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Annual Report 2021
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Report prepared by the OHEJP Communications Team at the University of Surrey
The One Health European Joint Programme (OHEJP) is a landmark partnership between 43 public health, animal health and food organisations and the Med-Vet-Net Association, a European Network of Excellence for Zoonoses research; the OHEJP spans 22 countries across Europe. It was launched in January 2018.

The main focus of the OHEJP is to reinforce collaboration between partners by enhancing collaboration and integration of activities by means of dedicated Joint Integrative Projects (JIPs), Joint Research Projects (JRPs), and through education and training in the fields of foodborne zoonoses, antimicrobial resistance and emerging infectious disease threats.

Through the OHEJP, there are opportunities for harmonisation of approaches, methodologies, databases and procedures for the assessment and management of foodborne zoonoses, emerging infectious disease threats and antimicrobial resistance across Europe, which will improve the quality and compatibility of shared information for policy decision making.

Although mainly covering animal health, public health and food safety, the OHEJP is set up in line with and supporting the One Health approach. The One Health concept recognises that human health is tightly connected to the health of animals and the environment, therefore the study of infectious diseases that may cross species and environmental barriers is imperative. One Health is an integrated, unifying approach that aims to sustainably balance and optimise the health of people, animals, and ecosystems. It recognises the health of humans, domestic and wild animals, plants and the wider environment (including ecosystems) are closely linked and inter-dependent.
OUR OBJECTIVES

The overarching objective of the OHEJP is to develop a collaborative European network of public research organisations with reference laboratory functions.

A key aim of the OHEJP is to integrate medical, veterinary and food scientists to address three key research domains: foodborne zoonoses, antimicrobial resistance and emerging infectious disease threats. Public health concerns of consumers and other stakeholders are also at the forefront of the consortium’s focus.

Key objectives:

• To bring together the major representatives of European scientific communities with expertise in foodborne zoonoses, antimicrobial resistance and emerging infectious disease threats.
• To implement scientific integrative and collaborative projects related to the prevention of foodborne zoonoses, antimicrobial resistance and emerging infectious disease threats.
• To stimulate scientific excellence by co-funding Joint Research Projects and Joint Integrative Projects that have the potential to enhance scientific knowledge and provide tools for disease surveillance at both the national and European level.
• To foster the harmonisation and standardisation of laboratory methods by bringing together scientific and technical expertise.
• To exchange and communicate with European and international stakeholders, first and foremost with the European Centre for Disease Control and Prevention (ECDC) and the European Food Safety Authority (EFSA).
ONE HEALTH EJP GOVERNANCE

A governing and management system was established at the beginning of the OHEJP, in January 2018.

The governing boards specific to the OHEJP include: The Project Management Team (PMT), Scientific Steering Board (SSB) and Programme Managers Committee (PMC).

There are also important contributions from members outside of the OHEJP and these include: The Programme Owners Committee (POC), the External Scientific Advisory Board (ESAB), the Stakeholders Committee (SC), the Ethics Advisors and National Mirror Groups.

The OHEJP Coordination Team are based at the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France.

The OHEJP Scientific Coordinator resides at Sciensano, the Belgian Institute for Health.

The Project Management Team consists of all the Work Package (WP) Leaders and Deputy Leaders.
The OHEJP consist of seven work packages (WP), all are targeted towards specific overarching needs and objectives of the OHEJP. Each work package ensures the alignment and integration in the implementation of the project. Visit the One Health EJP website for more information about the different work packages.

Coordination - Work Package 1
WP1 enables the successful functioning of the OHEJP and maintains the environment where scientists can effectively and actively collaborate.

Strategic Research Agenda - Work Package 2
WP2 is responsible for the Integrative Strategic Research Agenda of the OHEJP, which identifies research and integrative priority topics aligning to stakeholder needs.

Joint Research Projects (JRs) - Work Package 3
WP3 mainly supports the Joint Research Projects which carry out jointly prioritised research projects and stimulate collaboration and harmonisation across the projects and partner institutes.

Joint Integrative Projects (JIPs) - Work Package 4
WP4 is responsible for organising selection, supervision and evaluation of the Joint Integrative Projects and stimulating harmonisation across partner institutes and with other ongoing EU initiatives.

Science to Policy Translation - Work Package 5
WP5 ensures best use of the outcomes of the OHEJP through dissemination activities and dialogue with OHEJP stakeholders.

Education and Training - Work Package 6
WP6 develops and delivers innovative training platforms with a specific focus on One Health.

Sustainability - Work Package 7
WP7 explores operational means to sustain long-term research and innovation beyond the duration of the OHEJP.
What has the One Health EJP achieved in year four?
COORDINATION OF THE ONE HEALTH EJP

The coordination of the OHEJP involves overseeing the organisation (WP1), coordinating the Joint Integrative and Joint Research Projects (WP3 and WP4 respectively) and coordinating the Education and Training activities (WP6), in addition to carrying out all central communication activities.

What were the key coordination activities in year four?

• Communicating and collaborating with the OHEJP governance bodies: Coordination Team, Project Management Team, Scientific Steering Board, Programme Managers Committee and Programme Owners Committee.

• Successful and proactive monitoring of the OHEJP’s progress and reporting of scientific outcomes to the European Commission (EC) and Research Executive Agency (REA).

• Expansion of the OHEJP Consortium to now include 44 partners across 22 European countries, which facilitates more public health/animal health partnerships within EU member states.

• Funding extensions that had been granted to JIPs and JRP s due to to the COVID-19 pandemic enabled research to be successfully completed for 7 projects in 2021.

• The additional JIP, COVRIN, started in March 2021 that integrates research activities on SARS-CoV-2 across project partners.

• Continued coordination and communication to inform internal and external audiences of the OHEJP’s role and the One Health approach to the COVID-19 pandemic.

• Within the Work Package dealing with the Joint Integrative Projects WP4, the subtask of the One Health EJP SimEx was proposed to enable all partner institutes to participate in the conduction of a national-level One Health exercise during 2022.

• Ongoing support for the JIPs, JRP s, Education and Training activities and other OHEJP scientific events.

• Consortium member institutes organised the Annual Scientific Meeting (hybrid event – online and in Copenhagen), ASM Satellite Workshop - Online Software Fair, Summer School (online event), CPD Module - Digital innovations for One Health practitioners (online event). Four Short Term Missions were awarded. Whilst two were completed in 2021, one was postponed to 2022 and one was cancelled.

• Key OHEJP deliverables were submitted on time to the REA.

• The Communications Team have continued to develop and enhance communication throughout the consortium to stakeholders and external audiences. The OHEJP brand, website and social media platforms were used to inform audiences of all joint successes.
COMMUNICATING SUCCESS

The Communications Team sits centrally in the OHEJP Consortium in the Coordination Team (WP1), delivering communications and coordinating activities effectively to ensure the OHEJP achieves its goals and fulfils its potential.

The OHEJP Communications Team successfully contributed their expertise in year four:

- The OHEJP brand was strengthened by continuing to improve visibility across all platforms including meetings, workshops, Education and Training events, the OHEJP website and social media, the latter highlighted by the doubling of followers on Twitter and LinkedIn.
- Supported all OHEJP events, which were successfully conducted as either online or hybrid events in response to the COVID-19 pandemic.
- Further developed communication and dissemination tools for consortium members to support the dissemination of scientific outcomes and demonstrate impact.
- Supported OHEJP scientists in promoting the outputs and potential impacts of their research by disseminating this information on social media posts, internal and external newsletters, the website, and key documents.
- Created several interactive documents, including the first project brochure for METASTAVA, and provided case studies to showcase the OHEJP to scientific and non-scientific audiences across the globe.
- Highlighted the work of OHEJP PhD students by creating social media and blog posts about their activities and providing information in newsletters, including the 3-minute thesis competition and links to their publications. The #OHEJPphdlife campaign was launched in late 2021 to showcase a day in the life of individual PhD students.
- Provided a key presentation on “Communication in One Health” at the Summer School.
- Maintained the OHEJP Zenodo account to ensure that publications and deliverables are open access.
A key aim of the OHEJP is to identify Stakeholders’ needs to inform the Strategic Research Agenda (WP2), which ensures the scientific outcomes are useful, and the Strategic Research and Innovation Agenda (WP7), which supports the sustainability of the consortium. The impact of the OHEJP’s outcomes is maximised by targeted dissemination and dialogue with OHEJP Stakeholders (WP5).

What were the key outcomes for year four?

- Consolidated relationships with key EU stakeholders ECDC and EFSA, and other European and global stakeholders: European Environment Agency (EEA), European Medicines Agency (EMA), Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and the World Health Organization regional office for Europe (WHO-EURO). These stakeholders joined the bi-annual Stakeholders Committee meetings to discuss the impact and sustainability of the OHEJP.
- The public database OHEJP Outcome Inventory, which documents and widely shares all outcomes from OHEJP projects, was updated, and the user interface was improved.
- A series of Dissemination Workshops targeted at policy and decision makers at the national level, was initiated. These workshops depict the practical use of OHEJP-developed solutions, and the benefits that they are having in Europe.
- The ‘going global’ strategy was promoted, recognising the impact that OHEJP outcomes could have outside of Europe. This resulted, for example, in support to the WHO-Global Outbreak Alert and Response Network (GOARN) and to the Preventing Zoonotic Diseases Emergence (PREZODE) initiative.
- Strategic Research and Innovation Agenda is under development, which aims to define research needs beyond the lifetime of the OHEJP.
One Health EJP Scientific Outcomes
ONE HEALTH EJP SCIENTIFIC OUTCOMES

To date, the One Health EJP has co-funded 6 Joint Integrative Projects (JIPs), 24 Joint Research Projects (JPRs) and 17 PhD projects. In 2021, Continuing Professional Development Module, Summer School, the Annual Scientific Meeting, and the Annual Scientific Meeting Satellite Workshop and other workshops were organised. For 2021, four Short Term Missions were also awarded.

What were the key outcomes in the third year?

- The 6-month extensions granted to some projects enabled their successful completion despite the challenges due to the COVID-19 pandemic.
- The following JIPs were completed in 2021: ORION in June 2021 and COHESIVE in December 2021.
- The following JPRs were completed in June 2021: ARDIG, TOX-Detect, NOVA, ListAdapt and MoMIR-PPC.
- Established procedures continued to be followed to ensure that data and publications were open access.
- Over 60 peer-reviewed publications were published.
- The JIP COVRIN “SARS-CoV2 Research Integration and Preparedness” started in March 2021.
- The Third One Health EJP Annual Scientific Meeting was an extremely well-attended hybrid event with over 500 participants.
- One Health EJP PhD students’ research was showcased in the second Three-Minute Thesis (3MT) competition, the first-time students gave these presentations in a hybrid format.
- The One Health EJP Satellite Workshop as an Online Software Fair, delivered 7th June 202, was a very successful online event with over 50 international attendees.
- The first in a series of Dissemination Workshops took place on 27th October 2021. The topic was on Metagenomics, which was well-received by 143 participants. One Health EJP researchers discussed how their novel tools were practically applied in the field and explaining their benefits and impacts.
ORION - ended June 2021

The primary aim of the ORION project was to establish and strengthen collaboration and cross-disciplinary knowledge transfer to promote One Health disease surveillance. The ORION project consisted of 13 partners from veterinary and public health institutes from seven European countries.

The ORION project progressed to develop and optimise One Health resources, which were evaluated in several national One Health pilots and in a supra-national pilot with EFSA and ECDC. One main outcome of the ORION project was the creation and implementation the “One Health Surveillance (OHS) Codex” - a framework to provide users with guidance and resources that improve collaboration, mutual understanding, and knowledge exchange between the different OHS sectors. The OHS Codex was made available as a continuously updateable online resource here. An extendable OHS Codex structure was designed to cover four main principles, with each principle representing an area where stakeholders could implement specific actions for adopting the One Health paradigm. The OHS Codex integrated a broad spectrum of innovative solutions, resources, and findings (including lessons learned from national pilots) into one overarching framework. Some key resources encompassed in the OHS Codex include the One Health Report Annotation Checklist (OH-CRAC), National OHS Report Templates, the One Health EJP Glossary, an OHS Inspiration catalogue, the OHS Pathway Visualisation, the One Health Knowledge Base – Surveillance systems, the Sequencing for Surveillance (SfS) Handbook, the Health Surveillance Ontology and the One Health Linked Data Toolbox (OHLDT).

In several national One Health pilots, country specific solutions were established that created direct impact on cross-sector communication, collaboration, surveillance data exchange and interpretation. In the WP2-NGS pilot, the first cross-sector IRIDA NGS data analysis platform was established at the Norwegian Research and Education Cloud. This led to an increased collaboration and information exchange between the veterinary and public health sectors in Norway. The Danish pilots contributed to improved One Health reporting e.g., in the Salmonella and Campylobacter chapter in DANMAP 2019 report, the Danish national surveillance system for AMR and AMU and the establishment of a sequence-based Campylobacter surveillance system to be extended into a platform for real-time surveillance data and result sharing. The Swedish pilot led to the implementation of revised processes for generating the annual surveillance reports on zoonotic diseases, which improved cross-sectoral interoperability and FAIRness of surveillance results.
On international level, the ORION project performed various activities to share knowledge with international stakeholders (e.g., EFSA and ECDC), with involvement in dedicated pilot projects. Several ORION solutions were integrated into the Surveillance and Information Sharing Operational Tool (SISOT) of WHO/FAO/OIE. Dissemination continued with members of the ORION project presenting research results at the international conferences of ASM2021, the 6th World One Health Congress and the 39th meeting of the Scientific Network for Zoonoses monitoring data (EFSA) and conducting several webinars. Members also organised international workshops with other research projects and institutes, including the NGS workshop 2020, the One Health EJP CPD2021 module, the ASM2021 Satellite Workshop Software Fair. Eight scientific papers were published in peer-reviewed journals and several other publications are in preparation.

The ORION project contributed to the OHEJP Data Management Plan committee and initiated collaborations with other OHEJP projects (e.g., MATRIX, COHESIVE, RADAR, NOVA, BeONE), initiatives (e.g., RAKIP, SISOT, IRIDA, PAHO-WHO workshops) and both national and international research and development projects (e.g., SafeConsume, SEQ-TECH, SIGMA, COMBACTE-MAGNET EPI-Net network).

**JOINT INTEGRATIVE PROJECTS**

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COHESIVE - ended December 2021

The mission of the COHESIVE project was to develop sustainable One Health approaches for signaling, assessing, and controlling zoonoses at the national and regional level within EU countries and across borders.

The goal to support countries with setting up or strengthening human-veterinary-food-environmental collaborations, with the aim to improve the organisation of One Health signaling, risk assessment and response to zoonoses, has been achieved. This involved the development of a web-based guideline ‘One Health: Setting up a risk analysis system for zoonoses’. In the future, it will be coupled to the MedVetNet Association to maintain this resource. The guideline provides a stepwise approach to implement a complete or partial One Health risk analysis system (OHRAS). It gives information on the organisation of One Health activities (signaling, risk assessment, feasibility assessment, risk management and risk communication), input for Terms of References, suggestions for tools to use, shared experiences, and guidance on how to handle specific barriers. Several pilots were organised in countries to test the guidelines and gain feedback, with the pilots reaching step 3 (out of 5) of the implementation process in Norway and Portugal. Their impacts have led to cross-sectoral collaborations, built trust and relations, and created dialogue for improving the current situation.

Interviews with professionals working within public health, animal health and food safety were performed in six European countries, with the aim of identifying factors that contribute to well-functioning processes for sharing early signals of zoonotic events within and between countries. The interview findings highlighted the importance of informal personal contacts and the need for systems and solutions to data sharing. This scientific approach confirms the outcomes of the needs assessment be done before developing the guidelines. Since the identification of potential risk(s) is essential for One Health risk analysis, information may be obtained from horizon scanning (a technique for detecting developments through examination of potential threats), which led to COHESIVE developing an inventory and assessment of tools for horizon scanning. Two horizon scanning pilot exercises were conducted that identified potential drivers for emerging zoonotic risks, including an ageing population, global trade, increased personal travel and new consumption habits.

The cross-disciplinary dissemination of information between countries on the signals of zoonoses was delivered by COHESIVE in three digital workshops, which discussed issues around topics such as Brucella canis and zoonoses in pet rats. An informal group has been formed which aims to continue with this activity after the COHESIVE project ends.
JOINT INTEGRATIVE PROJECTS

Key outputs from the COHESIVE project have included several free and open-source web tools to improve the efficiency and detail of risk analysis, including risk assessment and outbreak management. Firstly, the development of the prototype COHESIVE Information System (CIS) that helps to integrate pathogen information (whole genome sequencing data and related metadata) from the human and veterinary sectors. The CIS provides graphical and interactive dashboards where spatial and temporal maps are combined with World Geodetic System analysis results beneficial for risk assessments. Three feasibility studies of such integration have been conducted among institutes in Italy, The Netherlands and Norway with demonstration versions of CIS available.

Secondly, the tracing web portal FoodChain-Lab web application (FCL Web) is a joint output of OHEJP COHESIVE and EFSA projects that helps to trace suspicious food items along complex global supply chains during foodborne incidents. It unifies tracing data visualisation, analysis and reporting in one modular framework, as used by several food safety authorities.

The third web application is shiny Rrisk, which supports risk assessors in conducting quantitative risk assessments and is part of the European Food Risk Assessment Training Programme (EU-FORA). Since there are many methods available for risk assessments, the development of an online decision support tool helps users decide on the most appropriate risk assessment method to use given their specific situation.

The COHESIVE project conducted a systematic review of economic analysis for foodborne zoonoses to better understand how economic factors can included in decision-making for conducting a risk analysis. This review categorised a range of economic analysis approaches and described advantages and disadvantages of each. The collation of this information will help improve and standardise future economic analyses.

A key aim of the COHESIVE project was to learn from past experiences with respect to zoonotic outbreaks. Q-fever in the UK was chosen to be retrospectively analysed and all UK-government publications relating to Q-fever were compiled and their data-sources tracked and mapped. A general European-level Q-fever surveillance map has been made in a similar way, using input from consortium members and available online publications. Process mapping through interviews with response teams following an outbreak was conducted to understand the reporting of Q-fever cases in the UK, which identified several gaps in the reporting structure. This information supported the designation of Q-fever as a reportable disease in the UK in 2021.
Several activities were performed within COHESIVE to share knowledge and collaborate with international stakeholders, such as EFSA, ECDC, FAO, WHO and OIE. EFSA was actively involved in the development of CIS, FCL Web and shiny Rrisk tools. The project worked closely with the Tripartite (FAO, WHO and OIE) who are developing the Surveillance and Information Sharing Operational Toolbox (SISOT). Several COHESIVE tools are integrated in this toolbox.

Overall, the COHESIVE project has disseminated acquired knowledge, developed useful tools, and built capacity both within and between countries. There is a broad application for these results and tools, with some already being used in practice, e.g., shiny Rrisk tool at BfR, the CIS in Italy and FCL Web internationally. The COHESIVE project has promoted the importance of sustainable One Health approaches in risk analysis and will be continued by other projects (e.g., MATRIX and BeOne) in conjunction with implementation activities.

**JOINT INTEGRATIVE PROJECTS**

Publications in 2021:


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CARE - year 2

The CARE project aims to develop new One Health concepts for External Quality Assurance (EQA) schemes for laboratories, reference materials, and quality and availability of demographic data.

During 2021, the CARE project updated and adjusted its activities to meet the deliverables and milestones. In 2021, three new partners joined the CARE consortium: ISCIII in Spain, BIOR in Latvia, and RUOKA in Finland. The quarterly meeting with all partners now includes participation by representatives of OHEJP MATRIX and EJP OH-HARMONY-CAP. The two lead organisations have participated in integrative activities, thereby promoting, and disseminating information about the OHEJP and related activities.

The coordination work focused on the first cross sectorial culture-based pilot EQA, which has been successfully executed. The EQA materials were dispatched from Sweden on 12th April 2021 and the results are being assessed with drafting of a report in process. The second pilot EQA, on typing/characterisation based on whole genome shotgun sequencing (WGS) has been re-scheduled, with the deadline for submitting the results now postponed. The third EQA on outbreak surveillance based on Whole Genome Sequencing data, has also been re-scheduled.

The inventory of available reference material has been finalised based on the need assessment document, which included more than 2500 reference material strains. This inventory is being converted into a database for which a web interphase is being developed to ensure accessibility and visibility of the reference material. Currently, the strategic research agenda and joint scientific projects are focused on the extended analyses of the identified genomes for antimicrobial resistance, virulence and other typing regimes, and the isolates for identification by MALDI-TOF. Institute Pasteur already has half of the genomes and links are in process for them to be stored in a public repository.

The planned risk assessment activities for the CARE project have progressed. More than 50 individuals from various institutions provided feedback for the risk assessment survey on relevant (meta-)data in relation to quantitative microbial risk assessments for science to policy translation. This information contributed to the quantitative microbial risk assessment analysis on data originating from the last five years and provided by 19 OHEJP members or stakeholders, including those from ORION and RADAR. The identified (meta-)data was shared with the strategic research agenda, the joint research projects and stakeholders to assist with increasing the accessibility of data and avoiding duplication. Current work includes developing a user guide for accessing relevant data for risk assessment and producing the R software suite package, rStain Select, to ease the process of selecting suitable reference strains.
based on exposure and risks. The intended outcomes for the user guide and R software are to contribute to and support the strategy developed by EU authorities to raise the awareness of relevant reference material microbial risk assessment analysis. The CARE project has discussed with the EFSA what type of data is necessary for risk assessment to be taken into consideration.

Future dissemination activities are planned. A joint venture application has been made for hosting the third One Health CPD module with the theme, Rapid diagnostics and harmonisation of diagnostic tests, in Autumn 2022. This year, OHEJP JIPs OH-HARMONY-CAP, CARE and MATRIX have launched a thematic call for original publications in the domain of integrated One Health surveillance in the Journal Frontiers in Public Health. This will highlight some of the intermediate achievements of these JIPs with a special focus on the integrative aspects of the projects’ methods and strategies.
OH-HARMONY-CAP - year 2

The **OH-HARMONY-CAP** aims to collect information on current capabilities, capacities, interoperability, and adaptability at both the National Reference Laboratory (NRL) and the primary diagnostic level, across Europe by developing an in-depth OHLabCap survey. The quantitative description of current and best practices and the development of harmonised protocols will identify the gaps and suggest future studies on how to best detect and characterise foodborne pathogens across the One Health sectors.

The project has been extended to December 2022, which enables participating partners to be able to test the chosen harmonised protocols. Additionally, OH-HARMONY-CAP has welcomed three new partners to the consortium, and it presently comprises of 18 partner institutes across 14 countries.

A pilot survey on current practices regarding capabilities, capacities and interoperability was previously conducted and a report was compiled. This information enabled the development of the benchmarking 59 question OHLabCap instrument, a Europe-wide in-depth survey on One Health laboratory interoperability, capability, and performance. This was launched in May 2021 by distributing it via several networks with participants activated in their network. The OHLabCap survey received 122 responses and now 32-FHI is analysing the responses with the preliminary data already presented to the consortium. Instead of three dimensions and 10 targets, the analyses will use four dimensions (adaptability, capability, capacity, and interoperability) and 15 targets to quantify the data.

The OH-HARMONY-CAP has identified a gap regarding sampling and analyses that are performed on behalf of our food business operators HACCP-based self-control programmes. This has led to the inclusion of an additional deliverable, with 39-SLV to organise a survey of this sector.

The project developed and launched an anonymous nine question survey using the EUSurvey tool. Surveys of relevant laboratories were designed to collate key information on current characterisation practices of chosen model pathogens. The relevant data was analysed by 26-Teagasc and 21-APHA to inform the second and third summary report on characterisation and data management. The technical report on the characterisation of methods used for Shiga-toxin-producing *Escherichia coli* (STEC) and Enterotoxigenic *Escherichia coli* (ETEC), *Cryptosporidium* and antimicrobial resistance in *Salmonella* and *Campylobacter* spp. in the European Union has been concluded.

The OH-HARMONY-CAP has established what characterisation, of the target pathogens is performed in public health, veterinary and food/feed/environmental testing laboratories throughout the EU.
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Also, harmonised this work to ensure the data required for food safety policy development and more efficient risk assessment/management is supported in the future. It examined current and best practice in the One Health sectors (public health, veterinary & food/environment testing labs) in ‘Sampling & testing’, ‘Characterisation of isolates’ and ‘Data management & harmonised reporting’ including the identification of current knowledge gaps and propose new studies and/or methods to fill them.

The laboratory protocols used in EU/EEA laboratories for the detection, characterisation, and typing of selected pathogens have already been collected. The evaluation of the collected laboratory protocols was completed, and the selection of harmonised protocols is ongoing. Experts will decide on the criteria, which will be used for ranking of the protocols. The ranking activity has been concluded, which is based on the evaluation tables on the original protocols collected. The ranked protocols have been sent to the EURs for comments and suggestions. Specifically, the ranked *Cryptosporidium* protocols will be sent for comments at the *Cryptosporidium* Reference Unit, Public Health Wales.

Dissemination activities for 2022 are planned. OH-HARMONY-CAP in collaboration with MATRIX and CARE have launched a research topic in Frontiers Public Health “One Health Surveillance in Practice: Experiences of Integration among Human Health, Animal Health, Environmental Health, and Food Safety Sectors” as a dissemination platform in 2022. The final meeting will be held in Copenhagen September 2022 which includes a joint workshop with CARE and MATRIX.

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MATRIX - year 2

MATRIX aims to advance the implementation of One Health Surveillance (OHS) in practice, by building on existing resources, adding value to them, and creating synergies among the animal health, public health, and food safety sectors. Learning from the experiences of the previous OHEJP integrative projects, MATRIX creates solutions for European countries while looking at the entire OHS pathway and recognising the existence of different national realities. The MATRIX solutions for OHS will be finalised in 2022.

During 2021, MATRIX finalised a review of commonalities and differences of various surveillance frameworks in animal health, public health, and food safety, which led to identify recommendations for developing a common sectorial framework. The problem-oriented approach of the project is reflected in the creation of hazard-specific tracks to ensure that the solutions that MATRIX develops are relevant to specific pathogens: in 2021, the surveillance chains for *Listeria*, *Salmonella*, *Campylobacter*, and Hepatitis E were mapped across a combination of sectors and countries. Also, available evidence about output-based surveillance was reviewed and internal inventories on relevant surveillance methodologies among MATRIX partners were completed.

Last year, a report was finalised on the necessary criteria to evaluate One Health capacities and capabilities for integrated surveillance in the development of the benchmarking tool, known as OH-EpiCap, to characterise, monitor and evaluate these factors. Also, work has progressed on designing a roadmap for integrated national OHS, and the *OHS Codex: The Knowledge integration platform* was adapted and extended to the MATRIX objectives and scope. This has been followed with continued training and dissemination activities.

The MATRIX project also aims to create user-driven dashboards for collaboration and decision making in OHS. In 2021, an online dashboard inventory and practical manual to facilitate the design and implementation of OHS dashboards using open-source tools were initiated.

MATRIX demonstrated an excellent response to the negative impact that the SARS-CoV-2 pandemic had on the project. The MATRIX Consortium revised the project plans based on the 6-month extension, which allows MATRIX to i) properly benefit from the results produced by other extended OHEJP projects and to ii) expand the synergies and links among the different WPs and sectors to achieve the project’s objectives under the best possible conditions.
In January 2021, the National Food Institute, Technical University of Denmark (12-DTU Food) left the MATRIX Consortium. Despite the scientific loss related to the specific DTU Food expertise in food safety, the Consortium managed to take over the related activities. This was the second withdrawal of a partner institute from MATRIX since the project started. Subsequently, a new partner, the Finnish Food Authority, Ruokavirasto (4S-RUOKA), joined the MATRIX project Consortium in June 2021.

In 2021, MATRIX activities caught up with the project's initial plans and, given the continuous availability of the expected resources including the 6-month extension, the project's objectives can be achieved.

**Publications in 2021:**


The COVRIN project focuses on One Health research integration of SARS-CoV-2 emergence, risk assessment and preparedness. The COVRIN research work has two main operational objectives: i) to identify drivers for the emergence and spread of SARS-CoV-2 and ii) to generate data and build models for risk assessment of SARS-CoV-2.

At the start of the project a scoping exercise was executed to avoid overlaps with other coronavirus projects. A primary research area was the development of detection methods for SARS-CoV-2 in animal reservoirs and hosts, and the environment. Data of SARS-CoV2 genome testing were shared and immunoassays methodologies were exchanged and harmonised. Approaches in the tasks on assessment of bioavailability were agreed and further developed.

Further research on SARS-CoV-2 characterisation, genome analyses and next generation sequencing of detected isolates and metagenomic sequencing of different samples were taken forward. At least two surveys were executed to make a summary description of protocols and to make an overview of the key steps of the bioinformatics analyses. A bioinformatics ring trial was prepared to harmonise bioinformatics pipelines. Regarding in vitro and ex vivo biological characterisation of circulating SARS-CoV-2 strains, cell line models have been collated and shared between partners. Animal model protocols have also been categorised and shared between partners. This will allow better analyses of virus traits related to zoonotic and/or reverse zoonotic transmission.

SARS-CoV-2 risk assessment and surveillance, formats/procedures for sampling and surveillance in wildlife, livestock and pets and the environment were evaluated and reported. Several workshops were organised to involve partners in the surveillance data collection. A review of surveillance activities in the different countries was produced. Risk factors for virus transmission in wildlife reservoirs, food producing animals and the environment are being studied, and analyses of transmissions in pets were produced. An overview of models and parameters to assess transmission in animals from a One Health perspective was reported.

From a One Health perspective, COVRIN research on coronavirus preparedness has initiated isolations of virus from different wildlife species. A report on relevant sample types and hot spots for coronavirus sampling was written to start on the evaluations of the impacts on ecological factors and interventions. During the second year of the COVRIN project more work will be done on the study of virus – host interactions and on the identification of drivers of virus emergence through evaluation of phylodynamics and cross-species interactions, with focus on zoonotic and reverse zoonotic aspects and adaptations. Generated data and research outcomes will be used to develop SARS-CoV-2 control strategies, intervention strategies, and prevention.

Publications in 2021:
NOVA - ended June 2021

The NOVA project aimed to develop new surveillance tools and methods for zoonotic foodborne diseases, and to harmonise and optimise the use of existing surveillance data. Zoonotic foodborne diseases constitute a serious one health problem throughout the world and are expected to stay high on the infectious diseases risk agenda, fuelled by factors such as globalised trade, intensified agricultural systems, international travel, and the continuous adaptation of known and arising pathogens and hazards. The collaborative structure of NOVA has addressed improvement of modern disease surveillance principles across Europe through many separate projects, organised into five main themes. Key results from each theme are described below.

The first theme considers aspects and issues in connection with performing One Health surveillance. NOVA found that surveillance across the food chain is insufficiently used and has potential to be developed further. Focus group studies highlighted a complex pattern of barriers and opportunities for One Health surveillance relating to data governance. A glossary of terms was developed to help overcome barriers between veterinary, food, and human surveillance activities.

The second theme examines the use of electronic traces for purchase of food for surveillance and outbreak investigations. NOVA project built an electronic web-based module for an online network to facilitate the use of information from electronic records of foods bought in shops for the investigation of foodborne outbreaks. Since methods of analyses of big datasets on supermarket purchases can automatically find vehicles and calculate odds ratio’s, the project recommends regularly using supermarket purchase information in the future for foodborne outbreak investigations. NOVA found that when foodborne outbreaks in care institutions occur, they are often preventable and sometimes traceable via electronic invoices food sales.

The third theme within NOVA examines developments of syndromic surveillance methods in veterinary and public health sectors. Different research activities within NOVA have led to the following findings, with the aim of improving outbreak detection in humans. Individual monitoring of Campylobacter detections in major broiler slaughterhouses proved effective for enhancing early detection of Campylobacter outbreaks in humans. In another study, parallel monitoring of indicators on cattle health, Salmonella detection in food, and human gastro-intestinal syndromes revealed simultaneous temporal abnormalities. Therefore, simultaneous alarms may be used to trigger and orientate field epidemiological investigations. In Norway, predictions based on models combining veterinary, meteorological and medical data were used to develop a dashboard to provide stakeholders with predictions of gastrointestinal outbreaks one week ahead of other systems.

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The fourth theme develops spatial risk mapping. The development of spatial disease distribution models for \textit{Salmonella} have identified provinces in Spain at risk, while considering the effect of potential risk factors. Risk-based surveillance of \textit{Salmonella}, based on a spatial stochastic disease spread model, was economically favourable. It relied on only one third of the samples used by traditional surveillance, although the capacity for case detection was also reduced. The NOVA project proposes that models and scripts developed for salmonellosis can be applied to other zoonotic diseases and many geographical regions in Europe, depending on data availability.

The final theme models the cost efficiency of surveillance programmes. Research within NOVA combined models for disease transmission (SIR) simulating emerging food safety issues with models simulating sampling schedules and models of laboratory procedures. Model analyses then determined the time such systems will need to detect an emerging infection – thereby providing a tool to optimise emerging risk surveillance systems. Modelling techniques can be used to make a holistic assessment of how new laboratory technologies can be used to improve the performance of surveillance systems before the technology is implemented in a population.

Overall, NOVA has contributed to the development of several novel methods that can be used to make surveillance and response more efficient and cost-effective. In parallel to strengthening of modelling and data frameworks, this project has explored and started to incorporate new data sources in everyday surveillance activities. NOVA project collaboration confirms that a multidisciplinary approach is needed to combine and understand information from the different surveillance components.

**Publications in 2021:**
LISTADAPT- ended June 2021

The LISTADAPT project aims to decipher the molecular mechanisms of adaptation seen in *Listeria monocytogenes* to its various ecological niches by comparing both phenotypic and genotypic data from a large, balanced sets of strains isolated from human clinical cases, animals, food and environment in several European countries.

Thanks to the collaboration between 21 partners institutes from the food, environment, veterinary and public health sectors, the LISTADAPT project compiled a dataset of 1,575 *L. monocytogenes* and their genomes. These strains were collected in 20 European countries and the researchers ensured that the dataset encompasses a large number of Clonal complexes (CC)s occurring worldwide, covers many diverse habitats and is balanced between ecological compartments and geographic regions. All the *L. monocytogenes* genomes collected by LISTADAPT are available to the scientific community (umbrella Bioproject submitted to European Nucleotide Archive (ENA)).

The LISTADAPT project also reported the first occurrence of a *L. monocytogenes* strain being isolated from a dolphin brain. This strain belongs to the clonal complex CC121, and genomic comparisons showed that it is closely related to 16 strains isolated from food. Like most of the food strains analysed in the LISTADAPT project, the dolphin strain includes genomic features (transposon Tn6188, plasmid pLM6179) both described as being associated with *L. monocytogenes* adaptation to the Food Processing Environment (FPE). It is likely that the infection of a dolphin by *L. monocytogenes* results from environmental contamination by anthropogenic activities.

Of the 1,575 *L. monocytogenes* strains collected, a subset of 200 were selected to be representative of 34 Clonal Complexes (CC) occurring worldwide and with a balance between three ecological niches: environment, animal and food. Phenotypic tests were performed to investigate the ability of these strains to survive in the soil and the strains were subsequently categorised into three groups according to their survival rate. Genotypic analyses were then performed using a Genome Wide Association Studies (GWAS) approach, to investigate genetic variations associated with the ability to survive in soil and it was found that survival in soil is linked to multiple genetic factors. A more in-depth study on strains from the same clonal complex or from the same origin successfully identified genomic variations in various transcriptional regulators and stress related genes as well as (pro)phage-related -genes associated to soil fitness.

Antimicrobial susceptibility testing was also an important part of the work done by the LISTADAPT
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project, with 11 antibiotics and 4 biocides being tested against the *L. monocytogenes* isolates collected in this project. Testing results revealed that strains isolated from food exhibited overall higher minimal inhibitory concentrations (MIC) for the following biocides: quaternary ammonium compounds and peracetic acid compared to strains isolated from animals and natural environments. Conversely, no significant differences were observed for MIC of antibiotics tested on strains from different niches.

Interestingly, repeated exposure to quaternary ammonium compounds (biocides) recurrently led to a decrease of susceptibility toward ciprofloxacin, a fluoroquinolone antibiotic largely used in human and veterinary medicine and considered as a critically important antimicrobial. Additionally, these lower levels of susceptibility to ciprofloxacin remained stable in most strains even after subcultures without biocide selection pressure, suggesting an adaptation involving modifications at the genetic level. Genomic analysis suggested that elements of the accessory genome were associated with biocide tolerance. Prophage-related loci and mobile genetic elements (Tn6188_qacH transposon and the pLMST6_emrC plasmid) were associated with *L. monocytogenes* tolerance to two quaternary ammonium compounds widely used in food processing and were widespread in the food strains collected during this study. Genomics elements encoding for surface proteins were also found to be associated with biocides tolerance and were found in *L. monocytogenes* strains isolated from animal and the environment. These results demonstrate the high adaptability of *L. monocytogenes* and the need to monitor the gene markers associated to biocide tolerance, in order to reduce the risk of contamination with biocide tolerant *L. monocytogenes* genotypes in food industrial settings.
**MoMIR-PPC - ended June 2021**

The MoMIR project aimed to develop new approaches to predict, identify and prevent the appearance of animal and human super-shedders based on immune response and gut microbiota composition. The project objectives were to define predictive markers that signal risk of animals and humans becoming *Salmonella* super-shedders, to identify immune and microbiota biomarkers to detect super and low shedder phenotypes in all species studied (human, pig, chicken, and mouse), to identify measures to prevent and control *Salmonella* super-shedders and to develop epidemic mathematical models for risk management. The project has achieved most of these objectives and have provided better understanding of the heterogeneity of *Salmonella* excretion that helps to predict and prevent these different shedding phenotypes.

The most important advances from the MoMIR project are highlighted:

The different shedding phenotypes have been observed in pigs and chickens. The interactions between the pathogen, the host response and the gut microbiota can explain these different shedding phenotypes. Studies to identify predictive biomarkers for *Salmonella* super-shedders were undertaken and several biomarkers have been identified based on the composition of chicken gut microbiota. The key role of animal-animal recontaminations in the spread of *Salmonella* infection at the flock level have been shown. They are related to the presence of super-shedders, which function as a pathogen reservoir. The variation in the bacterial virulence level cannot explain the super and low shedding phenotypes, and instead host factors and gut microbiota composition play a crucial role. Several prebiotics and probiotics have been successfully tested to decrease *Salmonella* colonisation under experimental and field conditions. Although the risk of human-to-human transmission of *Salmonella* from asymptomatic carriers is considered low, our study indicates a 10-fold higher rates (24%) of nontyphoidal *Salmonella* shedding than previously suggested. Several risk factors to become chronic carrier have been identified.

Original mathematical models of epidemics and methods of analysing intra-animal transmission data were developed. Additionally, a model of indirect transmission of bacteria between broilers was developed. The model can be used for designing and quantitatively assessing candidate bio-security based intervention strategies against indirect transmission of *Campylobacter* and *Salmonella*.

Studies determined several control strategies based on the importance of the ventilation in farms and the identification of the animals at risk. For that purpose, the MoMIR project identified several predictive biomarkers, based on gut microbiota composition and immune parameters. The identified biomarkers,
which still need to be further confirmed, can either predict the levels of *Salmonella* shedding (in pigs or in chickens) if animals are infected, or can differentiate between the *Salmonella* shedding levels. Studies also revealed the major factors associated with the long-term shedding of *Salmonella* in humans.

The MoMIR project determined several preventive measures based on the use of pre- and probiotics. Studies isolated, characterised, and tested the efficacy of probiotics and prebiotics for use in pigs and poultry to prevent salmonellosis, which were undertaken in experimental infections and in field conditions. This included testing the interventions in 72 640 chicks in Bulgaria and 130 000 chicks in Czech Republic. These probiotics and prebiotics can be used as an alternative to antibiotics and may help reduce AMR. Samples were also taken from several challenge and intervention studies for metagenomic analysis.

Finally, a draft inventory of relevant intervention measures against *Salmonella* in poultry has been developed and the cost effectiveness of probiotic intervention strategies has been calculated.
The **DiSCoVeR** project brings together experts from different disciplines (microbiology, bioinformatics, and epidemiology) and sectors (veterinary science, food safety, public health, and environmental health) from 19 institutions in 13 European countries to address the challenges of source attribution in an interdisciplinary manner. As there is no gold standard for source attribution, this project takes a comprehensive approach by applying several different methodologies and models in a comparative fashion.

Research in the first year mapped the existing knowledge gaps and recommended new studies and/or methods that are needed to fill them. Existing data was mapped by creating data inventories for the DiSCoVeR target pathogens of *Salmonella*, *Campylobacter*, Shiga-toxin producing *E. coli* (STEC) and Extended-spectrum beta-lactamase-producing (ESBL) *E. coli*. Data collection is ongoing, as some partners met delays in collecting new data from non-livestock and non-food sources and the completion date of project datasets was extended to February 2022. Data incorporates a broad range of reservoirs and sources, including those that are not traditionally part of existing monitoring and surveillance activities, such as pets (including reptiles), wildlife, and environmental sources.

Project work focuses on cataloguing, evaluating, and advancing existing methods for source attribution and developing novel methods/approaches for the critical assessment of source attribution models. Existing approaches being investigated include microbial subtyping, meta-analysis of case-control studies and outbreak data, and risk-assessment based methods. The source attribution estimates focus on *Salmonella*, *Campylobacter*, and Shiga-toxin producing *E. coli* and searching for antimicrobial resistance in these species.

For *Salmonella* and *Campylobacter*, several partners are developing Whole Genome Sequencing-based attribution models. Preliminary results indicate that the outputs are in line with previous subtyping approaches (phenotypic and MLST based), although the WGS-based models appear to have a higher predicting accuracy. Conventional subtyping approaches are also being applied for all the target pathogens, with the intention to make a multi-country model for at least *Salmonella*. A systematic literature review of recently published (2011-2021) case-control studies of sporadic *Salmonella* and *Campylobacter* infections was completed and will, together with data from previous reviews, form the basis for an overall meta-analysis. The completion of Multi-Country Comparative Exposure Assessments (CEA) of target pathogens in pets demonstrated the relative exposure of humans to pathogenic and antimicrobial resistant bacteria due to (in)direct contact with cats or dogs.
Dissemination of information was shared at an online stakeholder workshop in January 2021, which included around 20 participants from EFSA, ECDC, EURLS, DiSCoVeR and other relevant EJP projects. Scientific and grey literature reviews in combination with a survey of relevant national experts has led to the recent completion of the task to map currently existing control programmes of the target pathogens and AMR in humans, animals and the environment at the EU and national level.

The DiSCoVeR project has also created a secure space data sharing platform of scientidata.dk to share genomic sequences, with ongoing work to further expand this inventory.

**Publications in 2021:**
BIOPIGEE - year 2

The BIOPIGEE project aims to identify effective and cost-efficient biosecurity measures to control potentially zoonotic hepatitis E virus (HEV) and *Salmonella* occurrence in European pig farming. Nineteen partner institutes from 12 countries are collaborating to conduct comprehensive literature reviews, field and experimental studies, expert surveys, and risk modelling. The project will develop tools to limit pathogen load along the food chain, thus resulting in healthier animals and a safer food chain. The collated information will be disseminated to stakeholders (farmers, veterinarians, advisors) in illustrations, a support tool, and workshops.

The BIOPIGEE mid-term meeting took place as a virtual 2-day-event in March 2021 and a new task defined ‘biosecurity measures’ to harmonise understanding across project tasks.

A BIOPIGEE biosecurity questionnaire collected data from 262 pig farms in several partner European countries. A slaughterhouse online survey on best biosecurity practice was designed and carried out in seven participating countries, which included Germany, Czechia, Estonia, Italy, Austria, UK, and Netherlands. Sampling methods for slaughterhouses were agreed and bacteriological samples collected from two slaughterhouses in Czechia and Italy. The longitudinal field studies in the UK, Italy, and the Netherlands were initiated after being delayed due to COVID-19 pandemic.

A HEV infectivity assay was developed successfully to be used to detect infectious HEV in samples of different origin. It may be included in a method for assessing the persistence of infectious HEV in surface samples. A method on how to test effectivity of disinfectants using the HEV infectivity assay and the method for testing persistence of infectious HEV in surface microlayers were established. *Salmonella* isolates for testing were selected. The comparison of methods and planning of how to test effectiveness of disinfectants is ongoing.

Recent work has obtained pig movement data from UK and France, but data privacy issues with the UK data meant they could not be fully integrated in the time available. A potential source for similar data from Germany was identified. Time constraints meant it was not possible to obtain and consider data from Italy or Germany. Draft results of the network model with the French data have been produced, including simulations of some on-farm biosecurity interventions. Work is underway to adapt the previous *Salmonella* QMRA for HEV and integrate the network model with the QMRA.

For science to policy translation, the BIOPIGEE catalogue of biosecurity measures to reduce HEV and *Salmonella* prevalence was revised to ensure consistent data and safe updates. A systematic literature
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review and meta-analysis on the effectiveness of biosecurity measures against *Salmonella* and HEV in pig farms were carried out. In addition, literature on pathogenic *E. coli* was studied to complement knowledge about *Salmonella* based on the similarity of the pathogens. An expert scientific panel was expanded to incorporate knowledge of other stakeholders (e.g., practitioners, advisors). This panel assessed relevance of biosecurity measures to reduce *Salmonella* and/or HEV in pig herds in an online survey, and the results have now been analysed.

Illustrations of examples for good biosecurity were collected at the BIOPIGEE web group. Education, discussion and training activities of workshops and meetings were delayed or cancelled due to the pandemic. Two workshops have since gone ahead and a relevant conference the organisation of the third workshop is ongoing. A BIOPIGEE glossary was also developed.
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TOXOSOURCES - year 2

The TOXOSOURCES project focuses on the zoonotic parasite *Toxoplasma gondii* at the interface between humans, animals, food, and the environment. *Toxoplasma gondii* is a foodborne parasite that causes a high disease burden. The infection can be acquired by ingesting oocysts (environmental pathway) or tissue cysts (meat-borne pathway). The relative contributions of the two pathways to the infection and disease in humans remain unknown, partly due to a lack of appropriate methods. Environmental contamination with *T. gondii* oocysts is understudied and underestimated due to the lack of suitable harmonised sampling approaches and detection methods.

TOXOSOURCES investigates the relative contributions of the different sources and transmission routes to *T. gondii* infections using multidisciplinary approaches and new methods to yield robust estimates that can inform risk managers and policy makers.

The TOXOSOURCES consortium has collected data for a multicentre quantitative microbiological risk assessment for *T. gondii*. The data was sourced from a multicentre quantitative exposure survey and systematic literature reviews on *T. gondii* contamination of soil, water, fresh produce, and bivalve molluscs as well as on *T. gondii* prevalence in animals raised and hunted for human consumption as well as in cats, the definitive hosts of the parasite. An extensive literature review was performed to support the selection of the most suitable method to detect *T. gondii* oocysts in fresh produce, which led to the development, implementation, and validation of a Standard Operating Procedure, which is being applied in a multicentre survey on ready-to-eat salads. Bioinformatic selection of promising antigens for a novel serology method was finalised and recombinant expression of selected proteins was conducted, as part of exploring serology for detecting *T. gondii* infections caused by oocysts. An unprecedented effort of whole genome sequencing of *T. gondii* isolates was used to identify polymorphic marker regions for the establishment of a new typing method to detect within-genotype variation and detect outbreaks as well as imported and recombinant strains.

The outcomes of TOXOSOURCES will include quantitative and comparable estimates of the contribution of the main sources and transmission routes to *T. gondii* infections across Europe. A major outcome already reached is the collection and whole genome sequencing of a high number of *T. gondii* isolates across Europe.

The TOXOSOURCES consortium has actively disseminated its outcomes. Dissemination efforts have included scientific publications and presentations at conferences, workshops and webinars, and the establishment of several collaborations with other projects and networks.
JOINT RESEARCH PROJECTS: FOODBORNE ZOONOSES (FBZ)

TOXOSOURCES Publications in 2021:


Salmonellosis remains the second most common zoonosis in humans in the EU with an increasing incidence in some countries. The ADONIS project aims to investigate the potential underlying drivers for the change in Salmonella epidemiology in humans and poultry across the EU. This is done with a One Health approach where primary (poultry) production, human epidemiology, and pathogen genomics is considered. The recent project work for each level of investigation is summarised.

On the primary production level, measures implemented in the frame of the national control programs and information generated through these measures was evaluated using data from member states where strategic research agenda partners were located. This included analysis of audit reports from Salmonella National Control Programmes. Data on detection of Salmonella in poultry farms from one member state was used to identify factors associated with positive results in routine samplings, suggesting that farm, animal, and sampling associated factors are associated with increased chance of detection of Salmonella. The framework developed will be used to analyse data from another country in 2022.

On the epidemiology level, a detailed statistical analysis for the Netherlands and Belgium revealed that the incidence of S. Enteritidis decreased significantly in both countries until 2015 followed by a significant increase after 2015 for The Netherlands and an upward trend for Belgium. The increased occurrence of potential outbreaks and invasive infections since 2015 might partially explain the observed reversal of the trend.

The project investigated possible factors influencing the change in S. Enteritidis epidemiology. The performance of the surveillance programs in Belgium and the Netherlands was evaluated. Both surveillance systems are characterised by a relatively high population coverage, which marginally changed between the time-periods 2006-2012 and 2013-2020. This suggests that the epidemiological change cannot be explained by an alteration in surveillance systems. An exposure assessment of S. Enteritidis contamination of eggs, (with data mainly from Spain) revealed the absence of a correlation of total exposure (product of prevalence and microbiological load) with clinical incidence. This indicates that changes in exposure are unlikely to explain epidemiological change.

The project analysed the impact of the COVID19 pandemic on the incidence of Salmonellosis in The Netherlands, which decreased significantly after March 2020 by roughly half of the incidence in the years 2016-2019. There were higher fractions of invasive infections and increased relative proportion
of *S. Typhimurium* and decreased proportion of *S. Enteritidis*. This led to decreased contributions of laying hens and increased contributions of pigs and cattle as sources of human infections. The observed changes likely reflect a combination of reduced exposure to *Salmonella* due to implementation of public health restrictions on socialising and travel, and changes in healthcare-seeking and diagnostic behaviours.

In collaboration with the OHEJP JRP Discover, a source attribution study for *Salmonella* in the EU based on outbreak data using EFSA data was conducted. The most important food source was eggs (48%) followed by pork (10%), and (other) meat products (9%). Outbreaks caused by *S. enteritidis* and other less common serotypes were mostly attributed to eggs (58% and 20% respectively), whereas outbreaks caused by *S. Typhimurium* and its monophasic variant were mainly attributed to pork (37%). There was a significant increase in the number of outbreaks, by 5% on average per year between 2015 and 2019, which was mainly caused by an increase in Eastern Europe related to *S. Enteritidis*.

On the pathogen level, the project constructed a genome sequence inventory and a shared database to enable future analysis that will assess potential changes in the population biology of *S. Enteritidis* on EU scale. Preliminary results suggest a role of plasmid content. The analysis of the population structure of *Salmonella* in the EU has started, which involves a population-genetic description and phylodynamic analysis to identify recent clones that have emerged.

ADONIS project has created a framework for the multi-criteria decision analysis, which defines the criteria for the assessment of potential explaining determinants for the changing *Salmonella* epidemiology. Two surveys have been completed that determined the weighting of criteria according to their importance and scoring the different drivers based on the ranked criteria. Subsequent data modelling will be performed in 2022.
The BeONE project aims to develop an integrated surveillance dashboard in which molecular and epidemiological data for foodborne pathogens can be analysed, visualised, and interpreted interactively by experts across different sectors and disciplines. Surveillance of foodborne infections and outbreak distribution is primarily handled at the national or regional level. The cross-sectional and interdisciplinary datasets generated are complex, which requires the development of tools to facilitate data integration and analysis.

The BeOne project has created a test dataset consisting of whole genome sequences and related metadata across the four target pathogens: *Salmonella*, *Listeria*, Shiga-toxin producing *E. coli*, and *Campylobacter*. This is used to test different approaches of analysing and clustering bacterial genomes for surveillance and outbreak detection based on standardised guidelines for whole genome sequence data, metadata collection and ontologies that has been produced. The core of a database model has been designed and is awaiting addition of key epidemiological metadata to complete its development.

Additionally, BeOne is creating a common platform for data sharing between national and international institutions, which also includes a dashboard for visualising data. The dashboard and website development has been closely linked to a Danish project (SOFI), where the food authorities, DTU (as expert data processor of food and livestock samples) and SSI (public health Institute), collaborate on a common platform for integrated analysis of food and patient samples for outbreak detection. The dashboard currently provides a searchable and sortable data table that can merge and display data from multiple sources. Dashboard development is ongoing to include the visualisation of epi-curves and phylogenetic trees.

The BeONE dataset collection and curation was completed, with a total of 3,439 isolates. Quality control and assembly of the genomes is ongoing. An automated system for congruence analysis has been set up that is yet to undergo full evaluation. The project has also devised a conceptual model on genomic epidemiology, which aims to serve as a first step in developing and evaluating new algorithms for the detection of pathogens and outbreaks. The outbreak detection algorithm has been finalised and tested on *Listeria* and will subsequently be tested on the remaining target pathogens.

Overall, significant progress has been made with software development, data management and modelling, analysis pipelines and testing plans among different stakeholders. The project will ensure that its findings are sustainable and therefore the base code created during the project is available on GitHub, with full support and documentation.

**Publications in 2021**

ARDIG - ended December 2021

The ARDIG project aims to examine the dynamics of antibiotic administration and antimicrobial resistance (AMR) in humans, animals, food and the environment across six countries in Europe, using a One Health approach. This will provide a better understanding of the types of resistances, their prevalence and their variation in different populations over time, with the hope that this can contribute to the control of multi-drug resistant superbugs. Using a One Health approach will help to overcome the limitations in comparability between data from different sectors and countries.

The ARDIG project has identified that there is a lack of harmonisation on antimicrobial resistance (AMR) and antimicrobial usage (AMU) data in the livestock sector and on antimicrobial resistance in the human sector, across national and international surveillance systems in Europe. A One Health approach for AMU and AMR requires harmonisation in various aspects between systems in the human, animal and food sector. The ARDIG project has explored statistical methods to overcome the lack of harmonisation (such as the Net Reclassification Index (NRI)) and a detailed analysis of the trends in clinical data sets could be made across countries, in addition to enabling comparisons between clinical and non-clinical datasets. Significant differences in AMR between clinical and non-clinical isolates were identified within and between countries for different animal categories. Higher resistance levels in clinical isolates than in non-clinical isolates were found for calves, while the opposite was found in isolates from broilers chicken and turkeys. Decreasing resistance was found in all animal isolates between 2014 and 2017, suggesting that measures carried out against AMR including the reduction in AMU in each country achieved effective results. A workshop was held with experts in the field, including those from EFSA, EMA and ECDC and other European projects, to make recommendations for improved data collection and harmonisation for “One Health” surveillance strategies.

Both public health and animal health partners involved in the ARDIG project collected E. coli isolates from retrospective and prospective longitudinal studies, including E. coli isolates from urinary tract infections from local hospitals and from livestock. Several partners have also characterised E. coli isolates through EU harmonised surveillance programmes for AMR. A whole genome sequencing project (WGS) was also carried out to investigate the persistence of AMR in E. coli. During a whole genome sequencing workshop approximately 450 genome sequences submitted from partner institutes were subjected to five different pipelines used to investigate AMR: APHA SeqFinder/Abricate, PHE GeneFinder, WBVR, Ariba and ResFinder/PointFinder. AMR phenotypes were investigated for these isolates to compare AMR genotype to corresponding phenotype. A manuscript is in
preparation based on the AMR pipeline comparison work that was initiated in the workshop to assist in the harmonisation of in silico AMR gene prediction in future.

WGS of over 3000 isolates collected from longitudinal, as well as national surveillance and research by partners were also compared and showed that human isolates are generally more similar to each other than to those from animals, and vice versa; although several isolates from each compartment were much more varied. AMR genes, plasmids and mobile genetic elements of these >3000 multi-drug resistant *E. coli* isolates were characterised using the WGS data. Phylogenetic analysis and AMR profiling performed by ARDIG partners have helped identify possible transmission events or epidemiological links between MDR isolates from different compartments and countries. Also, plasmid characterisation has provided an overview of the types of AMR plasmids commonly circulating in these countries and compartments, the majority of which belong to the following types: *IncI*, *IncF* and *IncX*. A manuscript is being prepared of this work.

**Publications in 2021:**


Antimicrobial resistance (AMR) threatens global health. Current AMR pathogen detection methods are reliant on classic culture techniques. The development of new tools for rapid, real-time detection of pathogens and their resistance profile is much needed but requires robust protocols using minimal technical equipment that can be used outside of a laboratory environment. Additionally, metagenomic techniques could be an invaluable tool for diagnosis and assessment of microbial communities for potential pathogens and AMR or virulence genes. However, metagenomics cannot accurately associate individual genes in a community to specific organisms, which is a limitation for detection of AMR in pathogens.

The FARMED project aims to address these issues by assessing the feasibility of long-read sequencing to rapidly characterise the metagenome and resistome of on-site samples to provide investigators with the correct information to apply the most appropriate control measures.

Bioinformatics analysis of long read sequencing of a simple (pond water) or complex (animal faeces) matrix spiked with a defined microbial community, with DNA extracted using the preferred method in each institute, highlighted the differences of microbiomes achieved by each institute. Further work is currently underway to understand why these differences have occurred even though the ‘same’ samples were used. The consortium has selected three lab-based DNA extraction kits, which were assessed by a minimum of three institutes to determine the usability and consistency of these methods. To aid this assessment, the same commercially available defined community was used by all institutes. Institutes have applied one of these lab-based methods on sample matrices most relevant to their institutes and the data analysis is ongoing. Further work is still required to optimise and automate the DNA sequence analysis methods, such that the analysis can be performed on ‘basic’ computers by non-bioinformaticians.

Two instruments capable of on-site DNA extractions are being trialled for their suitability to produce DNA of sufficient quantity and quality for DNA sequencing. Preliminary analysis using these instruments has yielded DNA for single isolates suitable for long read sequencing, but current optimisation is underway to achieve the appropriate quality of metagenomic DNA. This is being combined with the Voltrax instrument, allowing on site sequence library preparation.

The FARMED project has produced a peer-reviewed publication and presented a poster highlighting the use of long read metagenome sequencing to characterise the microbiome, and identify the presence of genetically modified microorganisms, *Bacillus subtilis*, (containing AMR genes on the chromosome and on a plasmid) in a feed additive. Two oral presentations were given at the One Health EJP Dissemination Workshop on Metagenomics, on 27th October 2021.

**Publications in 2021:**
FULL-FORCE - year 2

The FULL-FORCE project aims to supply 17 EU partners with a technological toolbox and hands-on training in Single-Molecule Real-Time (SMRT) sequencing, and to apply this knowledge to six study cases and applications in metagenomics and AMR transmission models. Using this state-of-the-art technology, public health and veterinary labs will have the capacity to perform full-length sequencing and gain detailed insight in mobile genetic elements (MGEs) which carry antimicrobial resistance and virulence genes within, and across species.

During 2021, FULL-FORCE project activities included (i) the application of the Full Force Plasmid Assembly (FFPA) pipeline during an internal proficiency test, (ii) the start-up of the five study cases, and (iii) applications on metagenomics datasets. However, the strong laboratory focus that is inherent to the project set-up, continued to suffer from the pandemic. The physical progress meeting, which was planned during ECCMID 2021 in Vienna, was postponed to a hybrid meeting on 22nd November 2021 in Brussels.

The main goal of the Full Force project is to enable consortium partners to reach a sufficient technical level in plasmid sequencing. To maximize the output from this project, an easy-to-use software package (Full Force Plasmid Assembler) has been developed, which will automatically perform hybrid assemblies through the built-in UniCycler program from a combination of short and long sequence reads. This tool is currently being re-evaluated.

A proficiency test to assess each institute's capacity for SMRT sequencing is currently ongoing. Five multi-drug resistance E. coli strains were sent to 16 participating institutes.

Although five cases studies implementing long-read sequencing on existing datasets has suffered some delays, substantial progress has been made. In four of the five study cases, phylogenetic analyses on short-read datasets are ongoing to identify reference strains and plasmids for detailed study by long-read technology.

Metagenomics studies using long-read technology has advanced very well leading to publication of a manuscript on the plasmidome of sewage samples using methodology which was optimised during Full Force.

A protocol for harmonised bacterial conjugation has been established. The robustness of this method will be assessed with selected Full Force partners. The design and parameterisation of a transmission spread model of pAMR in the simulation framework SimInf is currently ongoing in collaboration between the consortium partners. Finally, the task to perform exposure assessment of horizontal and vertical transmitted AMR has been initiated and a first report completed.

Publications in 2021:


WORLDCOM - year 2

The WORLDCOM project aims to develop diagnostic tools linked with mobile referencing technology for detection of antimicrobial resistance (AMR) in zoonotic pathogens. The resulting technologies will enable rapid on-site detection of AMR in pathogens from animal and human populations, facilitating early investigation of emerging resistances and development of machine-learning algorithms for AMR prediction, beneficial for improving surveillance activities.

Extended-spectrum beta-lactamase (ESBL) enzymes produced by Enterobacteriaceae organisms confer resistance to beta-lactam antimicrobials presenting a major public health concern. Biomarker genes for highly prevalent ESBLs, cefotaximase (CTX-M), oxacillinase (OXA), Klebsiella pneumoniae carbapenemase (KPC) and New Delhi metallo-beta-lactamase (NDM), were selected as assay development targets for AMR marker detection. Loop-mediated isothermal amplification (LAMP) technology was selected for the detection of the selected AMR genes, due to its rapidity and ease of use. In Loop-primer endonuclease cleavage LAMP (LEC-LAMP) assays, the LAMP primers and reaction are modified to detect multiple targets in one tube. An internally controlled LEC-LAMP assay was developed for differential detection of closely related CTX-M variants, \( \text{bla}_{\text{CTX-M-1}} \) and \( \text{bla}_{\text{CTX-M-15}} \) associated with animal and human infection, respectively. A singleplex LEC-LAMP assay was also developed for differential detection of closely related OXA variants, \( \text{bla}_{\text{OXA-48}} \) and \( \text{bla}_{\text{OXA-181/232}} \), as a precursor to multiplex assay development. AMR LAMP assays have been developed and validated targeting important AMR genes (MCR-1, KPC, OXA-48, OXA-23 and VIM) that are associated with zoonotic bacterial infections. The assays can detect target genes within 10 min, with 100% sensitivity and specificity.

In relation to sample preparation, a rapid protocol for on-site DNA isolation was successfully developed and validated using confirmed CTX-M-1 positive porcine faecal samples. There has also been significant progress with water sample preparation for LAMP detection of pathogens and AMR genes. The method is rapid (30 min), with a detection limit of 10 cfu/mL. The method allowed consistent LAMP detection of all target AMR genes from tap and pond water spiked with different Gram-negative bacterial cultures within 35 min of sample preparation.

Targeted and whole-genome sequencing of AMR strains has progressed. Progress includes NGS data generated from 24 cephalosporin-resistant \( E. \ coli \) strains isolated from alpacas or llamas, and 138 cephalosporin-resistant \( E. \ coli \) strains isolated from pigs reared in Germany, containing a range of \( \text{bla} \) AMR genotypes. These included 390 \( E. \ coli \) isolates from diverse sources (110 from human, 144 from swine and 136 from broilers) and 43 \( E. \ coli \) isolates from river and wastewater, confirming the presence of CTX-M
genes and the colistin resistance gene *mcr-1* among these *E. coli* isolates. This information will be used for further improvement of LAMP assays.

The automated assessment of colorimetric LAMP assays for AMR genes has made significant progress, employing an image processing pipeline combined with a machine learning method. This procedure remains robust against background noise, making it suitable for on-site use as part of a mobile detection kit, and has been implemented as an Android smartphone app for portability. This includes automatically annotating these data and communicating the results with a cloud storage service.

**Publications in 2021:**
FED-AMR - year 2

Bacterial transformation contributes to the horizontal gene transfer (HGT) of antimicrobial resistance genes (ARGs) but there is a lack of empirical data on the impact of bacterial transformation in the environment. This is addressed through the FED-AMR project, which aims to demonstrate the importance of extracellular free DNA (exDNA) as an environmental reservoir for ARGs. ARG concentrations, diversity, mobility, and bacterial biodiversity is being determined in an annual longitudinal study covering a crop’s growing period. Different fertilisation and land management techniques, and environmental compartments are being investigated.

During the second year of the FED-AMR project, work has been done to coordinate the scientific and administrative matters between the project partners including scientific meetings and webinars, scientific discussions on results and data analysis and budgeting.

In the second year of the project, the last sampling round took place and all samples were processed. Sample cultivation, antimicrobial susceptibility testing (AST) and genomic characterisation of the collected bacterial isolates are being performed with a great progress being made. The FED-AMR bacterial collection is a diverse set of relevant bacteria, from several distinct reservoirs that will overall contribute to understand the dissemination of antimicrobial resistance (AMR) within the compartments, reinforcing the One Health approach. In addition, extracellular and total DNA have been analysed through 16S metagenomics (measurement of bacterial diversity) and through gene enrichment (characterisation the resistome but also the mobilome, and heavy metal resistance genes). Results derived from those analyses are being interpreted through statistical comparisons, with some initial results already presented at the OHEJP ASM2021 and other works accepted for poster presentation at ECCMID 2022 and OHEJP ASM2022. Whole Genome Sequencing (WGS) is being performed for selected isolates and important results were already obtained, from which an article and an MSc emerged. A scientific manuscript with the WGS results from the Austrian Open Air Laboratory (OAL) is being prepared.

The epidemiological survey of zoonotic C. difficile ribotypes across participant countries has finished, with evaluation of their dissemination, including those resistant to antimicrobials among humans, animals, and the environment, using the pig farm as proof of concept. Several sampling campaigns were conducted to enrich the collection of C. difficile isolates targeting several animal species, including previously uncharacterised for C. difficile, such as llamas and alpacas, and food, such as strawberries. Since the conclusion of these sampling campaigns, AMR characterisation of C. difficile isolates is ongoing. The obtained genomes from important ribotypes are being gathered for a genomic study.
Initial results were given as poster presentations in the OHEJP ASM2021. A scientific manuscript with the results from the Portuguese Open Air Laboratory (OAL) is being prepared.

Likewise, the analysis of antibiotics, elements and herbicides for all regions will be completed in May 2022 by the three specialised laboratories in Austria, UK, and Poland. Preliminary results were obtained, and quantifications will be concluded during the first semester of the third year.

Risk assessments are finalised for the in vitro gut model and the model has been established using pig faeces. A FED-AMR researcher has prepared the *E. coli* strain and DNA amplicon, encoding rifampicin resistance, to be used in the gut model and confirmed the ability of the *E. coli* strain to acquire AMR from the DNA amplicon in the absence of faeces. Current work focuses on optimising the conditions of the in vitro pig gut fermentation model to demonstrate optimum growth of the recipient strain and demonstrate the transfer of AMR using amplicons in the presence of faeces and environmental drivers, such as antibiotics and trace elements.

The protocol for a systematic review on factors influencing the prevalence of AMR in the environment was finalised. The protocol was submitted to *Environment International* and following extensive feedback, it has been changed to a “systematic evidence map”. The editors’ comments have been addressed and the revised version was resubmitted in February 2022. A second round of revisions were requested, and the protocol will be resubmitted in early May. The protocol has also been registered to OSF (https://osf.io/a8gv6/). Additionally, the formulation and validation of a mathematical model for the dynamics of microbial communities was conducted. A new model was devised, with other models explored for comparison purposes to refine the research questions. Finally, *in silico* data have been developed to validate the approach.

**Publications in 2021:**
INTRODUCTION

TOX-detect - year 3

The TOX-Detect project aimed to contribute to increase consumer health protection by filling critical knowledge gaps in the detection and characterisation of toxigenic bacteria and their toxins causing food-poisoning outbreaks.

All the partners’ institutes involved in the TOX-Detect project collected a large number of toxin-producing bacteria isolates from environmental, clinical, and food samples from different geographic locations. To characterise these strains, culture, RNA extraction, RNAseq assays and cytotoxicity assays were developed and optimised. Data analysis allowed gene expression and strain toxicity measurement, defining the most suitable biomarkers (known and new) to characterise the strains. PCR assays were subsequently developed for both *Bacillus cereus* and *Clostridium perfringens*. This work was realised thanks to optimised standard operating procedures shared between the institutes and resulted in the establishment of a reference collection of fully characterised strains for *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens*.

The reference strain collection was also used by the OH EJP TOX-Detect project to evaluate a panel of three methods: (i) Matrix-assisted laser desorption/ionization-time of flight (MALDI-ToF), (ii) Liquid chromatography–mass spectrometry (LC-MS) and (iii) enzyme-linked immunosorbent assay (ELISA) for the detection and characterisation of the three pathogens of interest and some of their toxins. These tools provided relevant information and data on toxin expression and production by toxigenic strains responsible for food-poisoning outbreaks. A total of 15 harmonised SOPs for all the targeted virulence factors and 6 dedicated methods were established and shared between all partners’ institutes. The robustness and efficiency of these SOPs and methods were demonstrated by Inter-lab tests prepared according to the ISO/IEC 17043 standard. A new MALDI-ToF library, containing 152 reference spectra (MSP) from 76 strains, was developed. Comparisons with commercialised databases showed high reliable scores for the differentiation of the three targeted species. This MALDI-ToF library was transferred to the European Union Reference network, through the organisation of an inter-laboratory test that generated more than 3,000 data and a high percentage of correct identifications (improved results compared with other current methods).

Pathogen-specific studies were also performed under this project. Methods were developed for the study and detection of staphylococcal enterotoxins types SEM, SEN and SEO and for *B. cereus* enterotoxins CytK2, hemolysin HlyII and Sphingomyelinase, as there is a lack in commercially available
JOINT RESEARCH PROJECTS: EMERGING THREATS (ET)

methods. For this purpose, recombinant toxins were produced and purified, due to the lack of toxin standards. Stable recombinant toxins were successfully produced for SEN, hemolysin HlyII and Sphingomyelinase and have been transferred between partners, to be used as standards for LC-MS methods development. ELISA and LC-MS methods were successfully developed and optimised for Bacillus enterotoxins. LC-MS method targeting staphylococcal enterotoxin type SEN was also successfully developed. These methods were transferred between TOX-Detect partners for inter-laboratory test. Toxicity and enterotoxin data obtained for Clostridium perfringens strains isolated from foodborne outbreaks in the European Union, also led to the development of RT-PCR and LC-MS techniques for the detection of this pathogen.

Results obtained in the TOX-Detect project enhance the knowledge about strains producing toxins. Methods developed could be used by reference laboratories (NRLs and EURL) for further development and foodborne outbreak investigation, especially when it comes to non-classical toxins. Finally, some fully characterised strains of Staphylococcus and Bacillus have been included in OHEJP CARE project.

Publications in 2021:
TELE-Vir - year 2

The TELE-Vir project aims to develop a fast point-of-evidence toolbox for identification and characterisation of emerging virus threats for human and/or domestic and wildlife animals. The TELE-Vir project is combining a suitable field-deployable point-of-care approach, and a direct upload of genomic, phenotypic, and epidemiological data into a user-friendly bioinformatics toolkit for fast identification and characterisation of new emerging virus threats. Existing point-of-care methods and tools are being developed, adapted, and expanded to create harmonised point-of-evidence protocol for field analysis. The protocol requires only a minimum of laboratory equipment and is designed to be compatible with MinION sequencing technology. Phenotypic and epidemiological data is being combined and integrated in the point-of-evidence toolbox to aid risk assessment and management, which is available to other interested national and international parties and shared with established networks.

The SARS-CoV-2 pandemic has a great impact on the TELE-Vir project due to national lockdowns, laboratories being closed and a worldwide shortage of basic laboratory reagents and equipment. However, this pandemic has also had a positive influence on the project. Project work demonstrated that the SARS-CoV-2 can be inactivated, other RNA viruses being non-infectious, and alternative methods for NA extraction (WP3) were developed, which corresponds to the development of a field-based protocol for MinION sequencing using a minimum of laboratory equipment (the point-of-evidence toolbox). The SARS-CoV-2 was successfully used as model virus in the TELE-Vir metagenomics toolbox. This approach will now be adapted and applied for DNA viruses, which is currently in progress.

The first annual meeting from the TELE-Vir project was held online at the end of January 2021. TELE-Vir members are in the process of planning the next TELE-Vir annual meeting together with a workshop where TELEVIR partners are trained using the point-evidence toolbox in June 2022. The project strategy focus is on RNA viruses, such as the newly emerged SARS-CoV-2, but also on DNA viruses to apply the TELE-Vir metagenomics approach. Research provided proof of concept for bioinformatic platforms using coronaviruses, and influenza viruses as main models. The pandemic has both given new opportunities in this area with overwhelming volumes of data becoming available, in addition to challenges caused by data quality, and logistical constraints and restrictions. The TELE-Vir platform has been built to enhance genome-based viral surveillance, by facilitating both sequencing data analysis and output navigation and interpretation.
Despite the challenges of the SARS-CoV-2 pandemic, the TELE-Vir consortium has shown impressive resourcefulness and adaptability, with deliverables and reports met and submitted. The dissemination of TELE-Vir research findings have included scientific publications, presentations at workshops, webinars, and conferences.

Overall, the experiences and problems encountered during the pandemic will be further used or translated to the development of the TELE-VIR point-of-evidence toolbox, which will help to control outbreaks of new emerging viruses in the future, at national, regional, European, and even global levels.
INTRODUCTION

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ACHIEVEMENTS

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JOINT RESEARCH PROJECTS: EMERGING THREATS (ET)

MEmE - year 1

The MEmE project is an international, multicentre collaborative project that aims to fill research gaps highlighted by international agencies (EFSA, ECDC, WHO) for the detection and control of zoonotic parasites *Echinococcus multilocularis* and *Echinococcus granulosus sensu lato* (s.l.), causing alveolar echinococcosis and cystic echinococcosis, respectively. The MEmE project focuses on harmonisation and validation of existing parasitological and molecular methods, in addition to the development and comparative assessment of innovative molecular tools to detect Echinococcus species in the food chain. Production of epidemiological data on the presence of *E. multilocularis* and *E. granulosus s.l.* eggs in the food chain focuses on vegetables and wild berries for human consumption and on canine faeces in selected endemic countries. This project provides a comprehensive set of integrative activities to harmonise procedures, improve the detection and produce epidemiological data on potential pathways of transmission of alveolar echinococcosis and cystic echinococcosis.

The MEmE project has produced Standard Operating Procedures and sampled different matrices from naturally or experimentally infected definitive and intermediate animal hosts. Validation of the parasitological Segmental Sedimentation and Counting Technique (SSCT) and molecular diagnostic (multiplex-PCRs and MC-RT-PCR assay) procedures to detect alveolar and cystic echinococcosis in different matrices along the food chain were completed.

Dissemination of MEmE research has included publications on the development and validation of new tools: i) Comparison of two DNA extraction methods and two PCRs for the detection of alveolar echinococcosis in stool samples; ii) Bayesian Analysis of three methods for diagnosis of cystic echinococcosis in sheep; iii) Microsatellite investigations of *E.granulosus* cysts; iv) Species detection of *E. granulosus* by novel probe-based real-time PCRs; v) Validated method based on PCR-RFLP and multiplex PCR assay for the identification of *E. granulosus* species; vi) Identification of *E. granulosus* G1/G3 by SNPs assays.

There are ongoing multicentre studies to produce data relevant for epidemiological assessments, which include the contamination of vegetables for human consumption by *E. multilocularis* and *E. granulosus* eggs, and prevalence of *E. multilocularis* and *E. granulosus* in dog faeces.

Publications in 2021:


PARADISE - year 2

The PARADISE project aims to deliver informative genotyping schemes and innovative isolation strategies applicable to food and environmental matrices for Cryptosporidium and Giardia parasites. These are important intestinal pathogens of humans and young animals, transmitted through direct and indirect routes, with a global distribution. Large outbreaks linked to contaminated water and food have been reported in Europe and elsewhere. The PARADISE project will generate data to allow the first large-scale analysis of the genomes of these organisms. Further, the project will deliver improved strain-typing schemes for better investigations of outbreaks and understanding of the epidemiology (e.g., zoonotic transmission). Finally, the work on the enrichment strategies should deliver tools enabling cost-effective investigation of food and environmental matrices, in perspective leading to improved risk assessment.

During the last two years, significant progress has been made on research-orientated activities.

Many partners in the PARADISE project contributed to the collection of C. parvum and G. duodenalis isolates across Europe for whole genome sequencing experiments. This has generated >100 new C. parvum genomes from humans and ruminants, and of >40 new G. duodenalis assemblage B genomes, mostly from humans. This represents the largest datasets available for these pathogens at present. Comparative genomics studies are in progress. The metagenomics work focused on both in silico and experimental approaches. The in silico work demonstrated the presence of parasite sequences in metagenomes from various matrices, using Cryptosporidium as a model. A paper was published that reported new analytical workflows that increase the specificity of detection. The experimental part focused on further testing of an amplicon-based platform for detection of eukaryotic pathogens. A paper was published that reported on the application of the platform for detection of zoonotic protozoa in pig faeces. Finally, the limit of detection of the two metagenomics approaches (amplicon-based and shotgun) is being explored by sequencing plant DNA spiked with parasite DNA at concentrations that mimic natural contamination.

Research activities identified candidate genomic regions endowed with high genetic variability for both C. parvum and G. duodenalis assemblage B, by comparison of >140 genomes and 18 genomes, respectively. Laboratory tests by PCR and Sanger sequencing were performed for all candidates and each parasite, with design of protocols for single and nested PCR. Based on the observed genetic variability and additional criteria, eight makers have been selected for C. parvum and seven for G. duodenalis assemblage B. Further testing, performed on about 500 C. parvum isolates and
**JOINT RESEARCH PROJECTS: EMERGING THREATS (ET)**

*G. duodenalis* assemblage B isolates, allowed estimating the discriminatory power of the typing schemes. For *C. parvum*, a comparison with a typing scheme based on Variable Number of Tandem Repeats, which was developed by an associated partner (CRU), has also been conducted. Ring tests involving all the Consortium partners will be organised shortly to establish the two typing schemes.

The work on nanobodies has led to the identification of several heavy-chain only antibodies (VHH) clones reacting against *Giardia duodenalis* cysts. Further validation of these promising candidates is under way. For *C. parvum*, screening of the cDNA library against parasite oysts is still in progress. The work on aptamers has seen a change in the candidate selection strategy as well as with the application of a high-throughput sequencing of DNA sequences from each SELEX round and each replica. A core of sequences enriched at the end of the SELEX process was identified for both *C. parvum* and *G. duodenalis*. Experiments to evaluate the binding properties and affinity of potential binders are underway. Finally, for the post-DNA extraction work, two hybridisation capture systems were designed for *Cryptosporidium* (18S rDNA and gp60 loci), and one for *Giardia* (beta giardin locus). Robust validation of the *Cryptosporidium* capture systems is currently ongoing in two laboratories.

**Publications in 2021:**

The IDEMBRU project aims to develop a toolkit for diagnostics, characterisation, and assessment of zoonotic potential of newly discovered species of the bacteria from *Brucella* genus. The project also examines the possibilities for the existence of new wildlife and environmental reservoirs of classical *Brucella* species. The *Brucella* spp. are one of the most pathogenic bacteria described, which historically have been used for bioterrorism. The IDEMBRU toolkit will integrate data across Europe to assess the risks for new sources and newly discovered strains of *Brucella* spp., which will assist in improving the public health strategies against this highly contagious disease.

In the second year, the IDEMBRU consortium reorganised and prioritised the tasks of genetic, phenotypic comparisons and characterisation of zoonotic potential of newly discovered species and isolated strains. All partners continued sample collections from wildlife, environment, and humans to assess the levels of contamination and provide scenarios to test the final toolkit within the One Health approach concept.

Sample collection started throughout Europe mainly from wildlife species found in forest, fresh water, and marine coastal habitats. The sample collection was organised with the wildlife protection networks and hunting organisations in each country. To optimise the usage of wildlife carcases from these organisations, the existing tissue samples already collected for other research projects have been prioritised. More than 1500 samples have been processed and analysed by molecular or bacteriological methods. Several environmental strains of bacteria similar to *Brucella* (such as *Ochrobactrum* spp.) have been isolated, but further genetic typing is needed to establish the links.

Currently, many European countries are experiencing the surge in cases of canine brucellosis caused by *B. canis*, with few human cases described as well. IDEMBRU has established a network with the COHESIVE project regarding the evaluation of presence of *B. canis* in dogs and humans to determine its zoonotic potential and pathogenic characteristics. The exposed groups have been identified such that serology sampling comes from individuals who are professionally more likely to be exposed to *Brucellae*. The members of two projects organised the first European workshop on *B. canis* in May 2021, which contributed to the joint white paper initiative on the current situation in Europe with recommendations to EU commission for further actions. Therefore, IDEMBRU project decided to focus a part of its work on *B. canis* as one of the evident emerging zoonoses from *Brucella* genus.

The evaluation of DNA extraction methods from complex matrices (water, soil, and faeces) was finalised at the end of 2021. Additionally, the DNA extraction from canine faeces was analysed, since
this type of samples barres its own complexities. The harmonised whole genome sequencing protocols were prepared, and first ring trials were completed. The project has added the environmental root commensal bacteria *Ocrobactrum* spp. to these analyses, since several species from this group are considered opportunistic pathogens and have been added to *Brucella* genus regarding their phylogenetic closeness. Although many isolated strains (especially from environmental samples and similar to *Brucella* morphologically), the molecular methods have not been able to detect them. This led to the construction of primers specific for *Ochrobactrum* spp. to distinguish between the two genera.

The next stages will validate a microfluidics PCR plate, so that the diagnostic and typing of *Brucella* and *Brucella*-like bacteria can be performed, even from a few organisms on multiple samples. The main advantage of the microfluidics system is that it can perform PCR with up to 96 primer pairs on up to 96 samples at one time. The project validated the protocols for RNA extraction, RNA sequencing, antimicrobial resistance as well as *in vitro* and *in vivo* assays. Due to the manufacturer's cessation of micronaut plate production, IDEMBRU decided to replace micronauts with MALDI-TOF MS high resolution. This decision will provide proteomic profiling of highly virulent strains, low virulent and non-zoonotic strains from bacterial media and cell culture infections. A complete proteomic profile will be created to help identify the virulence factors that can be used in new diagnostic and/or characterisation tests. The results obtained will be confirmed with other employed methods before being added to the final toolkit. The cell cultures for *in vitro* infection have been chosen, and pipeline for pathogenicity classification established for the first experiments to be conducted in 2022.

**Publications in 2021:**
**PUBLICATIONS 2021 - ENDED PROJECTS**

**AIR-SAMPLE**

**METASTAVA – ended December 2020**


**IMPART - ended December 2020**
**PhD Projects**

**One Health EJP PhD Programme Progress**

Between 2018 and 2021, 17 PhDs were co-funded by the One Health EJP Education and Training activities as part of Work Package 6. The research focus of the individual PhD projects falls within at least one of the three research domains of the OHEJP: foodborne zoonoses, antimicrobial resistance and emerging threats. An additional PhD was funded in 2019 which focuses on sustainability and lies in the field of social sciences and public health.

The PhD projects provide opportunities to explore and share skills, expertise and knowledge from the OHEJP consortium, therefore accelerating both the rate and quality of research in addition to developing the One Health scientific leaders of the future contributing to the sustainability of the One Health approach.

There is significant scope for inter-disciplinary networking among OHEJP partners in addition to the interaction with the JRPs and JIPs. The JRPs and JIPs have expertise that can support the PhD students and provide opportunities to explore and share skills and knowledge, accelerating both the rate and quality of the research. These interactions help to bring the physical, biological, and social sciences together, and allow greater flexibility in the PhD projects to ensure innovative hypothesis driven research.

Despite the challenges caused by the COVID-19 pandemic, our PhD students continued to advance their scientific research and work towards achieving their key deliverables and milestones. The students also participated at the Annual Scientific Meeting in June 2021, where they presented their projects and research through presentation of a scientific poster and an oral presentation at the three-minute thesis (3MT) competition, to an audience of around 500 scientists and professionals across the globe. To find more information about the PhD students, click here.

You can read their 12-month reports describing their progress between January to December 2020 here.
ECO-HEN

The ECO-HEN project commenced in February 2019. This PhD project studies the role of *Escherichia coli* (*E. coli*) from the intestinal microbiota in the spread of antimicrobial resistance (AMR). *E. coli* clonal strains are able to survive on the food chain and could spread AMR genes across bacteria due to its ability for horizontal transfer of genetic platforms (such as plasmids and integrons). The presence of AMR *E. coli* in animal intestinal microbiota such as pigs and broilers are well documented, however the dynamics of AMR *E. coli* populations in commercial table eggs production has been scarcely studied.

The main goal of the PhD is to fill the knowledge gap on the transmission dynamics of AMR *E. coli* in commercial laying hen production and to determine to what extent the table egg production poses a public health risk through food and/or environment contamination. To evaluate the risk for spread of AMR to humans and the environment, genomic data will be shared across electronic platforms available for the larger community of clinical and environmental microbiologists for comparative analyses. The results will also reveal what the effect is of reduced antimicrobial use on the AMR bacteria initially present in the day-one chicks.

In 2021, the reconstruction of plasmids which spread AMR genes from animal isolates to eggshell isolates was finalised. This work aimed to reconstruct the plasmids responsible for dissemination of AMR genes across isolates from different sources. It is widely recognised 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 therefore knowledge on the AMR plasmids is essential to describe the flow of AMR in different ecological niches. Different plasmids harbouring genes responsible for cephalosporin resistance have been identified and fully characterised.

The research focusing on the in-depth analysis of the surroundings of the trimethoprim resistance gene dfrA36, continued. The stability of the large plasmid harbouring dfrA36 was studied and it was found that after 100 generations, more than 80% of the cells lost the plasmid. The stability of this plasmid in the presence of antibiotic was also studied up to generation 20, with 20% of the cells lost the plasmid up to this generation, compared to 50% lost after 20 generations in the absence of selective pressure. This means that this large plasmid is lost over generations even with antibiotic pressure, although less abruptly in the presence of antibiotic in the medium.

In addition, the AMR flow between animals has been studied, by phylogenetic analyses of isolates from day-old chicks to pullets and laying hens. Based on phylogenetic distances and clones, mobile genetic elements have been studied for their transmission dynamics. Clonal transmission of resistance genes has been identified, in addition to transmission due to mobile genetic elements.
LIN-RES

The LIN-RES project commenced in January 2019, and focuses on the antimicrobial Linezolid, one of the last resort drugs used to fight human infections caused by multi-resistant Gram-positive bacteria such as *Streptococci*, *Staphylococci* and *Enterococci*. It is commercially available since 2000 and has not been licensed for use in animals. In 2008, the first instance of transferable resistance to Linezolid caused by the 23SrRNA methylase Cfr (Chloramphenicol Florfenicol Resistance) was reported in US Staphylococcal isolates from human infections. A second gene, *optrA*, conferring resistance to Linezolid and Phenicols whose sequence was first reported in 2015, is an ABC-type membrane transporter and works as an efflux pump. After its initial finding in China, it recently emerged in animal and human Enterococci/Staphylococci on both the American and European continents.

This PhD project aims to investigate the molecular basis, origin, transferability, and risk factors associated with Linezolid-resistance emergence in Gram-positive bacteria of both human and animal origin.

Between 2019 and 2020, 1325 faeces samples and 148 nasal swabs samples were collected from food-producing animals in Belgium for the official monitoring of antimicrobial resistance and were screened for linezolid resistant bacteria. All the linezolid resistant isolates were sequenced by whole genome sequencing (WGS), assembled and analysed. A cgMLST analysis was conducted to study the relatedness of these isolates and compare with published sequenced of linezolid-resistant isolates. Insertion sequences were identified in contigs carrying the linezolid resistant genes. A paper has been published in open access in the *Journal of Antimicrobial Chemotherapy* (https://doi.org/10.1093/jac/dkab376), and describes the diversity of linezolid-resistant isolates.

A study about antibiotic consumption at farm level is in the process of being finalised to look after a potential correlation between linezolid resistant gene occurrences and antibiotic consumption with a special focus on phenicol consumption (as linezolid resistant genes also confers resistance to phenicols).
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HME-AMR

There has been a substantial delay to the commencement of this project due to recruitment issues, followed by the COVID-19 pandemic. The work only commenced in February 2021.

A key element in managing antimicrobial resistance (AMR) in the One Health paradigm is to reduce the spread of resistance genes between microorganisms in the agri-food environment. Heavy metals occur ubiquitously in the agri-food environment and sometimes in high concentrations in soil. In food animal production, heavy metals such as zinc and copper are frequently added to animal feed to promote growth and health. Such heavy metals may not be fully absorbed from the animal gut and are excreted in faeces into the environment.

It is recognised that a One Health approach is required to tackle AMR, which includes the role of the environment, and the food production environment in particular. Very limited information is available regarding the impact that selective pressures such as heavy metals may have on the mobilisation of AMR and its potential transfer into the food chain. There is a clear need for more data on the impact of heavy metal concentrations in food production settings, and their potential impact on the co-selection and dissemination of AMR in the environment and food chain, and this is therefore the driver of this project.

The HME-AMR PhD project is investigating the role of heavy metals in the environment as a selective pressure for the dissemination of antimicrobial resistance.

The first experimental trial of the project is nearing completion. Field plots were set up in two geographically distinct regions, with zinc added to some plots and not to others at each site. Soil samples were taken at both sites (22 zinc added, 22 no zinc added) and returned to the lab. Each sample was analysed for the presence of Enterobacterales, ESBL-producing Enterobacterales, carbapenamase producing Enterobacterales and ciprofloxacin resistant Enterobacterales using culture-based standard operating procedures in place in the hosting institute. Presumptive isolates obtained from this sampling were identified by MALDI-TOF. The ones confirmed as Enterobacterales were then tested for their antimicrobial susceptibility by disc diffusion in accordance with EUCAST criteria and phenotypic profiles will be compared with genotypic profiles obtained from whole genome sequencing. Soil samples from each plot were also stored for later DNA extraction for metagenomics analysis.
The KENTUCKY project commenced in January 2020. Salmonella enterica serovar Kentucky (S. Kentucky) is a common causative agent of gastroenteritis in humans. It is one of most notorious Salmonella serotypes, as it is strongly associated with antimicrobial resistance (AMR). Ciprofloxacin-resistant S. Kentucky (CIPR S. Kentucky) belongs to a single sequence type (ST198), which acquired a variant of the Salmonella genomic island 1 (SGI1) conferring resistance to first-line antimicrobials (beta-lactams, aminoglycosides, sulphonamides, tetracyclines). In addition to resistance to Ciprofloxacin, S. Kentucky can gain additional antibiotic resistance determinants through the acquisition of locally circulating plasmid-borne genes, such as the ones coding for Extended Spectrum Beta-Lactamases (ESBLs), AmpC beta-lactamases and/or carbapenemases. Most recently, the situation has worsened, as the European Centre for Disease Prevention and Control (ECDC) has launched an Urgent Inquiry (UI-464) on a CIPR S. Kentucky ST198 strain carrying a chromosomally integrated blaCTX-M-14b gene encoding for cephalosporin resistance.

The insertion event was traced back to Malta, but the strain has already spread to Belgium, UK, The Netherlands and five other EU countries. To date, this clone is only reported in humans, as opposed to (for example) the CipS S. Kentucky ST152 clone widely found in poultry in the USA but rarely reported in humans.

The KENTUCKY PhD project aims to investigate (i) what explains the evolutionary success of the multidrug resistant S. Kentucky ST198 clone, and (ii) what is the mechanism of the integration (and potential further transfer) of the ESBL gene in its chromosome.

In 2021, the study of the genetic environment of the integrated blaCTX-M-14b gene and its mechanism of integration continued. This part of the study focused on the investigation of the IncHI-type, mega-plasmid carrying the ISECP1-blaCTX-M-14b transposition unit. Due to its clinical significance, the plasmid was chosen as a focal point for fluorescent tagging. Yet, wet-lab experiments backed with in silico analysis indicated that the plasmid is non-conjugable, most probably due to truncation in the transfer region. Thus, the prototype IncHI1 plasmid (R27), a self-transmissible plasmid with fully characterised conjugation machinery (Craig et al., 2000) and capable of transfer between members of the Enterobacteriaceae was chosen to continue the study. To investigate the transposition mechanism of ISECP1, fluorescent reporters were developed, based on plasmid partitioning systems. Fluorescence tagging was possible using the P1/ parS partitioning system (Nielsen et al., 2006).
The abundance of insertion sequences (IS) and AMR genes was also evaluated using bioinformatics tools (ResFinder, ISEScan, PlasmidFinder and RFPlasmid) on a dataset of *Klebsiella pneumoniae* assemblies downloaded from online databases. The IS family that presented the highest number of associations with different AMR types was IS6, which was particularly associated with aminoglycosides and beta-lactams, followed by IS91, IS1380 and IS5. Considering specifically beta-lactamases, the IS family that is most often associated with genes of this class of resistance was IS1182, followed by IS21, IS1380 and IS6. In particular, the genes blaCTX-M-15, blaCTX-M-3, blaKPC.3 seem to be often found close to many of the IS families present in the dataset. The IS1380 family, to which belongs the ISEcp1 element, can be found close to the following beta-lactam resistance genes, blaCTX-M-15, blaCTX-M-132, blaCTX-M-3, blaCTX-M-36, and blaKPC-2. The results obtained so far provide a detailed look into the mobilising capacity of Insertion sequence (IS) elements and AMR genes. In the next year, this mobilisation using the constructed fluorescently marked reporter strains will continue to be studied.
METAPRO

The METAPRO project is investigating the use of metagenomics and genomic approaches for the prevention of the spread of plazomicin resistance in humans, animals, and the environment.

Plazomicin is a novel semisynthetic aminoglycoside approved in June 2018 by the FDA that is used as a last resort antibiotic in complicated urinary tract infections (UTI) caused by multidrug resistant Gram-negative bacteria in humans. However, the expression of acquired 16S rRNA methyltransferases by bacteria results in complete resistance to plazomicin. In addition to resistance to this novel compound, bacteria harbouring these determinants show resistance to all clinically relevant aminoglycosides. Recent studies show that, despite not being as prevalent as other resistance mechanisms, these methyltransferases are already globally present, jeopardising the use of this critically needed antibiotic.

The PhD candidate was recruited in March 2020 and was involved in a network to diagnose COVID-19 in elderly homes in Madrid for the 6 first months of his PhD. During 2021, the sampling process started in pig and poultry farms and is ongoing for other ecological niches. A metagenomic analysis was performed on data collected in previous projects from pig and poultry in Spain by researchers from the host institute. This analysis detected the main plazomicin resistant determinants, the 16S rRNA methyltransferases, from samples of very different origins and the surroundings of these genes were analysed, to investigate which bacteria may act as reservoir of these important resistance determinants. It suggested that the Order Eubacterium may play a role in the maintenance of these resistance determinants.

This metagenomic analysis is currently being extended to samples from pig and poultry farms across Europe to enlarge the dataset. Human metagenomic data accessible via online databases are also being added to the study, to investigate the presence of 16S rRNA methyltransferases. Culture methods are also being investigated for the isolation of potential plazomicin resistant bacteria, to proceed with their Whole Genome Sequencing and identify more accurately the genetic environment of the methyltransferases.
PEMbo

Bovine tuberculosis (bTB), mainly caused by *Mycobacterium bovis*, is a zoonotic disease intimately and historically associated to cattle rearing. Although developing countries suffer the most from bTB, this disease remains a major problem in some developed countries. When cattle breeding developed into an established industry, strong control strategies were initially setup in Europe and other developed countries. In France, this resulted in a rapid decline in the number of infected herds, and in 2000, France obtained the official bTB free status from the EU. Despite considerable financial and social efforts against this disease, bTB continues to slowly but continuously rise and persist at regional levels.

The aim of the PEMbo project, a collaborative study between ANSES and INRA, is to better understand the complex biology of *M. bovis* through the study of the complete genomes of a large panel of isolates.

Ten representative *M. bovis* genotypes were selected from strains circulating in France. These selected strains were cultured (3-6 months for field strains) and their DNA was extracted using an optimised process. These strains representing ten different genotypes were then sequenced in MinION (Nanopore) and Illumina technology. Two of these strains were also sequenced at Genoscreen, Lille, with the PacBio technology. The results obtained were analysed using different assemblers for a same genotype and the most accurate one was selected. *De novo* assemblies were then performed with the best tools.

IS6110 is an insertion sequence of the *Mycobacterium tuberculosis* complex –to which *M. bovis* belongs-, with an important role in genome plasticity and in bacterial evolution caused by IS6110 transposition. It was previously demonstrated, in this PhD project, that IS6110 is present in multiple copies in endemic French *M. bovis* groups and could lead to phenotypic changes that explain their epidemiologic success. In 2021, it was discovered that some IS6110 interrupt or could regulate (via its strong promoter) some genes involved in virulence, host persistence or environmental stress resistance. The insertion positions seem stable in French endemic *M. bovis* groups over time and independently of the affected animal species.

Genomics analyses are also being performed on a larger panel of *M. bovis* short read sequences which represents the French *M. bovis* diversity, to better define the French clonal group. This aims to genetic targets explaining differential phenotypical characteristics of the different *M. bovis* groups.
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**MACE**

Cystic echinococcosis is a zoonotic parasite disease of significant public health concern in many parts of the world, with over 5,000 new cystic echinococcosis cases reported each year in South America. The burden, extending to include economic impacts, is mostly felt in subsistence livestock keepers and other rural and peri-urban populations where other health competing interests persist. Under reporting of the condition is large and the evidence to inform efficient surveillance and control approaches is scarce. The comparison of evidence is further complicated due to the large range of surveillance and control measures across all hosts, and their varied application across geographies.

The **MACE** project aims to inform the most efficient portfolio of surveillance options and interventions towards cystic echinococcosis control and elimination, accounting for the various risks, disease control capacities, and risk preferences across geographies. The project is novel in the joint application of mathematical modelling and economic evaluation, and in the active elicitation of risk attitudes towards cystic echinococcosis and related control measures to formally model their impact on the uptake of interventions and their efficacy. In this project, two highly cystic echinococcosis endemic areas will be targeted- Argentina (high incidence) and Albania (low incidence).

This PhD project started in January 2020. In 2021, a methodology for a new surveillance programme for cystic echinococcosis in dogs for Uruguay has been developed and will be implemented by the National Commission for zoonoses (NCZ). This framework is being extended and adapted to support current planning of a screening programme across the country, in collaboration with the Ministry of Agriculture. Due to changes in prioritisation since the beginning of the pandemic and a change in leadership in Uruguay, the collaboration with the Ministry of Agriculture has been put on hold indefinitely. The collaborations on the PhD project will have to change and will be established in Iran and Italy, for data sharing for the validation of the methodology.

A trip was made to Argentina, with a visit to the Argentine medical research institute under the Argentinean Health Ministry (ANLIS) in Buenos Aires and the regional unit of epidemiology and environmental health in the Andes area (URESA), in Rio Negro, with engagement activities and support of the sheep vaccination programme against CE.

A multi-host, individual based transmission model is currently being developed to, initially, simulate the dynamics of the disease between sheep and dogs. This will be extended to include different control options. Coding of the model has started, but not finished. Simulations still need to be finished.
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**DESIRE**

Brown and black rats carry a multitude of pathogens with public and veterinary health importance. Their potential to rapidly reach high population numbers creates unpredictable situations of high pathogen transmission risks. Rat populations are heavily affected by environmental changes such as urbanisation and climate change. A new phenomenon known as ‘greening’ and ‘blueing’ refers to improving living conditions and biodiversity in the city, and to combat heat; however, the consequences of these on rat-borne diseases is unknown.

Sustainable intervention is required to be directed to situations where the risk of transmission of pathogens creates human or veterinary risks. To perform risk assessment and mitigate the risks, a surveillance system is needed which has information both about the pathogen distribution as well as rat population developments.

The overarching aim of the DESIRE project is to design and test an effective surveillance system for rat-borne diseases, using the Netherlands as a test case. This project will provide evidence-based insights in four key elements of this surveillance system—monitoring of populations, monitoring of pathogens, risk assessment and intervention. The project will build onto existing surveillance activities and extend these by collaboration with international institutes.

In 2021 the PhD candidate has focused on the NGS analysis, literature review, field work, lab work and preparations for the population genetic study. The collection of monitoring data of rat populations by app continued in 2021. However, the number of reports is currently too low and too geographically clustered to be used for analyses. The monitoring app is running and will still continue, but the PhD will focus on a different study: a population genetics study, assessing the genetic relatedness of rat populations across a city, and studying the influence of green and blue areas on the dispersal of wild rats. In 2021, the study has been set up and samples have been collected. These will be analysed in 2022. The PhD student has familiarised herself with the analyses of the NGS data. These results have been verified with more traditional techniques in collaboration with the partner institute WBVR. This demanded more time than anticipated but improved the final result. Also, with the WBVR, the PhD student has studied the virome of a selection of rats. Currently, the PhD student is finishing the manuscript of this study, which will be submitted in the first months of 2022 to a peer-reviewed journal.

Additional to the initial work plan, a literature review on zoonoses of wildlife has started, for which several wildlife species that are commonly found in the urban environment have been selected. This should aid in prioritising research and funds and should give more insight in ecological mechanisms influencing the wildlife populations and/or the zoonotic pathogens.
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UDoFRIC

Since 2005, \textit{Campylobacter} has been the most reported gastrointestinal bacterial pathogen for people (campylobacteriosis), with an EU notification rate of 64.8 per 100,000 population in 2017.

Across European member states, antimicrobial resistance (AMR) monitoring in clinical \textit{Campylobacter} isolates has reported increasingly high levels of ciprofloxacin resistance. Ciprofloxacin is an antibiotic of the fluoroquinolone class and is regarded as critically important by the World Health Organisation, therefore this increase in resistance to Ciprofloxacin is a major public health concern. \textit{Campylobacter} originating from poultry are considered a main source of campylobacteriosis in people. The use of ciprofloxacin in broiler flocks has been linked to the development of resistance in \textit{Campylobacter} that can persist after use has ceased and is a potential source of resistance in human campylobacteriosis. In the EU, a ban of routine use of feed supplemented with antibiotics was implemented in 2006, however therapeutic use of fluoroquinolones in poultry remains an option.

The UDoFRIC project aims to exploit the archives of \textit{Campylobacter} and associated information from surveillance and research across the food-chain to investigate temporal trends in the development and diversity of fluoroquinolone resistance in UK and French broiler flocks. The project aims to examine the relationship between fluoroquinolone use in poultry and development of resistance, assess fitness benefits/costs of acquired resistance and determine if any specific fluoroquinolone resistant variants found in poultry are more or less likely to persist and cause disease in people. The data from this project would feed into the risk assessment for ongoing use of fluoroquinolone in poultry and consequent risks of fluoroquinolone resistance in clinical cases.

The PhD project started in March 2020. A literature review focussing on the background and previous research conducted on fluoroquinolone (FQ) resistance (FOR) in \textit{Campylobacter} has been completed. The first section of the literature review focused on \textit{Campylobacter} within broiler production systems, its impact on industry and on human health. Also, publications on \textit{Campylobacter} genetics, including virulence mechanisms, mutations, and gene transfer have been reviewed. The second section of the literature review has also been completed, with a distinct focus on FQs, their chemical structure and the differences between different types of FQs, their uses, importance and mechanisms of action. Then, the review describes the historic trends in the use of FQs and how this usage has developed over time.
Data has also been gathered and reviewed from six datasets taken from research and surveillance of broiler production systems from 1994 to 2020. These datasets include information on phenotypic resistance to antimicrobials, genomic data (including some whole genome sequence data) and epidemiological meta-data of 3,052 poultry *Campylobacter* isolates. Key work has been undertaken in the collation of the datasets included in this project. A sample size of isolates has been collected for years (1995 (n=96), 2008 (n=96) and 2020 (96)) where WGS information was missing, and work is ongoing to process this. Isolates sampled from 1995 have been profiled using MLST and we are currently working on building a fluoroquinolone resistant snps database for *Campylobacter* to determine genotypic resistance. From 1995, 2008 and 2020 there also is access to a sample list and outlined methods for MICs to determine phenotypic resistance profile to compare with genotypic resistance findings. Further work has been carried out in the analysis of previously characterised datasets to identify trends in resistance in several associated production variables (such as farming method, abattoir of slaughter etc). Initial observations follow that of previous studies, indicating that FQR in *Campylobacter* has increased over time and that certain clonal complexes and sequence types may be specifically associated with FQR. Similar analysis is needed on one final dataset the student has yet to acquire.

Training in Whole Genome Sequencing (WGS) and bioinformatics has been undertaken. Bioinformatics training has involved the building of a database of known antimicrobial resistance (AMR) genes and mutations for *Campylobacter*, and initial training to assemble genomes, to identify multi locus sequence types using SRST2 and detecting AMR genes and mutations using the APHA SeqFinder WGS analysis pipeline. The student has now categorised the first set of WGS information (1995 dataset, n=96 isolates) into MLST profiles, completed training in the use of APHA SeqFinder and is finalising the *Campylobacter* AMR database to determine a genotypic resistance profile. The student has also attended a week-long online course with the Antimicrobial Resistance EURL and an online training course of "Bioinformatics for Biologists: An Introduction to Linux, Bash Scripting, and R" with Wellcome Genome Campus Courses and Conferences to train in different detection methods of AMR and utilising WGS information.

*In vivo* experiments have begun at ANSES with the student relocating to conduct such experimentation. Four groups of specific pathogen-free (SPF) and *Campylobacter*-free chicks were inoculated with one of four FQ-susceptible *C. jejuni* strains. Two weeks post inoculation birds underwent enrofloxacin treatment, as per manufacturer’s instructions. Samples were taken at regular intervals throughout the experiment and *C. jejuni* positive samples were monitored for fluoroquinolone resistance. Four FQR isolates taken from samples at the latest point in the study were selected for use in competition trials.
WILBR

The propagation and spread of microorganisms resistant to antimicrobials is a global phenomenon that is affecting both human and animal health. Through the One Health agenda, the risks posed by the medical and veterinary sectors are being assessed and addressed through national and global initiatives and programmes. However, there has been limited focus both in Europe and globally on the role of the environment in propagating resistant microorganisms through inadequate treatment of contaminated/wastewater, or medical, biological and food waste, which may be dispersed further through wildlife such as wild birds.

Migratory birds, which represent ~40% of total birds in the world, can fly many thousands of kilometres often overwintering in Africa and Eurasia and returning to the northern hemisphere in spring. These birds add another level of complexity to identifying and controlling the routes for spread of antimicrobial resistance, as they often overwinter in countries or areas where they may be little information of resistance trends due to limited surveillance and diagnostic capacity, with the burden of AMR unknown.

To help provide an assessment of the environmental risk posed by AMR and identify management options with clear indicators of effectiveness, the WILBR project aims to understand the contribution of factors such as wild birds in the spread of AMR in the environment in general, and on livestock farms in particular.

The PhD student was successfully recruited to the WILBR project in February 2020. In 2021, a literature review has been written on the role of wild birds in dissemination and persistence of antimicrobial resistance (AMR) in the farm environment. The review includes sections on identifying the current situation regarding AMR in different environments; drivers for AMR; the role of vectors and the environment in persistence and dissemination of AMR; the role of AMR surveillance; and evaluation of different methodologies for identifying AMR, both by phenotype and genotype, in bacteria.

Pig and presumptive gull faecal samples were collected over three time points in October 2017, 2018, and 2019 from a low antimicrobial usage pig farm as part of the APHA’s work on the ARDIG project within the One Health European Joint Programme. These were grown on antibiotic free and antibiotic selective agar, and 632 E. coli were purified from pig and gull faeces (n=342 and n=290 respectively). 176 gull isolates (110 from T3, 66 from T5) that had not been subject to previous analyses were identified and underwent whole genome sequencing. WGS data for all isolates was run through APHA Seqfinder pipeline and Abricate to identify AMR determinants and plasmids. Seven allele MLST was
PhD PROJECTS

carried out using SRST2 to identify the *E. coli* Sequence Type (ST) of isolates, which was used as an indicator of *E. coli* diversity in pig and gull populations. A total of 91 different sequence types were identified from the isolates included in this study, with 63 and 57 STs identified in gull and pig isolates, respectively. Considering the SNP differences is the next step to understand if the same “clone” or strain has been transferred rather than an AMR plasmid. A core genome SNP alignment was generated from WGS data of all isolates using Snippy version 4.6.0 to compare the core genes that are present in all members of the same species, and a SNP distance matrix was generated using snp-dists version 0.7.0 to estimate the SNP differences between isolates to determine the levels of genetic variation in the ST744s, allowing any transmission and persistence of clones across host species and time points, on this farm, to be identified. Preliminary analysis allowed AMR profiles to be linked to ST types that are circulating in both wild bird and pig populations.

Another outdoor pig farm, known to have wild birds persistently present on farm, had been recruited for a longitudinal study and was visited in September 2021 where over 150 environmental swabs were collected. An opportunity to examine the dissemination of AMR in wild bird populations across Great Britain for a 12-month period through the APHA’s wild bird scanning surveillance programme is currently being pursued.
INTRODUCTION

Foodborne diseases remain an important cause of morbidity, mortality, and healthcare costs worldwide. The problem is expected to be exacerbated by population growth and the rising of antibiotic resistance. Furthermore, anthropogenic activities are constantly changing the environment, for example climate change, land use and socio-economic factors, which are a well-recognised driver of diseases. The environment can affect pathogen abundance, survival, and virulence, host susceptibility to infection as well as human behaviour. It is thought that the accelerating rate of global climate and other environmental change will impact the distribution, frequency, and patterns of established diseases as well as the emergence and re-emergence of new and old ones.

The EnvDis project aims to develop a tool to assess the public health risk of foodborne zoonoses based on information of relevant environmental factors. This will be done using *Salmonella*, for which the mechanism of transmission is relatively well-known, and will help validate the approach.

In April 2021, the updated daily human salmonellosis data was received from the UK Health Security Agency (UKHSA) (previously known as Public Health England), completing the disease data required for performing a thorough analysis for England and Wales. Together with other gathered data (such as altitude, detailed demographics with the number of residents by age and sex, year to year), an exploratory analysis of the data was performed confirming the notably higher prevalence of salmonellosis in the younger age groups (i.e. 0-4 years old). *S. Enteritidis* was confirmed as the predominant serotype over the other serovars, accounting for 54.11% of the cases.

On top of temperature, vapour pressure and cloud cover were found to potentially have a relevant influence over the incidence of salmonellosis, according to some papers (Park, Park and Bahk, 2018), (Aguilar, Herrero and Polo, 2010), (Busse et al., 2019). At the same time, air pressure and dewpoint were identified to be related with water activity in food commodities. Water activity plays an important role in the microbial growth in food and may be more relevant than atmospheric relative humidity of the environment. A conditional incidence of the combined effect of all these variables as well as the effect of extreme climate conditions is on progress. A short set of laboratorial experiments in a chicken gut model were performed to assess the impact of environmental perturbances over two bacterial populations (*E. coli* and *Salmonella* alone and in combination) when applied in a patterned fashion. A 48h-cyclic pH alteration was used as example. The outcomes will be a chapter of the thesis. A spatial analysis to determine the influence of the location over the cases of salmonellosis is also ongoing. Two country-level maps, each of which will display the reported incidence and the simulated incidence based
on its linkage with the relevant weather variables. They can be visually compared as a proxy of the role of the weather and the influence of the climate particularities of each region in the incidence of disease.

Since animals are the source of disease for humans, all available information was gathered to investigate relevant relationships, such as the proximity of animal premises to the disease events or the linkage of the peak of animal salmonellosis with human incidence. So far, yearly farm animal (cattle, pig, and poultry) numbers per county from APHA were collected. A conditional incidence analysis investigating the likelihood of salmonellosis given the presence of livestock/poultry will follow.
AptaTrich

Trichinellosis is a zoonosis caused by the consumption of raw or undercooked meat of animals (mainly pigs, wild boars, horses) infected with the nematode, *Trichinella* spp. To date, *Trichinella* remains in the top three of prioritised foodborne parasites in Europe and this parasite is still of major public health and economic importance at international level. Due to the very low *Trichinella* prevalence in pigs, a test specificity bordering 100% is needed, as false positive samples would need to be retested with a second serological method. Such tests can only be performed by specialised laboratories, making the testing logistics more complicated and expensive.

Therefore, new diagnostic methods with higher specificity and earlier detection are needed for prevention and to improve human disease detection. One such method is the use of aptamers. Aptamers are synthetic nucleic acids that fold into unique 3D conformations capable of binding pathogen antigens with remarkable affinity and specificity, thus, combining the ease of serological sampling, and the direct detection of the presence of the pathogen. Aptamers have successfully been used for the detection of parasites in fresh produce, including in aptamer-based biosensors.

The AptaTrich project is investigating the development of an aptamer-based detection system for *Trichinella* which would bypass the caveats associated with serological testing and enable specific and early detection in both human diagnostics and *Trichinella* monitoring programmes in pigs. The technique can also be combined with aptamers designed against other pig diseases (e.g., *Toxoplasma*, *Salmonella*, etc) making a wider future application possible.

A whole-larvae SELEX method has been established against *Trichinella spiralis* muscle larvae. The current protocol is continuously being improved to produce the highest quality aptamers possible. This includes making frequent alterations in the parameters and conditions of target-aptamer incubation. Selection of single stranded DNA-based aptamers specific for *T. spiralis* whole muscle larvae (ML) is ongoing. Using a highly variable and randomized ssDNA library, a whole-larvae SELEX method (Systematic Evolution of Ligands by Enrichment) is undergoing continuous improvement. As of now, 8 rounds of SELEX have been completed using 2 different protocols. Additionally, further selection in ongoing using an alternative protocol.

As is demonstrated in prior research literature, optimisation of sequence amplification by PCR, as well as the generation of single-stranded DNA (ssDNA) from double-stranded DNA (dsDNA) is of absolute importance for a successful selection procedure. In light of this, two different methodologies were
optimised and compared to determine the best method. Using biotinylated reverse primers, a Streptavidin magnetic bead method used the denaturing abilities of NaOH to liberate unlabeled ssDNA from the magnetic bead surface. Additionally, a lambda exonuclease enzyme was used to digest sequences amplified with phosphorylated reverse primers. A collaborative partnership has been established and several samples from various rounds of SELEX have been subjected to High-throughput sequencing (HTS) and subsequent bioinformatics analysis to determine possible sequence enrichment. From this, several sequences containing consensus motifs have been selected for synthesis and eventual binding affinity and specificity testing. By incorporating a 5'Fluorescein isothiocyanate (FITC) onto each sequence, binding affinity and specificity for the *T. spiralis* whole ML will be measured using confocal microscopy.

While results of qPCR suggest low sequence enrichment with minimal evolution, HTS and bioinformatics results using PATTERNITY.seq software demonstrate an interesting shift towards a certain consensus sequence present in the variable region. In addition, a number of sequences illustrating higher than average evolution possess a defined consensus motif of interest.

*Trichinella spiralis* ES proteins are currently awaiting proteomic analysis by LC-MS/MS for potential biomarkers and aptamer targets of interest. Following the production of *T. spiralis* muscle larvae Excretory/Secretory proteins, the student visited the Montreal partner laboratory at the Research Institute of the McGill University Health Centre (RI-MUHC) to analyse the samples by Liquid Chromatography-Mass Spectrometry (LC-MS). During the mission however, several problems were encountered with the equipment, ultimately resulting in a failure to analyse the samples without impact on the samples themselves. During the mission in Montreal, a review article surrounding the use of aptamers in parasitology-based diagnostics was completed. The protein samples are currently awaiting analysis by the partner institute.
VIMOGUT

Antimicrobial resistance (AMR) is a major public health concern, and many factors contribute to this, including the use of antimicrobials as growth promoters in livestock. It is essential to reduce prevalence of AMR in livestock to reduce the likelihood of antibiotic resistant bacteria passing through the food chain and to retain effective therapeutic treatment of the livestock itself. Preliminary results show that the microbiome of chickens colonised early in life by extended-spectrum beta-lactamase (ESBL) producing *E. coli* is less diverse than those of flock mates that are not colonised. This is supported by *in vivo* studies that have shown that competitive exclusion through probiotics is currently the most effective prevention strategy for colonisation by organisms harbouring ESBLs. However, this strategy has been tested with limited attention for chick age. Furthermore, in practice, probiotics are considered too expensive to use throughout the whole production cycle. An *in vitro* chicken gut model was developed at the APHA, UK as an alternative to study bacterial interactions in complex communities such as the microbiome. This system allows the evaluation of new treatment interventions at different stages of the microbiome development, without the ethical concerns and high cost of *in vivo* experiments.

The VIMOGUT PhD project investigates the chicken microbiome development of chickens on farms to determine if the reported microbial progression is reproducible between different production rounds and farms. In 2021, data analysis was performed for the *in vivo* component to complete the first manuscript titled “Succession in the caecal microbiota of developing broilers colonised by Extended-spectrum β-lactamase-producing *Escherichia coli*”, which was submitted in September 2021. This study describes the ESBL-*E. coli* prevalence and successional dynamics of the caecal microbiome of developing broilers in a commercial flock during their production life (age 0 to 35). Broilers were discriminated as ESBL-*E. coli* colonised or not by selective culturing. Using 16S rRNA gene sequencing, the richness, evenness and composition of the caecal microbiota of both broiler groups were compared and the combined role of age and ESBL status on the microbiota was assessed. In conclusion, the role of ESBL-producing *E. coli* in the successional dynamics of the cecal microbiome in developing broilers was assessed and it was demonstrated that the presence of ESBL-producing *E. coli* is associated with mild but consistent reductions in alpha diversity and transient microbial compositional differences. The clonal spread of ESBL-*E. coli* and point to the farm environment as a likely source for ESBLs were also highlighted in the research paper.

The *in vitro* chicken caecum model was adjusted and optimised to minimise the presence of oxygen in the culture media, underlined by the high presence of anaerobic bacteria in the microbiome. Tests to monitor the presence of oxygen in the system were performed with a different set of sensors. The first
experiments used two feed additive phytochemicals as an intervention strategy to test the system. 16S rRNA genes sequencing and microbiome analyses were performed for different test runs. Additional optimisation of the system included the addition of a second gas inlet that creates an overlay of nitrogen on top of the culture media in the bioreactor to prevent any oxygen from dissolving into the culture. After DNA isolation, 16S rRNA genes amplicon sequencing was performed, and the data was analysed. In both experiments, the relative abundance of phyla of the in vitro microbiome was relatively stable and consisted primarily of anaerobic bacteria, confirming that the in vitro system can maintain the main broilers caecal microbial communities.

An approach of fluorescent labelling of plasmids, which was previously carried out at the University of Copenhagen is planned. A short-term mission proposal was submitted for the student to visit this research group to create her own fluorescently labelled plasmids efficiently. The PhD student was granted the STM and will complete it in May 2022.
ToxSauQMRA

*Toxoplasma gondii* is an intracellular parasite and one of the most successful parasites worldwide. Humans, as intermediate hosts, can become infected with *T. gondii* through ingestion of oocysts (e.g. when handling soil or cat litter, or on unwashed vegetables) or tissue cysts in raw or undercooked meat. Pigs, like other livestock, can harbour tissue cysts following the ingestion of oocysts. Products such as raw cured meats are a possible source of *T. gondii* and pose a major issue to public health. For example, in 2015, France produced just over 108,000 tonnes of sausages and dry sausages, representing ~9% of the total tonnage of all sausages (FICT data, 2016), therefore monitoring the prevalence of *T. gondii* is essential.

The high prevalence of *T. gondii* infection in France in humans and the fact that the main mode of contamination is foodborne, justifies the conduct of a quantitative microbiological risk assessment (QMRA). In France, *T. gondii* seroprevalence estimates in various animal species (ovine, bovine, pig, horses) are available, enabling such an analysis, however knowledge gaps include the quantitative estimates concerning the distribution of parasites in the various parts (muscles) of a carcass and the reduction of the parasite load according to the cooking, preparation, or preservation, as identified by an expert group of ANSES in 2005.

The ToxSauQMRA project aims to answer the scientific question- “What is the attribution of the traditional raw pork products in the human *Toxoplasma gondii* infection?” based on three areas: (i) A thorough investigation of the preferential sites for *T. gondii* in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst). (ii) Evaluation of 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*. (iii) A quantitative microbiological risk assessment analysis to be conducted for the various raw pork products (dry sausage, dry ham, etc.).

The first part of the PhD work (investigation of the preferential sites for *T. gondii* in experimentally infested pig carcasses with two different stages), has been completed with the help of external partners: Institut du Porc (IFIP) and Université de Champagne-Ardennes (URCA). To be able to collect meat massively contaminated with *Toxoplasma gondii*, pigs were infected with a high dose (1000) of parasites (strain ME49) in pigs. These tests were carried out with 2 parasitic forms that may be at the origin of contamination in pigs: the oocyst (ingestion from the environment) and the tissue cyst (ingestion from infested meat). Three pigs were inoculated with oocysts and 3 others with tissue cysts. Serological monitoring of the serum of these pigs was carried out by Modified Agglutination Test (MAT) on a weekly basis until D30, then every 15 days until the end of the protocol (3-4 months/80-90 kg). After euthanasia of the MAT positive pigs, 4 tissues /pig were collected for analysis: heart, breast,
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shoulder and ham as tissues used in the manufacture of dry sausage. For each of the anatomical regions studied, the different muscles (4 for breast, 7 for shoulder and 13 for ham) were pooled. Some 40 supplementary muscles per carcass were individually collected, representing the most important anatomical regions. One hind leg was collected for each pig for dry ham production. To identify the predilection sites of *T. gondii* presence in pig tissues, mouse bioassays and quantitative PCR were performed on a part (200g) of the pooled sample per region. The remaining parts of the pools were used for industrial (salting, smoking, etc) processing. The analysis of tissue samples ([breast, shoulder, ham, heart + 40 supplementary muscles] x 6 pigs) by MC-PCR, delayed by the 1st and the 2nd confinement due to COVID19 sanitary crisis in France, has been completed this year, for both parasitic stages (oocysts and tissue cysts). This involved the digestion, DNA extraction and PCR for each individual tissue sample.

Dry sausages were manufactured on a pilot scale by IFIP, according to a protocol representative of those used by a commercial factory. Briefly, after mincing, the muscle pool was divided into seven portions (corresponding to the 7 combinations of nitrites (as sodium NaNO2 nitrites) and NaCl concentrations that was to be compared: 120 (maximum dose of nitrites mentioned in the Code of Practice), 60 and 0 ppm of nitrites combined with the usual dose of 26 g/kg NaCl or a reduced dose of 20 g/kg NaCl or 0 g/kg NaCl. For each of the tested formulations, 3 dry sausages were collected at different dates (D0, D2, D10, D20, D30 and D50). On each analysis date, IFIP has carried out a physico-chemical monitoring (pH, Aw, weight loss) and a count of the lactic flora from a dry sausage per formulation, in particular to check that the process is running properly. A total of 168 dry sausages were tested in this study. The dry hams were meant to be sent and manufactured by INRAE Corte (Corsica), using two traditional salting techniques (a long one: 2.5 days/kg and a short one: 1 day/kg). However due to a local strike in the harbour of Marseille, the hams arrived with 10 days of delay, causing the sanitary quality of meat to be questionable in terms of manufacturing. Therefore, a long salting technique has been applied only, with 300g of product that was taken at D30 and D90 due to Covid19 shutdown. The analysis of the dry sausages and ham for the presence of viable *T. gondii* by bioassay in mice that has been performed in the animal facility of URCA. In total 90 mouse bioassays were performed with the last data being collected 6 weeks after the last bioassay was performed. At the same time, the presence of *T. gondii* DNA in the inocula, resulting from the dry sausage digestion, was quantified by URCA using a (conventional) qPCR and by ANSES by MC-qPCR developed by Opsteegh et al., (2010).

A review of the prevalence data of *T. gondii* in pigs and pork products also started in 2021. Data from various studies involving long-last processing pork delicatessen (Jambon de Parme; Jambon Serrano, etc) started to be collated, building on the systematic reviews performed for EFSA and OHEJP TOXOSOURCES. This will be continued in 2022.
Hepatitis E virus is a zoonotic virus responsible for acute hepatitis E in humans in Western countries. The main route of HEV transmission in Europe is through consumption of raw or undercooked contaminated pork, which have been associated to clinical Hepatitis E. Molecular analyses showed that hepatitis E virus strains detected in pigs and humans in the same geographical region present high genetic identity, indicating that swine are the main source of infection for humans. Since 2014, an increase in clinical cases was observed in many EU member states including the Netherlands. During the summer of 2017 and 2018, a temporal drop was observed in acute hepatitis E clinical infections, after which the incidence went back up to similar numbers as had been observed before. These events have remained unexplained to this date.

To explain the predominance of certain hepatitis E virus variants and to explain differences in virulence, the TRACE project aims to characterise the hepatitis E variants and attempt to identify the virulence factors in hepatitis E virus strains detected in clinical patients, in the general population and in domestic swine. Given the high genetic diversity of hepatitis E virus, it is critical to obtain higher resolution genomic data (i.e. whole genome sequences) to gain more understanding on its molecular epidemiology and possible variations in its adaptive traits.

During 2021, the PhD student had to change the method of sample pre-processing (enrichment) required for whole genome sequencing due to the original enrichment product becoming unavailable from the supplier. The second method involving multi-primer enrichment was unsuccessful, which led to the PhD student starting a new method based on probe capture enrichment that required the development of a new probe set, thus delaying the research plan. Hepatitis E virus culture preceding sequencing may be considered to increase the amount of hepatitis E virus (the work on a culture method is part of the BIOPIGEE project). First results were promising, since whole genomes could be generated from the control sample and a positive faecal sample with a Ct of 24. Now the procedure needs to be optimised to generate whole genomes from samples with a Ct value up to Ct30.

These initial changes to methods, led to the delay of the phylodynamics work to analyse newly produced sequences. The PhD student has now set up a method for HEV analyses for timed-phylogeny of known sequences from the NCBI, which could be later applied to sequences generated in the PhD project.
**INTRODUCTION**

Whole genome sequencing allows the tracking of pathogenic strains and informs infection control, diagnostic and sometimes treatment strategies. To track strains globally, and as they spread between the environment, food, animals and humans, universal strain nomenclatures are necessary. The core genome Multilocus Sequence Typing (cgMLST) approach is an accurate, reproducible, and portable strain genotyping method that underlies widely used strain nomenclatures, in which groups are generally determined by single-linkage clustering. However, cgMLST groups are unstable due to the possibility of group fusion upon subsequent sampling. Recently, a new coding approach named LIN (Life Identification Number) was introduced by Marakeby et al. (2014). It provides a numerical code for each genome based on its similarity (estimated using the Average Nucleotide Identity, ANI) to the closest genome already encoded. As LIN codes are attributed to genome rather than groups, they are stable.

The aim of the **Codes4strains** project is to develop a novel genome-based genotyping approach taking the best of the two above classification approaches, i.e., combining the advantages of cgMLST (discrimination, standardisation) with those of the LIN code approach (complete stability). Thus, the aim is to develop and explore the strain classification utility of cgMLST-based LIN code (cgLINcodes) systems and compare the cgLINcodes approach with other existing classification approaches: the SNP address and multi-level single-linkage classifications (MLSL). The two important pathogens *Klebsiella pneumoniae* and *Escherichia coli* are used to develop and evaluate our approach.

The Codes4strains project had previously selected the pilot genome dataset for the pathogen Kp, developed procedures (bioinformatics, algorithmic) for a cgMLST-based LIN code system, and defined the cgMLST-based LIN code algorithm for bioinformatic implementation in Python. In 2021, analyses of LINcodes and their comparison with MLSL have been consolidated. A novel way to define the population structure of species, called MSTclust was developed. In addition, the inheritance algorithm to provide backwards compatibility of MLSL groups with previous nomenclature identifiers from 7-gene MLST has been optimised. Project work has also defined the optimal input order for LIN encoding, compared the ANI metric with the cgMLST metric and devised a method to identify recombination in cgMLST data. The MLSL identifiers have now been incorporated into the BIGSdb platform to make them publicly available. Finally, a manuscript on the cgLIN codes concept and its implementation in Kp with comparison with multi-level single-linkage classifications has been submitted for journal publication.

**PhD PROJECTS**

**Codes4strains**

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**Publications in 2021:**


The SUSTAIN project lies at the interface of social sciences and public health and aims to understand the political drivers and constraints for increased transboundary integration and institutionalisation of the One Health approach across EU member states. The challenges for implementing the One Health approach are complex political and institutional structures. Complex political structures emerge through various levels on which politics can be discussed, such as local, regional, national and international levels. Within and across these institutions, information infrastructure, collaboration and relationships can pose obstacles for implementing a One Health approach. Understanding the barriers and facilitators to change policy processes, and how these differ across sectors and across EU member states is important information for the future of One Health in the EU.

The SUSTAIN project work involves quantitative studies of databases and a survey addressed to institutions working on One Health topics, with a subsequent qualitative study that includes interviews and observations. These studies will inform different aspects, such as the current state of One Health institutionalisation in the EU and on a national level, and the One Health networks’ interactions and relationships.

In 2021, data analysis was started as planned for the SUSTAIN project, which followed on from the interview-based data collection conducted in Sweden and Italy during 2020. Data analyses included transcribing the interviews with experts from public health, veterinary and environmental agencies from Sweden and experts at institutes in Italy. Data gathering for the interviews was slowed down due to the Covid-19 pandemic. Due to travel restrictions, the final interviews were conducted in October 2021 with Italian veterinary experts. Most of the data analyses will be conducted in 2022.

During 2021, data analysis was also conducted for the NOVA project “Surveillance barriers and opportunities as perceived by med-vet-food experts”.

A survey on the governance of One Health was launched in March 2021. The survey is addressed to ministries, EU institutions, international organisations, and high-level members of staff from public health, veterinary, environment and food agencies. The online survey examined policy networks for One Health by understanding policy- and decision-making processes of One Health. The survey investigated political constrains for turning One Health topics into policy (science to policy) to shed light on opportunities for One Health policy integration. The analysis of the survey data started after the end of the survey in July 2021 and an article is being prepared.

The PhD student has authored three publications in peer-reviewed journals during 2021. She has also been invited to write a chapter for an edited book about One Health and AMR in the European Union to be published by Oxford University Press.

**Publications in 2021:**


One Health EJP
Dissemination Activities
The One Health EJP is committed to sharing results and knowledge from all activities.

Every OHEJP consortium member plays a key role in dissemination, and there are several ways this was achieved in 2021:

- OHEJP outcomes were regularly disseminated at key governance meetings, such as Scientific Steering Board meeting, Stakeholders Committee meeting, the Programme Management – Programme Owners Committee meeting.
- OHEJP was involved in several stakeholder activities, which included consultations, set up of new initiatives, joining pre-existing initiatives, and providing other ad hoc support e.g., contributed to a new One Health session in a module of ECDC fellowships.
- OHEJP contributed to the first dialogue meeting of the Partner Platform of the Regional One Health Coordination mechanism in November 2021.
- A series of Dissemination Workshops was initiated to showcase the impact of OHEJP outputs to national, European, and international decision makers and strategic planners. The first workshop was held in October 2021, which focused on metagenomics and led to the “One Health EJP Report of the Dissemination Workshop on Metagenomics.”
- Two targeted reports (zenodo.org/record/5906799 and zenodo.org/record/5906639) were created to ensure that key EU stakeholders are informed of the key scientific and integrative outcomes from the OHEJP.
- An additional thematic report “One Health Thematic Report on environmental aspects addressed in One Health EJP activities” was produced.
- The OHEJP Outcome Inventory was regularly updated, and its user interface improved. It acts as a repository for all OHEJP outcomes (JIPs, JRPs and PhD projects) and is a hub for information for both national and international stakeholders and both internal and external audiences.
- The Data Management Plan was further advanced, and an abundance of support has been provided to the Project Leaders of the OHEJP projects to ensure that data generated in their projects are FAIR (findable, accessible, interoperable, and reusable).
- The OHEJP website and social media platforms served as central platforms to share public events, news, research outcomes and key information to a global audience.
- The OHEJP issued regular newsletters, which contained highlights and links to scientific and collaborative activities. These newsletters were an important tool to disseminate news on a regular basis.
The OHEJP Annual Scientific Meeting provided a central platform for communication and dissemination of the scientific outcomes from the Joint Research and Joint Integrative Projects, in addition to the PhD projects. This event also facilitated collaboration with One Health experts both internal and external to our consortium.

The establishment of excellent relationships with key EU and international stakeholders facilitates translation of science to policy, in addition to ensuring there is no duplication of research between major European organisations.

The OHEJP disseminated important scientific results on a regular basis to the European Commission and the Research Executive Agency (REA), ensuring exchange of information and knowledge to maximise impact.

Organisation of Education and Training activities to aid in training the next generation of One Health researchers.
The Third One Health EJP Annual Scientific Meeting

Organising institutes: The Statens Serum Institut and National Food Institute at the Technical University of Denmark, Copenhagen.

Location: Hybrid event online and at DGI Byen, Copenhagen, Denmark.

Dates: 9th-11th June 2021

The third One Health EJP Annual Scientific Meeting was a great success as a hybrid event that welcomed over 500 participants from across the globe in Denmark and online. The three-day event highlighted the importance of an integrated, cross-sector approach to global health threats, which includes foodborne zoonoses, antimicrobial resistance and emerging threats, using the One Health approach. The wide variety of One Health research was shared in 6 keynote presentations from 9 speakers, 30 oral presentations, and over 160 poster presentations. A dedicated session was co-organised with the Med-Vet-Net Association on the topic of the importance of the environment in the One Health approach.

The event included an exciting three-minute thesis competition by One Health EJP PhD students. This gave the 17 PhD students the opportunity to enhance their communication skills by showcasing their research to this international audience.

The Keynote speakers focused on One Health topics highly relevant to the recent global challenges impacting societies and human and animal populations. Dr. Sara Tomczyk from Robert Koch Institute, Germany, discussed confronting antimicrobial resistance in times of a pandemic. Dr. Berit Müller-Pebody from Statens Serum Institut and Dr. Birgitte Borck Høg from the National Food Institute, Technical University of Denmark, provided a duo-talk on DANMAP, which is a long-lasting interdisciplinary collaboration. Prof. Maiken Cavling Arendrup and Dr. Rasmus Krøger Hare from Statens Serum Institut both explained the One Health aspects of azole-resistant Aspergillus fumigatus. Dr. Sylvain Brisse from Institut Pasteur, France, presented the One Health aspects of Klebsiella. Prof. Kate Millar and François Hirsch described supporting One Health researchers in ethical aspects. The final keynote talk by Prof. Mark Woolhouse from University of Edinburgh, UK, discussed what factors will cause the next pandemic.

The social media presence for this event generated a great international response with interactions between scientists and stakeholders globally.
Educating the next generation of One Health researchers
EDUCATION AND TRAINING

The Education and Training activities (WP6) develop and deliver innovative training platforms and materials with a specific focus on One Health. Our Education and Training activities uniquely bring together students, early-career researchers, and key experts with diverse expertise in the human, animal, and environmental health fields. These activities reinforce collaboration across multiple disciplines, and integration between the consortium member institutes and stakeholders (and beyond,) bringing these individuals together so that One Health knowledge and experiences can be shared, and collaborative engagements and future relationships can be formed for One Health activities.

In 2021, as global travel was still significantly impacted by the COVID-19 pandemic, WP6 continued to deliver training events online to provide unique opportunities for training in One Health and encourage collaboration and exchange of knowledge and experiences amongst scientists across the globe.

ONE HEALTH EJP SUMMER SCHOOL 2021

Our annual summer schools are an important component of the One Health EJP, as they provide One Health training opportunities for the next generation of One Health scientists from across Europe and worldwide.

Organising institutes: Italian National Institute of Health (ISS) and University of Surrey (UK).
Theme: Environmental issues in One Health – from risk assessment to surveillance
Location: Online
Dates: 26th July to 6th August 2021

Collaborative interactions: The programme was delivered by leading EU and international experts in public health, animal health and environmental health who emphasise the role of environmental issues in One Health and the issue of sustainability. This was the first international training event entirely devoted to environmental issues in One Health at a global level. The collaborative interactions between more than 50 experts from all over the world and 41 delegates from 12 countries across the globe provided opportunities for knowledge, skills, and competencies to be shared amongst individuals with different perspectives and experiences.
Participants attended from several countries within the EU such as Denmark, Italy, Germany, Spain, Portugal, Finland, and Belarus, but also from countries beyond the EU such as the UK, Armenia, Somalia, USA, and Nigeria. This diversity significantly enriched the experience of all those that participated and added value to the course. Some delegates have attended the OHEJP’s other training events since the summer school, and so this training event directly contributed to building a future consortium to support the sustainability of the OHEJP, and to extend the OHEJP outside the EU in alignment with our global initiatives.

Delegates: Bringing together delegates from a range of education levels and interdisciplinary backgrounds brought a diverse pool of experience and knowledge which facilitated unique multi-disciplinary and collaborative interactions. Delegates represented various stages of their career including bachelors, master’s students, PhD students, early career post-doctoral researchers and teaching lecturers. They belonged to multiple disciplines across the One Health domains including microbiology, veterinary medicine, ecology, toxicology, biotechnology, machine learning, human nutrition, human epidemiology and social sciences.

Programme: The global One Health concept emphasises the interdependence of human health with the health of animals, plants and sustainable ecosystems from a global perspective. This summer school programme aimed to understand and illustrate the role of environmental issues, and to discuss the multi-faceted aspects of environmental health such as risk assessment, the role of the ecosystem and related factors, the role of natural and man-made factors, the farm as an environmental modifier, and the issue of sustainability. The programme delivered an introduction to global One Health basics, factors related to the natural environment (ecosystem, wildlife etc.) and to the human-made environment involved in zoonoses and AMR, the environment-farm interface and how to go from the bench to the field and society. Delegates were provided with the opportunity to present their own experiences and ideas in the global One Health field which were subsequently discussed with the key experts delivering the Summer School.

To read more about this event, please view the programme and blog post on our website - click here.

View the full report here.
ONE HEALTH EJP CONTINUING PROFESSIONAL DEVELOPMENT MODULE

Continuing Professional Development (CPD) is the process of recording and reflecting on the skills, knowledge, and experience young scientists gain as they work. CPD includes formal or informal learning beyond any initial qualification or training undertaken. The OHEJP's CPD modules cover several themes in One Health and are targeted at Early Career Researchers who can apply the training they receive in their future careers and in the training of future junior researchers.

Organising institutes: German Federal Institute for Risk Assessment (BfR) (Germany)
Theme: Digital Innovation for One Health Practitioners
Location: Online
Dates: 15th to 19th February 2021

Collaborative interactions: The module consisted of sessions delivered by leading European experts on digital innovation tools that can be applied to scenarios from the domains of food safety, public health and animal health. The module provided an opportunity to bring together experts and 50 delegates from our consortium partner institutes, stakeholders and OHEJP alumni, from 15 European countries, to share knowledge and experiences. The diversity in educational background, experience and countries significantly enhanced the quality of the module and the experience of all those that participated.

Delegates: This training event was attended by 50 participants, from early-career researchers and PhD students, up to senior scientists, from across our consortium partner network. Participants from our stakeholders EFSA, also participated. Bringing together people from across the health disciplines made this module truly cross-disciplinary and highlighted the possible applications of digital innovation tools to the different domains of One Health. The delegate's educational backgrounds were diverse across the One Health fields and included biological sciences, veterinary medicine, food safety, social sciences, and public health, which helped to facilitate the sharing of One Health knowledge and expertise across different disciplines.

Programme: The module centred around the use of innovative open-source software solutions supporting risk assessment, zoonotic outbreak investigations and data interoperability. This training module introduced new tools and technologies for One Health researchers and professionals; specifically, solutions that support foodborne disease outbreak investigations, efficient surveillance data integration as well as the re-use of risk assessment models. The module provided joint plenary
presentation sessions, moderated interactive workshops with practical exercises, as well as an e-learning platform, that can still be used by participants to further improve their knowledge. For this, dedicated e-learning courses on the software FoodChain-Lab, the RAKIP and RADAR model repositories and on resources promoting the adoption of the Linked Open Data concept in One Health were developed. The e-learning platform was also used to measure knowledge gains and collect feedback from the workshop participants. In an increasingly digital world, we must use training opportunities such as these to harmonise One Health approaches, collaborative and share knowledge across all pillars of One Health. This event is an exemplary illustration of what can be achieved.

To read more about this event, view the programme and blog post on our website click here.
ONE HEALTH EJP FUNDED 4 SHORT TERM MISSIONS IN 2021

The COVID-19 pandemic still affected the success of these missions due to the disruption to travel plans. Two of four STMs took place in 2021. Of the remaining two STMs, one mission was cancelled and the remaining one has been postponed (and scheduled) in 2022.

STM 1: Start-up of an efficient sequencing facility
Theme: Skills Development Missions
Home Institute: The Norwegian Veterinary Institute, Norway
Mission Hosting Institute: Statens Serum Institut (SSI), Denmark
Duration of mission: 1 week

Background and Aim of mission: The project SEQ-TECH at The Norwegian Veterinary Institute (NVI) has acquired a platform of sequencing machines, pipetting robots and other equipment through 2019-2021 and now set-up for an automated flow-through of several hundred high throughput sequencing samples per week. Although the equipment was there, the routine workflow was not fully established, and the potential of the different machines not exploited. The aim of this mission was to develop skills on the management of a sequencing facility using an automated workflow. During this mission, routine whole genome sequencing (WGS) of bacteria and virus (SARS-CoV-2) were performed, the data quality control process was described and one-on-one meetings with key personnel were held.

For further details, read the full report here. Read the 2021 case studies here.
STM 2: CarbaPlasmid – Tracking endemic carbapenemase plasmids in human, animal and environmental isolates

Theme: One Health, AMR

Home Institute: NUI Galway, Ireland

Mission Hosting Institute: VISAVET, Universidad Complutense Madrid, Spain

Duration of mission: 1 month

**Aim and background of mission:** The aim of this mission was to develop skills in nanopore sequencing and hybrid sequence analysis, to characterise antimicrobial resistance plasmids for One Health epidemiological investigations. Carbapenemase-producing Enterobacterales (CPE) isolated from the natural environment, hospital wastewater, the hospital environment and hospital patients in Galway, Ireland, were analysed by hybrid sequencing analysis. Several techniques and applications for analysis of hybrid bacterial sequence data were learned. A harmonised hybrid sequence analysis pipeline was successfully transferred between One Health EJP partners Universidad Complutense Madrid (UCA) and NUI Galway.

This short-term mission developed capacity for long-read sequencing and hybrid sequence assembly in Irish OHEJP partner NUI Galway, which will also be used to support the surveillance function of the Irish National CPE Reference Laboratory. This expertise will be fundamental in ongoing and future collaborative AMR research projects with OHEJP partners and in the surveillance of endemic carbapenemase plasmids in a One-Health context in Ireland.

For further details, read the full report [here](#). Read the 2021 case studies [here](#).
The One Health EJP Annual Scientific Meeting Satellite Workshop

Each year, the OHEJP consortium holds an annual Scientific Meeting (ASM), showcasing the knowledge and scientific advances in the OHEJP Joint Research Projects and Joint Integrative activities, as well as One Health research undertaken by partners and collaborators. For each ASM, a satellite workshop focusing on one of the priority areas for the OHEJP has been organised. ASM satellite workshops provide opportunities for early career researchers to present short talks and discuss state-of-the-art with invited speakers presenting and discussing expert topics related to the sub-theme of the workshop. Satellite workshops are open to attend to all audiences who are working in the area of One Health. Priority is given to those who are registered as members of one of the OHEJP partner institutes.

Organising institutes: German Federal Institute for Risk Assessment (BfR), Germany
Theme: Online Software Fair
Location: Online
Dates: 7th 2021

Collaborative interactions: The workshop was organised and delivered by leading European experts, to inform One Health researchers and practitioners of new digital solutions and tools that can support generation, integration, and analysis of data across food safety, public and animal health. These tools could be game changers for disease surveillance, outbreak investigation, and/or risk assessment. The tools presented in the satellite workshop are being or have already been developed for application across all pillars of One Health, including human health, animal health, environmental health, and food safety. The workshop was a big success and a demonstration of knowledge and information exchange across One Health sectors. The event highlighted the One Health EJP's ability to bring together experts from across our European network and share tools to promote a sustainable One Health approach in the future.

Delegates: Over 70 delegates across 29 countries worldwide registered to attend – including PhD students, junior and senior researchers, bioinformaticians, epidemiologists, and other One Health professionals. Bringing together people from across the health disciplines made this module truly cross-disciplinary and highlighted the possible applications of digital solutions and tools to the different domains of One Health. The delegate's educational backgrounds were diverse across the One Health fields and included biological sciences, veterinary medicine, food safety, social sciences, and public health, which helped to facilitate the sharing of One Health knowledge and expertise.
**Programme:** The online software fair was split into two sessions. In the morning session, eight digital solutions and software tools were presented in the form of pitch presentations by members of our consortium from INIA (Spain), NIPH (Norway), NVI (Norway) and BfR (Germany). In the afternoon session, each software tool was presented in a breakout room. Delegates could join up to three breakout rooms to learn more and take part in discussions, interactive exercises and gain hands-on experience using these tools and innovative solutions and applying them to their area of expertise. All delegates were given access to the slides and recordings after the workshop, so they continue to be inspired and benefit from the content presented.

To read more about this event, view the programme and blog post on our website [click here](#).

View the full report [here](#).
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ONE HEALTH

PREVENT • DETECT • RESPOND

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