameworks and OH capacity
This WP takes place over the third, fourth, and fifth annual period of the EJP
WP start month: M25
WP end month: M54
WP Leader: 10-FLI
Deputy WP Leader: 13-SSI
WP participants: 1-ANSES, 10-FLI, 13-SSI, 17-UCM, 21-APHA, 27-ISS, 28-IZSAM, 30-RIVM, 31-WbvR, 32-FHI, 33-NVI, 34-PIWET, 36-INSA, 40-FoHM, 41-SVA.
Description of the WP: The aim of WP1 is to provide guidelines for adapting existing AH, PH, and FS surveillance frameworks into systems that can support sectorial surveillance of inter-sectorial hazards. This WP will therefore focus on existing relevant surveillance frameworks found within each of the three individual sectors and how these frameworks can be adapted and improved to support OHS of inter-sectorial hazards. Furthermore, this WP will continue and build upon the work already undertaken in COHESIVE and ORION, as well as previous and current initiatives, such as RISKSUR, HOTLINE, STOC-FREE, SIGMA, COMPARE, and EFFORT (Illustrated in Figure 4, Annex 6). WP1 will revise frameworks within each sector, and provide input for the cross-sectorial work carried out in all other WPs.

Task: WP1-T1: Generate an inventory of existing frameworks for surveillance of inter-sectorial hazards, by sector (AH, PH, and FS)
Task start month: M25
Task end month: M36
Task Leader: 10-FLI
Deputy Task Leader: 13-SSI
Task Participants: 1-ANSES, 10-FLI, 13-SSI, 17- UCM, 21-APHA, 27-ISS, 28-IZSAM, 30-RIVM, 32-FHI, 34-PIWET, 36-INSA, 40-FoHM, 41-SVA.
Description of the task: WP1-T1 will focus on identifying the institutions and their inter- and intra-sectorial collaborations within each of the participating countries, as well as identify operational existing frameworks used in each sector. The achievements of the previous integrative projects (ORION and COHESIVE) will be consolidated and summarized. As part of a requirement analysis phase, AH, PH, and FS agencies in the partner countries will revise this documentation of existing inventories of surveillance initiatives and frameworks, to identify any gaps and needs for further development/documentation. This WP will also liaise with ongoing projects, such as SIGMA, when performing such an inventory of existing frameworks.

WP2: Best-practices and multi-sectorial collaboration
This WP takes place over the third, fourth, and fifth annual period of the project
WP start month: M25
WP end month: M54
WP Leader: 28-IZSAM

Deputy WP Leader: 13-SSI
WP participants: 1-ANSES, 9-BfR, 10-FLI, 12-DTU, 13-SSI, 16-INIA, 21-APHA, 27-ISS, 28-IZSAM, 30-RIVM, 32-FHI, 33-NVI, 34-PIWET, 36-INSA, 41-SVA
Description of the WP: WP2 aims to provide a common framework across sectors with best-practices for data collection, analysis, and dissemination of surveillance results, and facilitate OH collaboration across sectors. The dissemination of results, in particular, has been extensively addressed in the first round of OHEJP projects (ORION and COHESIVE), therefore MATRIX will focus more on the other steps of the surveillance continuum, and how the results brought forward by ORION and COHESIVE can be brought into surveillance practice, connecting surveillance practice to outputs dissemination. While WP1 focuses on strengthening surveillance within each sector, to support OHS, WP2 is concerned with cross-sectorial collaboration and the operationalisation of OHS as a multi-sectorial activity. The outcome is to add more value to existing surveillance related processes, with a focus on loops of feedback among sector agencies in order to inform decision making, while simultaneously avoiding duplication and overlapping processes with systems already in place. Using information generated by individual agencies to inform surveillance decision across sectors is highly dependent on effective collaboration. Therefore, the development of best-practices will be strongly supported by the development of guidelines and compilation of effective strategies for multi-sectorial collaboration. This WP is linked to WP1 and will utilise the outputs of WP1 to identify cross-sectorial linkages in order to define a common framework for OHS of inter-sectorial hazards. This information will further be utilised by WP5 and WP6.

Task: WP2-T1: Mapping of the surveillance chain across all sectors for each hazard-track
Task start month: M25
Task end month: M30
Task Leader: 13-SSI
Deputy Task Leader: 32-FHI
Task Participants: 1-ANSES, 9-BfR, 10-FLI, 13-SSI, 16-INIA, 21-APHA, 27-ISS, 28-IZSAM, 30-RIVM, 32-FHI, 33-NVI, 34-PIWET, 36-INSA, 41-SVA
Description of the task: Some of this work is already planned within the first round of OHEJP projects - NOVA for instance plans to deliver such a mapping for a few specific hazards and countries. It is likely that these mappings will not cover all the hazards in the MATRIX “hazard tracks”, or relate to the same countries, but we will learn from their work, and complement where needed following their experience, in order to provide a full map of the surveillance chain for the hazard tracks for at least one country per hazard-track.

Task: 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
Task start month: M30
Task end month: M36
Task Leader: 41-SVA
Deputy Task Leader: 13-SSI
Task Participants: 1-ANSES, 9-BfR, 10-FLI, 12-DTU, 13-SSI, 16-INIA, 21-APHA, 27-ISS, 28-IZSAM, 30-RIVM, 32-FHI, 33-NVI, 34-PIWET, 36-INSA, 41-SVA
Description of the task: Once we have a full map of the surveillance chain for each sector, we will start identifying chain linkages e.g. which outputs of one surveillance sector could serve as inputs for decision making in another sector. The sectorial mapping of the surveillance activities will also provide a base for discussion on how sharing of information could be operationalised - timelines and mode of operation. This task will provide the input for discussions in the following task.

Task: 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.

Task start month: M33
Task end month: M48
Task Leader: 28-IZSAM
Deputy Task Leader: 41-SVA
Task Participants: 1-ANSES, 9-BfR, 12-DTU, 13-SSI, 16-INIA, 21-APHA, 27-ISS, 28-IZSAM, 30-RIVM, 32-FHI, 33-NVI, 34-PIWET, 36-INSA, 41-SVA
Description of the task: This task will develop best-practices for multi-sectorial collaboration to operationalise OHS. This will address data sharing, dealing with data sharing limitations, data analysis, and interpretation and dissemination of outputs. The work in this task will be hazard specific (hazard tracks). This work will gather the conclusions from the first round of OHEJP projects, in particular the integrative and NOVA (focused on surveillance), and consolidate them into a suggested framework of OHS. These will cover both the aspects of collaboration among people, as well as the technical aspects of implementation. In other words, a practical guide for concrete implementation of the results of previous projects, advancing OHS practice.

WP3: Output-based surveillance evaluation
This WP takes place over the third, fourth, and fifth annual period of the project
WP start month: M25
WP end month: M54
WP Leader: 21-APHA
Deputy WP Leader: 34-PIWET
WP participants: 12-DTU, UCPH, 17-UCM, 21-APHA, 28-IZSAM, 31-WbvR, 34-PIWET, 36-INSA, 41-SVA
Description of the WP: The aim of WP3 is to develop guidelines for the design, implementation, and evaluation of official controls within the food sector using output-based standards. Although the current legislative framework exist for countries to take an output-based standards approach to surveillance, previous work within the RISKSUR project identified, that few countries are implementing this approach. Most surveillance, control and monitoring programmes rely on input-based standards instead. In this context, WP3 will assess the suitability of using output-based standards for OHS using several different worked examples from the hazard specific tracks included in MATRIX. WP3 is linked with the food sector part of WP1 and WP2. The output of this WP will, where possible, be streamlined to correlate with the roles and messages of national risk managers and standard setting bodies.

Task: WP3-T1: Inventory of previous work and current practice
Task start month: M25
Task end month: M36
Task Leader: 21-APHA
Deputy Task Leader: 17-UCM
Task Participants: 17-UCM, 21-APHA, 31-WbvR, 34-PIWET, 36-INSA, 41-SVA
Description of the task: This task will gather information on output-based surveillance from previous work in the field, in particular, that generated in RISKSUR, COHESIVE, and ORION. RISKSUR and ORION have developed comprehensive glossaries of surveillance terms. These will serve as input to this task. Additional literature review for specific output-based surveillance activities outside RISKSUR and ORION will be carried out. Review of decision-support tools applicable for OHS from within and outside the EU will be included to widen the sources of experience contributing to the work package.

Task: WP3-T2: Identification of operational partners and stakeholders
Task start month: M25
Task end month: M54

Task Leader: 17-UCM
Deputy Task Leader: DTU
Task Participants: 12-DTU, 17-UCM, 21-APHA, 33-NVI, 34-PIWET, 36-INSA
Description of the task: Each participating country will as required select the appropriate members of AH, PH, and FS competent authorities, to reflect the heterogeneity in governance of official controls within different countries. Makeup of the operational partners will include stakeholders from design and implementation of 'official control' schemes. As the identification of operational partners is important to designing schemes suitable for the specific environment, this deliverable starts from the beginning of the project and is constantly reviewed through the life of the project.

WP4: EUEpiCap
This WP takes place over the third, fourth, and fifth annual period of the project
WP start month: M25
WP end month: M54
WP Leader: 1-ANSES
Deputy WP Leader: 23-UoS
WP participants: 1-ANSES, 10-FLI, 12-DTU, 13-SSI, 16-INIA, 23-UoS, 34-PIWET
Description of the WP: The objective of WP4 is to develop a benchmarking tool "EUEpiCap" to support performance monitoring and evaluation of OH surveillance activities across MS, by sector. In practice, OHS comprises the shared collection, collation, analysis and collaborative interpretation of information produced for and by surveillance systems from diverse sectors. A truly efficient OHS would ensure that surveillance inputs (data and resources) and outputs from systems that may vary widely in scope, objectives and methods/activities are accessible, comparable and easy-to-use by other sectors. This requires identifying and characterising the processes across AH, PH and FS sectors regarding practices and resources at each step of the surveillance system (e.g. governance and coordination, data collection, collation, analysis, interpretation and dissemination of data) in order to identify barriers and best-practices towards the implementation of OH surveillance. WP4 aims to develop a generic benchmarking tool, EUEpiCap, for characterizing, monitoring and evaluating surveillance capacity and capabilities within each sector (AH, PH, FS) that supports OHS. The tool will produce country-scale profiles of surveillance interoperability between sectors and highlight needs in surveillance capabilities and capacity. Comparison of results among systems and MS will allow identification of best-practices and making recommendations to facilitate the development and implementation of OHS approaches and improve existing OHS frameworks by providing targeted advice. This tool will not include an evaluation of laboratory capacities and capabilities, which are targeted by the existing EULabCap survey tool. Close collaboration is planned, including a common workshop, between MATRIX and the proposed OH-HARMONY-CAP for laboratory capacities if both projects are funded.

Task: WP4-T1. Review of existing methods for surveillance systems evaluation
Task start month: M25
Task end month: M36
Subtask Leader: 1-ANSES
Deputy Task Leader: 23-UoS
Task Participants: 1-ANSES, 10-FLI, 13-SSI, 16-INIA, 23-UoS, 33-NVI, 34-PIWET
Description of the task: The work will identify existing methods for surveillance systems evaluation from previous reviews (e.g. RISKSUR project for AH and PH sectors), international policy documents and standards, and other literature sources. We will also ask partners to provide results of any such evaluations previously applied in their countries. Previous reviews have identified up to 49 evaluation criteria. We will use different approaches to reduce this number of criteria to a manageable set while delivering a fair snapshot of OHS capabilities.

WP5: Outreach and roadmap
This WP takes place over the third, fourth, and fifth annual period of the EJP
WP start month: M25
WP end month: M54
WP Leader: 9-BfR
Deputy WP Leader: 41-SVA
WP participants: 9-BfR, 12-DTU, 13-SSI, 17-UCM, 21-APHA, 23-UoS, 27-ISS, 28-IZSAM, 30-RIVM, 32-FHI, 33-NVI, 34-PIWET, 36-INSA, 40-FoHM, 41-SVA
Description of the WP: The objective of WP5 is to create a roadmap for future development of national OH surveillance activities, specifically targeted at different levels of infrastructural and economic capacities. This OHS roadmap template will take into consideration: the heterogeneity of OHS capacities, resources, and developments across MS, as well as the OHS frameworks and solutions identified by WP1, WP2, WP3, and WP4. A high-level strategy that addresses opportunities for better communication, knowledge exchange and knowledge integration between OHS domains will be outlined in this WP. WP5 will set up a technical platform supporting knowledge exchange between the different MATRIX partners with close connection to the overarching EJP platform.

Task: WP5-T1: Perform a requirement analysis for national OH surveillance roadmaps
Task start month: M25
Task end month: M45
Task Leader: 13-SSI
Deputy Task Leader: 30-RIVM
Task Participants: 9-BfR, 12-DTU, 13-SSI, 17-UCM, 21-APHA, 23-Univ. Surrey, 27-ISS, 28-IZSAM, 30-RIVM, 32-FHI, 34-PIWET, 36-INSA, 40-FoHM, 41-SVA
Description of the task: The objective of this task is to assess the national barriers (e.g. in OHS capacity, resources and developments) preventing efficient OHS integration in the different EU Member States (MS). This task will perform interviews and/or surveys with experts/stakeholders from different MS. Moreover, this task aims at gathering intelligence and identifying needs for the development or implementation of national OHS roadmaps that could be integrated into the planned OHS roadmap template in order to support national activities along the OH objective. Further, it will outline the aspects to be prioritized in the development of the OHS roadmap template, taking into consideration OHS frameworks and solutions identified by WP1, WP2, WP3, and WP4. Lastly, this task aims at gathering intelligence about best-practices that could support the implementation and widespread application of the OHS roadmap template.

Task: WP5-T3:Knowledge-Integration Platform
Task start month: M25
Task end month: M54
Task Leader: 9-BfR
Deputy Task Leader: 41-SVA
Task Participants: 9-BfR, 13-SSI, 21-APHA, 27-ISS, 30-RIVM, 32-FHI, 33-NVI, 34-PIWET, 41-SVA
Description of the task: The objective of this task is to establish a web-based Knowledge-Integration Platform. This platform aims at providing means to facilitate and enhances interaction/collaboration between the OHS stakeholders (e.g. EFSA and ECDC) and project members (from different disciplines, institutions and countries). It will further offer resources (e.g. tools/technologies/features) that support collaborative collection, exchange, management and creation of knowledge. Given the improved knowledge exchange and collaboration means the Knowledge-Integration Platform will support three main objectives, namely 1) to identify stakeholder needs, 2) to enable collaborative and synergetic work and prevent duplication of work, and 3) to inform project members about past, ongoing and future OHS initiatives. The Knowledge-

Integration Platform will further facilitate the dissemination and permanent access to the generated project outcomes from all WPs, which are meant to benefit the OHS community.

Task: WP5-T4: Training and dissemination
Task start month: M25
Task end month: M54
Task Leader: 9-BfR
Deputy Task Leader:41-SVA
Task Participants: 9-BfR, 13-SSI, 17-UCM, 21-APHA, 27-ISS, 28-IZSAM, 32-FHI, 34-PIWET, 36-INSA, 41-SVA
Description of the task: This task will be concerned with knowledge dissemination and training. Knowledge dissemination and information about past, ongoing and future OHS initiatives and projects on EU level will be given trough "OHS Briefings" (e.g. webinars/meetings), which will be held regularly. These "OHS Briefings" support the aims of the Knowledge-Integration Platform (outlined in Task5.1) and will actively involve stakeholders, such as EFSA and ECDC. A dedicated sub-task for the creation of a web-portal is also closely linked to the Knowledge-Integration Platform and aims at making training material (e.g. tutorials, videos, and guidelines) that is created/collected during the project publicly and permanently available to internal and external OHEJP partners. Large resources will also be allocated to external trainings that are meant to share the generated knowledge and to train external experts in the adoption of the developed resources.

WP6: Decision and collaboration dashboards
This WP takes place over the third, fourth, and fifth annual period of the EJP
WP start month: M25
WP end month: M54
WP Leader: 41-SVA
Deputy WP Leader: 32-FHI
WP participants: 1-ANSES, 9-BfR, 10-FLI, 13-SSI, 17-UCM, 21-APHA, 28-IZSAM, 32-FHI, 33-NVI, 34-PIWET, 40-FoHM, 41-SVA
Description of the WP: WP6 will focus on visualisation of OHS inputs and outputs for the use of surveillance officials in PH, AH and FS. This will be achieved by the creation of dashboards, an information centre for collaboration and decision making in surveillance for specific hazards. The dashboards will include surveillance data from all three sectors to the extent that data accessibility agreements allow (timelines, level of aggregation and level of details). This WP will not build a data sharing platform, it will work on converting already available data into actionable information.
The ORION project has identified data sources that are already public or shared across sectors. The NOVA project is building automated routines for data analyses and visualization when trend monitoring is possible. The MATRIX project will build on these results to create an information centre where all data/information shared will have rich contextual meta-data/meta-information, allowing explicit consideration of possible pitfalls and biases when OH data is co-analysed, which is crucial for the right decision-making. Trend monitoring and signal detection will be performed and visualised jointly across sectors for specific hazards. The issue of multi-sectorial data analysis will be addressed specifically. The dashboards will include not only data and statistical outputs, but also all important surveillance information for specific zoonoses such as contact persons, workflow, and regulations, as such depicting the full OHS structure for that hazard.

Task: WP6-T1: Country-based Identification of existing cross-sectorial OHs activities
Task start month: M25
Task end month: M36
Task Leader: 40-FOHM
Deputy Task Leader: 41-SVA

Task Participants: 1-ANSES, 9-BfR, 10-FLI, 17-UCM, 21-APHA, 28-IZSAM, 32-FHI, 33-NVI, 34-PIWET, 40-FoHM, 41-SVA
Description of the task: Task leaders will gather the information from the first round of EJP projects that is relevant for this WP, in particular: the lessons learned in ORION regarding multi-agency collaboration and data interoperability in OH; documentation from COHESIVE on dissemination of surveillance results; and the achievements of NOVA recommending novel methods for analysis of surveillance data. Task leaders will draw a map for the work in the next two years, highlighting: (i) the knowledge and data sources available regarding multi-agency collaboration on data analysis for One-Health, and (ii) the gaps in knowledge, which should be further addressed.

Task:WP6-T2: Defining the content needs for cross-sectorial decision and collaboration dashboards
Task start month: M34
Task end month: M45
Task Leader: 41-SVA
Deputy Task Leader: 40-FOHM
Task Participants: 1-ANSES, 9-BfR, 17-UCM, 21-APHA, 28-IZSAM, 32-FHI, 33-NVI, 34-PIWET, 40-FOHM, 41-SVA
Description of the task: Each participating country (at least 2) will choose one or more zoonotic pathogens from the hazard-tracks to serve as the "case study" for dashboard construction. Surveillance officials in the sectors of AH, PH and Food safety (FS) will be invited to workshops to present the dashboard concept, and sparkle inter-agency discussions on the possibilities and problems that can be addressed with joint analyses and/or visualization of data. Design of the dashboards will be driven from the end user perspective, that is, what information does a surveillance official need on their everyday work, and in case of emergency? What kind of data can provide that information? This will lead to a specific discussion on what kind of contextual information must be presented with the data, so that the data can lead to right conclusions even across domains/agencies. Information gaps and methodological limitations, in particular, will be documented to allow the information presented to be correctly interpreted, from their original collection context, into the OH surveillance context. Technical and legal barriers associated with data sharing across sectors will be documented. Within the timeframe of MATRIX, it is not expected that new data agreements will be put into place. Rather, we will document current possibilities and constraints, and build upon achievements from the previous integrative projects.

Deliverables and Milestones Year 3

Deliverables		
Ref	Title	Due month
D-WP0.1	Data Management Plan - draft	M36
D-WP1.1	Report on the commonalities and differences of the different operational frameworks in AH, PH, and FS	M36
D-WP2.1	Mapping of the surveillance chain for all hazard tracks, and cross-sectorial linkages	M36
D-WP4.1	M36: WP4-D1. Report on the selected set of criteria for evaluation of epidemiological capacities.	M36
Milestones		
Ref	Title	Due month
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

2.4.2.1.36 PhD01-R1-AMR2-ECO-HEN

Reference to the Stategic Research Agenda (please refer to D2.7)	**AMR 2: Epidemiological studies into the dynamics of AMR in human and animal populations and the environment including horizontal gene transfer and selection of AMR**									
PhD Project Title	**Investigate the gaps in the transmission dynamics of AMR *E. coli* in commercial laying hen production**									
PhD Project Acronym	**ECO-HEN**									
PhD Supervising Organisation	**P17-UCM**			Deputy PhD Supervising Organisation			**P12-DTU**			
PhD Supervisor	**Miguel A. Moreno**			Deputy PhD Supervisor			**Valeria Bortolaia**			
PhD Start month	**M14**			Project End month			**M49**			
Participant number	1	2	4	5	6	7	8	9	10	11
Acronym of participant	Anses	Ages	Sciensano	NCIPD	NDRVMI	SZU	VRI	BfR	FLI	RKI
PersonXmonths[1] per participant:										
Participant number	12	13	14	15	16	**17**	18	19	20	21
Acronym of participant	DTU	SSI	UT	VFL	INIA	**UCM**	MVNA	INRA	IP	APHA
PersonXmonths per participant:						**14**				
Participant number	22	23	24	25	26	27	28	29	30	31
Acronym of participant	PHE	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR
PersonXmonths per participant:										
Participant number	32	33	34	35	36	37	38	39	40	41
Acronym of participant	FHI	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FoHM	SVA
PersonXmonths per participant										

Objectives

- Reveal to what extend the table egg production system represents a risk for spread of AMR to humans and the environment.
- Effect of reduced antimicrobial use in the production animals on the AMR bacteria initially present in one-day chicks.

Description of work

WP: **WP5. Reconstruction of plasmids spreading AMR genes from animals' to egg shell's isolates**
WP start month: M12

WP end month: M14
WP Leader: V. Bortolaia (DTU)
WP participants: DTU, UCM
Description of the WP: The objective of this WP is to reconstruct the plasmids responsible for dissemination of AMR genes across isolates from different sources. It is widely recognized that the epidemiology of certain AMR genes (e.g. those conferring resistance to critically important antimicrobials in human medicine such as third-generation cephalosporins and colistin) is linked mainly to AMR gene spread via plasmids rather than via bacterial clones and thus knowledge on the AMR plasmids is essential to describe the flow of AMR in different ecological niches.

WP: **WP6. Flow of AMR isolates between animals**.
WP start month: M15
WP end month: M20
WP Leader: M.A. Moreno (UCM)
WP participants: UCM, DTU
Description of the WP: 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.

Task: **T6.1. Checking on the isolates data base looking for non-sequenced isolates putatively needed for this WP.**
Task start month: M15
Task end month: M15
Task Leader: M.A. Moreno (UCM)
Deputy Task Leader: V. Bortolaia (DTU)
Task Participants: UCM, DTU
Description of the task: the phenotypic data base of *E. coli* isolates from animals will be checked looking for isolates non-sequenced that should be sequenced to improve understanding of the flow of AMR genes on the farm.

Task: **T6.2. WGS of the isolates identified on T6.1.**
Task start month: M16
Task end month: M20
Task Leader: Miguel A. Moreno (UCM)
Deputy Task Leader[1]: V. Bortolaia (DTU)
Task Participants: UCM, DTU
Description of the task[1]: Sequencing of isolates identified on the previous task.

Task[1]: **T6.3. Bioinformatic analysis of the WGS.**
Task start month: M15
Task end month: M20
Task Leader: M.A.Moreno (UCM)
Deputy Task Leader: V. Bortolaia (DTU)
Task Participants: UCM, DTU
Description of the task: new sequenced isolates will be examined using the same bioinformatic analysis that the previous isolates.

Task: **T6.4. Data analysis and scientific manuscript preparation.**
Task start month: M15
Task end month: M20
Task Leader: M.A. Moreno (UCM)
Deputy Task Leader: V. Bortolaia (DTU)

Task Participants: UCM, DTU
Description of the task: this task is devoted to analysis of data and preparation of a manuscript.

WP[1]: **WP7. Flow of AMR isolates from animals to egg shells**.
WP start month: M21
WP end month: M23
WP Leader: M.A. Moreno (UCM)
Deputy WP Leader: V. Bortolaia (DTU)
WP participants: UCM, DTU
Description of the WP[1]: WP7 takes place over the second and the third annual period of the OHEJP and is devoted to analysing relationships between AMR bacteria of laying hens and eggs.

Task[1]: **T7.1. Checking on the isolates data base looking for non-sequenced isolates putatively needed for this WP.**
Task start month: M21
Task end month: M21
Task Leader: M.A. Moreno (UCM)
Task Participants: UCM, DTU
Description of the task[1]: the phenotypic data base of *E. coli* isolates from eggs will be checked looking for isolates non-sequenced that should be sequenced to improve understanding of the flow of AMR genes on the farm.

Task[1]: **T7.2. WGS of the isolates identified on T7.1.**
Task start month: M22
Task end month: M23
Task Leader: M.A. Moreno (UCM)
Deputy Task Leader[1]: V. Bortolaia (DTU)
Task Participants: UCM, DTU
Description of the task[1]: Sequencing of isolates identified on the previous task.

Deliverables[1]		
Ref[1]	Title	Due month[1]
D-E14-5.1.	A list of plasmids putatively present on *E. coli* isolates	M14
D-E14-6.1	A list of *E. coli* from animals isolates to be sequenced	M15
D-E14-6.2.	Manuscript	M20
D-E14-7.1.	A list of *E. coli* from eggs to be sequenced	M21
Milestones[1]		
Ref[1]	Title	Due month[1]
M-E14-3	New sequenced *E. coli* isolates from animals	M20
M-E14-4	New sequenced *E. coli* isolates from eggs	M23

2.4.2.1.37 PhD02-R1-AMR2/3/6-LIN-RES

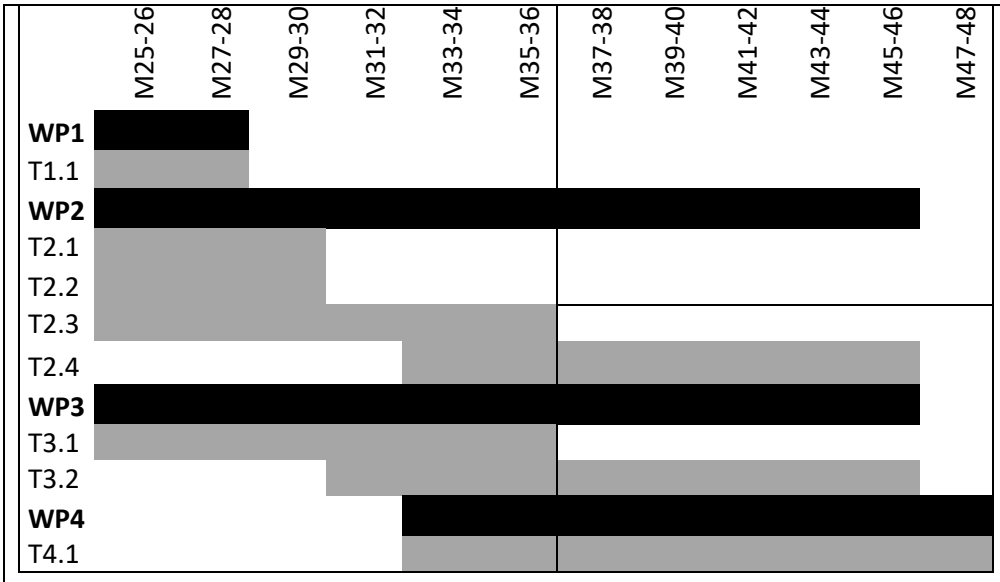
Reference to the Stategic Research Agenda (please refer to D2.7)	**AMR 2: Epidemiological studies into the dynamics of AMR in human and animal populations and the environment including horizontal gene transfer and selection of AMR** **AMR 3: Risk Assessment AMR** **AMR 6: Metagenomics and bioinformatics for detection and surveillance of AMR pathogens and determinants**									
PhD Project Title	**Investigation of the molecular basis, origin, transferability and risk factors associated with linezolid-resistance emergence in Gram-positive bacteria of human and animal origin.**									
PhD Project Acronym	**LIN-RES**									
PhD Supervising Organisation	**P4-Sciensano**			Deputy PhD Supervising Organisation			**P4-Sciensano**			
PhD Supervisor	**Dr. Cécile Boland**			Deputy PhD Supervisor			**Dr. David Fretin**			
PhD Start month	**M13**			Project End month			**M48**			
Participant number	1	2	**4**	5	6	7	8	9	10	11
Acronym of participant	Anses	Ages	**Sciensano**	NCIPD	NDRVMI	SZU	VRI	BfR	FLI	RKI
PersonXmonths[1] per participant:			**12**							
Participant number	12	13	14	15	16	17	18	19	20	21
Acronym of participant	DTU	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA
PersonXmonths per participant:										
Participant number	22	23	24	25	26	27	28	29	30	31
Acronym of participant	PHE	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR
PersonXmonths per participant:										
Participant number	32	33	34	35	36	37	38	39	40	41
Acronym of participant	FHI	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FoHM	SVA
PersonXmonths per participant										

Objectives

- Investigate the molecular basis, origin, transferability and risk factors associated with linezolid-resistance emergence in Gram-positive bacteria of human and animal origin.
-

Description of work[1]

SECOND ANNUAL PERIOD (OHEJP Y3)	THIRD ANNUAL PERIOD (OHEJP Y4)



WP[1]: **WP1- Sampling and Collection of bacteria**
WP start month: M13
WP end month: M28
WP Leader: Dr. Cécile Boland
Deputy WP Leader: Dr. David Fretin
WP participants: Sciensano, BfR and ANSES

Description of the WP[1]:
Mention introductorily: WP1 continues based upon what was achieved during the first annual period of the PhD (OHEJP Y2).
Collection of Gram-positive bacteria (mainly Staphylococci and Enterococci) resistant to Linezolid from animal and human origin and, if feasible, sampling of environmental bacteria from suspected "at risk" biotopes. The coordinator (Sciensano) will contact the other participants and other partners to gather this collection.

Task[1]: T1.1 Sampling and Collection of bacteria
Task start month: M13
Task end month: M28
Task Leader: Dr. Cécile Boland
Deputy Task Leader[1]: Dr. David Fretin
Task Participants: Sciensano, BfR and ANSES
Description of the task[1]: Collection of Gram-positive bacteria (mainly Staphylococci and Enterococci) resistant to Linezolid from animal and human origin and, if feasible, sampling of environmental bacteria from suspected "at risk" biotopes. The coordinator (Sciensano) will contact the other participants and other partners to gather this collection.

WP[1]: **WP2- Next Generation Sequencing**
WP start month: M13
WP end month: M46
WP Leader: Dr. Cécile Boland
Deputy WP Leader: Dr. David Fretin
WP participants: Sciensano

Description of the WP[1]:
Mention introductorily: WP2 continues based upon what was achieved during the first annual period of the PhD (OHEJP Y2) and will continue through the OHEJP Y3 and Y4.
Next Generation Sequencing (NGS) analysis of all the strains collected during the WP1 and that will be collected during investigation of risk factors at farm level (WP4).

Task[1]: T2.1 NGS resistance analysis
Task start month: M13
Task end month: M30
Task Leader: Dr. Cécile Boland
Deputy Task Leader[1]: Dr. David Fretin
Task Participants: Sciensano
Description of the task[1]: NGS resistance analysis (search of linezolid resistance genetic determinants (cfr, optrA, mutations in the 23SrRNA gene,...) and analysis of the genetic context of the resistance genes.

Task[1]: T2.2 NGS subtyping of the strains and associated host specificity
Task start month: M13
Task end month: M30
Task Leader: Dr. Cécile Boland
Deputy Task Leader[1]: Dr. David Fretin
Task Participants: Sciensano
Description of the task[1]: In silico subtyping and analysis of host specificity of the strains starting from NGS raw data.

Task[1]: T2.3 Research of genetic scars of horizontal transfer or recombination events
Task start month: M25
Task end month: M36
Task Leader: Dr. Cécile Boland
Deputy Task Leader[1]: Dr. David Fretin
Task Participants: Sciensano
Description of the task[1]: In depth in silico analysis to search for genetic scars of horizontal transfer or recombination events

Task[1]: T2.4 NGS resistance analysis from investigation of risk factors at farm level
Task start month: M33
Task end month: M46
Task Leader: Dr. Cécile Boland
Deputy Task Leader[1]: Dr. David Fretin
Task Participants: Sciensano
Description of the task[1]: NGS analysis of strains collected during the investigation of risks factors at farm level (farm owners, veterinarians, companion animals,…)

WP[1]: **WP3 - Transferability**
WP start month: M25
WP end month: M46
WP Leader: Dr. Cécile Boland
Deputy WP Leader: Dr. David Fretin
WP participants: Sciensano

Description of the WP[1]:

Mention introductorily: WP3 takes place over the second and third year of the PhD (OHEJP Y3 and Y4).
The transferability of the linezolid resistance genetic markers observed in NGS data (WP2) will be investigate more in depth by searching for inter- and intra-species mobility and transferability markers (plasmid incompatibility groups/conjugation markers/transposons). Some strains representative of the different plasmids observed in the collection of linezolid resistant bacteria will be used as donor strains for conjugation experiments (laboratory experiments aiming to demonstrate the transferability of the resistance markers and estimate transfer rates).

Task[1]: T3.1 in silico analysis of transferability
Task start month: M25
Task end month: M36
Task Leader: Dr. Cécile Boland
Deputy Task Leader[1]: Dr. David Fretin
Task Participants: Sciensano
Description of the task[1]: in silico search of inter- and intra-species mobility and transferability markers (plasmid incompatibility groups/conjugation markers/transposons)

Task[1]: T3.2 Laboratory experiments to demonstrate transferability of linezolid resistance genes
Task start month: M31
Task end month: M46
Task Leader: Dr. Cécile Boland
Deputy Task Leader[1]: Dr. David Fretin
Task Participants: Sciensano
Description of the task[1]: Laboratory experiments to demonstrate transferability of linezolid resistance genes and estimate transfer rates

WP: **WP4- Risk factors**
WP start month: M33
WP end month: M48
WP Leader: Dr. Cécile Boland
Deputy WP Leader: Dr. David Fretin
WP participants: Sciensano, BfR and ANSES

Description of the WP[1]:
Mention introductorily: WP4 takes place over the second and third year of the PhD (OHEJP Y3 and Y4).
This WP4 aims to investigate the risk factors associated with cfr/optrA occurrence at farm level (antimicrobial consumption history, gene carriage by farm owners, farm veterinarians and their companion animals, contacts with other farms).

Task: T4.1 risk factors associated with cfr/optrA occurrence investigation at farm level
Task start month: M33
Task end month: M48
Task Leader: Dr. Cécile Boland
Deputy Task Leader[1]: Dr. David Fretin
Task Participants: Sciensano, BfR and ANSES
Description of the task[1]: collection of antimicrobial consumption history of farms with previous observation of linezolid resistant bacteria and additional sampling in these farms (farm owners, farm veterinarians, companion animals,…)

Deliverables		
Ref	Title	Due month[1]
D-E27-2.3	Genetic resistance profiles and subtypes of strains sequenced until M36	M36
D-E27-2.4	Results of in silico analysis of genetic scars of horizontal transfer or recombination events	M36
D-E27-3.1	Results of in silico analysis of transferability	M36
D-E27-3.2	Poster or oral presentation at an international conference of the results of NGS analysis of linezolid resistant bacteria, including the in silico analysis of transferability.	M36
Milestones		
Ref[1]	Title	Due month[1]
M-E27-3	Synthesis of genetic resistance, subtyping and transferability markers analysis of the linezolid resistant bacteria collected.	M36
M-E27-4	Poster or oral presentation at an international conference of the results of NGS analysis of linezolid resistant bacteria, including the in silico analysis of transferability.	M36

2.4.2.1.38 PhD03-R2-AMR2.1-HME-AMR

Reference to the Stategic Research Agenda (please refer to D2.7)	**AMR2.1: Dynamics of AMR selection, clonal spread and horizontal gene transfer in humans, animals and the environment, including epidemiology of resistant microorganisms and antimicrobials in the environment and their (environment-mediated) spread**									
PhD Project Title	**Investigating the role of heavy metals in the environment as a selective pressure for the dissemination of antimicrobial resistance**									
PhD Project Acronym	**HME-AMR**									
PhD Supervising Organisation	**P26-TEAGASC**					Deputy PhD Supervising Organisation			**P25-NUIG**	
PhD Supervisor	**Kaye Burgess**					Deputy PhD Supervisor			**Dearbháile Morris**	
PhD Start month	**M22**					PhD End month			**M57**	
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	23	24	25	**26**	27	28	29	30	31	32
	UoS	OKI	NUIG	**TEAGASC**	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM				**12**						
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives:

- The objective for this work period is to compare the resistome in low and high metal containing soils using culture dependent and independent analyses.

Description of work:
Selective pressures drive bacterial populations to evolve and may promote the dissemination of AMR genes, in the human and animal gut, or in the environment. However, there is limited

information about the impact of selective pressures in the agri-food environment on HGT between microorganisms. One of the factors which can act as a selective pressure and can influence this HGT is the presence of heavy metals. Heavy metals occur ubiquitously in the agri-food environment and sometimes in high concentrations in soil. Very limited information is available regarding the impact of selective pressures such as heavy metals which may be present in the environment on the mobilisation of antimicrobial resistance and its potential transfer into the food chain.
Culture based analysis will screen for the presence of extended-spectrum beta-lactamase producing *Enterobacteriaceae,* fluoroquinolone resistant *Enterobacteriaceae* and carbapenemase producing *Enterobacteriaceae* on selective agars, using methodologies in place. Isolates will be identified by MALDI-TOF and screened for resistance to a panel of antimicrobials and heavy metals. A representative sample set from each study will be utilised in a shotgun metagenomic study to characterise the wider resistome.

Deliverables

Ref	**Title**	**Due month**
	Report on AMR bacteria present in soils of differing heavy metal content	M36

Milestones

Ref	**Title**	**Due month**
	SOP in place for sampling culture based analysis	M27
	Sequences of sufficient quality obtained from metagenomic analysis	M35

2.4.2.1.39 PhD04-R2-AMR2.1-KENTUCKY

Reference to the Stategic Research Agenda (please refer to D2.7)			**AMR 2.1: Dynamics of AMR selection, clonal spread and horizontal gene transfer in humans, animals and the environment, including epidemiology of resistant microorganisms and antimicrobials in the environment and their (environment-mediated) spread**							
PhD Project Title			**Exploring the evolutionary success of the antibiotic resistant Salmonella Kentucky ST198**							
PhD Supervising Organisation			**KENTUCKY**							
PhD Supervisor			**P4-Sciensano**			Deputy PhD Supervising Organisation			**P19-INRA**	
PhD Coordinator person name			**Ceyssens**			Deputy PhD Supervisor			**Doublet**	
PhD Start month			**M21**			PhD End month			**M57**	
Participant	1	2	**4**	6	7	8	9	10	11	12
	Anses	Ages	**Sciensano**	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM			**12**							
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	23	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives:

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).

In this project, we will investigate (i) what explains the evolutionary success of the multidrug resistant S. Kentucky ST198 clone, (ii) what is the mechanisms of the integration (and potential further transfer) of the ESBL gene in its chromosome, and (iii) Are there genetic determinants of different human-animal host ranges in epidemic S. Kentucky ST198 and ST152?

Description of work:

We hypothesize that the success of the MDR S. Kentucky ST198 is either due to (i) understudied accommodation of genetic elements encoded within mobile genetic elements (MGE) and their interaction with the core and accessory genome, or (ii) altered expression of a core genome-encoded virulence factor.
In this project, we will therefore perform a detailed examination of four representative strains of *S.* Kentucky:

- S. Kentucky ST198, CIP^R (isolated from a Belgian patient)
- S. Kentucky ST152, CIP^S (commensal isolate from poultry, widespread in USA)
- S. Kentucky ST198:$bla_{CTX\text{-}M\text{-}14b}$ (i.e. chromosome-encoded), CIP^R, CTX^R (isolated from a Belgian patient)
- S. Kentucky ST198 p$bla_{CTX\text{-}M14\text{-}b}$ (i.e. plasmid-encoded), CIP^R CTX^R (isolated from a Belgian patient)

In Y3, their genome (including plasmidome) will be completely reconstituted using hybrid assemblies with short- and long-read (MINion) sequence data. This will allow a complete image of core and accessory genome for these strains. Using this data, will be perform the following analyses at **Sciensano** (BEL):

- Sequence data from other French and Belgian CIP^R ST198 strains are already available among partners and will be downloaded from GenomeTrakr, Enterobase (Warwick) and the ENGAGE project databases for phylogenetic clustering
- Detailed examination of the chromosomal integration site of the ESBL gene in *S.* Kentucky ST198::$bla_{CTX\text{-}M\text{-}14b}$

Based on these results, we will initiate to investigate epidemically successful associations between identified MGEs and *S.* Kentucky will be by analysing conjugative efficiencies, cell biology transfer dynamics, maintenance (addiction systems), incompatibility/exclusion, cooperation (i.e. conjugative mobilization) and fitness in the different genetic backgrounds. This part will be performed at **INRA** (FR).

Deliverables		
Ref	**Title**	**Due month**
D4	Protocol for long-read sequencing designed and implemented	32
Milestones		
Ref	**Title**	**Due month**
M2	S. Kentucky strain and genome collection completed	30
M3	Completion of hybrid assemblies of four S. Kentucky strain	36

2.4.2.1.40 PhD05-R2-AMR2/6.1/ET5-METAPRO

Reference to the Stategic Research Agenda (please refer to D2.7)			**AMR 2: Epidemiological studies into the dynamics of AMR in human and animal populations and the environment including horizontal gene transfer and selection of AMR** **AMR 6.1: Development of NGS-based tools for surveillance of AMR in Enterobacteriaceae in animals, humans and the environment** **ET 5: Ecology of emerging pathogens**							
PhD Project Title			**Metagenomics and genomic approaches for the prevention of the spread of plazomicin resistance in humans, animals and the environment**							
PhD Project Acronym			**METAPRO**							
PhD Supervising Organisation			**P17-UCM**			Deputy PhD Supervising Organisation			**P23-UoS**	
PhD Supervisor			**Bruno Gonzalez Zorn**			Deputy PhD Supervisor			**Roberto La Ragione**	
PhD Start month			**M22**			PhD End month			**M60**	
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	**17**	18	19	20	21	22
	SSI	UT	VFL	INIA	**UCM**	MVNA	INRA	IP	APHA	PHE
PM					**12**					
Participant	23	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives:

- Execute the sampling in Spain
- Metagenomic analysis of Spanish samples
- Isolation and genomic analysis of Enterobacteria harbouring plazomicin resistance determinants
- Design of the sampling in the United Kingdom

Description of work:

Sewage samples of the 8 different hotspots selected will be taken. Sequencing of the metagenome of the different samples will be performed and the prevalence of plazomicin resistance determinants will be assessed. Further analysis of the metagenomic data obtained will be carried out. Simultaneously, isolation of enterobacteria in plazomicin selective media will be undertaken. Up to ten enterobacteria from each site will be subjected to MALDI-TOF for their identification and to antimicrobial resistance profiling. Bacteria differing in either characteristic will be subjected to Whole Genome Sequencing (WGS) using a hybrid long read-short read approach.

As performed in Spain, how and where the sampling will be executed will be analysed. Relevant information on antimicrobial resistance and antimicrobial usage of the area will be collected and studied.

Deliverables		
Ref	**Title** N/A	**Due month**
Milestones		
	Title	**Due month**
	Sampling in Spain	MAR 2020
	Metagenome sequencing	JUN 2020
	Enterobacteria isolation and WGS	SEP 2020
	Analysis of the Spanish genomic and metagenomic data	DEC 2020
	United Kingdom sampling design	DEC 2020

2.4.2.1.41 PhD06-R1-ET5-PEMbo

Reference to the Stategic Research Agenda (please refer to D2.7)	**ET 5: Ecology of emerging pathogens**									
PhD Project Title	**From genotype to phenotype: patho-evolution of French strains of *Mycobacterium bovis*.**									
PhD Project Acronym	**PEMbo**									
PhD Supervising Organisation	**P1-ANSES**			Deputy PhD Supervising Organisation				**N/A**		
PhD Supervisor	**Boschiroli Maria Laura**			Deputy PhD Supervisor				**N/A**		
PhD Start month	**M22**			Project End month				**M58**		
Participant number	**1**	2	4	5	6	7	8	9	10	11
Acronym of participant	**Anses**	Ages	Sciensano	NCIPD	NDRVMI	SZU	VRI	BfR	FLI	RKI
PersonXmonths[1] per participant :	**12**									
Participant number	12	13	14	15	16	17	18	19	20	21
Acronym of participant	DTU	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA
PersonXmonths per participant:										
Participant number	22	23	24	25	26	27	28	29	30	31
Acronym of participant	PHE	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR
PersonXmonths per participant:										
Participant number	32	33	34	35	36	37	38	39	40	41
Acronym of participant	FHI	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FoHM	SVA
PersonXmonths per participant										

Objectives

- Obtaining reference sequences by Illumina / PacBio sequencing of strains belonging to main clonal groups
- Identification of genomic events (insertion / deletion or broad sequence polymorphism (LSP)) by comparison study of genetic variation: analysis focused on the study of 182 virulence genes, genes involved in envelope biosynthesis and the study of excreted antigens.
- Study of the antigenic variation: biochemical and lipidomic analyses of the strains

Description of work[1]

WP2: Genome sequence analysis

WP2 start month: M9
WP2 end month: M24
WP2 Leader: Boschiroli Maria Laura
Deputy WP2 Leader : Lorraine Michelet
WP2 participants: PhD student
Description of the WP2: The genomic data (87 genomes) already available at the NRL will be supplemented by sequencing (Illumina) additional strains in order to extend the panel of isolated strains from various hosts, from different foci of different locations but also of "modern" versus "old" strains. An overall analysis will be performed by comparing these data with the new reference sequences generated in WP1 to identify variants (SNPs) as well as other genetic events (deletion, insertions and LSPs). A genome-wide analysis will be also carried out focusing on the selection of 182 virulence genes. Strains showing interesting profiles or particular mutations profiles in those genes will be selected to for further *in vitro* analysis (WP3).

Task-2.2: Genomic markers analysis
Task-2.2 start month: M13
Task-2.2 end month: M24
Task-2.2 Participants: PhD student
Description of the task-2.2: Potential SNPs and insertion/deletion (in/del), which differentiate the selected strains will be identified. SNP will be screened and sorting using information in the whole genome including intergenic, synonymous and non-synonymous mutations. The total number of nonsynonymous and synonymous sites in the SNP-affected genes will be determined and sorted using Bionumerics software 7.0 and command line. Values of dN/dS will be calculated according to the formula (N/n)/(S/s), in which N = the number of nonsynonymous SNPs, n = the number of nonsynonymous sites, S = the number of synonymous SNPs, and s = the number of synonymous sites. A dN/dS calculation will be used to compare selection pressures at an individual isolate level. Concatenated SNPs will be used to create a maximum likelihood phylogenetic tree using MEGA 4.1 software. A selection of 182 virulence genes, involved in envelope biosynthesis and excreted antigen (targeted genes: lipoprotein (lppO, lpqT, lpqG, lprM, lppA and rpfA), genes involved in host tropism (PE-PGRS genes and PPE genes), the ESAT-6 family of secreted antigens (Mb3919c and Mb3935c), the genes involved in the variability of parietal lipids, polyketide synthases (pks) and transporter (mmpSL)) will be deeply analysed. SNPs identified in these genes will be criteria for the selection of strains which will be further characterized in WP3.

WP3: Analysis of the antigenic variability: biochemical and lipidomic studies
WP3 start month: M21
WP3 end month: M30
WP3 Leader: Biet Franck
Deputy WP3 Leader: Boschiroli Maria Laura
WP3 participants: PhD student, Thierry Cochard
Description of the WP3: The strains selected according to their potential for antigenic variation (WP2) and their epidemiological characteristics will be analysed by biochemical approaches.

Task-3.1: Protein profiles determination
Task-3.1 start month: M21
Task-3.1 end month: M30
Task-3.1 Participants: PhD student, Thierry Cochard
Description of the task-3.1: analysis of protein profiles by SDS PAGE Western blot using a collection of sera from animals of different infectious status

Task-3.2: Lipidomic analyses

Task-3.2 start month: M21
Task-3.2 end month: M30
Task-3.2 Participants: PhD student, Thierry Cochard
Description of the task-3.2: lipid analyses by thin layer chromatography coupled with mass spectrometry analyses.

WP4: Valorisation and dissemination of results
WP4 start month: M17
WP4 end month: M36
WP4 Leader: Boschiroli Maria Laura
Deputy WP4 Leader: Lorraine Michelet
WP4 participants: PhD student
Description of the WP4: This WP is dedicated to the dissemination of all the results obtained in the other WPs to the scientific community, either by the publication of articles in international open access journalsor by the participation at international scientific meetings. Writing of the PhD manuscript is the final step of the project.

Task-4.1: Publication of results
Task-4.1 start month: M21
Task-4.1 end month: M36
Task-4.1 Participants: PhD student
Description of the task-4.1: writing of scientific papers for publication in international open access journals and publication of genomic data in international databases.

Task-4.2: Communication
Task-4.2 start month: M17
Task-4.2 end month: M34
Task-4.2 Participants: PhD student
Description of the task-4.2: Participation at international scientific meetings to present the results of the different WP.

Deliverables[1]		
Ref[1]	Title	Due month[1]
D-E5-2	Second steering committee report ●	M30
D-E5-3	Oral communication at a congress ♦	M30
D-E5-4	Publication in an international journal (in prep or submitted) ■	M36
Milestones[1]		
Ref[1]	Title	Due month[1]
M-E5-4	Mapped genome	M28
M-E5-5	Strain selection for WP3	M32
M-E5-6	SNP matrix	M36
M-E5-7	List of SNP in virulence genes	M36

2.4.2.1.42 PhD07-R2-ET2.1-MACE

Reference to the Stategic Research Agenda (please refer to D2.7)	**ET 2.1: Evaluation of early detection methods for emerging threats**									
PhD Project Title	**Mathematical models and economic evaluation for cystic echinococcosis control and elimination**									
PhD Project Acronym	**MACE**									
PhD Supervising Organisation	**P23-UoS**					Deputy PhD Supervising Organisation			**P27-ISS**	
PhD Supervisor	**Joaquin Prada**					Deputy PhD Supervisor			**Adriano Casulli**	
PhD Start month	**M21**					PhD End month			**M57**	
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	**23**	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM	**13**									
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives

1) Improve surveillance design and optimise delivery for control of CE in Argentina

Description of work

The purpose of this objective is to assess the best combination of surveillance streams, targeting all the stages of the transmission pathway (dog, sheep, people, and contaminated environment) that would ensure accurate and efficient detection and prediction of CE.
The first task will be to develop a robust mechanistic individual-based model hat accurately describes the biological processes of CE transmission, which will be initially validated using a dataset

from Rio Negro, Argentina (provided by our collaborators). The model will be based on state-of-the-art models of CE transmission, which will integrate the animal component life-cycle (sheep-dog), with detailed disease progression in the human hosts.
The student will then run different control and surveillance scenarios, to better understand surveillance needs and assess different control strategies.

Deliverables

Ref	Title	Due month
MACE.Y3.1	Draft of publication with the control scenarios	M32
MACE.Y3.2	Model of CE validated in Argentina	M32

Milestones

Ref	Title	Due month
MACE.Y3.A	Fitted model of CE to data from Rio Negro	M30
MACE.Y3.B	Simulations of different control scenarios	M32

2.4.2.1.43 PhD08-R2-ET5.1/4.1/FBZSH3-DESIRE

Reference to the Stategic Research Agenda (please refer to D2.7)	**ET 5.1: The role of wildlife in the ecology of potentially zoonotic emerging threats** **ET 4.1: Host factors associated with increased susceptibility to infection and disease of emerging threats with undefined routes of transmission** **FBZ-SH 3: Source attribution of bacterial foodborne zoonoses and antimicrobial resistance considering also the environment and non-livestock reservoirs (e.g. pets and wildlife) as sources**									
PhD Project Title	**Developing Evidence-based Surveillance for emergIng Rat-borne zoonoses in changing Environments**									
PhD Supervising Organisation	**DESIRE (Developing Evidence-based Surveillance for emergIng Rat-borne zoonoses in changing Environments)**									
PhD Supervisor	**P30-RIVM**				Deputy PhD Supervising Organisation		**P31-WbvR**			
PhD Coordinator person name	**Miriam Maas**				Deputy PhD Supervisor		**Wim van der Poel**			
PhD Start month	**M21**				PhD End month		**M68**			
	1	2	4	6	7	8	9	10	11	12
Participant	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM*										
	13	14	15	16	17	18	19	20	21	22
Participant	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
	23	24	25	26	27	28	29	**30**	31	32
Participant	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	**RIVM**	WbvR	FHI
PM								**12**		
	33	34	35	36	37	38	39	40	41	
Participant	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

* PM are supervision and technical support, and is in kind

Objectives:
In the first year of the PhD, the PhD candidate will design and start the field studies in which the subsequent years the samples and data will be collected. Furthermore, the PhD candidate will learn to perform the necessary laboratory analyses (training) and will develop new methods

Description of work:
1)
The mobile application will have been launched several months before the start of the PhD, so data collection is only at the start. PhD student will develop a modelling framework to analyse the data later in PhD. The PhD student will use field data from e.g. camera traps to reflect on the value of the mobile app data. This comparison of camera trapping data with data collected in the mobile application will be used in Y4 for D2. If the data collected with the mobile applications reflects the relative rat abundance well, the mobile application may also be used in other European countries. If the data collected by the application does not reflect the rat relative abundance, it will be optimized.
2)
Initial laboratory analysis of pathobiome of brown and black rats will be performed on available samples. The results of this analysis will serve as input for follow-up studies in urban environments. The PhD candidate will set-up these follow-up studies in Y2 (i.e. the first PhD year).
3)
Data necessary for the risk-map analysis will be determined and studies to collect the data will be initiated.
4)
The PhD student will assess various methods that are used for greening of cities, in view of the effect on rat populations.

The PhD candidate will join the Wageningen University International graduate school for Agricultural Sciences (WIAS) for courses and training.

Deliverables N/A

Milestones

Ref	Title	Due month
M1	Pathobiome analysis: list of pathogens and protocols	M27(March)
M2	Field study set-up	M30 (June)

2.4.2.1.44 PhD09-R2-FBZSH3/AMR2.1-UDOFRIC

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ-SH 3: Source attribution of bacterial foodborne zoonoses and antimicrobial resistance considering also the environment and non-livestock reservoirs (e.g. pets and wildlife) as sources. AMR2.1: Dynamics of AMR selection, clonal spread and horizontal gene transfer in humans, animals and the environment, including epidemiology of resistant microorganisms and antimicrobials in the environment and their (environment-mediated) spread**									
PhD Project Title	**Understanding the development of fluoroquinolone (FQ) resistance in *Campylobacter* present in broilers and the risks of FQ resistance persisting through the food-chain to cause disease in people**									
PhD Project Acronym	**UDoFRiC**									
PhD Supervising Organisation	**P21-APHA**					Deputy PhD Supervising Organisation			**P1-ANSES**	
PhD Supervisor	**John Rodgers**					Deputy PhD Supervisor			**Isabelle Kempf/Katell Rivoal**	
PhD Start month	**M21**					PhD End month			**M57**	
	1	2	4	6	7	8	9	10	11	12
Participant	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
	13	14	15	16	17	18	19	20	**21**	22
Participant	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	PHE
PM									**12**	
	23	24	25	26	27	28	29	30	31	32
Participant	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
	33	34	35	36	37	38	39	40	41	
Participant	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives:

1) Literature review of FQ resistance in Campylobacter
2) Collation of FQ resistance data in both UK and French Broilers and other livestock and environmental sources and identification of data-gaps in temporal sequence and source.
3) MIC determination for Campylobacter for phenotypic characterisation to fill data gaps.
4) WGS sequence of Campylobacter and predicted resistance determination to complete data gaps
5) Description of the diversity of FQ resistance and acquisition of resistance variants over time.
6) Describe relationship between WGS and phenotype for FQ resistance.

Description of work:

1) Literature review of FQ resistance in Campylobacter
   A thorough review of the scientific literature on Campylobacter with a focus on antimicrobial resistance and the persistence of Campylobacter in the food-chain.
2) Collation and description of available data on FQ resistance in both UK and French Broilers and other livestock and environmental sources.
   Available archives and reports on FQ resistance data will be compiled for broilers from UK, France, data from other locations and from other sources (host species) will be investigated to compile a comprehensive collection of data for further analysis. Key gaps in temporal data or for particular sources will be identified. Where possible gaps will be filled using available isolates and the generation of novel WGS or phenotypic data.
3) MIC determination for Campylobacter for phenotypic characterisation to fill data gaps.
   Competence in bacterial culture and MIC testing methodology will be acquired. Isolates identified for phenotyping will be MIC tested.
4) WGS sequence of Campylobacter and predicted resistance determination to complete data gaps
   Competence in WGS methodology including sample preparation, processing and data handling and analysis will be acquired. Isolates identified for WGS will be processed tested.
5) Description of the diversity of FQ resistance and acquisition of resistance variants over time.
   A full description of resistance genes present for FQ in the collated dataset will be undertaken, this report will examine the temporal trends in the acquisition of these resistance genes. The diversity of resistance genes present in Campylobacter from difference locations or sources will also be described.
6) The relationship between WGS and phenotype for FQ resistance.
   Using the available WGS and phenotyping data, the relationship between WGS and MIC phenotype will be explored. This will examine the performance of resistance predicting pipelines, relationship between MIC and WGS may be examined.

Deliverables

Ref	Title	Due month
1	Literature review of FQ in Campylobacter	Feb 2020
2	PhD Annual review	Sept 2020
3	Description of the diversity of FQ resistance and acquisition of resistance variants over time.	Nov 2020
4	Report on the relationship between WGS and phenotype.	Dec 2020

Milestones

Ref	Title	Due month
1	Completion of literature review	Feb 2020

2	Completion of data collation and identification of data gaps	March 2020
3	Completion of training in bacteriology and MIC	March 2020
4	Completion of training in WGS and bioinformatics	June 2020
5	Completion of phenotypic and WGS data generation to fill data-gaps	Sept 2020
6	PhD Annual Review	Sept 2020
7	Description of the diversity of FQ resistance and acquisition of resistance variants over time.	Nov 2020
8	Report on the relationship between WGS and phenotype.	Dec 2020

2.4.2.1.45 PhD10-R2-FBZSH3/AMR2.1-WILBR

Reference to the Stategic Research Agenda (please refer to D2.7)			**FBZ-SH 3: Source attribution of bacterial foodborne zoonoses and antimicrobial resistance. AMR 2.1: Dynamics of AMR selection, clonal spread and horizontal gene transfer.**							
PhD Project Title			**Contribution of wild birds to AMR in the environment and on farm.**							
PhD Project Acronym			**WILBR**							
PhD Supervising Organisation			**P21-APHA**			Deputy PhD Supervising Organisation			**P41-SVA**	
PhD Supervisor			**Muna Anjum**			Deputy PhD Supervisor			**Stefan Borjesson**	
PhD Start month			**M21**			PhD End month			**M57**	
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	17	18	19	20	**21**	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	**APHA**	PHE
PM									**14.9**	
Participant	23	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives for Y1 of PhD:

7) Literature review of antimicrobial resistance in Escherichia coli in livestock and wild birds on farm.
8) Contacting farmers and identifying farms for sampling.
9) Designing a sampling framework for sampling both livestock and wild bird faeces for a longitudinal study
10) Designing a questionnaire for capturing antimicrobial usage and cleaning/disinfection practices on farm.

11) Designing microbiological protocols for isolation of E. coli harbouring resistance to important antimicrobials.
12) Sampling on farm and isolating AMR E. coli from livestock and wild life faeces.

Description of work:

1) Literature review of antimicrobial resistance in Escherichia coli in livestock and wild birds on farm: A thorough review of the scientific literature on Campylobacter with a focus on antimicrobial resistance and the persistence of Campylobacter in the food-chain.
2) Contacting farmers and identifying farms for sampling: There is a list available within APHA Dept of Epidemiological Studies (DES) of livestock farms in the UK. The student will be expected to liaise with colleagues in DES to help identify suitable outdoor and indoor farms for sampling and seek their permission.
3) Designing a sampling framework for sampling both livestock and wild bird faeces for a longitudinal study: The student will work with both colleagues in DES and the APHA Wild Life team to design a sampling framework which will be based on the size of the farm (both its area and livestock population).
4) Designing a questionnaire for capturing antimicrobial usage and cleaning/disinfection practices on farm: A questionnaire will be designed to capture on-farm practices that may help in co-selection and persistence of AMR E. coli on farm.
5) Designing microbiological protocols for isolation of E. coli harbouring resistance to important antimicrobials: The APHA Dept of Bacteriology has extensive experience in this area and will work closely with colleagues in the AMR/enterics team to update/adapt current protocols that are already in place.
6) Sampling on farm and isolating AMR E. coli from livestock and wild life faeces: It is expected that the student will perform their first sampling visit to farms which will be accompanied by more experienced colleagues from within DES or Bacteriology, as well as the Wild Life team. Faecal samples collected will be brought back to the laboratory at Weybridge for purification of AMR E. coli.

Deliverables

Ref	Title	Due month
1	Literature review of AMR on-farm and wild birds	Feb 2020
2	PhD Annual review	Sept 2020

Milestones

Ref	Title	Due month
1	Completion of literature review	Feb 2020
2	Identification of farms for sampling	March 2020
3	Completion of sampling protocol and questionnaire design	March 2020
4	Completion of microbiological protocol	March 2020
5	Completion of first on-farm sampling and E. coli purification	Sept 2020
6	PhD Annual Review	Sept 2020

2.4.2.1.46 PhD11-R1-FBZ4/5- EnvDis

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ 4: Source attribution and transmission routes** **FBZ 5: Epidemiological studies: risk factors and dynamics**									
PhD Project Title	**Environment and Foodborne Zoonosis: Linking Mechanism and Phenomenology**									
PhD Project Acronym	**EnvDis**									
PhD Supervising Organisation	**P23-UoS**			Deputy PhD Supervising Organisation				**P23-UoS**		
PhD Supervisor	**Giovanni (Gianni) Lo Iacono**			Deputy PhD Supervisor				**Alasdair (Alex) Cook**		
PhD Start month	**M24**			Project End month				**M60**		
Participant number	1	2	4	5	6	7	8	9	10	11
Acronym of participant	Anses	Ages	Sciensano	NCIPD	NDRVMI	SZU	VRI	BfR	FLI	RKI
PersonXmonths[1] per participant:										
Participant number	12	13	14	15	16	17	18	19	20	21
Acronym of participant	DTU	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA
PersonXmonths per participant:										
Participant number	22	**23**	24	25	26	27	28	29	30	31
Acronym of participant	PHE	**UoS**	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR
PersonXmonths per participant:		**12**								
Participant number	32	33	34	35	36	37	38	39	40	41
Acronym of participant	FHI	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FoHM	SVA
PersonXmonths per participant										

O1.

Objectives (for all years)

- **O1.** Can we identify and access "big data" – existing information that can be interrogated to yield new evidence for decision-making in One Health? The generation of new analytical approaches would provide tools that could then be adapted to specific animal or human health issues where environment plays a key role in aetiology.
- **O2.** Can we identify the key environmental processes triggering and propagating zoonoses?
- **O3.** Can we disentangle the role of animal, human (including socio-economic factors) and environmental factors in zoonoses?

O4. Can we identify the delay between variations in the environment (e.g. increase in the temperature or behavioural change) and the occurrence of a foodborne outbreak?
O5. How can we quantify their impact on Animal and Public Health?

Description of work[1]

WP1
WP start month: January 2019 (13M)
WP end month: December 2021 (48M)
WP Leader Gianni Lo Iacono
Deputy WP Leader: Alex Cook
WP participants: PhD Student
Description of the WP[1]:
To develop a general tool to assess the risk of infectious diseases (in particular zoonosis) when we have information of relevant environmental factors.

Task[1]: ii) Develop a process based model for Salmonellosis based on statistical patterns found in year 1; Start to repeat tasks done in year 1 for Leptospirosis
Task start month: 25M
Task end month: 36M
Task Leader: Gianni Lo Iacono
Deputy Task Leader[1]: Alex Cook
Task Participants: PhD Student, Alex Cook, Gianni Lo Iacono

Description of the task[1]: Develop a process based model for Salmonellosis based on statistical patterns found in year 1; Start to repeat tasks done in year 1 for Leptospirosis
Sub-Task[1]: Develop a process based model for Salmonellosis based on statistical patterns found in year 1
Sub-Task start month: 25M
Sub-Task end month: 36M
Sub-Task Leader: Gianni Lo Iacono
Deputy Task Leader[1]: Alex Cook
Task Participants: PhD Student, Alex Cook, Gianni Lo Iacono

Sub-Task[1]: Start to repeat tasks done in year 1 for Leptospirosis
Sub-Task start month: 30M
Sub-Task end month: 36M
Sub-Task Leader: Gianni Lo Iacono
Deputy Task Leader[1]: Alex Cook
Task Participants: PhD Student, Alex Cook, Gianni Lo Iacono

Deliverables[1]

Ref[1]	Title	Due month[1]
D-XX-6.4 (Optional)	Attending Relevant Training courses (this is optional and it might happen in year 2 depending on the student's needs.)	12
D-XX-6.5	Presentation of findings (e.g. conferences, internal school seminars)	12
D-XX-6.6	End of Year Confirmation Report	12

Milestones[1]

Ref[1]	Title	Due month[1]

M-XX-6.5	Develop an environmental-driven compartmental process based model for Salmonellosis, link the model with the statistical patterns found in year 1	6
M-XX-6.6	Produce relevant output of the model	12
M-XX-6.7	Start to repeat tasks done in year 1 for Leptospirosis	12

2.4.2.1.47 PhD12-R2-FBZSH9-AptaTrich

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ-SH 9: Better tools for detection and investigation of foodborne outbreaks, including antimicrobial resistant pathogens, as well as economic assessments of potentially increased cluster detection through whole genome sequencing**									
PhD Project Title	**Development of an aptamer-based test for *Trichinella* detection**									
PhD Project Acronym	**AptaTrich**									
PhD Supervising Organisation	**P1-Anses**					Deputy PhD Supervising Organisation			**P9-BfR**	
PhD Supervisor	**Gregory Karadjian**					Deputy PhD Supervisor			**Anne Mayer-Scholl**	
PhD Start month	**M22**					PhD End month			**M57**	
Participant	**1**	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM	**15.5**									
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	23	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives:

- To select aptamers on *T. spiralis* whole larvae
- To produce stage specific proteins and apply protein-SELEX

Description of work:

In year 3, all the work will be done at Anses laboratory of Maisons-Alfort.
Selection of a DNA-based aptamers set specific for *Trichinella spiralis* (*T. spiralis*) whole Muscle Larvae (ML) will be performed using a DNA library by SELEX method (Systematic Evolution of Ligands

by Enrichment) at ANSES. Intact *T. spiralis* whole ML will be incubated with the DNA library for multiple rounds (>10) with a progressive decrease in ML number and interaction time at each round. Negative selection will be performed to avoid cross-reactivity using larvae from related organisms (i.e. *Toxocara* spp., *Strongyloides stercoralis*, …).
As it is very important to select aptamers against early and late *T. spiralis* expressed proteins, recombinant stage specific proteins will be expressed and protein-SELEX method will be applied on these recombinant proteins.
At each round of the two SELEX methods, the quantity of the different aptamers selected will be evaluated by qPCR, using the number of melting peaks to quantify. The number of cycles to obtain less than ten aptamers by selection will be defined.
During each cycle round, a PCR step will allow to amplify the aptamers in each set including a FAM staining. At the fifth cycle, the capacity of the aptamers to bind Trichinella larvae will be assayed by contact and looked at confocal microscope.

Deliverables

Ref	Title	Due month
D-2	Aptamers set on whole larvae is selected	29
D-3	Aptamers set on stage specific proteins is selected	36

Milestones

Ref	Title	Due month
M-3	The aptamers bind to Trichinella spiralis ML	36

2.4.2.1.48 PhD13-R2-FBZ8/AMR2-VIMOGUT-AMR

Reference to the Stategic Research Agenda (please refer to D2.7)			**FBZ 8: Model Systems (in vitro and in vivo) to study host/food – microbe interactions** **AMR 2: Epidemiological studies into the dynamics of AMR in human and animals populations and the environment including horizontal gene transfer and selection of AMR**							
PhD Project Title			***In VItro* and *in VIvo* analyses and MOdulation of the chicken GUT microbiome to combat AMR.**							
PhD Project Acronym			**VIMOGUT-AMR**							
PhD Supervising Organisation			**P31-WbvR**			Deputy PhD Supervising Organisation			**P21-APHA**	
PhD Supervisor			**Michael Brouwer**			Deputy PhD Supervisor			**Muna Anjum**	
PhD Start month			**M21**			PhD End month			**M68**	
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	23	24	25	26	27	28	29	30	**31**	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	**WbvR**	FHI
PM									**12**	
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives:

- Perform 16S barcode sequencing and analysis on chicken caecal samples collected before the start of VIMOGUT-AMR.
- Write a manuscript describing the initial results for the relationship between chicken gut microbiome maturation and ESBL *E. coli* gut colonisation.
- Collect chicken caecal samples to validate preliminary results on different farms/flocks.

- Perform literature search for intervention strategies that can be tested *in vitro* for ESBL *E. coli* colonisation and plasmid spread through the microbiome.
- Perform initial test runs on the *in vitro* chicken gut model to determine CFU for reliable ESBL colonisation.

Description of work:

The student will perform 16S barcode sequencing on a set of chicken caecal samples that was collected longitudinally on one chicken farm to describe the relationship between the maturation of the microbiome and the colonisation of the gut by ESBL *E. coli*. Caecal samples from additional farms and flocks will also be collected during this year to repeat this study. During this sampling, a mixed pool stock will also be collected to provide the *in vitro* chicken gut model with harmonised material for seeding during the project.
The *in vitro* chicken gut model will be assembled and tested. During the test runs, an accurate number will be determined to colonise the model with ESBL *E. coli*. The read-out parameters for the model will be determined using classical culture-based microbiology techniques as well as 16S barcode sequencing that is also used for the *in vivo* studies.
A literature study will be performed to determine the most promising intervention strategies for gut colonisation by ESBL *E. coli* and for transfer of ESBL plasmids within the microbiome.

Deliverables

Ref	Title	Due month
D1	Manuscript on preliminary findings for the relationship between chicken gut microbiome maturation and ESBL colonisation.	36

Milestones

Ref	Title	Due month
M4	Perform 16S barcode sequencing on currently collected samples.	27
M5	Perform analysis of initial 16S experiment	30
M6	Perform initial test runs on *in vitro* gut model to determine CFU for reliable ESBL colonisation.	
M7	Write manuscript on initial results relationship between chicken gut microbiome maturation and ESBL gut colonisation.	36

2.4.2.1.49 PhD14-R2-FBZ4-ToxSauQMRA

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ 4: Source attribution and transmission routes**									
PhD Project Title	**Study of the tropism and persistence of Toxoplasma gondii: from pork carcass to sausage and dry ham, a quantitative risk assessment**									
PhD Project Acronym	**ToxSauQMRA**									
PhD Supervising Organisation	**P1-ANSES**					Deputy PhD Supervising Organisation			**P30-RIVM**	
PhD Supervisor	**Pascal BOIREAU**					Deputy PhD Supervisor			**Joke VAN DER GIESSEN**	
PhD Start month	**M22**					PhD End month			**M57**	
Participant	**1**	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM	**8.36**									
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	23	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives:
"What is the attribution of the traditional raw pork products in the human Toxoplasma infection? " Following the scientific question, the present research project aims to respond, 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).

Description of work:
The work is organized into 3 work packages (WPs) with tasks (T). The 3-year project spans over four Annual Periods (Y2-Y5) and is described in four Work Plans.

WP1 - Investigation of *T.gondii* predilection sites in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst)
WP start month M22 - WP end month M36
WP participants ANSES, Institut du Porc, Université de Champagne-Ardennes

WP1-T1: Experimental infection of pigs
Task start month M22 - Task end month M25

WP1-T1 takes place over the second and the third year of the project. During Y3, the main focus is collecting the samples from the experimentally infected pigs. After euthanasia, 4 tissues will be 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 muscle groups (4 for breast, 7 for shoulder and 13 for ham) will be pooled. Some 40 supplementary muscles per carcasses will be collected, representing the most important anatomical regions. One back leg will be collected for each pig for dry ham production.

WP1-T2: Predilection sites of *T.gondii* in pigs
Task start month M26 - Task end month M36
WP1-T2 takes place over the third year of the project. During Y3, the main focus is to identify the predilection sites of *T.gondii* presence in pig tissues. Therefore, mouse bioassays, quantitative PCR and magnetic capture PCR will be performed on a part of the pooled sample per tissue. The remaining parts of the pools will undergo industrial (salting, smoking, etc) processing (WP2).

WP2 - Assessment of the persistence of *T. gondii* during the production and storage of dry sausages and dry ham
WP start month M26 - WP end month M42
WP participants ANSES, Institut du Porc, Université de Champagne-Ardennes, INRA Corte

WP2-T1: Manufacture of dry sausages and dry ham
Task start month M26 - Task end month M31
WP2-T1 takes place over the third year of the project. During Y3, the main focus is the manufacture of the dry sausages and dry ham.
The manufacture of dry sausages will be carried out on a pilot scale by IFIP, according to a protocol representative of those used by a commercial factory. The dry hams will be manufactured by INRA Corte, using two traditional salting techniques (a long one : 2.5 days/Kg and a short one: 1 day/Kg). Briefly, after mincing, the muscle pool will be divided into seven portions (corresponding to the 7 combinations of nitrites (as sodium NaNO2 nitrites) and NaCl concentrations that will 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 will be collected at different dates (D0, D2, D10, D20, D30 and D50).
On each analysis date, IFIP will carry 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 will be 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).
For dry ham, 300g of products will be taken at D0, D30, D60 and D90 for both salting formulations (long and short). A total of 24 samples (4 times x 2 recipes x 3 pigs per recipe) will be taken for this type of product.

WP2-T2: Assessment of the persistence of viable *T. gondii* in pork delicatessen
Task start month M27 - Task end month M42
WP2-T2 takes place over the third and the fourth year of the project. During Y3, the main focus is the analyse of the dry sausages for the presence of viable T. gondii by bioassay in mice (animal facility URCA). The presence of T. gondii DNA in the inocula will be quantified by T. gondii by qPCR (URCA) of the remaining trypsin digests and MC-PCRq (ENVA).

Deliverables

Ref	Title	Due month
D-PhD-ToxSauQMRA-WP1	Final report on the experimentally infection of the pigs (serological monitoring, rectal temperature, weight) and predilection sites of T.gondii	M36

Milestones

Ref	Title	Due month
M-PhD-ToxSauQMRA-01	Pig infection experiment is terminated. Weekly/monthly weight and rectal temperature are collated and presented in a graph. Samples are collected and prepared for testing and dry sausage and ham processing.	M27
M-PhD-ToxSauQMRA-02	Serological testing of pigs is finalised and weekly levels of Igs for are collated in a graph.	M30
M-PhD-ToxSauQMRA-03	Testing of tissue samples prior to processing is finalised and quantitative PCR, Magnetic-capture PCR and mouse bioassay data are collected in tables.	M36
M-PhD-ToxSauQMRA-05	Testing of dry sausage samples is (almost) finalised and quantitative PCR and mouse bioassay data are starting to be collected in tables	M36

2.4.2.1.50 PhD15-R2-FBZ5-TRACE

Reference to the Stategic Research Agenda (please refer to D2.7)	**FBZ 5: Epidemiological studies: risk factors and dynamics**									
PhD Project Title	**Tracking the public health hazard of foodborne Hepatitis E**									
PhD Project Acronym	**TRACE (TRAcking publiC health risk of hepatitsE)**									
PhD Supervising Organisation	**P30-RIVM**					Deputy PhD Supervising Organisation		**P31-WbvR**		
PhD Supervisor	**Eelco Franz**					Deputy PhD Supervisor		**Wim van der Poel**		
PhD Start month	**M21**					PhD End month		**M68**		
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	17	18	19	20	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA	PHE
PM										
Participant	23	24	25	26	27	28	29	**30**	**31**	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	**RIVM**	**WbvR**	FHI
PM								**1.58**	**15.29**	
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives:

Construction of timed-phylogeny of HEV based on core-genome SNP analysis in order to infer time-points of evolutionary divergence of different HEV types

Description of work:

1. To generate whole genome sequences from HEVs, positive samples with high titers are needed. Since most of the available HEV positive samples are not from acute phase diseased and do not contain high amounts of virus, we will first work on vast sample (pre)processing (enrichment) to make whole genome sequencing possible. To enrich the sample for HEV we will use different methods. To eliminate the host and bacterial DNA we will optimise a DNA depletion method, this will be done using a DNAse treatment on the samples containing HEV RNA. After the DNA depletion

samples still contain a lot of messenger RNA which may interfere with virus amplification, most of this mRNA is 18S (host) and 16S (bacterial). With a specific primer RNA-DNA hybrids will be generated which subsequently can be depleted with RNase H. Using specific primers or random primers the whole sequence of the HEV virus will be generated. In case the method still lacks sensitivity we may also consider HEV culture to increase the amount of HEV virus (a culture method is under development in a related project).

2. 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

Deliverables

Ref	Title	Due month
D1	Publication on sample processing protocol for HEV RNA positive target samples of different origin and associated deep sequencing procedure for HEV from such samples	36
D2	Publication on HEV dynamics including information about the geographical origin of predominant virulent strains and identification of genetic traits changed over time	36

Milestones

Ref	Title	Due month
N/A		

2.4.2.1.51 PhD16-R2-FBZ2/AMR6.1-Codes4strains

Reference to the Stategic Research Agenda (please refer to D2.7)			**FBZ 2: Development and harmonisation of NGS-based methods for detection and tracing of FBZ agents, emerging threats and AMR determinants** **AMR 6.1: Development of NGS-based tools for surveillance of AMR in Enterobacteriaceae in animals, humans and the environment**							
PhD Project Title			**Tracking bacterial pathogens through sources, geography and time using stable phylogenetically-informative genome codes**							
PhD Project Acronym			**Codes4strains**							
PhD Supervising Organisation			**P20-IP**			Deputy PhD Supervising Organisation		**N/**		
PhD Supervisor			**Sylvain Brisse**			Deputy PhD Supervisor		**N/A**		
PhD Start month			**M22**			PhD End month		**M57**		
Participant	1	2	4	6	7	8	9	10	11	12
	Anses	Ages	Sciensano	NDRVMI	SZU	VRI	BfR	FLI	RKI	DTU
PM										
Participant	13	14	15	16	17	18	19	**20**	21	22
	SSI	UT	VFL	INIA	UCM	MVNA	INRA	**IP**	APHA	PHE
PM								**12**		
Participant	23	24	25	26	27	28	29	30	31	32
	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR	FHI
PM										
Participant	33	34	35	36	37	38	39	40	41	
	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FOHM	SVA	
PM										

Objectives:
To develop the LINcode approach and compare with cgMLST and SNapperDB approaches on large genomic datasets

Description of work:
1st quarter: we will evaluate the cgMLST schemes for K. pneumoniae and E. coli on the pilot genome dataset; define cgMLST schemes to be used

2nd quarter: collection of full dataset of genomic sequences (based on publicly available ones) and cgMLST scanning to define allelic profiles; and Development of LINcodes algorithm

3rd quarter: SNapperDB analysis of Ecoli genomes.

4th quarter: constitution of SNApperDB database for K. pneumoniae and analysis of K pneumoniae genomes and comparison of LINcodes with cgMLST and SNapperDB approaches**.**

Deliverables

Ref	**Title**	**Due month**
D3.1	cgMLST schemes for Kp and Ecoli	3
D3.2	LINcodes algorithm defined and implemented on full dataset	6
D3.3	SNapperDB Ecoli implemented for full dataset	9
D3.4	SNapperDB Kpneumoniae implemented for full dataset	12

Milestones

Ref	**Title**	**Due month**
M2.1	cgMLST schemes defined for Kp and Ecoli	3
M2.2	LINcodes algorithm defined	6
M2.3	SNapperDB databases set-up for both pathogens	12

2.4.2.1.52 ASM Satellite Workshop 2020

Workshop Title	**Systems mapping for risk analysis of zoonoses**									
Workshop Acronym	**Symraz**									
Workshop Organising Institute	**P30-RIVM**			Deputy Workshop Organising Institute				**N/A**		
Workshop Coordinator	**Kitty Maassen**			Deputy Workshop Coordinator				**N/A**		
Workshop Start Month	**M21**			Workshop End Month				**M33**		
Participant number	1	2	4	5	6	7	8	9	10	11
Acronym of participant	Anses	Ages	Sciensano	NCIPD	NDRVMI	SZU	VRI	BfR	FLI	RKI
PersonXmonths per participant:										
Participant number	12	13	14	15	16	17	18	19	20	21
Acronym of participant	DTU	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA
PersonXmonths per participant:										
Participant number	22	23	24	25	26	27	28	29	**30**	31
Acronym of participant	PHE	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	**RIVM**	WbvR
PersonXmonths per participant:									**0,2**	
Participant number	32	33	34	35	36	37	38	39	40	41
Acronym of participant	FHI	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FoHM	SVA
PersonXmonths per participant										

Objectives

- Offer people the opportunity to make a systems map that can be used as a first step into setting up a structured organisation to deal with (new and emerging) zoonoses in a One Health approach
- Learn about systems thinking, which is usable in many complex situations
- Bring together different domains/disciplines within and between countries

Description of work

WP1: Satellite workshop 'Systems mapping for risk analysis of zoonoses
WP start month: M21
WP end month: M33
WP Leader: Kitty Maassen
Deputy WP Leader

WP participants: Several of the COHESIVE partners
Description of the WP:
Every country has to deal with new and emerging zoonoses. A large outbreak can happen unpredicted, such as the Q-fever outbreak in The Netherlands. So, there is a clear need to be prepared. However, many countries have not organized the risk analysis (from signalling, via risk assessment to risk management and risk communication) in a One Health fashion.
In the JIP COHESIVE a guideline is made to support countries in a practical manner to set up or strengthen the collaboration between vet-med-food (-environment) in the area of risk analysis of zoonoses. A first step is to determine what the exact goal is of the collaboration and what the outcome is of this One Health initiative. The next step is to get a good impression of the system in which this initiative is placed. Because of the fact that trying to deal with new and emerging zoonoses is the goal, it is inherently a complex system dealing with a wicked problem. Once the system, including its interactions/dependencies, is clear, it will be easier to determine how to really organize the risk analysis for (new and emerging) zoonoses. As stated before, a guideline will become available to support this process.
In the COST action NEOH (Network for Evaluation of One Health) several tools were developed to evaluate One Health initiatives based upon systems thinking. A major characteristic of systems thinking is the idea of all components being interconnected and that there are all kind of dependencies. In order to get insight in the (complex) system the system can be mapped. In NEOH a systems mapping tool has been developed specifically to evaluate One Health initiatives. In Cohesive we would like to offer countries who would like to organize their risk analysis for (new and emerging) zoonoses a tool to map their system with the aim to set up a One Health risk analysis structure. Together with Simon Ruegg from the University of Zurich, who was the driving force behind the NEOH evaluation tools, we would like to make the NEOH tool fit for this purpose within Cohesive. At the university of Zurich a first project with respect to a *Brucellosis* program in Kazakhstan was promising using the tool in a setting up fashion.
In this WP we will organize a workshop in which we will provide EJP partners the opportunity to make a systems map for their own country. Preferably, the goal should be the (further) development of a risk analysis structure for new and emerging zoonoses at the national or regional level, but it can also be used for other One Health initiatives which a taking place in a complex system.
Having a systemic approach giving insight in (one of) the complex system we all work in will not only be beneficial for the specific goal we determine here, but learning about systems thinking will be beneficial for other wicked problems within the area of One Health and beyond.
The workshop:
What: Define a One Health initiative for your country and make a related systems map. Preferably an initiative to organize how to deal with new and emerging zoonoses (risk analysis structure), but other One Health initiatives are also possible.
Who: Experts in the area of risk analysis of zoonoses. Policy makers or other risk managers with responsibilities around zoonotic infectious diseases. If another One Health initiative is chosen, actors involved in that initiative can perform the exercise. Although it can be done alone, preferably this exercise is done with more than one person per country, preferably from different domains. Early career researchers (ECR) are especially invited to participate. Those interested in this workshop from a research point of view wanting to set up a One Health initiative/project or from a social sciences point of view interested in systems thinking. They will be the ones to present the results from the exercise.
Maximum number of participants: Not determined but probably around 25-30
When and where: The day before the ASM 2020 starts at the location of the ASM
Time: Start 9am – 17 pm
Program: Before the meeting an assignment will be given to prepare for the workshop (~2-4 hours). The workshop will be led by Simon Ruegg (invited from University of Zurich). The program will

consist of an introduction to systems thinking. Explanation of the tool. Go and fill in the tool together with people from your country. From previous experience it is known that this will yield intensive discussions. Making the map. Present to each other and have general discussion on the results. How to continue (use the results).
Information/materials (E-learning): Background information as well as in depth scientific information will be made available as well as the tools used during the workshop as well as additional tools
Further reading: https://www.wageningenacademic.com/doi/suppl/10.3920/978-90-8686-875-9 (specifically chapter 3, free download)

Task: T1-1 Preparation of the program
Task start month: M22
Task end month: M33
Task Leader: Kitty Maassen
Deputy Task Leader:
Task Participants: RIVM, (ECRs from) COHESIVE partners TBC, Simon Ruegg (University of Zurich)
Description of the task: In this task we will prepare the exact program for the workshop. Including the preparation of material to be used before, during and after the workshop as well as define the assignment to be send to the participants as preparation for the workshop

Task: T-1.2 Coordination and communication of the workshop
Task start month: M22
Task end month: M33
Task Leader: Kitty Maassen
Deputy Task Leader:
Task Participants: UoS, RIVM, (ECRs from) other partners TBC
Description of the task: In this task all practical issues around the workshop will be organised. This includes i.e. sending a save the date, making promotion materials, aligning with ASM etc. A meeting will be organized to determine the exact activities and make a time line.

Deliverables		
Ref	Title	Due month
D-WS-1.1	Timeline with activities around the program	M23
D-WS-1.2	Timeline with coordination and communication activities	M22
D-WS-1.3	Finalized program and assignment	M31
D-WS-1.4	Workshop in Prague	M33
Milestones		
Ref	Title	Due month
M-WS-1	Workshop in Prague	M33

2.4.2.1.53 Summer School 2020

Summer School Title	**Summer School Global One Health, Basic Concepts and Operationalization**									
Summer School Acronym	**GOH**									
Summer School Organising Institute	**P31-WbvR**			Deputy Summer School Organising Institute			**N/A**			
Summer School Coordinator	**Wim van der Poel**			Deputy Summer School Coordinator			**N/A**			
Summer School Start Month	**M34**			Summer School End Month			**M34**			
Participant number	1	2	4	5	6	7	8	9	10	11
Acronym of participant	Anses	Ages	Sciensano	NCIPD	NDRVMI	SZU	VRI	BfR	FLI	RKI
PersonXmonths per participant:										
Participant number	12	13	14	15	16	17	18	19	20	21
Acronym of participant	DTU	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA
PersonXmonths per participant:										
Participant number	22	23	24	25	26	27	28	29	30	31
Acronym of participant	PHE	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	**WbvR**
PersonXmonths per participant:										**1.8**
Participant number	32	33	34	35	36	37	38	39	40	41
Acronym of participant	FHI	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FoHM	SVA
PersonXmonths per participant										

Objectives

- Offer people the opportunity to make a systems map that can be used as a first step into setting up a structured organisation to deal with (new and emerging) zoonoses in a One Health approach
- Learn about systems thinking, which is usable in many complex situations
- Bring together different domains/disciplines within and between countries

Description of work

WP6: Summer School Global One Health
WP start month: M34
WP end month: M34
WP Leader: Roberto LaRagione
Deputy WP Leader: Wim van der Poel

WP participants: Internal and external partners EJP One Health
Description of the WP:
The course will focus on the Global One Health approach with focus on healthy, safe and sustainable food systems. In this wider multidisciplinary approach all possible effects of interventions on the health of humans, animals, plants and the environment, will be weighed while taking ecosystem sustainability into account. “Global One Health” will use multiple disciplines to seek transnational solutions for improving the health of humans, animals and plants worldwide.
Learning goals will include: a) Understand basic concepts in health research, b) Understand connections and conflicts of health research, c) Ability to implement a multidisciplinary approach in health research d) Operationalization of Global One Health: Understand and design surveillance strategies. Understand and design risk assessment and risk management approaches. Recognise methodology and organisation of effective disease outbreak control.
The course will include a visit to the Wageningen Bioveterinary Research laboratory facilities and/or Laboratories or animal facilities at the Wageningen Campus and/or the Utrecht University Veterinary Faculty clinics.
We will organise social events including a welcome drink on day 1 and dining outs in city centre of Wageningen or Utrecht.
Maximum number of participants: We will aim for 30 participants
When and where: The last two weeks of August 2020 at the Wageningen Campus and/or the Utrecht University
Time: Start 9am – 17 pm
Program:
Before the start of the Summer School preparatory materials will be send to the delegates.
The summer course will start with a number of scientific presentations on One Health issues, relevant diseases, important scientific skills and approaches:
Lectures will include:

- An introduction into Global One Health (Prof. Wim van der Poel, Wageningen University)
- Emerging zoonoses (Prof. Wim van der Poel, Wageningen University)
- Ethics of One Health (Prof. Marcel Verweij, Wageningen University)
- Ecological and epidemiological literacies in modern agriculture and agronomy (Prof. Jean Francois Guegan, Institute for Research on Sustainable Development France)
- Community centered control of tropical infections (Prof. Michelle van Vught, University of Amsterdam)
- One Health economics (Prof. Henk Hogeveen, Wageningen University)
- Connecting data analysis and modelling in infectious diseases (Prof. Mart de Jong, Wageningen University)

In the summer course we will also include a number of interactive assignments. Students will work together on such assignments in small groups. This will stimulate interactions between researchers with different back grounds. Results of the work will be presented to the whole group of students.
To stimulate international collaboration we will engage with the One Health Commission and the One Health Platform, also to seek sponsorship and to engage with the 2020 One Health conference, which will be held, in Edinburgh, 14-18 June 2020. The idea will be to connect with young scientist activities at this conference.
In the second week of the course, the students will work on an important One Health theme and write a report about their study of this One Health theme.
In this summer course, the aim will be primarily on PhD students and students with a Master degree in a health related discipline.

Task: T1-1 Preparation of the Summer School program
Task start month: M21
Task end month: M33

Task Leader: Wim van der Poel
Deputy Task Leader:
Task Participants: UoS, NCOH, Lecturers from different EJP partners
Description of the task: In this task we will prepare the program for the summer school. This will Include the preparation of all materials to be used before, during and after the workshop. Also preparation of assignments to be send to the participants prior to the workshop

Task: T-1.2 Coordination and communication of the Summer School
Task start month: M22
Task end month: M34
Task Leader: Wim van der Poel
Deputy Task Leader: UoS
Task Participants: WbvR, UoS, NCOH, Lecturers from different EJP partners
Description of the task: In this task all practical issues around the summer school GOH will be organised. This includes i.e. sending a save the date, the making of promotion materials, making a webpage, A number of skype meetings will be organized to further develop the course and make presentation materials etc..

Deliverables		
Ref	Title	Due month
D-GOH-1.1	Timeline of activities to develop the program	M23
D-GOH-1.2	Timeline of coordination and communication activities	M24
D-GOH-1.3	Finalized program and drafts of assignments	M31
D-GOH-1.4	Summer School in the Netherlands	M34
Milestones		
Ref	Title	Due month
M-GOH-1	Summer School in the Netherlands	M34

2.4.2.1.54 Communication and Media workshop

CaM Title	**How Science Achievements Get to People and Help a Better Life**									
CaM Acronym	**CaM workshop**									
CaM Organising Institute	**P6-NDRVMI**			Deputy CaM Organising Institute			**N/A**			
CaM Coordinator	**Hristo Daskalov**			Deputy CaM Coordinator			**N/A**			
CaM Start Month	**M21**			CaM End Month			**M34**			
Participant number	1	2	4	5	**6**	7	8	9	10	11
Acronym of participant	Anses	Ages	Sciensano	NCIPD	**NDRVMI**	SZU	VRI	BfR	FLI	RKI
PM					**2**					
Participant number	12	13	14	15	16	17	18	19	20	21
Acronym of participant	DTU	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA
PM										
Participant number	22	23	24	25	26	27	28	29	30	31
Acronym of participant	PHE	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	RIVM	WbvR
PM										
Participant number	32	33	34	35	36	37	38	39	40	41
Acronym of participant	FHI	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FoHM	SVA
PM										

Objectives

- To learn about the concept and importance of a communications strategy and to learn how best to use social media platforms such as twitter, and LinkedIn from a scientific perspective in the area of food safety and public health. The sessions will cover strategy, effective communication and marketing essentials to provide researchers with a rounded skillset.

- To equip researchers with the skills, knowledge and practices to be able to communicate their science appropriately and effectively for public media such as to the general public, stakeholders and media journalists; and for private media i.e. tailored and targeted information for a specific audience.

- The workshop will provide extensive information with the assistance of specialists from the Bulgarian Food Risk Assessment Center, playing the role of a national focal point EFSA for communicating risks of all kinds and their effects on public health. Food safety from the farm to the mass of consumer, broken through the lens of the latest scientific advances, will be the essence of the discussions in the proposed seminar.

Description of work

WP1: One Health EJP Communication and Media Workshop: How Science Achievements Get to People and Help a Better Life
WP start month M21
WP end month M34
WP Leader: Hristo Daskalov
Deputy WP Leader: Roberto La Ragione
WP participants: Hristo Daslakov, Mihail Milanov, BFSA PR specialist (We are waiting for new person at the moment) Vladimir Jeglov, Donka Popova, Roberto La Ragione, Dan Horton, Piyali Basu, Jade Passey, New Communication Officer (to be named)
Description of the WP:
Introducing the general public and all stakeholders to the achievements of science and their real meaning for people's lives plays a key role in enhancing food safety and public health. The communication of the new knowledge received with the wide range of stakeholders and organizations (private and state) is no less important than the process of knowledge acquisition itself. The need for public support and understanding is indisputable, especially on such a sensitive issue as food safety with regard to zoonotic agents, antimicrobial resistance and emerging biological hazards to human and animal health.
The European Union brings together over 500 million inhabitants living in 28 different countries with a different mentality, specific features that resolve the crises in a different way, including those related to food safety and health in the broadest sense. At the same time, the influence of the media in any form (television, radio, the Internet, direct public events, etc.) is indisputable for creating a relationship and gaining confidence from the audience in terms of conveying scientific knowledge.
The question of how to convey accurate information whilst also capture the minds and souls of people is key to any communication. This is the main goal of the organized workshop of scientists from the organizations participating in the scientific project.
Duration: 2 days
Theme: "Communication and Media: How Science Achievements Get to People and Help a Better Life".
Dates: October 2020 (specific dates TBC)
Where: Sofia, Bulgaria.
Program: This workshop will consist of sessions delivered by leading European experts in this field, practical and interactive workshops and sessions, and will uniquely bring together the participants' practical experience facilitating subsequent discussion which will allow assessment of the risk communication practices and the role of the private and public media (including social media strategies) in this process.
Information/materials (E-learning): Background information will be made available on the One Health EJP website as well as the tools used during the workshop.

T1.1 Preparation of the program
Task start month M21
Task end month M28
Task Leader: Hristo Daskalov
Deputy Task Leader: Roberto La Ragione
Task Participants: Hristo Daslakov, BFSA PR specialist (We are waiting for new person at the moment), Vladimir Jeglov, Georgi Georgiev, Iliyan Kostov, Donka Popova Roberto La Ragione, Dan Horton, Piyali Basu, Jade Passey, New Communication Officer (to be named)

Description of the task: In this task we will prepare the final program for the workshop. This will include the preparation of material to be used during the workshop. Preparation for accreditation should be started by M25.

T1.2 Coordination and communication of the workshop
Task start month M21
Task end month M34
Task Leader: Hristo Daskalov
Deputy Task Leader: Roberto La Ragione
Task Participants: Hristo Daslakov, Mihail Milanov, Violeta Borisova, Gergana Krumova-Valcheva, Roberto La Ragione, Dan Horton, Piyali Basu, Jade Passey, New Communication Officer (to be named)
Description of the task: In this task all practical issues around the workshop will be organised such as securing the venue, pre-booking accommodation, and other logistical arrangements. This also includes the communication activities for the event sending a save the date, making promotion materials, aligning with the OHEJP branding and liaising with the Communications Team at Surrey. A meeting will be organised to determine the exact activities and to produce a time line.

Deliverables		
Ref	Title	Due month
D-CaM-1.1	Timeline with activities around the program	M23
D-CaM-1.2	Timeline with coordination and communication activities	M24
D-WS-1.3	Finalised program	M26
D-WS-1.4	Workshop in Sofia, Bulgaria	M34
Milestones		
Ref	Title	Due month
MS-CaM-1	Save the Date	M26
MS-CaM-2	Flyer	M28

2.4.2.1.55 CPD module 2019

CPD Title			**Outbreak Preparedness**							
CPD Acronyme			**CPD 2019**							
CPD Organising Institute			**P30-RIVM**			Deputy CPD Organising Institute			**N/A**	
CPD Coordinator			**Dorothee Roβkamp**			Deputy CPD Coordinator			**N/A**	
CPD Start Month			**M22**			CPD End Month			**M28**	
Participant	1	2	4	5	6	7	8	9	10	11
	Anses	Ages	Sciensano	NCIPD	NDRVMI	SZU	VRI	BfR	FLI	RKI
PM										
Participant	12	13	14	15	16	17	18	19	20	21
	DTU	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA
PM										
Participant	22	23	24	25	26	27	28	29	**30**	31
	PHE	UoS	OKI	NUIG	TEAGASC	ISS	IZSAM	IZSLER	**RIVM**	WbvR
PM									**2.2**	
Participant	32	33	34	35	36	37	38	39	40	41
	FHI	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FoHM	SVA
PM										

Objectives

- To organize and host the 2019 CPD module on outbreak preparedness

Description of work

WP1: Organizing and hosting a CPD module on outbreak preparedness
WP start month: October 2019 (M22)
WP end month: April 2020 (M28)
WP Leader: Dorothee Rosskamp, sr. policy advisor for preparedness
Deputy WP Leader:
WP participants: Aura Timen, Corien Swaan, Kitty Maassen, Laura van Vossen, Jeanette de Boer
Description of the WP1

The National Coordination Centre for Communicable Disease Control will organize and host a CPD module on outbreak preparedness in line with the OHEJP CPD curriculum. As agreed with the WP6

project manager, the 2019 CPD outbreak preparedness module will be held in the first trimester of 2020.

Task 1: To organize and host the CPD module on outbreak preparedness
Task start month approx. October 2019 (M22)
Task end month approx. April 2020 (M28)
Task Leader: Dorothee Roβkamp
Deputy Task Leader:
Task Participants: Aura Timen, Corien Swaan, Kitty Maassen, Laura van Vossen, Jeanette de Boer
Description of the task:
The module itself will be two days in length and directed at Early Career researchers. An outline of the course content and an estimated timeline of the course days is provided in the module proposal form. This working plan is divided in three sub tasks: pre-event activities, execution of the 2-day module, and post-event activities.

Sub-Task 1: Pre-event activities – Organizing the program
Six months prior to the event, the LCO will initiate pre-event activities. In collaboration with WP6, the LCO will work on the program organization. This includes the development of a suitable course curriculum, establish accreditation (tbd with WP6), promote the event via various channels, and pre-book the venue and catering.
Sub-Task start month: October 2019 (M22)
Sub-Task end month: March 2020 (M27)
Sub-Task Leader: Dorothee Rosskamp
Deputy Sub-Task Leader:
Sub-Task Participants: Aura Timen, Corien Swaan, Kitty Maassen, Laura van Vossen, Jeanette de Boer

Sub-Task 2: Execution of the CPD module on outbreak preparedness

During the event, the LCO will be responsible for the set-up of the venue and the smooth operation of the event. The course curriculum will be a combination of short interactive lectures and case studies related to the outbreak management of zoonotic diseases.
Sub-Task start month: March/April 2020 (tbd)
Sub-Task end month: March/April 2020 (tbd)
Sub-Task Leader: Dorothee Roβkamp
Deputy Sub-Task Leader:
Sub-Task Participants: Aura Timen, Corien Swaan, Kitty Maassen, Laura van Vossen, Jeanette de Boer

Sub-Task 3: Post-event activities – Evaluation and event report
Post-event activities will include the analysis of the course evaluation and the development of an activity report, which can be used for the justification and promotion of future CPD curriculum activities. Evaluation and activity report will be finalized, the latest, one month after the event has finished.
Sub-Task start month: March/April 2020 (M26-M27)
Sub-Task end month: April 2020 (M28)
Sub-Task Leader: Dorothee Roβkamp
Deputy Sub-Task Leader:
Sub-Task Participants: Aura Timen, Corien Swaan, Kitty Maassen, Laura van Vossen, Jeanette de Boer

Deliverables		
Ref	Title	Due month
D-CPD2019-1.1.1	Overview of educational curriculum (including topics of the lectures/ case studies to be addressed), speaker proposed and time schedule for the 2-day module	M22
D-CPD2019-1.1.1	Request of accreditation	M22
D-CPD2019-1.1.1	Pre-booking of venue and arrangement of catering/social network dinner	M22
D-CPD2019-1.1.1	Development of a promotion campaign plan and advertising channels	M23
D-CPD2019-1.1.1	Development of a CPD module flyer/brochure and promotional text to be distributed via various channels (website, newsletter, email invitations)	M23
D-CPD2019-1.1.1	Development of an information package for participants and selection of promotional merchandise	M24
D-CPD2019-1.1.1	Development of course evaluation form	M24
D-CPD2019-1.1.2	Execution of the 2-day CPD module	M28
D-CPD2019-1.1.3	Analysis of the course evaluation	M28
D-CPD2019-1.1.3	Development of an activity report	M28
Milestones		
Ref	Title	Due month
MS-CPD2019-1	Official announcement/invitations to the CPD module	M24
MS-CPD2019-2	Execution of the CPD module	M27-M28
MS-CPD2019-3	Provision of the activity report	M28

2.4.2.1.56 Short Term Missions 2020

STM Title	**Short Term Missions 2020**									
STM Acronym	**STM 2020**									
STM Beneficiaries	**P1-ANSES** **P32-FHI** **P27-ISS** **P41-SVA**			Deputy STM Beneficiaries			**N/A**			
STM Coordinator	**N/A**			Deputy STM Coordinator			**N/A**			
T&E Start month	**M25**			Project End month			**M36**			
Participant number	**1**	2	4	5	6	7	8	9	10	11
Acronym of participant	**Anses**	Ages	Sciensano	NCIPD	NDRVMI	SZU	VRI	BfR	FLI	RKI
PersonXmonths per participant:	**0.2**									
Participant number	12	13	14	15	16	17	18	19	20	21
Acronym of participant	DTU	SSI	UT	VFL	INIA	UCM	MVNA	INRA	IP	APHA
PersonXmonths per participant:										
Participant number	22	23	24	25	26	**27**	28	29	30	31
Acronym of participant	PHE	UoS	OKI	NUIG	TEAGASC	**ISS**	IZSAM	IZSLER	RIVM	WbvR
PersonXmonths per participant:										
Participant number	**32**	33	34	35	36	37	38	39	40	**41**
Acronym of participant	**FHI**	NVI	PIWET	INIAV	INSA	IISPV	IC	SLV	FoHM	**SVA**
PersonXmonths per participant	**0.6**									**1**

Objectives

- To complete the six short term missions described below to share sceitific expertise, knowledge, facilities and methodologies to harmonise approaches in the area of Foodborne Zoonoses (FBZ), Antimicrobial Resistance (AMR), and Emerging Threats (ET). Reports for each of these short term missions will be provided, and summaries and testimonials from each of these missions will be made available on the One Health EJP website.

Description of work

Task: T1.1 STM 2020_1: Genetic variability of tick-borne viruses and identification of new antiviral targets
Task start month M28

Task end month M30
Task Leader: Migné Camille, ANSES (Fr)
Deputy Task Leader:
Task Participants: Migné Camille
Description of the task:

When the mission will start in April 2020, samples from the *in vitro* and *in vivo* experiments (serial and alternating passages of viruses: cell culture, ticks, organs tick, blood samples) will be available. This will allow Camille to start immediately her studies including sample treatments and sequencing:

1. RNA extractions from each sample using a guanidinium isothiocyanate extraction procedure (as described by Attoui *et al.,* 2000). dsRNA will be prepared by differential precipitation of ssRNA using 2M LiCl and recovery of the dsRNA from the supernatant by precipitation with isopropanol. (Month 1)
2. Sample preparation for sequencing: (Month 1 and 2)
    a. Full genome sequencing using standard single-primer amplification techniques (full-length amplification of cDNAs: FLAC). For that purpose, dsRNA genome segments will be individually purified using agarose gel electrophoresis techniques, followed by gel extraction. Genome segments will be reverse-transcribed into cDNA using FLAC (as described by Maan *et al.,* 2007), 2/ resulting cDNAs will be further PCR amplified.
    b. Denovo sequencing of the full length genome using NGS will also require methods of library preparations to be learnt.
    c. Combination of FLAC and NGS have been developed in the lab of Prof mertens and will be extremely useful for the processing and analyses of isolates generated during the experimental passages of the viruses.
3. Sequencing will be performed in platforms using both the standard Sanger chemistry as well as NGS chemistries. (Month 2-3)
4. Sequence data analyses for each sample: 1/ assembly into “contig” using the SepManII sequence analysis package (Lasergene version 5), 2/ Multiple alignments of consensus sequences will be performed using Clustal X (Version 2.0), Clustal Omega and MAFFT, to ensure proper alignment. (Month 3)

To summarize, all the in vitro RNA samples (infected cell lines) will be prepared in advance in the home laboratory (ANSES) before the short term scientific mission of Camille Migné. Nevertheless sample preparation of infected ticks and blood collected from infected mice will be prepared in ANSES but RNA extractions from this small volumes will be manage into the host Institute (University of Nottingham) where Camille will learn this specific technic of RNA extraction. Then sample preparation for Next generation sequencing on all those samples (in vitro and in vivo experiments) will be manage in the University of Nottingham and data analysis through specific bioinformatics pipelines will be run also in the University of Nottingham. All the in vitro samples and a first part of in vivo samples will be prepared into Nottingham and Camille Migné will go back with all the protocols she will learn, to be able to manage in ANSES the same experiments (RNA extractions on small samples, sample preparation for next generation sequencing, data analysis). Of course collaboration will continue between both partners after the short term scientific mission through the thesis of Camille Migné and through other collaborative projects managed by Camille Migné PhD Supervisors (Dr Sara Moutailler and Dr Houssam Attoui) as the PALEBLUE project (in progress), CovetLab and EJP new calls.

Task: T1.2 STM 2020_2: Study of the interactions between STEC and human gut microbiota in the ARtificial COLon (ARCOL) model
Task start month M25
Task end month M27
Task Leader: Paola Chiani, ISS (It)
Deputy Task Leader:
Task Participants: Paola Chiani, ISS (It)
Description of the task:

The project described in this application is based on the realization of two important experiments to be carried out at the Universitè Clermont Auvergne. Each experiment will involve one age condition described above (infant or adult) and two STEC strains selected among the ISS collection, including:

1. An O26 Shiga toxin-producing *E. coli* strain, which represents one of the most prevalent serogroup in the human infections occurring in Italy. In particular, we decided to consider for the study a STEC 026:H11 strain harbouring the *stx2a* gene subtype, which caused an outbreak in 2015 in a nursery in the province of Rome. Indeed we already conducted a preliminary study where stool samples from the outbreak were undergone to a metagenomic analysis, with the aim to investigate the taxonomic composition of the gut microbiota, either in presence and in absence of STEC infection.
2. A STEC strain that does not possess the locus of enterocyte effacement (LEE), in order to evaluate the capability of a LEE-negative STEC strain to efficiently colonize the intestinal tract, thus investigating possible changes occurring in the gut microbe population. Notably such strains should harbour a phage carrying the *stx2a* gene subtype, matching those harboured by the 026:H11 STEC strains cited above.

The two STEC strains will be sent to the Universitè Clermont Auvergne for the experimental work as stab agar cultures. Each experiment mentioned above, will take at least one month, comprising the following steps:

- One week is required for the preparation of the ARCOL model. ARCOL is a one-stage fermentation system (Applikon, Schiedam, The Netherlands) that integrates the main parameters of the *in vivo* human colonic environment, including pH, temperature, supply of ileal effluents, retention time and anaerobiosis maintained by the sole activity of the resident microbiota. The model will be set-up to simulate the intestinal conditions of subjects belonging to the considered age groups (infant or adult), with an external compartment simulating the colonic mucosal phase.
- Faeces from healthy volunteers (infant or adult) who had no history of antibiotic or probiotic treatment 1 month before the beginning of the experiment will be prepared under anaerobic conditions and inoculated into the bioreactors. Samples will be collected prior to inoculation (directly from the faeces from infant and adult) and immediately after inoculation into the bioreactors.
- Following a one-week stabilisation phase, the two strains (STEC O26 and STEC LEEnegative) will be administered into two bioreactors. Samples from each bioreactor will be collected daily, up to 15 days from the *E. coli* inoculum in the luminal phase for SCFA analyses. Gas from the atmospheric phase will be also analysed every day.
- Samples will be collected from the luminal and mucosal phases (from mucin beads) before STEC inoculation and after 15 days post-inoculation of the STEC strains in the model and total DNA will be extracted.

The DNA will be also extracted from fresh faeces, before the introduction in ARCOL, and from the fermentation medium collected at the end of the stabilisation phase, before the *E. coli* inoculum.
The experiments listed in this proposal will require a period of two months (excluding analysis) to be spent at the Université Clermont Auvergne (Clermont-Ferrand, France), in order to be trained on the ARCOL system and to carry out the experimental work presented above.
The metagenomic sequencing and the related analysis will be performed at ISS, after the completion of Paola's STM.

Task: T1.3 STM 2020_3: Skills Development Mission in Bioinformatics
Task start month M27
Task end month M28
Task Leader: Dinah Deborah Seligsohn, SVA (SE)
Deputy Task Leader:
Task Participants: Dinah Deborah Seligsohn, SVA (SE)
Description of the task:

Activities for expertise development
The planned educational activities involve a combination of lecture and hands-on training on basic bacterial genomics and specifically genomic analysis of generated sequence data from previously collected samples (GBS isolates from camel milk samples) under supervision of trained experts.
The topics covered will include:
• Background on comparative genomics:
- molecular typing methods vs whole genome sequencing

• Bacterial diversity:
- mutation vs recombination rates
- core genome, accessory genome and pangenome

• Introduction to whole genome sequencing: o from sample to sequence (DNA extraction, library preparation, PCR)
- different sequencing technologies available
- possibility to participate in an Oxford Nanopore MinION sequencing of GBS strains

• Introduction to bioinformatics: o working with the Unix command line
- introduction to the Terminal

• Analysing sequenced data: o raw reads polishing (ConDeTri)
- methods for genome assembly - read mapping vs *de novo* assembly (*de novo* assembly with SPAdes for Illumina data and with Miniasm for Oxford Nanopore data)
- quality control of genome assemblies (QUAST)
- dealing with recombination (Gubbins)
- genome annotation (Prokka)

• Introduction to phylogenetics: o multiple core genome alignment (Parsnp, Snippy, PRANK)
- substitution models
- tree estimation (RAxML-NG)
- tree visualisation and annotation (iTol, FigTree, SplitsTree)

During this skills development mission, specific analysis outcomes will be:
1. Determine the sequence type (ST) of the sequenced isolates.
2. Evaluate the presence of genes related to antibiotic resistance (AMR).

3. Assess the variability in gene content among isolates (i.e. core vs. accessory genes).
4. Explore the diversity of GBS within and between camel herds in Kenya.

Activities to stimulate multilateral collaborations
As a part of the visit, the applicant will participate in fortnightly science group meetings at the One Health Research into Bacterial infectious Diseases (OHRBID) lab, at the Institute of Biodiversity, Animal Health and Comparative Medicine. This provides a possibility to connect with the rest of the research group. As part of these meetings, the applicant will give an oral presentation of her research project to colleagues at the hosting institute to introduce herself to other researchers, receive input and promote scientific discussion.
The applicant will also be partaking in meetings with the shared interest group of Disease Ecology at the host institute to broaden her knowledge and develop new research links, and wherein further opportunities will be available to receive input on ongoing research. The applicant will attend weekly seminars at the Institute of Biodiversity, Animal Health and Comparative Medicine (visiting lecturers) and weekly seminars given by PhD students at the hosting institute. This will give her exposure to a broad range of research in ecology and veterinary medicine, both within the Institute and with external academic institutions.
The hosting institute will arrange social activities outside the curriculum for the applicant to join.

Task: T1.4 STM 2020_4&5: Knowledge exchange and collaboration for improving Syndromic Surveillance of One Health concern
Task start month M27
Task end month M27
Task Leaders: Gry Marysol Grøneng, FHI (No); Gunnar Rø, FHI (No)
Deputy Task Leader:
Task Participants: Gry Marysol Grøneng, FHI (No); Gunnar Rø, FHI (No)
Description of the task:

Day 1- APHA
On day one, we are planning to visit APHA and exchange knowledge of the surveillance systems in Norway and UK with special emphasis on One Health. This will include the work both APHA and NIPH are doing in COHESIVE and the further planned work.
The day will especially include tasks in COHESIVE WP3 (Towards and EU zoonoses structure). The overall aim of the work package is to bring together existing risk analysis and epidemiological tools, skills and facilities that are required for an One-Health surveillance in Europe. The UK researchers are for instance undertaking a reverse mapping approach to determine the data and communication structures that exist for zoonotic pathogen signalling. Using a mixture of interviews and internal process documents to document the infrastructure surrounding the production of regular reports and alerts on potentially zoonotic pathogens. The mission will support specifically the following COHESIVE tasks:

Task: T-3.1 Explore current ways for exchanging signals between countries and cross disciplines – pathway analysis
Task: T-3.2 Select tools for Horizon scanning and signal detection
Task: 3.3 Retrospective systems analysis of detection of outbreaks.
Task: 3.4 Pilot One-Health Early Warning System

These are tasks that both NIPH and APHA are working on and where collaboration and knowledge exchange can significantly advance the work on the tasks.
Day 2- Travelling between the institutes.

Day 3 – PHE
On day three we are planning to visit PHE to learn about the public health surveillance systems in the UK and their infrastructure and use. PHE have several structures running on a regular basis including Remote Health Advice (calling NHS telephone number for medical advice), GP in hours advice (monitoring planned/appointment visits to doctors), ED visits (monitoring visits to emergency departments in hospitals) and GP out of hours (monitoring contacts to doctors out of hours, non-emergency). The visit will also include a presentation of the Norwegian Syndromic Surveillance System (NorSySS) and its infrastructure and use. The use in One Health surveillance and further possibilities will be of high importance.
Visiting PHE will be of great importance for the further work in both COHESIVE and NOVA. For COHESIVE this falls under the same work packages as tasks as for day 1, while for NOVA this mainly is related to Task 3.2 and 3.3 in WP 3 (Syndromic surveillance). With the objective of assessing whether syndromic surveillance in animal and food sectors could be a valuable add-on to the current or future surveillance of food borne diseases (FBD) in humans. The expected impacts are a better understanding of the interplay between animal and human health and an improvement of the prediction of human FBD outbreaks.
Task 3.2. Univariate syndromic surveillance development for FBD (and AMR)
Task 3.3. Evaluation of multivariate syndromic surveillance for FBD
Overall the activities proposed for this short-term mission will support the development of the syndromic surveillance system in Norway, work on WP3 in both NOVA and COHESIVE and support knowledge transfer and learning across countries.
The costs will include airplane tickets from Norway to London, train transportation from airport to Weybridge (APHA), from Weybridge to Birmingham (PHE) and from Birmingham to the airport. In addition there is a need for two nights accommodation for this short term mission. The included budget is for 2 persons (Gunnar Rø and Gry M Grøneng).

Task: T1.4 STM 2020_6: Cross-domain and cross-country collaboration to develop multivariate syndromic surveillance

Task start month M26
Task end month M26
Task Leaders: Wiktor Gustafsson, SVA (SE)
Deputy Task Leader:
Task Participants: Wiktor Gustafsson, SVA (SE)
Description of the task:
Joint monitoring of disease signals across animal and public health is not just a problem of being able to join datasets from these two domains. It also incurs significant statistical challenges for implementation of detection algorithms that can account for, and extract evidence of, multiple data streams. Both institutes have devoted significant resources to solving this issue, and this mission will be an opportunity to share their expertise, identify best solutions, and jointly proposed a methodology that can be adopted by other countries to advance early disease detection and situational awareness of food-borne diseases.
This STM will be an opportunity to share experiences so far, and establish a person connection to continue the collaboration remotely, until the end of the NOVA project.

<u>Day 1-2: Getting familiar with each other's current data sources and univariate monitoring codes</u>
Both institutes developed codes in the statistical programming language R, and make their work publicly available on GitHub. Data analysis can, therefore, readily exchange codes. However, understanding someone else's codes without a personal "walk through" can be very hard, especially without having access to their data sources. These 2 days will be an opportunity for data analysis to explain to each other their work so far.

Day 3: Reviewing candidate data sources for multivariate surveillance and potential indicators to be combined.
As part of the NOVA project, we have discussed data sources, but are not able to share directly. With a visit in person, we can see each other's data structure without directly sharing data. More importantly, data analysts will be able to include in the discussion those producing and/or generating the data, to get their insights-

Day 4:Visit to the Norwegian Veterinary Institute, who is also a collaborator
This visit will extend the discussions from the previous day to one more institution involved in the OHEJP consortium, and advance the OH aspects of the collaboration.

Day 5-6: Sharing current ideas and preliminary methods developed for implementation of multivariate syndromic surveillance
Both institutes have already started thinking about this problematic, but multivariate syndromic surveillance is an open area of research. By sharing their current experience, both in successes and failures, it will be possible to make a plan for which analyses algorithms to test on the next step.

Day 7-9: "hackathon" – testing some of the pre-formulated codes for multivariate syndromic surveillance with each other's data
These will be 3 active days of attempting to implement and run some analyses codes. Attempting to use each others' analyses algorithms on their own and the collaborators' data, analysts will be able to advance towards developing solutions that are robust, and can be further developed for use within their institutions and eventually other countries.

Day 10: Gathering conclusions, needs for further development, and making a plan to continue the collaboration remotely.
Wrap up and exchanging tasks to continue the work.
Analysts will use their current computers, and NIPH will make available a desk for the visiting analyst from SVA. All analyses will be performed using free software.
This mission, therefore, incurs in no extra costs for the institutions, except for the direct traveling costs, and the indirect costs of travel management within the home institute.

Deliverables		
Ref	Title	Due month
D-STM-1.1	STM 2020_1 Mission	M30
D-STM-1.2	STM 2020_2_Mission	M27
D-STM-1.3	STM 2020_3_ Mission	M28
D-STM-1.4	STM 2020_4&5_ Mission	M27
D-STM-1.6	STM 2020_6_ Mission	M26
Milestones		
Ref	Title	Due month
MS-STM-1	STM 2020_1_Report and Testimonial	M31
MS-STM-2	STM 2020_2_Report and Testimonial	M28
MS-STM-3	STM 2020_3_Report and Testimonial	M29
MS-STM-4&5	STM 2020_4&5_Report and Testimonials	M28
MS-STM-6	STM 2020_6_Report and Testimonial	M27

2.4.2.2 List of programmed activities

Activity	Lead Beneficiary N°	Acronym lead beneficiary	PM	Start Month	End Month
WP1-Coordination and Management	1	Anses	97,60	25	36
WP2-Strategic Research Agenda	30	RIVM	4,50	25	36
WP3-Management of Joint Research Projects	4	Sciensano	15,00	25	36
WP4-Management of Joint Integrative Projects	41	SVA	15,57	25	36
WP5-Science to policy translation	9	BfR	25,33	25	36
WP6-Training and Education	23	UoS	15,50	25	36
WP7-Sustainability	27	ISS	12,00	25	36
JRP01-R1-AMR1-IMPART	31	WbvR	17,82	25	30
JRP02-R1-AMR2-ARDIG	21	APHA	112,44	25	36
JRP03-R1-AMR3-RADAR	30	RIVM	60,26	25	30
JRP12-R2-AMRSH5-FARMED	31	WbvR	79,48	25	36
JRP13-R2-AMRSH5-WORLDCOM	25	NUIG	117,65	25	36
JRP14-R2-AMR2.1-FULL-FORCE	04	Sciensano	119,02	25	36
JRP15-R2-AMR2.1-FED-AMR	02	Ages	159,31	25	36
JRP05-R1-ET1-TOXdetect	01	Anses	82,56	25	36
JRP16-R2-ET2.2-TELE-Vir	13	SSI	99,94	25	36
JRP17-R2-ET2.2-IDEMBRU	01	Anses	119,18	25	36
JRP18-R2-ET1.1-MEmE	27	ISS	114,92	25	36
JRP19-R2-ET1.1-PARADISE	27	ISS	98,55	25	36
JRP06-R1-FBZ1-NOVA	41	SVA	168,88	25	36
JRP07-R1-FBZ2-LISTADAPT	01	Anses	55,52	25	30
JRP08-R1-FBZ2-METASTAVA	04	Sciensano	31,27	25	30

Activity	Lead Beneficiary N°	Acronym lead beneficiary	PM	Start Month	End Month
JRP09-R1-FBZ3-AIR-SAMPLE	12	DTU	31,01	25	30
JRP10-R1-FBZ3-MoMIR-PPC	19	INRA	98,98	25	36
JRP11-R1-FBZ4-MedVetKlebs	20	IP	63,84	25	36
JRP20-R2-FBZSH3-DISCoVeR	12	DTU	173,22	25	36
JRP21-R2-FBZ3.1-BIOPIGEE	09	BfR	168,84	25	36
JRP22-R2-FBZ4.1-TOXOSOURCES	13	SSI	120,98	25	36
JRP23-R2-FBZSH5-ADONIS	30	RIVM	149,20	25	36
JRP24-R2-FBZSH9-BeONE	13	SSI	89,66	25	36
JIP01-R1-IA1.1-ORION	09	BfR	202,27	25	36
JIP02-R1-IA1.2-COHESIVE	30	RIVM	143,93	25	36
JIP03-R2-IA2.3-CARE	12	DTU	190,47	25	36
JIP04-R2-IA2.2-OH-HARMONY CAP	13	SSI	126,35	25	36
JIP05-R2-IA2.1-MATRIX	13	SSI	184,57	25	36
PhD01-R1-AMR2-ECO-HEN	17	UCM	14,00	25	36
PhD02-R1-AMR2/3/6-LIN-RES	04	Sciensano	12,00	25	36
PhD03-R2-AMR2.1-HME-AMR	26	TEAGASC	12,00	25	36
PhD04-R2-AMR2.1-KENTUCKY	04	Sciensano	12,00	25	36
PhD05-R2-AMR2/6.1/ET5-METAPRO	17	UCM	12,00	25	36
PhD06-R1-ET5-PEMbo	01	Anses	12,00	25	36
PhD07-R2-ET2.1-MACE	23	UoS	1,00	25	36
PhD08-R2-ET5.1/4.1/FBZSH3-DESIRE	30	RIVM	9,00	25	36
PhD09-R2-FBZSH3/AMR2.1-UDOFRIC	21	APHA	12,00	25	36
PhD10-R2-FBZSH3/AMR2.1-WILBR	21	APHA	14,90	25	36
PhD11-R1-FBZ4/5- EnvDis	23	UoS	0,00	25	36

Activity	Lead Beneficiary N°	Acronym lead beneficiary	PM	Start Month	End Month
PhD12-R2-FBZSH9-AptaTrich	01	Anses	9,50	25	36
PhD13-R2-FBZ8/AMR2-VIMOGUT-AMR	31	WbvR	0,00	25	36
PhD14-R2-FBZ4-ToxSauQMRA	01	Anses	8,36	25	36
PhD15-R2-FBZ5-TRACE	30	RIVM	4,87	25	36
PhD16-R2-FBZ2/AMR6.1-Codes4strains	20	IP	12,00	25	36
ASM Satellite Workshop 2020	30	RIVM	0,20	25	33
Summer School 2020	31	WbvR	1,80	34	34
Communication and Media workshop	06	NDRVMI	2,00	25	34
CPD module 2019	30	RIVM	2,19	25	28
Short Term Missions 2020	ANSES (01) – ISS (27) - FHI (32) - SVA (41)		1,80	25	36

2.4.2.3 Annual Deliverables List

Deliverable N°	Deliverable name	WP N°	Acronym of lead beneficiary	Dissemination level	Est. Del. Date
D1.8	Annual report on the internal and external newsletter produced during the second year	1	Anses	PU	31 Jan 2020
D1.9	Complete version of annual report for stakeholders n°2	1	Anses	PU	31 May 2020
D1.10	Summary progress report year 3	1	Anses	PU	30 Sep 2020
D1.11	Annual work plan for the fourth year	1	Anses	PU	30 Sep 2020

Deliverable N°	Deliverable name	WP N°	Acronym of lead beneficiary	Dissemination level	Est. Del. Date
D1.24	Ethical review report for Y2	3	Sciensano	PU	29 Feb 2020
D3.11	2nd periodic report on ongoing JRP	3	Sciensano	CO	31 Jan 2020
D3.12	Abstract book for 2nd Annual Scientific Meeting (ASM)	3	Sciensano	PU	30 Apr 2020
D3.13	Final JRP report, after expert evaluation (#1)	3	Sciensano	PU	31 Jul 2020
D3.14	Report on the recently started projects, 2nd call	3	Sciensano	CO	31 Aug 2020
D4.16	Report from supportive start-up meeting, 2nd call	4	SVA	CO	31 Jan 2020
D4.17	2nd periodic report on ongoing JIPs	4	SVA	PU	31 Jan 2020
D4.18	Report from 5th cogwheel workshop	4	SVA	PU	30 Apr 2020
D4.19	Guidelines for evaluation of final reports	4	SVA	CO	31 Oct 2020
D4.20	Report from 6th cogwheel workshop	4	SVA	PU	31 Oct 2020
D4.21	Report from thematic meeting III	4	SVA	PU	31 Dec 2020
D5.8	Third annual report on dissemination activities to international stakeholders	5	BfR	PU	31 Dec 2020

Deliverable N°	Deliverable name	WP N°	Acronym of lead beneficiary	Dissemination level	Est. Del. Date
D6.2	Report on communications workshop and media training	6	Surrey	PU	31 Dec 2020
D6.6	Report n°1 of the annual short term missions completed also uploaded onto the EJP webpage.	6	Surrey	PU	29 Feb 2020
D6.7	Report n°2 on one workshop per year associated with ASM (with WP1,3,4 and 5)	6	Surrey	PU	31 Dec 2020
D6.8	Reports of the 10 short term missions per year completed also uploaded onto the EJP webpage	6	Surrey	PU	31 Dec 2020
D7.1	Report of the end user Stakeholders´ Needs and Expectations	7	ISS	PU	31 Dec 2020

2.5 Participation in Annual Work Plan activities

Beneficiaries concerned : P01-Anses, P04-Sciensano, P06-NDRVMI, P08-VRI, P09-BfR, P10-FLI, P11-RKI, P12-DTU, P13-SSI, P14-UTARTU, P15-VFL, P16-INIA, P17-UCM, P18-MVNA, P19-INRA, P20-IP, P22-PHE, P23-UoS, P24-OKI, P25-NUIG, P23-ISS, P27-IZSAM, P28-IZSLER, P32-FHI, P33-NVI, P34-PIWET, P35-INIAV, P36-INSA, P37-IISPV, P38-IC, P39-SLV, P40-FoHM	
Does the participant plan to subcontract certain tasks (please note that core tasks of the programme should not be subcontracted) (article 13 of MGA)	**N**
Does the participant envisage that part of its work is performed by linked third parties (article 14 of MGA)	**N**
Does the participant envisage the use of in-kind contribution provided by third parties (articles 11 and 12 of MGA)	**N**
Does the participant envisage the provision of financial support to third parties (article 15 of MGA)	**N**

2.5.1 P02-AGES

Does the participant plan to subcontract certain tasks (please note that core tasks of the programme should not be subcontracted) (article 13 of MGA)	**N**
Does the participant envisage that part of its work will be performed by linked third parties (article 14 of MGA)	**N**
Does the participant envisage the use of in-kind contributions provided by third parties (articles 11 and 12 of MGA)	**Y**
AGES will use of the in-kind contribution, provided by the following third party under Articles 11 and 12 of the GA: **Activity FBZ2.2 JRP-BIOPIGEE**: University of Veterinary Medicine Vienna (Vetmeduni Vienna - VMU) will provide personnel having specific skills to implement the following tasks: WP2 (1 PM): VMU will contribute to the development and application of the biosecurity protocol, where input from recent work completed will be given and data from the recent study will be used. WP4 (1 PM): VMU will provide the information to be collected within WP2 for the economic assessment, to be conducted in Y2 and Y3. WP5 (1 PM): VMU will contribute to the data integration from WP2 and 4 into the catalogue of biosecurity measures. This builds on previous work which has been done at Vetmeduni Vienna.	
Does the participant envisage the provision of financial support to third parties (article 15 of MGA)	**N**

2.5.2 P07-SZU

Does the participant plan to subcontract certain tasks (please note that core tasks of the programme should not be subcontracted) (article 13 of MGA)	**Y**
WP3, T3.4: 2020 Annual Scientific Meeting organisation: P07-SZU will organise and host the 2019-ASM. In order to ensure a very well organised meeting it will hire a Professional Conference Organiser (PCO). Justification! There is a considerable resource effort needed to organise a major professional congress (250 international delegates expected). At the host institute (SZU, P07) there are no in-house staff with the resource time available or the necessary experience in this type of event organisation, therefore the host (SZU) will hire a Professional Conference Organiser (PCO) to undertake the organising and administration of the congress. Their role will be to set up and manage the website, set up and manage registration, marketing and promotion, attract sponsorship, delegate queries, finances and budget, production of conference materials, running of event at the venue and the social programme. The process to recruit the PCO is fully in line with national procurement rules. The PCO fee structure will be set out in a contract with the host organisation.	
Does the participant envisage that part of its work will be performed by linked third parties (article 14 of MGA)	**N**
Does the participant envisage the use of in-kind contributions provided by third parties (articles 11 and 12 of MGA)	**N**
Does the participant envisage the provision of financial support to third parties (article 15 of MGA)	**N**

2.5.3 P21-APHA

Does the participant plan to subcontract certain tasks (please note that core tasks of the programme should not be subcontracted) (article 13 of MGA)	**N**
Does the participant envisage that part of its work will be performed by linked third parties (article 14 of MGA)	**N**
Does the participant envisage the use of in-kind contributions provided by third parties (articles 11 and 12 of MGA)	**Y**

To support the two PhDs co-supervising by APHA, the institute will use article 11 of the Model Grant Agreement.
APHA is not an academic institution and as such is not in position to hire PhD student. However, the institute was implicated in co-supervising several PhD students to completion.

- **PhD-R2-FBZSH3/AMR2.1-WILBR**

1. The PhD student will be based at APHA, but the student will be registered at University of Exeter
2. The PhD student will perform the required work as expected in the work plan described for the activity PhD-R2-FBZSH3/AMR2.1-WILBR in section 0

- **PhD-R2-FBZSH3/AMR2.1-UDOFRIC**

1. The PhD student will be based at APHA, but the student will be registered at University of Warwick.
2. The PhD student will perform the required work as expected in the work plan described for the activity PhD-R2-FBZSH3/AMR2.1-UDOFRIC in section 2.4.2.1.44

- For both, PhDs:

1. APHA will therefore have to administer the fee for the PhD studentship as well as the stipend (the salary) through the University of Exeter and the University of Warwick.
2. The exact amount of financial support is calculated according to the official rates used by the University of Exeter and the University of Warwick to hire PhD students.
3. The category of persons to receive the funds are students engaging in a PhD cycle.
4. Student selection will be undertaken by the supervisory team according to APHA and University of Exeter guidelines and eligibility, and to include the following criteria:
    a. Demonstrated intention to pursue a career in at least one area of zoonotic disease: prevent-detect-respond, improve integration and preparedness
    b. Demonstrated enthusiasm for a One Health approach
    c. Demonstrated experience or potential for international integration

Does the participant envisage the provision of financial support to third parties (article 15 of MGA)	**N**

2.5.4 P30-RIVM

Does the participant plan to subcontract certain tasks (please note that core tasks of the programme should not be subcontracted) (article 13 of MGA)	**N**
Does the participant envisage that part of its work will be performed by linked third parties (article 14 of MGA)	**N**
Does the participant envisage the use of in-kind contributions provided by third parties (articles 11 and 12 of MGA)	**Y**
RIVM and UMCU are active in One Health research together with other leading parties to forge an open innovation network named Netherlands Centre for One Health (NCOH). In this centre Dutch One Health knowledge is brought together with the goal to create added value by centralizing the specific knowledge of the partners (http://ncoh.nl/). As previously agreed between the REA and RIVM in course of 2018, RIVM envisages the use of the following in-kind contributions, provided by the following third parties under Articles 11 and 12 of the GA: • **Activity JRP03-Radar**: University Medical Centre Utrecht (UMCU) will provide personnel having specific knowledge of AMR. Involved in several WPs of the project, UMCU, provides particular support to the second WP of the project, dealing with transmission models, as WP deputy leader. Within this WP, the university will be particularly involved in the communication of results, and this work will form part of a PhD project within NCOH. • **Activity JRP11-MedVetKlebs**: University Medical Centre Utrecht (UMCU) will provide personnel having specific knowledge of Klebsiella. The university, through the NCOH network, will be particularly involved in WP3 – Genomics and Modeling – on activities related to the analyses and genomic sequences.	
Does the participant envisage the provision of financial support to third parties (article 15 of MGA)	**N**

2.5.5 P31-WbVR

Does the participant plan to subcontract certain tasks (please note that core tasks of the programme should not be subcontracted) (article 13 of MGA)	**N**
Does the participant envisage that part of its work will be performed by linked third parties (article 14 of MGA)	**N**
Does the participant envisage the use of in-kind contributions provided by third parties (articles 11 and 12 of MGA)	**Y**

WbvR, UU, EMC and WU are active in One Health research together with other leading parties to forge an open innovation network named Netherlands Centre for One Health (NCOH). In this centre Dutch One Health knowledge is brought together with the goal to create added value by centralizing the specific knowledge of the partners (http://ncoh.nl/).
As previously agreed between the REA and WbvR in course of 2018, WbvR envisages the use of the following in-kind contributions, provided by the following third parties under Articles 11 and 12 of the GA:

- **Activity JRP01-IMPART**: University Utrecht (UU), Faculty of Veterinary Medicine, will provide strains resulted from sample material from its diagnostic microbiology laboratory for small animal, research results obtained with these strains and small animal microbiology expert knowledge. These activities are centred around the diagnostic apparatus of the veterinary microbiology diagnostic centre of the faculty of Veterinary Medicine, Utrecht. Therefore the in-kind contributions will be executed at the third party's premises

- **Activity JRP08-METASTAVA**: Erasmus Medical Centre (EMC) will provide personnel having specific expertise in metagenome analysis. These analyses will be carried out in EMC's equipped with adequate materiel and qualified personnel for the performance of the tasks related to the project.

- **Activity JRP10-MoMIR-PPC**: Wageningen University (WU) will provide personnel that is able to do the WU part of the research. The WU, which is particularly involved in WP3 of the project, carrying out the modelling activities, will provide the project with human resources as well as material resources.

- **Activity JRP15-FULL FORCE**: University Utrecht (UU), will contribute to the project with the provision of human resources. The scientists involved in the project are specialised in infectious diseases and animal health.

- **Activity JRP20- DISCoVeR**: University Utrecht (UU), will provide personnel having specific expertise, with a focus on analysis of bacterial (meta-) genomes for comparative genomics, molecular epidemiology and linking bacterial genotype to phenotype.

Does the participant envisage the provision of financial support to third parties (article 15 of MGA)	**N**

2.5.6 P41-SVA

Does the participant plan to subcontract certain tasks (please note that core tasks of the programme should not be subcontracted) (article 13 of MGA)	**N**
Does the participant envisage that part of its work will be performed by linked third parties (article 14 of MGA)	**N**
Does the participant envisage the use of in-kind contributions provided by third parties (articles 11 and 12 of MGA)	**Y**
SVA will use article 11 of the Model Grant Agreement to carry out the T7.4: Making the bridges between EJP's beneficiaries and stakeholders sustainable. The task of drafting of a detailed plan for an EU-wide analysis of current work processes, shared infrastructures, reference documents and guidelines across the multifacted OH interface (environmental health-animal health-public health) will be conducted partly in the form of a PhD project in collaboration with Roskilde University (DK), Department of Social Sciences and Business. The contribution from Roskilde University will be to cover a part of PhD salary and tuition fees. The PhD student will be based at Roskilde University.	
Does the participant envisage the provision of financial support to third parties (article 15 of MGA)	**N**

2.6 Resources to be committed

2.6.1 Summary effort table

2.6.1.1 Overarching activities

Étiquettes de lignes	WP1-Coordination	WP2-SRA	WP3-JRPs management	WP4-JIPs management	WP5-Science to Policy translation	WP6-T&E management	WP7-Sustainability	Total général
P01-Anses	47,50							47,50
P02-Ages	0,50							0,50
P04-Sciensano	6,50		14,00	1,25				21,75
P06-NDRVMI	0,50							0,50
P07-SZU	0,50							0,50
P08-VRI	0,50							0,50
P09-BfR	0,50				13,33			13,83
P10-FLI	0,50							0,50
P11-RKI	0,50							0,50
P12-DTU	0,50							0,50
P13-SSI	0,50		1,00		12,00			13,50
P14-UT	0,50							0,50
P15-VFL	0,50							0,50
P16-INIA	0,50							0,50
P17-UCM	0,50	2,25						2,75
P18-MVNA								
P19-INRA	0,50							0,50
P20-IP	0,50						1,50	2,00
P21-APHA	0,50							0,50
P22-PHE	0,50							0,50
P23-UoS	26,60					14,00		40,60
P24-OKI	0,50							0,50
P25-NUIG	0,50							0,50
P26-TEAGASC	0,50							0,50
P27-ISS	0,50						4,50	5,00
P28-IZS AM	0,50							0,50
P29-IZS LER	0,50							0,50
P30-RIVM	0,50	2,25						2,75
P31-WbvR	0,50					1,50		2,00
P32-FHI	0,50							0,50
P33-NVI	0,50			1,00				1,50
P34-PIWET	0,50							0,50
P35-INIAV	0,50							0,50
P36-INSA	0,50			3,90				4,40
P37-IISPV	0,50							0,50
P38-IC								
P39-SLV	0,50							0,50
P40-FoHM	0,50							0,50
P41-SVA	0,50			9,42			6,00	15,92
Total général	**97,60**	**4,50**	**15,00**	**15,57**	**25,33**	**15,50**	**12,00**	**185,50**

2.6.1.2 Training and education activities

Étiquettes de lignes	CaM-2020	CPD-2019	CPD-2020	PhD10-R2-FBZSH3/AMR2.1-WILBR	PhD11-R1-FBZ4/5-EnvDis	PhD12-R2-FBZSH9-AptaTrich	PhD13-R2-FBZ8/AMR2-VIMOGUT-AMR	PhD14-R2-FBZ4-ToxSauQMRA	PhD15-R2-FBZ5-TRACE	PhD16-R2-FBZ2/AMR6.1-Codes4strains	PhD1-R1-AMR2-ECO-HEN	PhD2-R1-AMR2/3/6-PhDLIN-RES	PhD3-R2-AMR2.1-HME-AMR	PhD4-R2-AMR2.1-KENTUCKY	PhD5-R2-AMR2/6.1/ET5-METAPRO	PhD6-R1-ET5-PEMbo	PhD7-R2-ET2.1-MACE	PhD8-R2-ET5.1/4.1/FBZSH3-DESIRE	PhD9-R2-FBZSH3/AMR2.1-UDOFRIC	SS-2020	STM-2020	WS-2020	Total général
P01-Anses						9,50		8,36								12,00					0,20		30,06
P04-Sciensano												12,00		12,00									24,00
P06-NDRVMI	2,00																						2,00
P17-UCM											14,00				12,00								26,00
P20-IP										12,00													12,00
P21-APHA				14,90															12,00				26,90
P23-UoS																	1,00						1,00
P26-TEAGASC													12,00										12,00
P27-ISS																					0,00		0,00
P30-RIVM		2,19							1,58									9,00		1,80		0,20	14,77
P31-WbvR									3,29														3,29
P32-FHI																					0,60		0,60
P41-SVA																					1,00		1,00
Total général	**2,00**	**2,19**		**14,90**		**9,50**		**8,36**	**4,87**	**12,00**	**14,00**	**12,00**	**12,00**	**12,00**	**12,00**	**12,00**	**1,00**	**9,00**	**12,00**	**1,80**	**1,80**	**0,20**	**153,62**

2.6.1.3 Joint Research Projects/ Joint Integrative Projects

Étiquettes de lignes	JIP1-R1-IA1.1-ORION	JIP2-R1-IA1.2-COHESIVE	JIP3-R2-IA2.3-CARE	JIP4-R2-IA2.2-OH-HARMONY CAP	JIP5-R2-IA2.1-MATRIX	JRP10-R1-FBZ3-MoMIR-PPC	JRP11-R1-FBZ4-MedVetKlebs	JRP12-R2-AMRSH5-FARMED	JRP13-R2-AMRSH5-WORLDCOM	JRP14-R2-AMR2.1-FULL-FORCE	JRP15-R2-AMR2.1-FED-AMR	JRP16-R2-ET2.2-TELE-Vir	JRP17-R2-ET2.2-IDEMBRU	JRP18-R2-ET1.1-MEmE	JRP19-R2-ET1.1-PARADISE	JRP1-R1-AMR1-IMPART	JRP20-R2-FBZSH3-DISCoVeR	JRP21-R2-FBZ3.1-BIOPIGEE	JRP22-R2-FBZ4.1-TOXOSOURCES	JRP23-R2-FBZSH5-ADONIS	JRP24-R2-FBZSH9-BeONE	JRP2-R1-AMR2-ARDIG	JRP3-R1-AMR3-RADAR	JRP5-R1-ET1-TOXdetect	JRP6-R1-FBZ1-NOVA	JRP7-R1-FBZ2-LISTADAPT	JRP8-R1-FBZ2-METASTAVA	JRP9-R1-FBZ3-AIR-SAMPLE	Total général
P01.5-ANSP																													
P01-Anses			23,50	6,20	3,15	2,50	4,86			5,00		2,00	21,15	22,00	6,50	3,83	12,50	15,00	13,50	21,70		2,71	11,50	32,76	13,37	29,75	5,99		259,47
P02-Ages		7,67					2,25				11,44							11,00		4,50						3,83			40,69
P04-Sciensano	8,00	2,70						9,80		12,19		7,00								7,04				5,50	16,90		4,09		73,22
P06-NDRVMI						12,43							19,00					27,00											58,43
P07-SZU											14,10								3,70										17,80
P08-VRI						13,00						0,00			4,00		16,00	11,00	13,50							4,00		2,60	64,10
P09-BfR	39,00	22,10		6,00	19,85			6,00		6,50	1,75		10,00		0,30	3,60	11,80	24,00	1,00		11,00	8,00	15,00	10,00	7,00				202,90
P10-FLI	21,00	1,50			8,40				6,80		8,40		8,40	9,00					9,00		0,60				0,00		6,80		79,90
P11-RKI															14,00			13,00	6,10		0,60	27,00			36,00				96,70
P12-DTU	16,00	1,25	17,50		2,50		0,55	5,00		6,92						0,00	14,10		1,10		4,00		2,00		16,00			20,61	107,53
P13-SSI	23,67		19,00	29,50	12,00		6,17	6,00		10,50	8,00	29,00		9,90	9,05	0,00	7,40		12,10	15,00	26,00				15,00				238,29
P14-UT									16,00		16,00			15,50															47,50
P15-VFL														5,40				7,90											13,30
P16-INIA					3,00					4,00		3,10													7,00				17,10
P17-UCM			13,00		8,00	7,50		20,00	23,30								4,00		9,10	16,00		28,00			8,00				136,90
P18-MVNA																													
P19-INRA			19,60			9,45	5,05			9,10														22,00		9,81			75,01
P20-IP			3,00				19,06				1,25									14,40		4,00		9,30					51,01
P21-APHA	7,34	6,86	1,62	15,30	3,39			11,81		6,22			9,97			1,87	8,57	9,55		7,92	0,20	14,21	3,10		6,65				114,58
P22-PHE	6,80	1,08														1,05	1,25			1,25	1,00	9,00			5,30				26,73
P23-UoS					8,40	3,00			12,80		12,96	9,70			5,65				3,75			6,04							62,30
P24-OKI															2,00														2,00
P25-NUIG							1,59		31,35		18,00																		50,94
P26-TEAGASC				2,40													2,60												5,00
P27-ISS			6,00	13,50	4,50	7,75		2,60		8,50				11,60	25,60		12,00	6,00	15,30	4,20			3,75						121,30
P28-IZS AM		49,00	10,00		27,00		12,31	13,00				7,50	12,25					9,00			4,50				2,50	11,56		4,00	162,62
P29-IZS LER		9,00	19,00			20,50						1,00																	49,50
P30-RIVM	9,33	14,00	6,50	3,80	8,50		12,00			3,69				2,46	3,34	1,85	7,73	0,62	9,39	10,08	10,67		19,42		3,20				126,58
P31-WbvR	3,63	3,06	4,40		3,00	14,45		5,27		11,47			1,11			2,75	4,96	7,52	1,00	5,50		3,48	1,14				12,39		85,13
P32-FHI	10,50	8,36		5,07	10,94	8,40											10,28		3,10		0,58				11,34				68,57
P33-NVI	12,00	4,05		2,00	3,89					3,00	2,81	1,33		7,00	0,30	3,30	3,85	6,50	1,94		1,50	10,00	4,35	3,00	1,62	8,13		0,70	81,27
P34-PIWET			36,00	4,83	9,65					8,21	9,60	7,00		11,70	3,90	1,90	8,00	12,25	6,50	9,50	6,00							3,10	138,14
P35-INIAV				5,45									25,30	9,16	2,51		8,80		7,30	10,45									68,97
P36-INSA				14,00	17,40				27,40	13,47	55,00	26,31	12,00	2,70			23,13		1,50	21,66	23,01								237,58
P37-IISPV																													0,00
P39-SLV		3,00	2,90	0,80											0,40				1,00							0,22			8,32
P40-FoHM	10,00	1,60	3,50	3,50	10,00					2,40					1,00										2,50				34,50
P41-SVA	35,00	8,70	4,95	14,00	21,00					7,85		6,00		8,50	20,00	4,42	16,25	8,50	1,10						16,50		2,00		174,77
Total général	**202,27**	**143,93**	**190,47**	**126,35**	**184,57**	**98,98**	**63,84**	**79,48**	**117,65**	**119,02**	**159,31**	**99,94**	**119,18**	**114,92**	**98,55**	**24,57**	**173,22**	**168,84**	**120,98**	**149,20**	**89,66**	**112,44**	**60,26**	**82,56**	**168,88**	**67,30**	**31,27**	**31,01**	**3 198,65**

2.6.2 Other major cost items (equipment, goods and services, travel & subsistence, meetings organisation)

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
P01.5-ANSP	**0,00**		**0,00**		**1 200,00**		**0,00**	
WP3	**0,00**		**0,00**		**1 200,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
P01-Anses	**10 200,00**		**282 957,00**		**99 969,00**		**27 129,00**	
WP1	**0,00**		**0,00**		**32 650,00**		**923,00**	
WP1-Coordination and Management	0,00		0,00		32 650,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 CT/REA meeting 1 in Brussels (BE) CT/REA meeting 2 in Brussels (BE) CT/REA meeting 3 in Brussels (BE) CT/REA meeting 4 in Brussels (BE) Various (SC, ESAB, etc.) ESAB members (EU) invitation to ASM 2020 ESAB members (US) invitation to ASM 2020 CCP meeting PMC meeting 2-2020 at ISS (IT) M29 for director	923,00	Financial meeting 3-2020 at Anses (FR) M18
WP3	**10 200,00**		**275 457,00**		**46 749,00**		**22 818,00**	
ASM T&S-2020	0,00		0,00		3 000,00	5 ASM 2020 FOC delegates	0,00	
JRP01-R1-AMR1-IMPART	0,00		17 500,00	Consumables to perform laboratory work and software APC	1 500,00	Project meeting	0,00	
JRP02-R1-AMR2-ARDIG	0,00		29 500,00	Lab consumable reagents, sequencing APC	3 000,00	Regular meetings with partners in Europe	0,00	
JRP03-R1-AMR3-RADAR	0,00		16 900,00	Complementary sequencing costs APC	0,00		0,00	
JRP05-R1-ET1-TOXDETECT	0,00		10 000,00	Reagents and consumables for interlab tests, shipment of samples to participants (WP4) APC	6 000,00	Consoritum meetings	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP06-R1-FBZ1-NOVA	0,00		4 300,00	APC	3 300,00	Consoritum meetings	0,00	
JRP07-R1-FBZ2-LISTADAPT	0,00		31 200,00	Consumables for whole genome sequencing , microbiology (pipets, cryovials, Flasks, storage media) shipment parcels Consumables for whole genome sequencing , microbiology (pipets, cryovials, Flasks, storage media) shipment parcels Consumables for whole genome sequencing , microbiology (pipets, cryovials, Flasks, storage media) shipment parcels APC	6 125,00	Project meetings	0,00	
JRP08-R1-FBZ2-METASTAVA	0,00		0,00		0,00		0,00	
JRP10-R1-FBZ3-MOMIRPPC	0,00		4 500,00	Bacterial, serological and molecular analysis ACP	1 800,00	Final meeting	0,00	
JRP11-R1-FBZ4-MedVetKlebs	0,00		10 307,00	Methods, sampling, strain characterization APC	6 400,00	Consoritum meetings	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		15 200,00	Lab reagents for SMRT sequencing (incl. Proficiency test)	1 680,00	Workshop SMRT sequencing	0,00	
JRP17-R2-ET2.2-IDEMBRU	5 700,00	Data server Microscop	22 400,00	Transport of DNA (UN 2814) Consumables for phenotyping Consumables for data management Consumables for coordination Cloud rental	0,00		3 268,00	Organization kick-off meeting
JRP16-R2-ET2.2-TELE-Vir	0,00		0,00		974,00	Kick-off meeting	0,00	
JRP18-R2-ET1.1-MEmE	4 500,00	Animal specific cage (fox Em) Equipement magnetic	26 700,00	Foxes (n=6) Reagents for molecular studies International courrier for the shipment of samples	700,00	Project meeting	19 550,00	Kick-off meeting organisation
JRP19-R2-ET1.1-PARADISE	0,00		32 600,00	Molecular biology reagents and Kits for NGS Consumables: Kits for cysts extraction, DNA or protein purification Consumables for Molecular biology Cloning and sequencing Shipment of samples to partners	1 400,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		8 050,00	Bacteriology (6 months field investigations)	1 200,00	Kick-off meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		0,00		2 550,00	Kick-off meeting Project meetings	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		26 800,00	Survey Consumables	2 900,00	Kick-off meeting Workshop	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		19 500,00	Sequencing 400 SE isolates Bacteriology	4 220,00	Kick-off meeting Farm visits Project meetings	0,00	
WP4	**0,00**		**6 000,00**		**15 870,00**		**3 388,00**	
JIP03-R2-IA2.3-CARE	0,00		6 000,00	Consumable APC	3 600,00	Kick off meeting	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		3 170,00	Kick off meeting Annual meeting	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		0,00		9 100,00	Kick-off meeting Biannual meeting Meetings with stake-holders	3 388,00	Organisation WP4 meeting
WP6	**0,00**		**1 500,00**		**4 700,00**		**0,00**	
PhD06-R1-ET5-PEMbo	0,00		0,00		1 500,00	Conferences	0,00	
PhD14-R2-FBZ4-ToxSauQMRA								
PhD12-R2-FBZSH9-AptaTrich	0,00		0,00		0,00		0,00	
STM-2020	0,00		1 500,00	Consumables Bench fees	3 200,00	Travel and subsistence costs of the short mission	0,00	
P02-Ages	**0,00**		**85 741,00**		**6 967,00**		**3 480,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**83 741,00**		**4 167,00**		**3 480,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP07-R1-FBZ2-LISTADAPT	0,00		3 308,00	Consumabels for whole genome sequencing APC	0,00		0,00	
JRP11-R1-FBZ4-MedVetKlebs	0,00		5 933,00	Methods, sampling, PCR, strain characterization APC	667,00	Consoritum meetings OHEJP ASM2020	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP15-R2-AMR2.1-FED-AMR	0,00		56 500,00	Cultivation "DNA isolation (Standard-kits and special procedure for free DNA isolation)" 16S amplicon sequencing Shot gun sequencing Gene enrichment; gene capture probes qPCR; qPCR arrays Sequencing (WGS and/or Sanger) Samples collection, transport Antibiotics quantification "Soil microcosm, competence regulons: gene expression, RNA isolation, RT PCR " Consumables	0,00		3 480,00	Kick-off meeting organisation
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		0,00		600,00	Kick-off meeting Project meetings	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		18 000,00	WGS	1 700,00	Kick-off meeting	0,00	
WP4	**0,00**		**2 000,00**		**200,00**		**0,00**	
JIP02-R1-IA1.2-COHESIVE	0,00		2 000,00	APC	200,00	Annual COHESIVE meeting Workshops	0,00	
P04-Sciensano	**5 500,00**		**145 591,00**		**54 800,00**		**2 200,00**	
WP1	**0,00**		**4 500,00**		**14 200,00**		**900,00**	
WP1-Coordination and Management	0,00		4 500,00		14 200,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 CT meeting at Anses 1 CT meeting at Anses 2 CT meeting at Anses 3 CT meeting at Anses 4 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting Various (SC, ESAB, etc.)	900,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4
WP3	**3 500,00**		**124 641,00**		**27 500,00**		**1 300,00**	
ASM T&S-2020	0,00		0,00		3 000,00	5 ASM 2020 FOC delegates	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP05-R1-ET1-TOXDETECT	1 500,00	LC-MS/MS maintenance costs Thermomixer accesories	8 000,00	Consumables for method transfer (preparation of the materials, shipping of materials to partners, customs), media and regents for confirmation microbiology techniques Production of Lab-Reference materials APC	2 000,00	Consoritum meetings	0,00	
JRP06-R1-FBZ1-NOVA	2 000,00	Laptop Usage Budget	4 000,00	Software License Budget APC	10 000,00	Consoritum meetings 1 "Combined NOVA WP meeting & integration workshop"	0,00	
JRP08-R1-FBZ2-METASTAVA	0,00		15 000,00	Publication costs Sequencing costs, molecular biology reagents, preparation libraries, preparation samples etc.	1 500,00	Project meetings, networking with other projects, and exchange visits	0,00	
JRP12-R2-AMRSH5-FARMED	0,00		19 000,00	Lab consumables sequencing	1 100,00	Annual meeting	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		36 906,00	Lab reagents for SMRT sequencing (incl. Proficiency test) Lab reagents for MGE investigation ENA database costs	5 250,00	Workshop SMRT sequencing	0,00	
JRP16-R2-ET2.2-TELE-Vir	0,00		13 622,00	Sequencing kits (2 kits)(Oxford Nanopore) Flowcells (12 pieces)(Oxford Nanopore) Other reagents and enzymes qPCR reagents	500,00	Kick-off meeting	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		23 113,00	Administration (publications, posters Lab reagents for WGS sequencing Lab reagents for typing	1 400,00	Kick-off meeting	0,00	
WP3-Management of Joint Research Projects	0,00		5 000,00	Reviewers fee	2 750,00	Meetings with JRPs	1 300,00	Annual JRPs/JIP meeting relating to Task 3.2.1 General support along the JRPs
WP4	**2 000,00**		**6 000,00**		**11 000,00**		**0,00**	
JIP01-R1-IA1.1-ORION	1 000,00	Laptop usage	3 000,00	Software licences APC	5 000,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	1 000,00	Laptop usage	3 000,00	Software licence APC	5 000,00	Annual COHESIVE meeting Combined WP meeting & workshop	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP4–Management of Joint Integrative Projects	0,00		0,00		1 000,00	Workshop organisation	0,00	
WP6	**0,00**		**10 450,00**		**2 100,00**		**0,00**	
PhD02-R1-AMR2/3/6-LIN-RES	0,00		10 450,00	Next generation sequencing Sampling and analysis of environmental samples (not included in the monitoring campaign) Laboratory experiments to assess transferability	1 000,00	International conference	0,00	
PhD04-R2-AMR2.1-KENTUCKY	0,00		0,00		1 100,00	Travel to INRA (P19) for a short mission	0,00	
P06-NDRVMI	**11 700,00**		**40 817,00**		**39 800,00**		**19 300,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**11 700,00**		**35 667,00**		**36 600,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 800,00	3 ASM 2020 FOC delegates	0,00	
JRP10-R1-FBZ3-MOMIRPPC	0,00		18 667,00	Consumables for laboratory study; reagents; feed and feed production; animal experiments Publication	9 900,00	Travel to 2 poultry farms for the experiments Final meeting	0,00	
JRP17-R2-ET2.2-IDEMBRU	2 700,00	Microplate centrifuge equipment Microplate ELISA reader	7 000,00	Consumables for sampling and serological analyses Transport of strains (UN 2814)	13 900,00	Kick-off meeting Collection of samples on field	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	9 000,00	Frizer at -80C degree	10 000,00	Reagents, consumables and materials for Bateriology ; Feed, feed preparation ; Animal experiment ; Publication.	11 000,00	Kick-off meeting Project meetings Travel to pig farms	0,00	
WP6	**0,00**		**5 150,00**		**600,00**		**19 300,00**	
CaM-2020	0,00		5 150,00	Financial support for speakers and tutors for Communication And Median workshop Accreditation for participants	600,00	Costs of the speakers and tutors	19 300,00	Organization of the two-day CaM workshop in Sofia
P07-SZU	**0,00**		**10 900,00**		**8 230,00**		**75 000,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**10 900,00**		**5 630,00**		**75 000,00**	
ASM org-2020							75 000,00	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		5 100,00	Sample containers Sample packaging Sample shipping Sater sampling	3 030,00	Kick-off mettings Project meetings	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		5 800,00	PCR kits, primers, chemicals, plastics, pipettes, laboratory animals, sequencing services	1 400,00	Kick-off meeting	0,00	
P08-VRI	**0,00**		**52 200,00**		**17 430,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**52 200,00**		**14 830,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP07-R1-FBZ2-LISTADAPT	0,00		2 000,00	Publications from material from Y2	1 180,00	Project meetings	0,00	
JRP09-R1-FBZ3-AIRSAMPLE	0,00		2 000,00	APC	0,00		0,00	
JRP10-R1-FBZ3-MOMIRPPC	0,00		0,00		1 800,00	Final meeting	0,00	
JRP16-R2-ET2.2-TELE-Vir	0,00		0,00		350,00	Kick-off meeting	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		6 200,00	Reagents and kits (for PCR and qPCR) Reagents for testing enrichment methods	1 400,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		10 000,00	Laboratory consumables, data analysis, administrative consumables Laboratory consumables (chemical reagents, isolation kits, plastics etc.)	3 600,00	Kick-off meeting Project meetings	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		10 000,00	Adminstrative and laboratory consumables (laboratory plastic, sample collection, culture media etc.) Farm visits	3 200,00	Kick-off meeting Project meetings	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		22 000,00	Multicenter QMRA for T. gondii Testing of methods for the detection of T. gonndii on fresh produce Supply of T. gondii oocysts	2 100,00	Kick-off meeting	0,00	
P09-BfR	**0,00**		**89 640,00**		**60 245,00**		**11 745,00**	
WP1	**0,00**		**0,00**		**5 500,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		5 500,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**85 640,00**		**21 028,00**		**11 745,00**	
ASM T&S-2020	0,00		0,00		1 800,00	3 ASM 2020 FOC delegates	0,00	
JRP01-R1-AMR1-IMPART	0,00		7 000,00	WP1,2,3 (culture media, antimicrobial test reagents, other consumables) APC	438,00	Project meeting	0,00	
JRP02-R1-AMR2-ARDIG	0,00		6 200,00	WP 3 (culture media, antimicrobial test reagents, other consumables) WP3 (Costs for sequencing) APC	1 400,00	Project meeting WP meeting (WP1 for model discussion)	0,00	
JRP03-R1-AMR3-RADAR	0,00		2 000,00	APC	2 000,00	Consortium Meetings	0,00	
JRP05-R1-ET1-TOXDETECT	0,00		12 000,00	Reagents for Toxin-development Reagents for Antibody-development Media for PT trial preparation "Media, reagents for micro- and molecular biological testings (e.g. kits etc)" Lab consumables (e.g. microtitration plates etc) APC	2 000,00	Consoritum meetings	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP06-R1-FBZ1-NOVA	0,00		2 000,00	APC	1 000,00	Consoritum meetings	0,00	
JRP12-R2-AMRSH5-FARMED	0,00		22 000,00	Hi-C sequencing MinIon Sequencing Illumina shotgun Sequencing	1 100,00	Annual meeting	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		4 000,00	Lab consumables (NGS)	700,00	Kick-off meeting	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		12 500,00	Lab reagents for SMRT sequencing (incl. Proficiency test)	840,00	Workshop SMRT sequencing	0,00	
JRP17-R2-ET2.2-IDEMBRU	0,00		15 000,00	Lab reagents Sequencing (RNASeq) Shipment of strains	1 400,00	Kick-off meeting	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		200,00	Transport costs for sending samples	1 400,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		0,00		450,00	Kick-off meeting	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		2 740,00	Conference registration Publication cost Consumables (tubes and packaging)	2 100,00	Kick-off meeting Project meetings Conferences	10 470,00	Kick-off meeting organisation Workshop organisation
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		0,00		3 300,00	Kick-off meeting 4 day laboratory training workshop Interlaboratory exchange visit	0,00	
JRP24-R2-FBZ-BeONE	0,00		0,00		1 100,00	Kick-off meeting	1 275,00	Development hackathon meeting
WP4	**0,00**		**4 000,00**		**16 317,00**		**0,00**	
JIP01-R1-IA1.1-ORION	0,00		2 000,00	APC	5 667,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		2 000,00	APC	4 000,00	Annual COHESIVE meeting Workshops	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		1 400,00	Kick off meeting Annual meeting	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		0,00		5 250,00	Kick-off meeting Biannual meeting WP2 meeting	0,00	
WP5	**0,00**		**0,00**		**17 400,00**		**0,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP5-Science to Policy translation	0,00		0,00		17 400,00	Consolidation of results 1 meeting relating to Task 5.2.3 Scientific support to enhance exploitation of results relating to Task 5.3.2 Stakeholders Committee meeting relating to Tasks 5.4 and 5.1 Dissemination/collaboration activity relating to Task 5.4 Dissemination/collaboration activity relating to Task 5.4	0,00	
P10-FLI	**0,00**		**131 907,00**		**41 450,00**		**8 400,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**124 074,00**		**21 950,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP06-R1-FBZ1-NOVA	0,00		2 000,00	APC	1 000,00	Consoritum meetings	0,00	
JRP08-R1-FBZ2-METASTAVA	0,00		30 655,00	Laboratory consumables for sampling, sample preparation, and sequencing	0,00		0,00	
JRP13-R2-AMR2-WORLDCOM	0,00		7 619,00	Consumables	3 750,00	Kick-off Meeting Biannual Meeting	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		8 800,00	Consumables Conference participation fees	4 500,00	Kick-off meeting Interlaboratory exchange Conferences	0,00	
JRP17-R2-ET2.2-IDEMBRU	0,00		4 500,00	Consumables for genotyping Consumables for biotyping	1 800,00	Kick-off meeting	0,00	
JRP18-R2-ET1.1-MEmE	0,00		28 600,00	Consumables Participation fees	5 750,00	Kick-off meeting Interlaboratory exchange Conferences	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		34 400,00	Survey Consumables	3 300,00	Kick-off meeting Interlaboratory exchange visit	0,00	
JRP24-R2-FBZ-BeONE	0,00		7 500,00	Whole-Genome-Sequencing of 50 Salmonella isolates from cattle	650,00	Kick-off meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP4	**0,00**		**7 833,00**		**16 900,00**		**8 400,00**	
JIP01-R1-IA1.1-ORION	0,00		5 333,00	Costs for data collection, literature costs, costs for publications, consumables APC	5 000,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		2 000,00	APC	4 000,00	Annual meeting WP meeting Closing symposium	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		500,00	FLI Participation fee costs	7 900,00	Kick-off meeting Biannual meeting FLI Participation in Scientific conference FLI Interlaboratory exchange meeting	8 400,00	Organisation WP1 meeting
P11-RKI	**0,00**		**52 200,00**		**14 200,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**52 200,00**		**11 600,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP02-R1-AMR2-ARDIG	0,00		3 000,00	Cost for publication fees in peer reviewe journals APC	2 000,00	Meeting with project partners	0,00	
JRP06-R1-FBZ1-NOVA	0,00		2 000,00	APC	3 100,00	Consoritum meetings	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		19 800,00	DNA extraction and shipping of samples Typing marker selection/development Nanobody development Lama immunisation	600,00	Kick-off meeting	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		4 900,00	Labotaratory disposibles e.g. culture media, tuber and micrtiter plates, disinfectants for testing	700,00	Kick-off meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		22 500,00	Commercial recombinant proteins Oligonucleotides Pcr reagents Cloning reagents Growth media Sequencing Plastic material Antibodies Purification reagents Shipping reagents to partners	700,00	Kick-off meeting	0,00	
JRP24-R2-FBZ-BeONE	0,00		0,00		3 300,00	Kick-off meeting Project meetings	0,00	
P12-DTU	**0,00**		**81 075,00**		**22 574,00**		**5 075,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**76 075,00**		**13 050,00**		**3 500,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP01-R1-AMR1-IMPART	0,00		6 200,00	Consumables APC	1 100,00	Project meeting Workshop	0,00	
JRP03-R1-AMR3-RADAR	0,00		3 500,00	Publication costs	2 750,00	2 WP meetings	0,00	
JRP06-R1-FBZ1-NOVA	0,00		2 000,00	APC	3 200,00	Consoritum meetings	0,00	
JRP07-R1-FBZ2-LISTADAPT								
JRP09-R1-FBZ3-AIRSAMPLE	0,00		2 500,00	APC	1 500,00	Project meetings	0,00	
JRP11-R1-FBZ4-MedVetKlebs	0,00		667,00	APC	800,00	Consoritum meetings	0,00	
JRP12-R2-AMRSH5-FARMED	0,00		30 000,00	MinIon Sequencing Illumina Sequencing	1 400,00	Annual meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP14-R2-AMR2.1-FULL-FORCE	0,00		31 208,00	Lab reagents for SMRT sequencing (incl. Proficiency test) Lab reagents for MGE investigation Computing for MGE investigations	0,00		0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		0,00		0,00		3 500,00	Kick-off meeting organisation
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		0,00		0,00		0,00	
JRP24-R2-FBZ-BeONE	0,00		0,00		1 100,00	Kick-off meeting Project meetings	0,00	
WP4	**0,00**		**5 000,00**		**6 924,00**		**1 575,00**	
JIP01-R1-IA1.1-ORION	0,00		2 000,00	APC	3 274,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		2 000,00	APC	750,00	Annual COHESIVE meeting Workshops	0,00	
JIP03-R2-IA2.3-CARE	0,00		1 000,00	Kits and reagents for WGS	700,00	WP1-WG meetin	1 575,00	Kick-off meeting organisation
JIP05-R2-IA2.1-MATRIX	0,00		0,00		2 200,00	Kick-off meeting Biannual meeting	0,00	
P13-SSI	**0,00**		**181 909,00**		**50 054,00**		**7 800,00**	
WP1	**0,00**		**0,00**		**5 500,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		5 500,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**153 742,00**		**26 487,00**		**7 800,00**	
ASM T&S-2020	0,00		0,00		2 400,00	4 ASM 2020 FOC delegates	0,00	
JRP01-R1-AMR1-IMPART	0,00		0,00		800,00	Project meeting	0,00	
JRP06-R1-FBZ1-NOVA	0,00		2 000,00	APC	1 000,00	Consoritum meetings	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP11-R1-FBZ4-MedVetKlebs	0,00		12 723,00	Lab consumables for culturing, PCR and genome sequencing Food sampling	467,00	Consoritum meetings	0,00	
JRP12-R2-AMRSH5-FARMED	0,00		22 000,00	Reagents and consumables for sample preparation, culturing, spiking, DNA purification, MinION and Illumina sequencing Reagents and consumables for sample preparation, Voltrax, DNA purification, MinION sequencing	1 400,00	Annual meeting	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		45 000,00	Lab consumables Lab costs for sequencing	1 400,00	Kick-off meeting	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		19 720,00	Lab reagents for SMRT sequencing (incl. Proficiency test) Lab reagents for Workshop Lab reagents for proficiency test	0,00		4 200,00	Workshop organiszation
JRP16-R2-ET2.2-TELE-Vir	0,00		0,00		1 000,00	Kick-off meeting	0,00	
JRP18-R2-ET1.1-MEmE	0,00		8 000,00	Laboratory consumables	8 250,00	Kick-off meeting European Multicolloquium on Parasites (Serbia 2020) Training (exchange of key staff members)	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		6 300,00	Laboratory consumables Shipment cost	1 400,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		13 100,00	Genome sequencing of isolates Lab consumables for sampling, culturing	120,00	Kick-off meeting	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		17 977,00	Survey and related costs Genotyping primers and laboratory consumables	1 900,00	Workshop	3 600,00	Kick-off meeting organisation
JRP23-R2-FBZSH5-ADONIS	0,00		6 000,00	Genome sequencing of isolates	1 400,00	Kick-off meeting	0,00	
JRP24-R2-FBZ-BeONE	0,00		922,00	Test server rental	4 950,00	Kick-off meeting Development hackathon meeting	0,00	
WP3-Management of Joint Research Projects								
WP4	**0,00**		**28 167,00**		**14 667,00**		**0,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JIP01-R1-IA1.1-ORION	0,00		6 167,00	Consumables to perform microbiology/laboratory work Consumables to perform epidemiological work APC	2 667,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP03-R2-IA2.3-CARE	0,00		10 000,00	Consumables (WGS, shipping, media etc)	1 000,00	Kick off meeting WP1-WG meeting	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		10 000,00	Laboratory Consumables	8 000,00	Kick off meeting Annual meeting Meeting with MATRIX project	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		2 000,00	Publication fees	3 000,00	Kick-off meeting Biannual meeting	0,00	
WP5	**0,00**		**0,00**		**3 400,00**		**0,00**	
WP5-Science to Policy translation	0,00		0,00		3 400,00	Stakeholders Committee meeting relating to Tasks 5.4 and 5.1 Dissemination/collaboration activity relating to Task 5.4 Dissemination/collaboration	0,00	
P14-UT	**6 000,00**		**73 300,00**		**16 000,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**6 000,00**		**73 300,00**		**13 400,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP13-R2-AMR2-WORLDCOM	6 000,00	Computer workstation for improved bioinformatics pipelines	33 500,00	Lab plastic Enzymes, molecular biology kits, lab chemicals, minor lab equipment (e.g. pipettes) etc Sequencing cost of bacterial genomes	2 200,00	Kick-off Meeting Biannual Meeting	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		18 800,00	Consumables Conference participation fees	7 450,00	Kick-off meeting Interlaboratory exchange Conferences	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP18-R2-ET1.1-MEmE	0,00		21 000,00	Consumables for molecular studies	2 550,00	Kick-off meeting European Multicolloquium on Parasites (Serbia 2020) Training (exchange of key staff members)	0,00	
P15-VFL	**0,00**		**14 200,00**		**7 750,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**14 200,00**		**5 150,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP18-R2-ET1.1-MEmE	0,00		6 700,00	Reagents and consumables Disposal of carcasses of definitive hosts Shipping of samples	2 550,00	Kick-off meeting Training (exchange of key staff members)	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		7 500,00	Materials directly for performance of analyses- reagents, culture media, consumables, gloves, disinfectants etc Materials directly for performance of analyses- reagents, culture media, consumables, gloves, disinfectants etc Local transportation	1 400,00	Kick-off meeting Project meetings	0,00	
P16-INIA	**0,00**		**37 960,00**		**8 380,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**35 960,00**		**4 880,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP06-R1-FBZ1-NOVA	0,00		4 000,00	Costs of stationery and computer science, program licences, scientific publications, ciberclouds… APC	1 000,00	Consoritum meetings	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP14-R2-AMR2.1-FULL-FORCE	0,00		12 750,00	Reactives for SMRT Reactives for plasmid DNA isolation Others: plastics and buffers	1 680,00	Workshop SMRT sequencing	0,00	
JRP16-R2-ET2.2-TELE-Vir	0,00		19 210,00	MinIT (Oxford Nanopore) MinION starter pack (advanced)(Oxford Nanopore) Nucleic acid extraction kits Molecular biology reagents Other consumables, external services	1 000,00	Kick-off meeting	0,00	
WP4	**0,00**		**2 000,00**		**900,00**		**0,00**	
JIP05-R2-IA2.1-MATRIX	0,00		2 000,00	Costs of stationery and computer science, program licences, cibercloud…	900,00	Participation to annual MATRIX meeting 2020	0,00	
P17-UCM	**0,00**		**144 489,00**		**27 800,00**		**0,00**	
WP1	**0,00**		**0,00**		**5 500,00**			
WP1-Coordination and Management	0,00		0,00		5 500,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting		
WP2	**0,00**		**0,00**		**2 500,00**		**0,00**	
WP2-SRA	0,00		0,00		2 500,00	Meetings related to Task 2.2.2: strategic interactions	0,00	
WP3	**0,00**		**133 639,00**		**14 650,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 800,00	3 ASM 2020 FOC delegates	0,00	
JRP02-R1-AMR2-ARDIG	0,00		34 207,00	Molecular Biology, High throughput sequencing (Illumina and PacBio) APC	3 000,00	Meeting with project partners	0,00	
JRP06-R1-FBZ1-NOVA	0,00		8 650,00	Software lisences Publication APC	1 000,00	Consoritum meetings	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP10-R1-FBZ3-MOMIRPPC	0,00		4 482,00	Reagents, consumables and material for molecular Biology and sequencing; Immunology (MALDI TOF) Animal experiments Publication	1 500,00	Final meeting	0,00	
JRP12-R2-AMRSH5-FARMED	0,00		6 000,00	Sequencing material for MinIon, Illumina Sequencing and Lab material for molecular Biology Sampling and on site testing	1 000,00	Annual meeting	0,00	
JRP13-R2-AMR2-WORLDCOM	0,00		23 000,00	Software Laboratory testing, moelcular biology tools, DNA extraction kits, genome sequencing Sample collection, laboratory testing	2 100,00	Kick-off Meeting Biannual Meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		10 000,00	Lab consumables for sampling, culturing	1 100,00	Kick-off meeting	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		34 300,00	Consumables for WP2 needed for surveys and collect data Consumables needed for WP3 (DNA extraction, PCR, package delivery) Consumables for Task 4.2. Development of a novel stage-specific antigen-based ELISA to diagnose oocysts and/or bradyzoite driven infections. Consumables for WP5 (generation of high qyuality DNA, PCR-RFLP and microsatellite genotyping)	2 050,00	Kick-off meeting Workshop	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		13 000,00	Reagents; consumables and material for Salmonella analysis; WGS; Publication	1 100,00	Kick-off meeting	0,00	
WP4	**0,00**		**6 850,00**	Consumables needed for WP3 (DNA extraction, PCR, package delivery)	**3 650,00**		**0,00**	
JIP03-R2-IA2.3-CARE	0,00		6 850,00		1 450,00	Kick off meeting WP1-WG meeting	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		0,00	Consumables for Task 4.2. Development of a novel stage-specific antigen-based ELISA to diagnose oocysts and/or bradyzoite driven infections.	2 200,00	Kick-off meeting Biannual meeting	0,00	
WP6	**0,00**		**4 000,00**		**1 500,00**		**0,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
PhD-R1-E14-ECO-HEN	0,00		4 000,00	Consumables for WP5 (generation of high qyuality DNA, PCR-RFLP and microsatellite genotyping)	1 500,00	Conferences	0,00	
PhD-R2-E2-METAPRO	0,00		0,00		0,00		0,00	
P18-MVNA	**0,00**		**0,00**		**4 900,00**		**0,00**	
WP1	**0,00**		**0,00**		**1 900,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		1 900,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 CCP meeting	0,00	
WP3	**0,00**		**0,00**		**3 000,00**		**0,00**	
ASM T&S-2020	0,00		0,00		3 000,00	5 ASM 2020 FOC delegates	0,00	
P19-INRA	**0,00**		**109 809,00**		**26 479,00**		**900,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**107 837,00**		**20 679,00**		**900,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP05-R1-ET1-TOXDETECT	0,00		31 000,00	Culture media, Laboratory glasswares and chemicals for microbiology and proteomic, small equipments (chromatography columns…) (WP3, WP5) Molecular biology and small equipment (WP4, WP5) APC	4 000,00	Consoritum meetings	0,00	
JRP07-R1-FBZ2-LISTADAPT	0,00		16 912,00	Laboratory glassware, Listeria enrichment and selective media. PCR products for molecular identification/confirmationL, Biofilm Ring Test kits for biofilm screenings, small equipement (micropipette) Molecular biology reagents, plasticware, bacteriology culture media	7 894,00	Project meeting Isopol conference	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP10-R1-FBZ3-MOMIRPPC	0,00		30 050,00	Reagents, consumables and material for molecular Biology and sequencing; Immunology (MALDI TOF) Animal experiments Publication	4 350,00	Workshops	900,00	Final meeting
JRP11-R1-FBZ4-MedVetKlebs	0,00		11 275,00	Molecular biology reagents, purification kits, culture media and other plasticware	1 555,00	Consoritum meetings OHEJP ASM2020	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		18 600,00	Consumables for SMRT sequencing Consumables for functional characterization of MGE	1 680,00	Workshop SMRT sequencing	0,00	
WP4	**0,00**		**1 972,00**		**3 200,00**		**0,00**	
JIP03-R2-IA2.3-CARE	0,00		1 972,00	Software, costs of data storage Software, costs of data storage Consumables for strain characterization analysis (Bacteriology, Sequencing, Mass Spectrometry) Consumables for IT development	3 200,00	Kick off meeting IT meeting with MIRRI/EOSC in one european country	0,00	
P20-IP	**0,00**		**105 650,00**		**26 467,00**		**0,00**	
WP1	**0,00**		**0,00**		**5 500,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		5 500,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**93 471,00**		**16 667,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 800,00	3 ASM 2020 FOC delegates	0,00	
JRP02-R1-AMR2-ARDIG	0,00		17 000,00	Sequencing kits (Illumina) Microbiology media Molecular biology (enzymes, primers, Q-PCR) Antibiotics and reactif for susceptibility determination Pastics (tups, tubes, plaques) antibodies publication APC	2 000,00	Participation to a scientific meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP05-R1-ET1-TOXDETECT	0,00		12 000,00	Consumables for LC-MS, data storage (WP3) Reagents for molecular biology; consumables for molecular biology and bacteriology (WP2) APC	4 000,00	Consoritum meetings	0,00	
JRP11-R1-FBZ4-MedVetKlebs	0,00		44 000,00	Methods, sampling and characterization ; Illumina & PacBio sequencing APC	7 567,00	Consoritum meetings	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		9 975,00	Lab consumables Lab costs for culturing, sequençing, AMR	300,00	Kick-off meeting	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		10 496,00	DNA preparation and High Throughput Sequencing Sequencing Analysis Phenotypic experiments	1 000,00	Kick-off meeting	0,00	
WP4	**0,00**		**12 179,00**		**2 100,00**		**0,00**	
JIP03-R2-IA2.3-CARE	0,00		12 179,00	Sequencing and characterization	2 100,00	Kick off meeting	0,00	
WP6	**0,00**		**0,00**		**0,00**		**0,00**	
PhD-R2-E22-codes4strains	0,00		0,00		0,00		0,00	
WP7	**0,00**		**0,00**		**2 200,00**		**0,00**	
WP7-Sustainability	0,00		0,00		2 200,00	WP 7 coordination activity meeting will be held in Paris and in Rome Meeting with other WPs leaders for specific sustainability issues, in particular with WP4 leader, WP5 leader Attendance at the 2020 Stakeholders meeting to start introducing sustainability strategies to the stakeholders	0,00	
P21-APHA	**8 213,00**		**210 295,00**		**47 910,00**		**13 091,00**	
WP1	**0,00**		**0,00**		**1 900,00**		**4 575,00**	
WP1-Coordination and Management	0,00		0,00		1 900,00	SSB meeting 4-2020 at INIAV (PT) M27 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	4 575,00	SSB meeting 3-2020 at APHA (UK) M33
WP3	**8 213,00**		**143 948,00**		**23 671,00**		**2 700,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP01-R1-AMR1-IMPART								
JRP02-R1-AMR2-ARDIG	0,00		11 733,00	Cost for culture media, molecular biology kit, WGS	4 500,00	Meeting with project partners Scientific conference	0,00	
JRP03-R1-AMR3-RADAR	0,00		0,00		594,00	Consortium Meetings	0,00	
JRP06-R1-FBZ1-NOVA	0,00		7 500,00	Cloud computing	2 620,00	Consoritum meetings	0,00	
JRP12-R2-AMRSH5-FARMED	8 213,00	VolTRAX V2	26 400,00	Consumables Nanopore Flow Cell (R9.4.1) (12x) Nanopore Rapid Barcoding Kit (5 kits) Agencore ampure beads Cloud Storage Seq consumables	3 360,00	Annual meeting	2 700,00	Project meeting
JRP14-R2-AMR2.1-FULL-FORCE	0,00		24 480,00	Nanopore Flow Cell (R9.4.1) (12x) Nanopore Rapid Barcoding Kit (5 kits) Cloud Storage	1 440,00	Workshop SMRT sequencing	0,00	
JRP17-R2-ET2.2-IDEMBRU	0,00		26 242,00	DNA extraction kits Molecular typing reagents WGS reagents RNAseq reagents Cloud computing Transport of strains (UN2814)	1 400,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		19 433,00	Consumables Farm (VTEC) Consumables Lab (VTEC) Consumable extract (VTEC) Consumables Seq (CSU) Cloud storage Consumables Salmonella	2 347,00	Kick-off meeting Project meetings	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		0,00		4 360,00	Kick-off meeting Project meetings Travel to farms	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		28 160,00	Consumable media Consumable (sequen) Claud storage	1 300,00	Kick-off meeting Project meetings	0,00	
JRP24-R2-FBZ-BeONE	0,00		0,00		550,00	Kick-off meeting	0,00	
WP4	**0,00**		**48 847,00**		**18 162,00**		**5 816,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JIP01-R1-IA1.1-ORION	0,00		5 548,00	Budget for procuring specialist IT solutions such as cloud computing, online code repositories and online collaboration sites APC	2 550,00	ORION consortium meeting Combined ORION WP meeting & integration workshop Meeting with PHE	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		4 365,00	Cloud computing time / Collaboration hosting site costs APC	5 913,00	Annual COHESIVE meeting WP meeting Workshops	0,00	
JIP03-R2-IA2.3-CARE	0,00		4 693,00	Consumables (standard laboratory supplies including buffers, sequencing reagents)	879,00	Kick off meeting	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		30 741,00	Consumables Dynabeads (ThermoFisher scientific) Seq consumables Seq consumables Cloud Storage	5 520,00	Annual meeting	5 816,00	Organisation kick off meeting
JIP05-R2-IA2.1-MATRIX	0,00		3 500,00	Specialist IT, e.g. cloud computing time and storage	3 300,00	Kick-off meeting Biannual meeting WP planning meeting	0,00	
WP6	**0,00**		**15 500,00**		**4 210,00**		**0,00**	
PhD-R2-E18-WILBR	0,00		5 500,00	Lab Consumables/annum PhD student fees at UoE	3 710,00	Sampling wildlife Sampling Phd Colloboration PhD	0,00	
PhD-R2-E9-UDoFRiC	0,00		10 000,00	Consumables PhD student fees at Uni	500,00	Start up meeting	0,00	
P22-PHE	**3 000,00**		**16 495,00**		**15 410,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**1 000,00**		**12 495,00**		**8 650,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP01-R1-AMR1-IMPART	0,00		1 800,00	Laboratory consumables APC	1 000,00	Project meeting	0,00	
JRP02-R1-AMR2-ARDIG	0,00		8 000,00	Laboratory consumables costs APC	1 650,00	Attendance at meetings and presentation of work at conferences	0,00	
JRP06-R1-FBZ1-NOVA	1 000,00		2 695,00	APC	1 500,00	Consoritum meetings	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP20-R2-FBZSH3-DISCoVeR	0,00		0,00		1 100,00	Kick-off meeting	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		0,00		1 100,00	Kick-off meeting	0,00	
JRP24-R2-FBZ-BeONE	0,00		0,00		1 100,00	Kick-off meeting	0,00	
WP4	**2 000,00**		**4 000,00**		**4 160,00**		**0,00**	
JIP01-R1-IA1.1-ORION	2 000,00		2 000,00	APC	2 900,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		2 000,00	APC	1 260,00	Annual COHESIVE meeting Workshops	0,00	
P23-UoS	**0,00**		**98 598,00**		**35 400,00**		**3 000,00**	
WP1	**0,00**		**0,00**		**8 500,00**		**3 000,00**	
WP1-Coordination and Management	0,00		0,00		8 500,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director	3 000,00	CCP meeting
WP3	**0,00**		**87 213,00**		**12 700,00**		**0,00**	
ASM T&S-2020	0,00		0,00		3 600,00	6 ASM 2020 FOC delegates	0,00	
JRP02-R1-AMR2-ARDIG	0,00		9 333,00	General laboratory consumables and Consumable costs for in vitro models APC	3 000,00	Meeting with project partners Scientific conference	0,00	
JRP10-R1-FBZ3-MOMIRPPC	0,00		11 080,00	General laboratory consumables and consumables for in vitro assays	0,00		0,00	
JRP13-R2-AMR2-WORLDCOM	0,00		22 000,00	Consumables - General laboratory consumables APHA – Sequencing	1 000,00	Kick-off Meeting	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		18 000,00	Consumables	1 400,00	Kick-off meeting	0,00	
JRP16-R2-ET2.2-TELE-Vir	0,00		6 800,00	Consumables	1 500,00	Kick-off meeting	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		20 000,00	Collecting and shipping samples for NGS Consumables for developing and testing typing schemes	750,00	Kick-off meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		0,00		1 450,00	Kick-off meeting Training in oocyst recovery	0,00	
WP4	**0,00**		**0,00**		**4 900,00**		**0,00**	
JIP05-R2-IA2.1-MATRIX	0,00		0,00		4 900,00	Kick-off meeting Biannual meeting WP-4 meeting WP-5 meeting	0,00	
WP6	**0,00**		**11 385,00**		**9 300,00**		**0,00**	
PhD-R1-E26-EnvDis	0,00		5 500,00	Registration fees 2st year Other consumables: e.g. books, subscriptions to relevant services (e.g. data service such as https://knoema.com/), printing material (posters for meeting, leaflets for stakeholders and partners as Zoetis)	3 800,00	International conferences	0,00	
PhD-R2-E8-MACE	0,00		5 885,00	PhD student fees	2 800,00	Conferences	0,00	
WP6-T&E management	0,00		0,00		2 700,00	Attendance at meetings relating to management of WP6	0,00	
P24-OKI	**0,00**		**1 300,00**		**5 200,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**1 300,00**		**2 600,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		1 300,00	Reagents for PCR and sequencing Shipment costs	1 400,00	Kick-off meeting	0,00	
P25-NUIG	**0,00**		**37 132,00**		**7 500,00**		**350,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**37 132,00**		**4 900,00**		**350,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP11-R1-FBZ4-MedVetKlebs	0,00		17 232,00	Methods, sampling, strain characterization, sequencing Sample purchase, processing, bacterial isolation and characterisation and sequencing APC	800,00	Consoritum meetings	0,00	
JRP13-R2-AMR2-WORLDCOM	0,00		13 000,00	Research consumables	2 200,00	Kick-off Meeting Biannual Meeting	350,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		6 900,00	Gene enrichment probes for 16 reactions Molecular biology reagents	700,00	Kick-off meeting	0,00	
P26-TEAGASC	**0,00**		**85 791,00**		**8 839,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**75 605,00**		**2 900,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		75 605,00	Sequencing of Campylobacter, Salmonella and AMR isolates Lab plastics and consumables	1 700,00	Kick-off meeting	0,00	
WP4	**0,00**		**0,00**		**2 064,00**		**0,00**	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		2 064,00	Kick off meeting Annual meeting	0,00	
WP6	**0,00**		**10 186,00**		**1 275,00**		**0,00**	
PhD-R2-E12-HME-AMR	0,00		10 186,00	Laboratory consumables, sequencing kits	1 275,00	Travel and subsistence costs - project meetings, sample collection, OHEJP meetings	0,00	
P27-ISS	**0,00**		**255 165,00**		**41 150,00**		**10 135,00**	
WP1	**0,00**		**0,00**		**4 500,00**		**4 675,00**	
WP1-Coordination and Management	0,00		0,00		4 500,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33	4 675,00	PMC meeting 2-2020 at ISS (IT) M29 for director
WP3	**0,00**		**230 165,00**		**22 275,00**		**5 460,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
ASM T&S-2020	0,00		0,00		2 400,00	4 ASM 2020 FOC delegates	0,00	
JRP03-R1-AMR3-RADAR	0,00		2 000,00	APC	0,00		0,00	
JRP10-R1-FBZ3-MOMIRPPC	0,00		25 570,00	Reagents, consumables and material for molecular Biology and sequencing; Immunology (MALDI TOF) Animal experiments Publication	4 000,00	Final meeting	0,00	
JRP12-R2-AMRSH5-FARMED	0,00		7 500,00	Reagents and consumables for metagenomics and sample preparation Reagents and consumables for benchmarking experiments	1 400,00	Annual meeting	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		23 870,00	Lab reagents for SMRT sequencing (incl. Proficiency test) Lab reagents for MGE investigation	1 750,00	Workshop SMRT sequencing	0,00	
JRP18-R2-ET1.1-MEmE	0,00		30 500,00	Consumables for molecular studies Publication fees	5 550,00	Kick-off meeting European Multicolloquium on Parasites (Serbia 2020) Training (exchange of key staff members)	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		50 000,00	Publication cost Reagents (PCR, kits, Sanger sequencing) for testing markers Cost of NGS sequencing	0,00		5 460,00	Kick-off meeting Organisation
JRP20-R2-FBZSH3-DISCoVeR	0,00		19 000,00	Sequencing of STEC stains Cost for Sampling description and isolation of STEC strains from pet, wid animals, environment	2 025,00	Kick-off meeting	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		9 300,00	Plasticware, personal protection equipment (PPE), reagents for molecular biology and sequencing (RT-PCR, Realtime-RT-PCR), ELISA screening kit, commercial antibodies.	1 400,00	Kick-off meeting Project meetings	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		50 000,00	Consumables International courrier Cost of NGS sequencing	2 350,00	Kick-off meeting Interlaboratory exchange visit	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		12 425,00	Lab reagents and services for WGS sequencing	1 400,00	Kick-off meeting	0,00	
WP4	**0,00**		**22 000,00**		**6 725,00**		**0,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JIP03-R2-IA2.3-CARE	0,00		22 000,00	Sequencing reagents for pilot PTs Molecular biology reagents for pilot PTs Media and reagents for microbiologal tests in pilot PTs	1 400,00	Kick off meeting WP1-WG meeting	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		3 750,00		0,00	
JIP05-R2-IA2.1-MATRIX	0,00		0,00		1 575,00	Kick-off meeting Biannual meeting	0,00	
WP6	**0,00**		**0,00**		**3 250,00**		**0,00**	
STM-2020	0,00		0,00		3 250,00	Travel and subsistence costs of the short mission	0,00	
WP7	**0,00**		**3 000,00**		**4 400,00**		**0,00**	
WP7-Sustainability	0,00		3 000,00	Tool for on line surveys and interviews. This will be used to contact and solicit different stakeholder groups and experts to collect information and opinions that will be translated in sustainability strategies	4 400,00	WP 7 coordination activity meeting will be held in Paris and in Rome Meeting with other WPs leaders for specific sustainability issues, in particular with WP4 leader, WP5 leader Attendance at the 2020 Stakeholders meeting to start introducing sustainability strategies to the stakeholders	0,00	
P28-IZS AM	**12 000,00**		**91 836,67**		**19 399,67**		**5 763,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**78 336,67**		**10 949,67**		**2 100,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP06-R1-FBZ1-NOVA	0,00		2 000,00	APC	2 000,00	Consoritum meetings	0,00	
JRP07-R1-FBZ2-LISTADAPT	0,00		15 946,67	Reagents (lab test including NGS) APC	1 666,67	Project meetings	0,00	
JRP09-R1-FBZ3-AIRSAMPLE	0,00		7 000,00	Materials and reagents for WP1 and WP2 activities APC	650,00	Project meetings	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP11-R1-FBZ4-MedVetKlebs	0,00		16 000,00	Methods, sampling, strain characterization APC	833,00	Consoritum meetings	0,00	
JRP12-R2-AMRSH5-FARMED	0,00		9 000,00	Reagents and consumables for metagenomics and sample preparation DNA extraction	1 400,00	Annual meeting	0,00	
JRP17-R2-ET2.2-IDEMBRU	0,00		8 640,00	Reagents for sampling and bacteriological experiments Reagents and materials for WGS Reagents and materials for molecular biology Reagents and materials for molecular biology Shipment of live emerging Brucella strains	1 400,00	Kick-off meeting	0,00	
JRP16-R2-ET2.2-TELE-Vir	0,00		9 750,00	Utensils Nucleic Acid Isolation Kits Other reagents and enzymes library preparation kits (12 kits)	0,00		2 100,00	Organization Kick-off meeting
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		10 000,00	Plastic PCR reagents Culture media&reagents Biological reference materials	700,00	Kick-off meeting	0,00	
JRP24-R2-FBZ-BeONE	0,00		0,00		1 100,00	Kick-off meeting	0,00	
WP4	**12 000,00**		**13 500,00**		**5 850,00**		**3 663,00**	
JIP02-R1-IA1.2-COHESIVE	12 000,00	One year mortgage of IT infrastructure	6 000,00	Cloud services APC	2 500,00	Annual COHESIVE meeting Closing symposium	0,00	
JIP03-R2-IA2.3-CARE	0,00		7 500,00	Plastic PCR reagents Culture media&reagents Biological reference materials	700,00	Kick off meeting	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		0,00		2 650,00	Kick-off meeting WP1 meeting WP planning meeting	3 663,00	Organization biannual meeting WP2 meeting
P29-IZS LER	**0,00**		**33 878,00**		**12 450,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**31 878,00**		**6 650,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP10-R1-FBZ3-MOMIRPPC	0,00		31 878,00	Reagents, consumables and materials for Bateriology ; Sample analysis by standard diagnostic PCRs Other reagents and enzymes Shipments (on dry ice) Animal experiment Cell Biology & Immunology Publication costs	3 950,00	Final meeting	0,00	
JRP16-R2-ET2.2-TELE-Vir	0,00		0,00		1 500,00	Kick-off meeting	0,00	
WP4	**0,00**		**2 000,00**		**3 200,00**		**0,00**	
JIP02-R1-IA1.2-COHESIVE	0,00		2 000,00	APC	1 500,00	Annual COHESIVE meeting Thematic workshops	0,00	
JIP03-R2-IA2.3-CARE	0,00		0,00		1 700,00	Kick off meeting	0,00	
P30-RIVM	**0,00**		**188 360,00**		**116 983,00**		**40 014,00**	
WP1	**0,00**		**0,00**		**5 500,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		5 500,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP2	**0,00**		**0,00**		**2 500,00**		**0,00**	
WP2-SRA	0,00		0,00		2 500,00	Meetings related to Task 2.1.2: Development of the SRA and Task 2.2.2: strategic interactions	0,00	
WP3	**0,00**		**76 278,00**		**25 783,00**		**10 464,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
ASM T&S-2020	0,00		0,00		1 800,00	3 ASM 2020 FOC delegates	0,00	
JRP01-R1-AMR1-IMPART	0,00		1 872,00	Lab Materials APC	1 000,00	Project meeting	0,00	
JRP03-R1-AMR3-RADAR	0,00		2 000,00	APC	0,00		0,00	
JRP06-R1-FBZ1-NOVA	0,00		2 000,00	APC	8 750,00	Consoritum meetings	0,00	
JRP11-R1-FBZ4-MedVetKlebs	0,00		13 005,00	Sampling, strain characterization APC	1 000,00	Consoritum meetings	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		16 667,00	Sequencing costs	1 500,00	Workshop SMRT sequencing	0,00	
JRP18-R2-ET1.1-MEmE	0,00		0,00		1 400,00	Kick-off meeting	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		400,00	Shipment costs	1 400,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		0,00		3 333,00	Kick-off meeting Project meetings	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		0,00		0,00		0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		17 000,00	Survey Open acces publishing costs	2 800,00	Kick-off meeting WP meeting	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		16 667,00	Sequencing costs	2 800,00	Project meetings	5 000,00	Kick-off meeting Organisation
JRP24-R2-FBZ-BeONE	0,00		6 667,00	Sequencing costs	0,00		5 464,00	Kick-off meeting Organisation
WP4	**0,00**		**67 667,00**		**41 850,00**		**0,00**	
JIP01-R1-IA1.1-ORION	0,00		22 000,00	NGC sequencing APC	1 000,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		43 667,00	Reserved for EJP partners not already in the COHESIVE consortium Meeting costs APC	30 000,00	Annual meeting and workshops Participation of OHEJP consortium partners not already in the COHESIVE consortium	0,00	
JIP03-R2-IA2.3-CARE	0,00		2 000,00	Chemicals	2 150,00	Kick off meeting Interim meetings	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		3 600,00	Kick off meeting Annual meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JIP05-R2-IA2.1-MATRIX	0,00		0,00		5 100,00	Kick-off meeting Biannual meeting WP-1 meeting WP-2 meeting	0,00	
WP6	**0,00**		**44 415,00**		**41 350,00**		**29 550,00**	
CPD-2019	0,00		8 500,00	Externalexpert/speaker Simulation game Merchandise Attendence certificates for participants Accreditation for participants	21 250,00	Costs of participants invited as part of the OHEJP consortium	4 550,00	Costs of organizing the meeting of the Continuing Professional Development Module
PhD-R2-E17-DESIRE	0,00		18 000,00	Bench fee (Lab material) Sequencing costs Education WIAS	500,00	Conferences	0,00	
PhD-R2-E5-TRACE	0,00		0,00		0,00		0,00	
SS-2020	0,00		15 415,00	Welcome drinks, Social dinner Delegate information materials Handbook Merchandise Accreditation Certificates Marketing Registration fee reimbursement for consortium members Presentation materials Contingencies	11 000,00	External speakers travel & subsistence	23 000,00	Summer School organisation costs
WS-2020	0,00		2 500,00	Communications and training materials	8 600,00	Costs of the WorkShop team for the preparation of the meeting that will be held in Prague during the ASM 2020	2 000,00	Costs of organizing the Satellite WorkShop
P31-WbvR	**14 000,00**		**188 126,00**		**48 019,00**		**1 620,00**	
WP1	**0,00**		**0,00**		**8 550,00**		**0,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP1-Coordination and Management	0,00		0,00		8 550,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**14 000,00**		**176 926,00**		**33 069,00**		**0,00**	
ASM T&S-2020	0,00		0,00		3 000,00	5 ASM 2020 FOC delegates	0,00	
JRP01-R1-AMR1-IMPART	0,00		9 345,00	Lab Materials	4 125,00	Project meeting	0,00	
JRP02-R1-AMR2-ARDIG	0,00		22 550,00	Lab disposables, media and molecular kits APC	1 000,00	Meeting with project partners Scientific conference	0,00	
JRP03-R1-AMR3-RADAR	0,00		0,00		1 000,00	Consortium Meetings	0,00	
JRP08-R1-FBZ2-METASTAVA	14 000,00		55 556,00	Materials WbvR	4 344,00	Collaboration meetings	0,00	
JRP10-R1-FBZ3-MOMIRPPC	0,00		12 500,00	Materials	2 250,00	Final meeting	0,00	
JRP12-R2-AMRSH5-FARMED	0,00		25 450,00	Reagents and consumables for sample preparation, culturing, spiking, DNA purification, MinION and Illumina sequencing	550,00	Annual meeting	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		30 000,00	Lab reagents for SMRT sequencing (incl. Proficiency test) Lab reagents for MGE investigation Other lab materials and publication costs	2 100,00	Workshop SMRT sequencing	0,00	
JRP17-R2-ET2.2-IDEMBRU	0,00		5 000,00	Software expensis Renting of computational resources	1 400,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		0,00		2 800,00	Kick-off meeting Project meetings	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		4 400,00	Consumables for sampling Post costs for sending samples to lab Travelling to farms Unforeseen Consumables Virus culture	7 700,00	Kick-off meeting Project meetings	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		0,00		700,00	Kick-off meeting	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		12 125,00	Salmonella isolates sequencing; sampling on farms	2 100,00	Kick-off meeting	0,00	
WP4	**0,00**		**11 200,00**		**6 400,00**		**1 620,00**	
JIP01-R1-IA1.1-ORION	0,00		2 000,00	APC	1 500,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		3 200,00	Literature Office needs Meeting costs APC	2 000,00	Annual COHESIVE meeting Workshops	0,00	
JIP03-R2-IA2.3-CARE	0,00		5 000,00	Labmaterials	1 400,00	Kick off meeting	1 620,00	One day meeting for the planning of pilot PT's WP1
JIP05-R2-IA2.1-MATRIX	0,00		1 000,00	Consumables and disposables laboratory	1 500,00	Kick-off meeting	0,00	
WP6	**0,00**		**0,00**		**0,00**		**0,00**	
PhD-R2-E16-VIMOGUT	0,00		0,00		0,00		0,00	
PhD-R2-E5-TRACE	0,00		0,00		0,00		0,00	
WP6-T&E management	0,00		0,00		0,00		0,00	
P32-FHI	**0,00**		**98 601,00**		**45 007,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**75 934,00**		**9 900,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP06-R1-FBZ1-NOVA	0,00		2 000,00	APC	0,00		0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP10-R1-FBZ3-MOMIRPPC	0,00		36 802,00	Consumables to lab Whole genome Sequencing of Salmonella isolates Microbiome sequencing Serotyping and AST (7 antibiotics) of Salmonella isolates Stool sampling kits for DNA Extraction Stool sampling kits for Salmonella culture Postage - sampling kits- tur/retur Postage - microbiome samples to sequence center on ice tur/retur Prize for participation (lottery) APC	4 750,00	Final meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		22 132,00	Whole genome sequencing of 175 STEC	2 600,00	Kick-off meeting	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		15 000,00	Survey	700,00	Kick-off meeting	0,00	
JRP24-R2-FBZ-BeONE	0,00		0,00		650,00	Kick-off meeting	0,00	
WP4	**0,00**		**22 667,00**		**27 867,00**		**0,00**	
JIP01-R1-IA1.1-ORION	0,00		18 667,00	Consumables to perform laboratory work APC	6 667,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		2 000,00	APC	5 000,00	Annual COHESIVE meeting WP meeting Workshops	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		3 600,00	Kick off meeting Annual meeting	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		2 000,00	APC	12 600,00	Kick-off meeting WP1 meeting WP planning meeting	0,00	
WP6	**0,00**		**0,00**		**4 640,00**		**0,00**	
STM-2020	0,00		0,00		4 640,00	Travel and subsistence costs of the short mission	0,00	
P33-NVI	**0,00**		**143 295,00**		**60 712,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**126 295,00**		**34 744,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP01-R1-AMR1-IMPART	0,00		5 501,00	APC	1 800,00	Project meeting	0,00	
JRP02-R1-AMR2-ARDIG	0,00		22 000,00	Consumables to perform laboratory work APC	4 500,00	Meeting with project partners	0,00	
JRP03-R1-AMR3-RADAR	0,00		8 077,00	Lab consumables	0,00		0,00	
JRP05-R1-ET1-TOXDETECT	0,00		8 000,00	Maldi-ToF reagents and growth media, shipment of strains to partners APC	2 000,00	Consoritum meetings	0,00	
JRP06-R1-FBZ1-NOVA	0,00		3 500,00	Open access for one publication in international refereed journal (WP2 or WP3) APC	2 745,00	Consoritum meetings	0,00	
JRP07-R1-FBZ2-LISTADAPT	0,00		11 194,00	Laboratory consumables	2 300,00	Conference - Food Micro, IAFP or EJP Project meetings	0,00	
JRP09-R1-FBZ3-AIRSAMPLE	0,00		4 000,00	APC	2 024,00	Project meetings OHEJP ASM 2020	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		6 300,00	Lab supplies for sample analysis Lab reagents DNA isolation of faecal samples Lab reagents for sample/DNA storage until analysis Supplies for sample collection (pigs) Lab reagents for DNA isolation of bacterial isolates Article publication cost Administration costs	1 700,00	Kick-off meeting	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		13 200,00	Lab reagents for SMRT sequencing (incl. Proficiency test)	2 625,00	Workshop SMRT sequencing	0,00	
JRP16-R2-ET2.2-TELE-Vir	0,00		0,00		2 000,00	Kick-off meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP18-R2-ET1.1-MEmE	0,00		14 650,00	Consumables	3 050,00	Kick-off meeting Training (exchange of key staff members)	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		300,00	Shipment costs	1 400,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		21 993,00	Lab reagents for screening for STEC, isolation and WGS	1 400,00	Kick-off meeting	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		6 380,00	Labotaratory disposibles e.g. culture media, tuber and micrtiter plates, disinfectants for testing	1 400,00	Kick-off meeting Project meetings	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		1 200,00	Consumables	3 200,00	Kick-off meeting Workshop	0,00	
JRP24-R2-FBZ-BeONE	0,00		0,00		1 400,00	Kick-off meeting	0,00	
WP4	**0,00**		**17 000,00**		**23 368,00**		**0,00**	
JIP01-R1-IA1.1-ORION	0,00		12 000,00	Sequencing at a sequencing facility Contracting of compute/storage at a HPC facility APC	7 000,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		5 000,00	Contracting of compute resources and storage at a HPC facility APC	5 000,00	Annual COHESIVE meeting WP meeting Workshops	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		3 468,00	Kick off meeting Annual meeting	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		0,00		7 900,00	Kick-off meeting WP1 meeting WP planning meeting	0,00	
WP4–Management of Joint Integrative Projects	0,00		0,00		0,00		0,00	
P34-PIWET	**6 000,00**		**161 020,00**		**37 050,00**		**2 820,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**6 000,00**		**121 020,00**		**22 850,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 800,00	3 ASM 2020 FOC delegates	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP01-R1-AMR1-IMPART	0,00		12 000,00	Reagents, consumables, services APC	550,00	Project meeting	0,00	
JRP09-R1-FBZ3-AIRSAMPLE	0,00		4 600,00	Culture media and reagents for reference cultivation of Campylobacter. Disposable materials for bacteriological sampling and storage. Reagents for identification and typing of isolated Campylobacter. Cansumables such as laboratory plastics, gloves and other accessories. Cost of samples collection and shipments across the partner laboratories. Cost of DNA isolation and sequencing, data analysis.	1 500,00	Project meetings	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		14 000,00	Chemical reagents and analytical standards of antibiotics Disposables - Laboratory equipment (vials, tubes, filters, laboratory glass, blenders, equipment for sample storage, laboratory stirrer, needles, syringes, laboratory pipettes, cartridges) LC-MS/MS analytical columns and consumables for LC-MS/MS system Services of equipment required for samples analysis	1 100,00	Kick-off meeting	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		8 100,00	Lab reagents for SMRT sequencing (incl. Proficiency test) Disposables (i.e. plastics)	1 750,00	Workshop SMRT sequencing	0,00	
JRP16-R2-ET2.2-TELE-Vir	0,00		5 000,00	Analitical test reagents and kits Disposables (i.e. plastics)	1 000,00	Kick-off meeting	0,00	
JRP18-R2-ET1.1-MEmE	0,00		20 870,00	Analitical test reagents and kits Disposables (i.e. plastics) Services (i.e. sampling, shipment)	1 750,00	Kick-off meeting Training (exchange of key staff members)	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		300,00	Services (i.e. sampling, shipment)	1 100,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		7 150,00	Reagents Disposables Services Sequencing reagents	4 800,00	Kick-off meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP21-R2-FBZ3.1-BIOPIGEE	6 000,00	Centrifuge	16 500,00	Analitical test reagents and kits Disposables (i.e. plastics) Services (i.e. sampling, shipment) Sampling costs	1 400,00	Kick-off meeting	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		15 000,00	Survey Analitical test reagents and kits	3 600,00	Kick-off meeting Workshop	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		17 500,00	Analitical test reagents and kits Disposables (i.e. plastics) Services (i.e. sampling, shipment)	1 400,00	Kick-off meeting	0,00	
JRP24-R2-FBZ-BeONE	0,00		0,00		1 100,00	Kick-off meeting	0,00	
WP4	**0,00**		**40 000,00**		**11 600,00**		**2 820,00**	
JIP03-R2-IA2.3-CARE	0,00		40 000,00	Analitical test reagents and kits Disposables (i.e. plastics) Services (i.e. sampling, shipment)	1 400,00	Kick off meeting	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		3 000,00	Kick off meeting Annual meeting	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		0,00		7 200,00	Kick-off meeting WP-2 meeting WP-3 meeting Scandinavian-Baltic Society for Parasitology's congress (Lithuania 2021)	2 820,00	WP3 Workshop
P35-INIAV	**12 850,00**		**107 680,00**		**18 560,00**		**4 575,00**	
WP1	**0,00**		**0,00**		**1 900,00**		**4 575,00**	
WP1-Coordination and Management	0,00		0,00		1 900,00	SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	4 575,00	SSB meeting 4-2020 at INIAV (PT) M27
WP3	**12 850,00**		**95 180,00**		**13 660,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 800,00	3 ASM 2020 FOC delegates	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP17-R2-ET2.2-IDEMBRU	0,00		40 000,00	Reagents and consumables for serological and bacteriological diagnosis Reagents and consumables for molecular typing methods Reagents and consumables for molecular typing methods Reagents and consumables for sequencing Shipment of live emerging Brucella strains	1 400,00	Kick-off meeting	0,00	
JRP18-R2-ET1.1-MEmE	6 000,00	Thermocycler	13 000,00	Reagents and consumables	3 800,00	Kick-off meeting Training (exchange of key staff members)	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		4 500,00	Reagents and Consumables	1 100,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		16 000,00	Reagents and consumables for WGS Reagents and consumables for metagenomics WP3 (Task2)	1 760,00	Kick-off meeting	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		13 000,00	Survey	2 200,00	Kick-off meeting Workshop	0,00	
JRP23-R2-FBZSH5-ADONIS	6 850,00	MinION nanopore sequencing device Laptop computer for MinION	8 680,00	Reagents and consumables for WGS	1 600,00	Kick-off meeting	0,00	
WP4	**0,00**		**12 500,00**		**3 000,00**		**0,00**	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		12 500,00	Laboratory Consumables	3 000,00	Kick off meeting Annual meeting	0,00	
P36-INSA	**10 550,00**		**159 000,00**		**45 175,00**		**0,00**	
WP1	**0,00**		**0,00**		**5 500,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		5 500,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP3	**10 550,00**		**148 700,00**		**18 000,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 800,00	3 ASM 2020 FOC delegates	0,00	
JRP13-R2-AMR2-WORLDCOM	0,00		23 500,00	Lab reagents for Whole genome and target sequence of bacterial pathogenes and AMR genes Lab reagents for multiplex	2 050,00	Kick-off Meeting Biannual Meeting	0,00	
JRP15-R2-AMR2.1-FED-AMR	0,00		31 300,00	Lab reagents for metagenomics and WGS Lab costs for sequencing	3 400,00	Kick-off meeting	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		24 400,00	Lab reagents for SMRT sequencing (incl. Proficiency test) Lab reagents for MGE investigation	875,00	Workshop SMRT sequencing	0,00	
JRP17-R2-ET2.2-IDEMBRU	0,00		27 000,00	Reagents and consumables for molecular diagnosis Reagents and consumables for bacteriological diagnosis Reagents and consumables for molecular typing Reagents and consumables for whole genome sequencing Reagents and consumables for whole genome sequencing Reagents and consumables for MLVA and MLST	1 400,00	Kick-off meeting	0,00	
JRP16-R2-ET2.2-TELE-Vir	4 750,00	2 labtops	0,00		1 000,00	Kick-off meeting	0,00	
JRP18-R2-ET1.1-MEmE	0,00		2 000,00	Reagents and consumables	2 250,00	Kick-off meeting Training (exchange of key staff members)	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		20 000,00	Lab consumables for sampling, isolation and phenotypic characterization Lab costs for sequencing	2 025,00	Kick-off meeting	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		0,00		700,00	Kick-off meeting	0,00	
JRP23-R2-FBZSH5-ADONIS	0,00		20 500,00	Consumables; DNA extraction and quantification WGS Phenotypic tests	1 400,00	Kick-off meeting	0,00	
JRP24-R2-FBZ-BeONE	5 800,00	2 laptops with strong computational capacity for bioinformatics	0,00		1 100,00	Kick-off meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP4	**0,00**		**10 300,00**		**21 675,00**		**0,00**	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		4 000,00	Communication with national partners National network meetings Publication	6 675,00	Kick off meeting Annual meeting Participation in EJP annual scientific meeting	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		6 300,00	Contacts with national partners Actions to the project progress updates at AMR level Actions to the inventories of surveillance initiatives and frameworks at AMR level Actions to map the surveillance chain for hazard tracks, cross-sectorial linkages, and construction of practical guidelines and strategies for implementation of results, mainly at AMR level Actions to gather information and to designing schemes suitable for the specific environment, mainly at AMR level	13 500,00	Kick-off meeting Biannual meeting WP-1 meeting WP-2 meeting WP-2 meeting	0,00	
WP4–Management of Joint Integrative Projects	0,00		0,00		1 500,00	Project monitoring Annual JRP/JIP meeting in Brussels (BE)	0,00	
P37-IISPV	**0,00**		**0,00**		**3 800,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**0,00**		**1 200,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
P38-IC								
P39-SLV	**0,00**		**4 500,00**		**12 450,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**0,00**		**3 250,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP07-R1-FBZ2-LISTADAPT								
JRP19-R2-ET1.1-PARADISE	0,00		0,00		1 350,00	Kick-off meeting	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		0,00		700,00	Kick-off meeting	0,00	
WP4	**0,00**		**4 500,00**		**6 600,00**		**0,00**	
JIP02-R1-IA1.2-COHESIVE	0,00		2 000,00	APC	2 500,00	Annual COHESIVE meeting Workshops	0,00	
JIP03-R2-IA2.3-CARE	0,00		2 500,00	Delivery services Consumables (plastics etc) Substrates and reagents	1 100,00	Kick off meeting	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		3 000,00	Kick off meeting Annual meeting	0,00	
P40-FoHM	**0,00**		**29 017,00**		**18 610,00**		**0,00**	
WP1	**0,00**		**0,00**		**2 600,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		2 600,00	SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**15 850,00**		**4 180,00**		**0,00**	
ASM T&S-2020	0,00		0,00		1 200,00	2 ASM 2020 FOC delegates	0,00	
JRP06-R1-FBZ1-NOVA	0,00		2 000,00	APC	600,00	Consoritum meetings	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		11 350,00	Lab reagents for SMRT sequencing (incl. Proficiency test)	1 680,00	Workshop SMRT sequencing	0,00	
JRP19-R2-ET1.1-PARADISE	0,00		2 500,00	Reagents Shipping	700,00	Kick-off meeting	0,00	
WP4	**0,00**		**13 167,00**		**11 830,00**		**0,00**	
JIP01-R1-IA1.1-ORION	0,00		8 667,00	Software (eg licences) Contracted analysis APC	3 330,00	ORION consortium meeting Combined ORION WP meeting & integration workshop	0,00	
JIP02-R1-IA1.2-COHESIVE	0,00		2 000,00	APC	2 400,00	Annual COHESIVE meeting Closing symposium	0,00	
JIP03-R2-IA2.3-CARE	0,00		2 500,00	Delivery services Consumables (plastics etc) Substrates and reagents	1 900,00	Kick off meeting Planning meeting pilot PT	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		1 800,00	Kick off meeting Annual meeting	0,00	
JIP05-R2-IA2.1-MATRIX	0,00		0,00		2 400,00	Kick-off meeting Biannual meeting	0,00	
P41-SVA	**1 500,00**		**209 360,00**		**91 920,00**		**8 383,00**	
WP1	**0,00**		**0,00**		**5 500,00**		**0,00**	
WP1-Coordination and Management	0,00		0,00		5 500,00	PMT meeting 1 PMT meeting 2 PMT meeting 3 PMT meeting 4 SSB meeting 4-2020 at INIAV (PT) M27 SSB meeting 5-2020 at APHA (GB) M33 PMC meeting 2-2020 at ISS (IT) M29 for director CCP meeting	0,00	
WP3	**0,00**		**176 610,00**		**23 660,00**		**0,00**	
ASM T&S-2020	0,00		0,00		2 400,00	4 ASM 2020 FOC delegates	0,00	
JRP01-R1-AMR1-IMPART	0,00		3 500,00	Microdilution plates, antbiotcs, cultivation agar/medium, molecular microbiology, refrence strains, transporation,	3 000,00	Project meetings Conference	0,00	
JRP06-R1-FBZ1-NOVA	0,00		18 000,00	Conference fees Annual assembly Costs for mandatory revision APC	5 000,00	Consoritum meetings	0,00	
JRP08-R1-FBZ2-METASTAVA	0,00		10 000,00	Sequencing related costs APC	1 000,00	Project meeting, workshop and dissemination	0,00	
JRP14-R2-AMR2.1-FULL-FORCE	0,00		21 750,00	Lab reagents for SMRT sequencing (incl. Proficiency test)	3 510,00	Workshop SMRT sequencing	0,00	
JRP16-R2-ET2.2-TELE-Vir	0,00		18 000,00	Library preparation kits (12 kits)(Oxford Nanopore) Flowcells (12 pieces)(Oxford Nanopore) Utensiles Other reagents and enzymes	1 500,00	Kick-off meeting	0,00	
JRP18-R2-ET1.1-MEmE	0,00		7 250,00	Plastics, reagents and consumables	700,00	Kick-off meeting	0,00	

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
JRP19-R2-ET1.1-PARADISE	0,00		54 300,00	Reagents for parasite isolation and DNA extraction etc Shipping of samples for genome sequencing (to ANSES) Bioinformatic software Reagents for MLST scheme development. DNA extraction, PCR and Sanger seq etc Shipping of samples to other main laboratories in T3.2 Reagents for development of hybridization capture probes	1 400,00	Kick-off meeting	0,00	
JRP20-R2-FBZSH3-DISCoVeR	0,00		39 910,00	Reagents for isolation and sequencing	1 650,00	Kick-off meeting	0,00	
JRP21-R2-FBZ3.1-BIOPIGEE	0,00		3 900,00	Computer for PhD TBR Computer for Stefan Widgren Expenses for 10 farm visits including reimbursement for farmers	2 800,00	Kick-off meeting Project meetings	0,00	
JRP22-R2-FBZ4.1-TOXOSOURCES	0,00		0,00		700,00	Kick-off meeting	0,00	
WP4	**1 500,00**		**29 500,00**		**54 735,00**		**8 383,00**	
JIP01-R1-IA1.1-ORION	0,00		12 000,00	Adobe connect license Training materials Workshop organization (local, food, etc) APC	8 960,00	ORION consortium meeting Combined ORION WP meeting & integration workshop Veterinary Epidemiology and surveillance conference	0,00	
JIP02-R1-IA1.2-COHESIVE	1 500,00		12 000,00	Rental of workshop facilities, lunches and refreshments for workshop APC	10 000,00	Annual COHESIVE meeting WP meeting Workshops	0,00	
JIP03-R2-IA2.3-CARE	0,00		4 000,00	Laboratory consumables Delivery costs Sequencing and MALDI-TOF	3 900,00	Kick off meeting Planning meeting pilot PT EJP annual meeting	0,00	
JIP04-R2-IA2.2-OH-HARMONY CAP	0,00		0,00		2 250,00	Kick-off meeting	4 920,00	Organisation 2nd Y3 meeting
JIP05-R2-IA2.1-MATRIX	0,00		0,00		3 625,00	Kick-off meeting Biannual meeting	3 463,00	Organisation kick-off meeting

Étiquettes de lignes	Somme de Direct equipment costs	Justification	Somme de Direct other goods and services costs	Justification	Somme de Direct travel and subsistence costs	Justification	Somme de Direct meeting organisation costs	Justification
WP4–Management of Joint Integrative Projects	0,00		1 500,00	License Adobe Connect	26 000,00	Project monitoring Implementation of integrative missions Workshop organisation Annual JRP/JIP meeting	0,00	
WP6	**0,00**		**0,00**		**5 625,00**		**0,00**	
STM-2020	0,00		0,00		5 625,00	Travel and subsistence costs of the short mission	0,00	
WP7	**0,00**		**3 250,00**		**2 400,00**		**0,00**	
WP7-Sustainability	0,00		3 250,00	Tuition fees for the PhD student working on substitutability issues within the OHEJP project	2 400,00	Participation of the PhD student to various meetings and conferences	0,00	
GRAND TOTAL	**101 513,00**		**3 759 794,67**		**1 226 239,67**		**320 710,00**	

3 Planned activities for the fourth year

3.1 WP1

Task 1.1: Management of EC contractual obligations
The CT will supervise the preparation and submission of the deliverables due from M37 to M48 to the REA. At the beginning of the period the CT will carry out the annual technical and financial reporting. Also, the CT will coordinate the preparation, compilation and submission to the EC of the Summary Progress Report (SPR) for year 4 and the Annual Work Plan (AWP) for Year 5. The CT will also coordinate and manage the fourth Amendments to the Grant Agreement. All the above activities will be carried out in liaison with the REA project officers.

Task 1.2 Project management
The CT members will exchange on a weekly basis via teleconferences to ensure that administrative, financial and contractual requirements are met. The CT and the PMT will have monthly teleconference to ensure a smooth management and coordination of the WP activities. The CT will regularly liaise with the REA project officers and report any issue that may arise.

Task 1.3: Organisation of EJP management and governance meetings
The Support Team (ST) will ensure the organisation of all the project governance bodies meetings in regards to the identified planning. There will be 2 SSB, 3 PMT, 1 POC and 1 PMC meetings organised.

Task 1.4: Communication tools
Subtask 1.4.0: Communication Groups: Relationships with the CCP network will continue to be developed in the fourth year. A face-to-face meeting with the CCP network will be organised again in the fourth year. The CCP mandate will be in place and therefore the roles and responsibilities of the CCPs will be clear, which will therefore improve communication across the consortium.

Subtask 1.4.1: Website: The website will continue to be developed throughout the lifetime of the OHEJP to meet the needs of the consortium and the OHEJP stakeholders. Content for all of the pages will be updated on a regular basis. In particular the Education and Training, news and events and Joint Research and Joint Integrative pages will be closely monitored to ensure that the content is up to date and clearly showcases the events throughout the One Health EJP. The users experience of the website will also be monitored and addressed accordingly.

Subtask 1.4.2: Internal communications: Newsletters: Consortium newsletters will be disseminated every three months in February, May, August and November 2021. These newsletters will provide updates regarding events, meetings, JIP/JRP progress, PhD project progress, funding opportunities and any other information that the consortium requests the Communications Officer disseminates. These newsletters will continue to be sent using MailChimp which allows the Communications Officer to monitor the success of each newsletter. Additionally, this service facilitates the monitoring of mailing lists. The external newsletters will be disseminated every six months in June and December 2021. This newsletter will be sent to the consortium, in addition to the external mailing list generated on the One Health EJP website. These newsletters will be targeted to a wider audience, including the general public.

Subtask 1.4.3: External Communications: Press Releases: Press releases for the One Health EJP ASM will be published each year (these are the responsibility of the local organisers). This press release will also be published on the One Health EJP website and advertised on social media platforms. If press releases for other events, such as WP6 events are appropriate, these will also be the responsibility of the local

organisers and will be advertised by the Communications Officer on the OHEJP website and social media platforms.

Subtask 1.4.4: External Communications: Professional social networks: The One Health EJP social medial platforms and the One Health EJP website continue to grow in popularity and are continuously monitored to ensure that this success continues to increase. The key objectives of the social media platforms are to increase the number of followers, engagements and impressions from the content posted. Advertising and live tweeting of One Health EJP events such as the ASM, meetings, events and funding opportunities will raise the profile of the One Health EJP and encourage engagement online. A key focus will be to raise the profile of the OHEJP events and encourage participants of events, WP6 activities, the ASM etc to actively tweet and engage on social media during events. Furthermore, to use Twitter and LinkedIn to post about their experiences of these events.

Subtask 1.4.6: External Communications: Merchandise and Branding: The Communications Officer will continue to increase the visibility of the One Health EJP brand. This will be achieved through close communication with JIP/JRP project leaders, PhD supervisors, PMT and the consortium to ensure that the branding of any outputs meets the needs of the OHEJP. Merchandise will continue to be sent to our partner institutes and the ASM and other OHEJP events will be excellent platforms to share our merchandise. Merchandise should also be offered at the OHEJP Summer School, CPD workshop, ASM Satellite workshop, ASM and all meetings across the consortium.

Subtask 1.4.7: Annual and final report: The annual report for Year 4 will be completed in this period. The content for this report will be created by the Communication Officer and validated with PMT before being sent to an external communication company to create an attractive report with the content provided.

3.2 WP2

WP2 will continue its activities to provide input for the development of the strategic research and innovation agenda (SRIA) in WP7. Relevant scientific developments, including the emergence of zoonotic threats and opportunities for scientific innovations will be monitored. For this, the output from OHEJP projects (e.g. deliverables, scientific publications) will be screened as well as relevant information from external sources. Furthermore, strategic interactions with other EU projects and initiatives, such as JPIAMR and JAMRAI, will be further explored and encouraged, with the aim to create synergies and avoid duplication (also through cogwheels workshops, WP4). Moreover, the web based database of relevant EU research projects and related initiatives will be regularly updated.

3.3 WP3

Task 3.1: Drawing up of guidelines for submission, selection and evaluation of JRP proposals as well as request of extension of accepted JRPs. Task with input from WP4 (Joint Integrative Projects) and with WP6 (Education and Training)
All guidelines concerning WP3 have been delivered in Y1 and Y2

Task 3.2: Supervision of the JRP in the first round of projects
Subtask 3.2.1 Periodic follow up of activities in the JRP consortia
As in Y1, Y2 and Y3, WP3 team will continue to have a close formal and informal contact with project leaders, i.e. through videoconferences or via their consortium meetings. This subtask helps in creating constructive collaborations among partners and thus facilitate integration.

Subtask 3.2.2 Follow-up of recently started projects
This task will be finalised in Y3.

Subtask 3.2.3: Mid- and end-term evaluations; monitoring of the KPI, deliverables and milestones detailed in the annual JRP reports
As each year, project leaders of first and second call projects will be requested to submit reports, i.e. the final reports for 3 year projects from the first call, possibly after having received a 6 month extension (i.e. subtask 3.2.4), and the 9- and 12-months reports from the second call projects.
All reports will be discussed in the SSB and validated by the Programme Managers Committee in the autumn of Y4.

Subtask 3.2.4: End-term evaluations by external experts: final report
See Subtask 3.2.3.

Task 3.3: Organisation of a second round of projects and their supervision. Task with help from WP4.
This task and its subtasks is finalised in Y2.

Task 3.4: Organisation of annual scientific meetings (ASM) where results from JRP are presented
Subtask 3.4.1: Protocol for organisation of annual scientific meetings
This subtask is finalised in Y1.

Subtask 3.4.2: Organisation of ASM, scientific part
The organisation of the ASM2021 will have started in 2020 and that of the meeting in 2022 should take a start in Y4. Most of the practical issues will be arranged in Y3.

3.4 WP4

During the fourth year, the WP4 commits to the following:

- To continue supervision of implementation and progress of JIPs, and evaluation of the merit of ongoing JIPs from first call of the EJP, following previously elaborated guidelines for evaluation of JIPs. To recognize opportunities to discontinue or redirect activities in JIPs from second call, based on the outcome of evaluations.
- To continue facilitation of internal integration of the resources developed, and support alignment with other similar activities in EC member states in collaboration with WP2, WP5 and WP6. To avoid redundancies and make use of previous and ongoing developments.
- To maintain integration of JIP output among EJP beneficiaries, namely of who are not primary participants in the respective JIPs at the time when a funding decision is taken.
- To continue the support of implementation of data management plan developed in each JIP project.
- To maintain continuous encouragement for networking among JIP and JRP partners, namely organizing the 7 th and 8th cogwheel workshops to allow key actors and relevant partners within the EJP from JRPs or JIPs projects to identify synergies, joint priorities and opportunities for collaboration.
- To continue support function for integration of additional partners in ongoing JIPs, through new integrative missions.
- To organize the 4th thematic integrative meeting between JRPs and/or JIP, to facilitate integration across domains, within themes, apart from ASMs, that will be in collaboration with WP3.
- 3rd periodic report on ongoing JIPs (M37)

- Report from 7th cogwheel workshop (M40)
- Report on evaluation of finalised JIPs, 1st round (M42)
- Report from 8th cogwheel workshop (M46)
- Report from 4th thematic meeting (M48)
- Simulation exercise (proposed)

3.5 WP5

During Y4, WP5 will continue the close interaction with the Key EU stakeholders and strengthen the interaction with the national as well as international stakeholders. The dissemination strategy will be discussed, continuously amended and activities broadened, and thus used to efficiently disseminate the outcomes of the One Health EJP to the various stakeholders. Preparations for the Stakeholder Conference (Y5) will start. Sustainability will be discussed with WP7. Input for the SRIA will be given from the work performed on stakeholders needs.

3.6 WP6

During the fourth year, the WP6 will co-ordinate the following activities:

- Work Package 6 will continue to coordinate, support and monitor the 16 PhD studentships which were selected to be funded in Y1 and Y2. WP6 will routinely liaise with the Principal Investigators (PIs) awarded the funding and their respective PhD students to ensure WP6 receives the relevant project progress updates, and any dissemination activities are reported on our website and are also reported to the coordination team via the Internal Events Survey. To report the progress of the project, PhD students will submit a summary report to WP6 on an annual basis. As the projects progress, PhD students will also be asked to participate in smaller activities including encouraging the use of the private space of the website, showcasing their projects at the ASM every year through various styles of presentations, getting involved in informal events at the ASM and contributing content to their dedicated project page on the website and for the OHEJP social media channels to raise the profiles of the PhD student cohort and their achievements. The PhD students will join students attending or participating in the remaining WP6 activities to build a new generation of 'One Health' scientists.

- WP6 will also monitor the progress of the STMs selected in Y3. These missions will take place throughout Y4, and once the missions are completed, individual reports will be produced. These reports will be used to inform the content for both Deliverable 6.14 and the webpage dedicated for STMs in 2021.

- WP6 will also review and amend the protocol (if necessary) for selecting the STMs for Y5. WP6 will then co-ordinate this call following the validated steps, and will select the successful awardees of the STM funding. This will be communicated with the consortium as described on the protocol, and the website will be updated with a webpage dedicated for STMs in 2022.

- WP6 will also review and amend the protocols (if necessary) for selecting the organisers of the fourth and final satellite workshop, Summer School and CPD module for Y5. WP6 will then co-ordinate the call ensuring the validated steps described in the protocol are followed.

- WP6 will support the organisers for the third ASM Satellite Workshop, Summer school selected in Y3. These events will be held in the fourth year.
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- WP6 will produce the deliverable reports for D6.10, D6.11, D6.12, and D6.13.

3.7 WP7

Among the WP7 activities planned for the 4th year are the following:

- Refinement of the survey on stakeholders needs and expectations, by extending it to non-EU bodies.

- Comtinuous monitoring and assessment of funding opportunities, within and beyond Horizon Europe, also exploiting the leverage effect of the EJP ativities and outputs.

- Drafting of sustainability plan for aftermath sustainability, to be challenging by the Consortium governance and stakeholders.

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