IMAGE: WIKIMEDIA

Annual Report 2020

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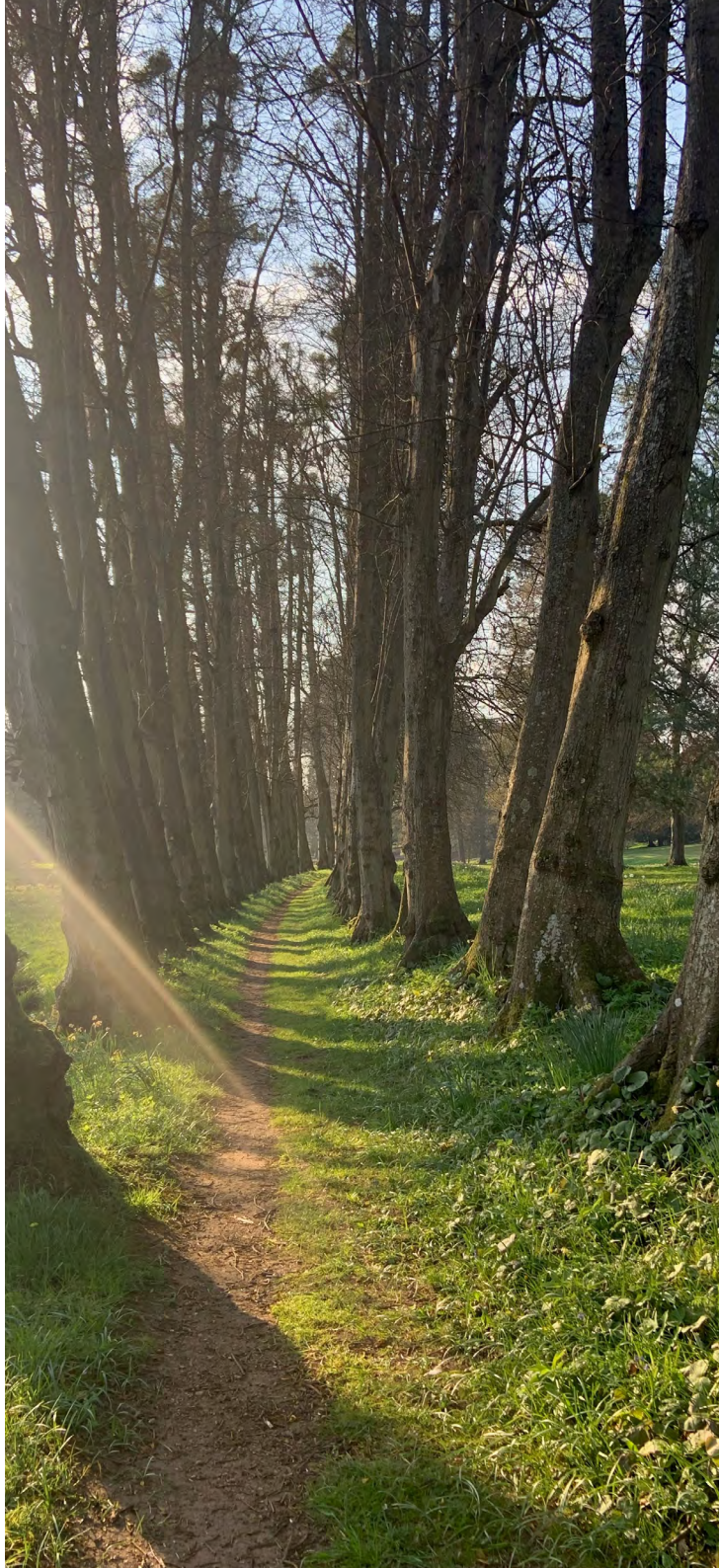


UNIVERSITY OF SURREY

Report prepared by the OHEJP Communications Team at the University of Surrey

WHAT IS THE ONE HEALTH EJP?

The One Health European Joint Programme (OHEJP) is a landmark partnership between 44 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.

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. The main focus of the OHEJP is to reinforce collaboration between partners by enhancing collaboration and integration of activities by means of dedicated Joint Research Projects (JRPs), Joint Integrative Projects (JIPs) 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.

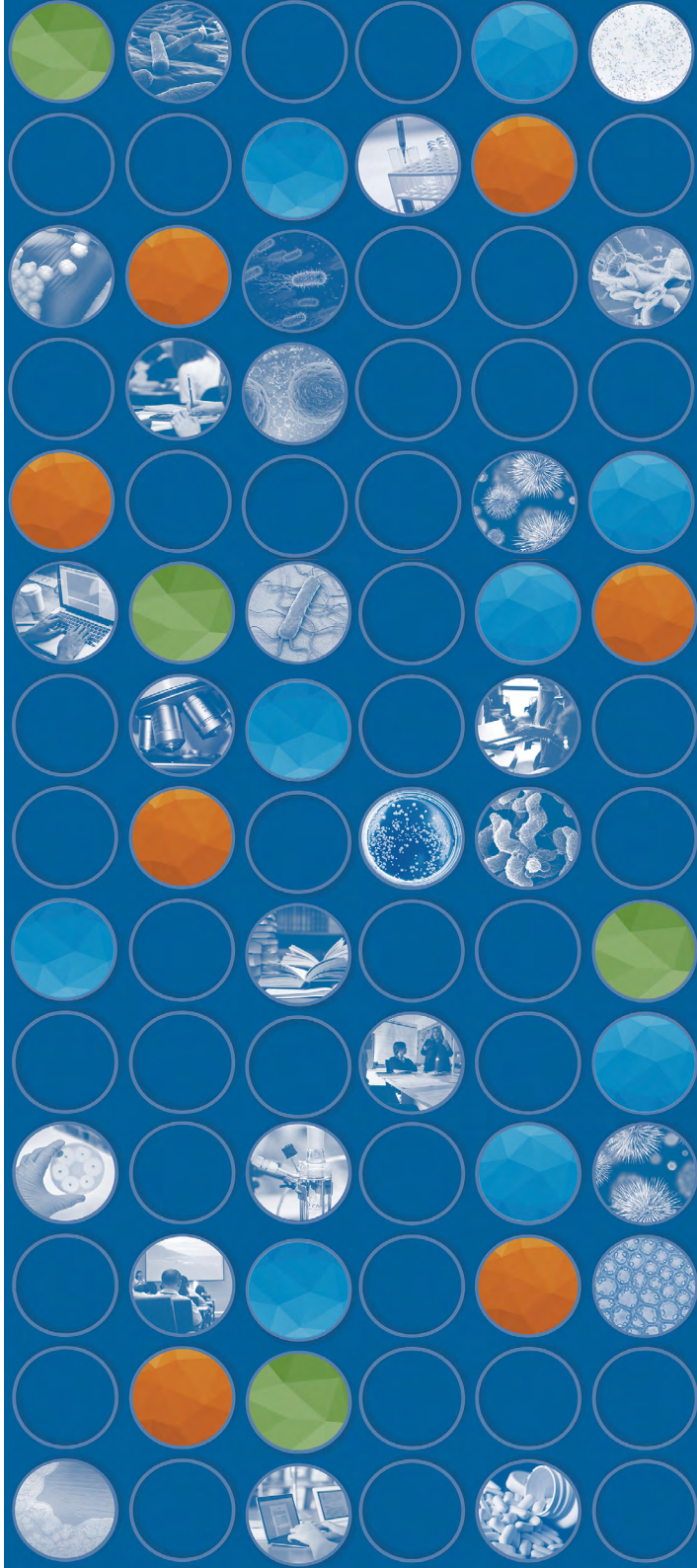
OUR OBJECTIVES

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 topics: 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.

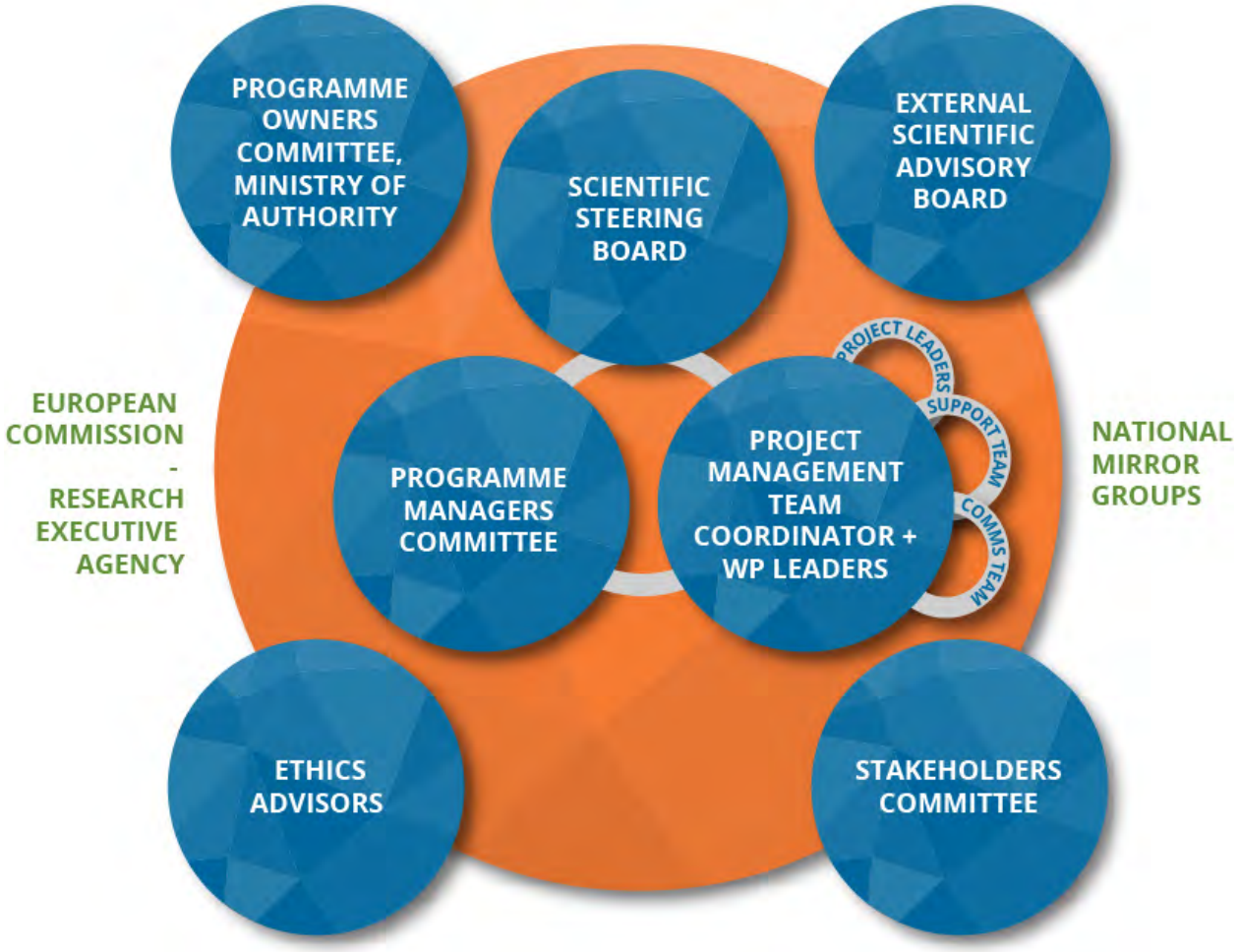
The governing boards specific to the OHEJP include: The Project Management Team (PMT), Scientific Steering Board (SSB) and Programme Managers Committee (PMC).

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.



ONE HEALTH EJP STRUCTURE

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 (JRPs) - 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

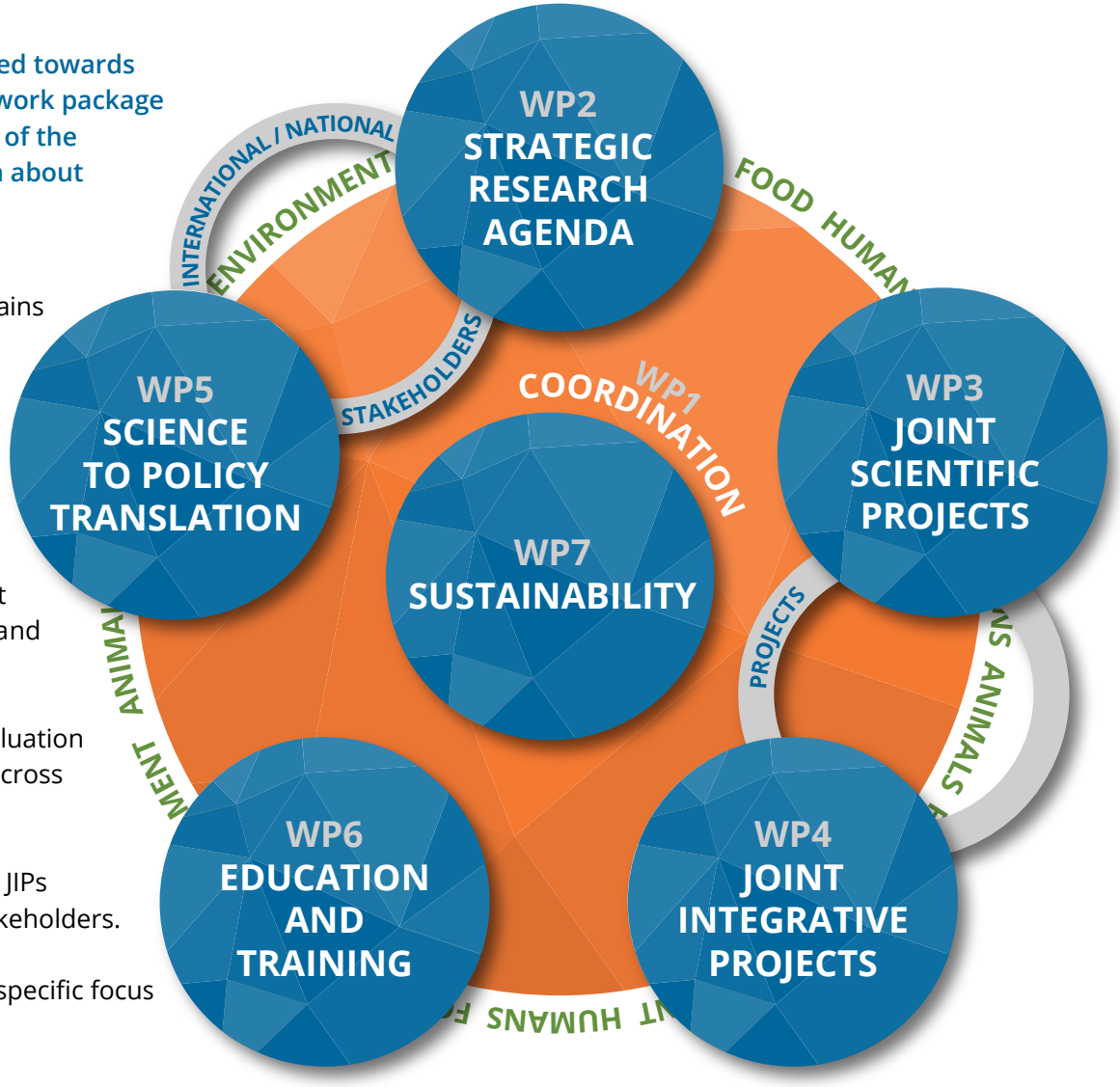
WP5 ensures best use of the scientific outcomes of the JRPs and JIPs through dissemination activities and networking with OHEJP stakeholders.

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 three?

IMAGE:PIXABAY

COORDINATION OF THE ONE HEALTH EJP

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

What were the key coordination activities in year three?

- 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).
- Preparing to expand the OHEJP consortium to include six additional European partners to complete more public health/animal health partnerships within the EU member states.
- A coordinated response to the COVID-19 pandemic to inform internal and external audiences of the OHEJP's role and the One Health approach to the pandemic.
- Ongoing support for the JRPs, JIPs, Education and Training activities and other OHEJP scientific events.
- The internal calls to organise the 2021 OHEJP activities were launched: the Annual Scientific Meeting (ASM) and ASM Satellite Workshop, Summer School, CPD Module, and Short Term Missions were launched. These will be hosted by one of the consortium member institutes.
- Co-ordination and management of 36 overarching OHEJP deliverables were submitted to the REA.
- The Communications Team have developed and enhanced communication throughout the consortium to stakeholders, and external audiences. The OHEJP brand, website and social media platforms were used to inform all audiences of all joint successes.
- Improved dissemination and publication procedures for key outcomes were determined and implemented to ensure consortium members were supported in communicating their research and impact.

IMAGE:PIXABAY

COMMUNICATING SUCCESS

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

The OHEJP Communications Team achieved several successes in year three:

- The OHEJP brand was strengthened by improving visibility across all platforms including, meetings, workshops, Education and Training events, the OHEJP website and social media.
- Supported all OHEJP events and facilitated the migration of physical events to virtual events in response to the COVID-19 pandemic.
- Developed communication and dissemination tools for consortium members to support the dissemination of scientific outcomes and demonstrate impact.
- Created several interactive documents and case studies to showcase the OHEJP to scientific and non-scientific audiences across the globe.
- Presented at the OHEJP Communication and Media Workshop in October 2020, delivering training and expertise in digital communications, creative communications and branding when organising events.
- Attended the virtual World One Health Congress in November 2020 which enabled the OHEJP to be visible to a global audience.
- Supported stakeholders in their work and social media campaigns, which included EU-JAMRAI's Antimicrobial Resistance Symbol and the European Commission's #ScienceFromHome campaigns.
- Maintained the OHEJP Zenodo account to ensure that publications and deliverables are open access.

IMAGE:PIXNIO

TRANSLATION OF SCIENCE TO POLICY

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 supports the sustainability of the consortium (WP7). The impact of the OHEJP's outcomes is maximised by targeted dissemination and communication to OHEJP Stakeholders (WP5).

What were the key outcomes for year three?

- Consolidated relationships with key EU stakeholders ECDC and EFSA, and other European and global stakeholders by expanding the stakeholder network: The European Medicine's Agency (EMA), European Environment Agency (EEA), 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), all joined the OHEJP Stakeholders Committee.
- The OHEJP Outcome Inventory was further developed to document and share all outcomes from the OHEJP projects. This has been widely shared with stakeholders and internal and external audiences.
- Stakeholders' needs were regularly monitored to ensure that the OHEJP is at the forefront of current and topical research interests, which enables quick responses from the consortium and high levels of engagement with needs across Europe and globally.
- Strategic interactions with EU projects and initiatives were further developed and shared with the OHEJP consortium. The new projects added include: VEO, SAFECONSUME, ZODIAC, GNA NOW, EVA-GLOBAL and ENOVAT.
- Three Cogwheel Workshops in 2020 were designed to strengthen relationships between the OHEJP and selected identified EU projects: InfAct, JPI-AMR and SAFECONSUME.
- A Strategic Research and Innovation Agenda is under development which aims to define research needs beyond the lifetime of the OHEJP.

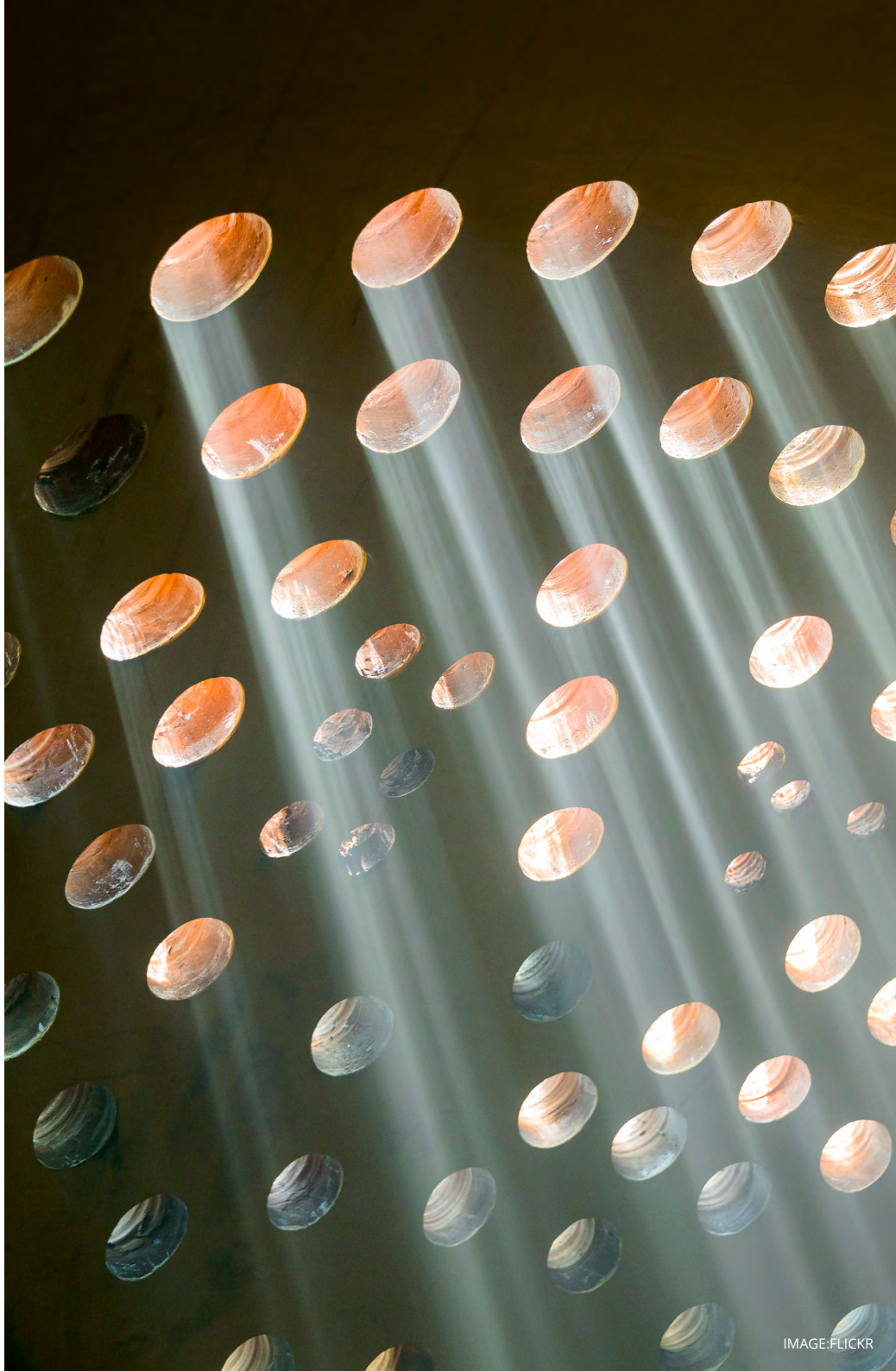


IMAGE:FLICKR

RESPONSE TO COVID-19

The third year of the One Health EJP was significantly impacted by the COVID-19 pandemic and many of our partner institutes and research groups turned their attention to surveillance and management during the pandemic. As a result, many of the projects funded in the first round of funding in 2018 were extended into 2021 which will enable the research to be completed.

The OHEJP Project Management Team and Communications Team responded to the pandemic in a timely manner and ensured that communication to internal and external audiences was clear and reflected the OHEJP's response to the pandemic.

The Communications Team published a COVID-19 newsletter in March 2020 to update audiences on how the consortium and its partner institutes were turning their efforts and using their collaborations to take a One Health approach to the pandemic. A Latest News page was also set up on the One Health EJP website to inform audiences in real time and to act as a hub for key information.

An additional Joint Integrative Project, COVRIN, was proposed to use the strengths of the consortium to make a significant impact during the pandemic. This project will begin in March 2021.

IMAGE:PIXABAY

One Health EJP Scientific Outcomes

IMAGE:WIKIMEDIA

ONE HEALTH EJP SCIENTIFIC OUTCOMES

To date, the One Health EJP has funded 24 Joint Research Projects (JPRs), 5 Joint Integrative Projects (JIPs) and 17 PhD projects. In 2020, the Continuing Professional Development Module, Summer School and Communication and Media Workshop were all funded and hosted virtually, and in addition, 2 Short Term Missions were funded before travel restrictions were implemented.

What were the key outcomes in the third year?

- Support for extensions of some projects funded in 2018 was established and projects were granted a 6-month no cost extension.
- Information was collected regarding the synergies between OHEJP projects to encourage collaboration and integration across the multitude of different activities.
- Procedures were established and followed to ensure data and publications were open access.
- The following projects were completed in December 2020: IMPART, RaDAR, AIR SAMPLE and MedVetKlebs.
- A total of 52 peer reviewed publications were published.
- A call for an additional JIP was launched in light of the COVID-19 pandemic. The proposal "SARS-CoV2 Research Integration and Preparedness" (COVRIN) was completed and evaluated by the REA steering group.
- The Second One Health Annual Scientific Meeting attracted a record audience as an online conference.
- The first 3 Minute Thesis (3MT) competition for the One Health EJP PhD students was organised virtually at the Annual Scientific Meeting.

IMAGE: MJ RICHARDSON

ORION - year 3

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

Having successfully moved through the first two project phases of "improvements and new resources" and "evaluation", the ORION project progressed into the third phase which encompassed the implementation of national One Health pilots in addition to the continuous improvement of resources developed during the second phase.

One aim of the ORION project was to create the One Health Surveillance Codex- a high level framework for harmonised, cross-sector descriptions and categorisation of surveillance data covering all surveillance phases and all knowledge types. A first version of the One Health Surveillance Codex was publicly released, presented during ASM2020 and a corresponding publication was submitted to the One Health journal.

A new tool was developed to maintain and exploit the OHEJP Glossary, enabling automatic matching of a user-defined document and a set of online glossaries. Also, the One Health Consensus Report Annotation Checklist (OH-CRAC) was extensively validated, improved and applied in several pilot applications. A new web-based service was created that supports the adoption of OH-CRAC by end-users.

The ORION project also created the One Health Surveillance Knowledge Hub, a cross-domain inventory of currently available data sources, methods / algorithms / tools, that support One Health surveillance data generation, data analysis, modelling and decision support. The input into this hub is the combined efforts of the One Health knowledge bases EPI, Integration and NGS. The knowledge base EPI was significantly improved in Y3, from the content as well as from the technical side, with resource investment into the preparation and implementation of national pilots (with some pilots being delayed due to COVID-19). Shiny web applications were created to provide public access to the user-friendly surveillance systems inventory as well as the methods and tools inventory.

The content of the web-based community (knowledge base) NGS handbook continued to be enriched and new functional NGS data analysis pipelines in collaboration with the IRIDA project were created.

Publications in 2020:

Joensen, KG., Kiil, K., Gantzhorn, MR.,Nauerby, B., Engberg, J., Holt, HM., Nielsen, HL.,Petersen, AM., Kuhn, KG.,Sandø, G.,Ethelberg, S.,Nielsen, EM. (2020). Whole-Genome Sequencing to Detect Numerous *Campylobacter jejuni* Outbreaks and Match Patient Isolates to Sources, Denmark, 2015–2017. *Emerging Infectious Diseases*, 26(3), 523-532. DOI: https://dx.doi.org/10.3201/eid2603.190947.

Houe, H., Nielsen, SS., Nielsen, LR., Ethelberg, S., Mølbak, K. (2020). Opportunities for Improved Disease Surveillance and Control by Use of Integrated Data on Animal and Human Health. *Frontiers in Veterinary Medicine*, 6: 301, pp. 1-8. DOI: https://doi.org/10.3389/fvets.2019.00301.

IMAGE:PIXABAY

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Also, the first cross-sector IRIDA NGS data analysis platform at the Norwegian Research and Education Cloud successfully implemented.

In January 2020 an ORION pilot workshop took place to synchronise the work done in the different country specific pilots under the overarching One Health Surveillance Codex framework. A generic template for evaluating impact and outcomes of each pilot was established, as well as a strategy for how lessons learned from the individual pilots can be captured and communicated. The national integration pilots contributed to improved One Health reporting, e.g. in the *Salmonella* chapter of the DANMAP 2019 report. Further development work focused on establishing the conceptual and technical foundations for innovative data interoperability solutions related to the linked open data concept. During the national pilot, this concept was promoted and new processes with improved collaboration among the national One Health sectors were established. This led to a significantly improved understanding on each sector's surveillance activities and results. Specifically, new instructions and guidelines were produced that will be used in future reporting practices, which also pave the way for the application of data interoperability solutions developed by the ORION project.

Dissemination continued with members of the ORION project presenting research results at the ASM2020 and the 6th World One Health Congress. Several scientific publications were submitted or are in the final preparation phase. ORION organised an OHEJP internal Cogwheel workshop and an online (knowledge base) NGS workshop.

IMAGE:PIXABAY

COHESIVE - year 3

The COHESIVE project aims to strengthen the human-veterinary-food collaboration, with the ultimate goal of improving risk assessment, communication and exchange of information and data, and bridging the gaps in risk-analysis in the public health, food safety and veterinary health sectors, by enhancing collaboration on all zoonotic threats.

The project worked closely with the Tripartite Guide to Addressing Zoonotic Diseases in Countries to determine the national One Health approaches that could be established and/or strengthened in European countries.

During the past year, the COHESIVE project has continued to gather information to further build on the creation of web-based European guidelines regarding the implementation of the Tripartite Guide. Several pilots have been planned in Belgium, Portugal, Norway and Italy to go through the first steps of the guidelines, with three countries established as a core group and a stakeholder analysis has been partly performed. However, due to COVID-19 the systems mapping workshops were all delayed.

Another key aim of this project is to develop an online decision tool to help the user decide on the most appropriate method to use when tasked with conducting a risk assessment for a specific situation. Version 1 of the tool is complete and is live. The tool is designed so that it can be updated as new risk assessment resources emerge and tool improvements will continue to be made as they are identified through feedback in the project.

Interviews with professionals working within food safety, public health and veterinary medicine at central, regional or local levels were performed in six countries, with the aim of identifying factors that contribute to well-functioning systems or processes to share signals of zoonotic events within and between countries. The thematic analysis of the interview data has been finalised at the national level and a joint thematic analysis is ongoing.

A follow-up of the horizon scanning pilot exercise regarding One Health that took place in November 2019 has been conducted; horizon scanning is a technique for detecting developments through examination of potential threats. The main drivers for One Health were political and decision-making behaviour, people and consumer behaviour, science and innovations, market behaviour, new and re-emerging pathogens or threats and climate change.

IMAGE:FLICKR

A key aim of the COHESIVE project is to learn from past experiences with respect to zoonotic outbreaks. Hantavirus and Q-fever in the UK were chosen to be retrospectively analysed and all UK-government publications relating to Hantavirus and Q-fever have been 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. Following an initial systems mapping, with a specific focus on Q-Fever, several potential improvements for surveillance in England have been identified and will be highlighted to animal health surveillance colleagues in the beginning of 2021.

Building on the findings within the COHESIVE project, a pilot One Health Early Warning System has been started. The aim is to share low threshold potential signals for pathogens with One Health impacts across multiple countries within the consortium, building relationships between countries and starting a forum that can continue post-project. Unfortunately, due to the outbreak of highly pathogenic avian influenza across Northern Europe the first meeting has been further postponed until early 2021.

The development of a tool for systematic cost-benefit analysis (CBA), with the aim of having a common method making CBA comparable across countries that is applicable to zoonotic pathogens, is underway with a literature review of CBA methods. As CBA can vary due to country specific conditions, affecting both costs and benefits, the aim is to produce a report focusing on the similarities, differences, strengths and weaknesses of different models and methods.

The COHESIVE project also aims to develop a prototype information system at the national level, allowing different databases to be interoperable. A survey has been conducted to gather detailed information on existing databases and information systems for whole genome sequence data management and analysis adopted or available among countries. A demo version of the COHESIVE prototype information system (CIS) has been produced and available for Italy. The feasibility studies in The Netherlands, Italy and Norway are ongoing. The Italy feasibility study is almost finished, while the others have some delays.

The development of the tracing web portal - FoodChain-Lab Web – (FCL Web) has advanced further and is available in production mode. A JavaScript Object Notation-based data exchange format allows for analysing delivery data from a data collection mask developed in a national project in FCL Web.

IMAGE:FLICKR

CARE - year 1

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.

In the first year of the project, a mapping review was conducted, identifying available and existing EQAs offered to the National Reference Laboratories for zoonotic bacterial agents and antimicrobial resistance from both the public health and veterinary health sectors. Developing new EQA schemes that can be used cross-sectorally and thereby used to evaluate the capacity to manage foodborne problems from a One Health perspective, is an important part of the CARE project. This review served as guidance for discussions within the CARE project for setting up new pilot EQA schemes expanding to whole genome sequencing, which for many laboratories in the EU is still a novel technology.

Progress has also been observed identifying the priority pathogens for which reference material should be collected. A list of nine pathogens, all known to be responsible for foodborne human infections, were agreed upon among partners. The target list will serve as a basis for which gaps will be identified in available reference material to cover all known serotypes, genotypes and generally pathogenic variants of the selected pathogens which are relevant for human health, also including AMR mechanisms and associated metadata. A questionnaire was disseminated to CARE partners to inquire about available reference material. A large number of available reference materials was reported, and work will continue with this material.

The final agreement and layout of the reference material database structure catalogue was delivered, followed by a synthesis document listing partner expectations, a list of existing software and technical choice.

The planned risk assessment activities are progressing as planned, initially identifying whom to be surveyed including the target audience from the EFSA network for microbial risk assessment. A small literature review was conducted to establish criteria to assess the data quality and accessibility in the field of risk assessment. The target group of risk assessors was approached with a developed questionnaire to query criteria to assess the data quality and accessibility as well as what type of risk assessment data and associated data to record. The outcome allowed the CARE project to establish a roadmap for sharing information for pathogens and AMR useful for exposure/risk assessment with already established and existing other initiatives.

IMAGE:FLICKR

OH-HARMONY-CAP - year 1

The OH-Harmony-CAP project aims to collect information on current capabilities, capacities and interoperability at both the National Reference Laboratory (NRL) and the primary diagnostic level. The quantitative description of current and best practices and the development of harmonised protocols will identify and possibly close knowledge gaps and suggest future studies of how best to detect and characterise foodborne pathogens across the One Health sectors.

A pilot survey on current practices with regard to capabilities, capacities and interoperability has been conducted and a report summarising the results was compiled. These results will enable development of the benchmarking OHLabCap instrument, a Europe-wide in-depth survey on One Health laboratory interoperability, capacity, and performance. This instrument will be repeatable and sustainable at EU and national level. One new and important observation that emerged during the analysis of the OHLabCap pilot survey was related to adaptability *i.e.*, the capacity and ability to adjust preparedness, methodology and organisation of each laboratory. In light of the COVID-19 pandemic, it became increasingly apparent that adaptability was more relevant than ever, and additional focus will be placed on adaptability in the development of the instrument in the coming years.

The first technical report created by the OH-Harmony-CAP project provided descriptions of the current and best practices for sampling and testing for Shiga toxin-producing *E. coli* (STEC), Enterotoxigenic *Escherichia coli* (ETEC), *Cryptosporidium* and antimicrobial resistance (AMR) in *Salmonella* and *Campylobacter*. This information will contribute to the development of harmonised protocols across the different laboratories and different EU member states. The first step to producing recommendations on how to harmonise the methodology for the detection and typing of these model pathogens was undertaken with the collection of laboratory protocols which are in use in the EU/EEA laboratories. This will provide an overview of the protocols adopted for the detection, characterisation and typing of the selected pathogens and the outcome will be a list of protocols for the detection, characterisation and typing of the selected pathogens in the EU/EEA NRLs.

IMAGE:FLICKR

MATRIX - year 1

The MATRIX project aims to advance the implementation of One Health Surveillance (OHS) in practice, by building on existing resources, adding value to them and creating synergies across all pillars of One Health. The MATRIX project acknowledges that each country has different infrastructural and economic realities with respect to setting up functional OHS, and all outputs from the MATRIX project will take this into consideration.

The project was met with a few challenges during the first year, especially during the first 6 months: the sudden and severe emergence of COVID-19 had a significant impact on the work planned within the MATRIX project. However, many institutes were able to continue after a short delay and as a result of the pandemic many have even strengthened national level communications by creating an opportunity for laboratories and epidemiologists from the animal health and food safety sectors to help support the epidemic control work carried by the public health institutes in their country.

To identify and describe the commonalities and differences of the various operational frameworks in animal health, public health and food safety, the MATRIX project collaborated with the ORION project in developing and populating an inventory of surveillance systems across the different sectors of project member's countries. A report has been compiled that describes the commonalities and differences between the individual surveillance components across sectors. Responsibility for the future continuation of, collecting and creating an inventory of current surveillance frameworks developed in the ORION project was agreed.

Mapping of food chain disease surveillance started through country-based case studies. This process took hazards and the associated food chains to analyse across at least two different countries. The case studies selected were *Salmonella* and the pork meat food chain, *Listeria* and dairy products, *Campylobacter* and the poultry meat food chain and Hepatitis E and the wild boar meat food chain. To collect information about the surveillance in place for these case studies online questionnaires were created and distributed across the human, animal and food sectors.

A literature review of output-based surveillance studies was started. Several of the partners have completed internal inventories of methodologies or ongoing work that complement the work package in the area of case-finding and evaluation of existing surveillance systems for antimicrobial resistance (AMR). More activities are planned in the second half of the project.

IMAGE:SNAPPYGOAT

A preliminary comparative analysis of the criteria used in previously developed surveillance tools (ECOSUR, NEOH, and RISKSUR) was conducted to characterise the overlap and differences between those tools. This comparison aimed to identify a set of strategic criteria which would be included in EU-EpiCap tool. These criteria would consider governance and organisation of multi-sectoral collaborations, technical characteristics of the collaboration, functional characteristics, surveillance short and intermediate-term outcomes.

Initial data collection has begun for the analysis for a One Health Surveillance Roadmap and the analysis of resources from other One Health EJP projects has started. In addition, the collection of requirements for the technical infrastructure of the Knowledge-Integration Platform (KIP) was performed and it was decided to extend the One Health Surveillance Codex developed by the ORION project. Developing this platform will link project outcomes and resources (e.g. tools/technologies/features) as well as support knowledge exchange between different MATRIX partners and stakeholders.

The work to facilitate the development of dashboards that enable data-driven surveillance to be carried out as an inter-sectorial activity has, thus far, been restricted to Sweden and Norway agencies. Focus will be given to cementing achievements of previous projects, such as the dashboards developed in the NOVA project and the open data publishing routines created in the ORION project.

IMAGE:SNAPPYGOAT

NOVA - year 3

The NOVA project aims to develop new surveillance tools and methods, and to harmonise and optimise the use of the existing surveillance data. The project consists of 19 institutes across 10 countries. This collaborative structure makes it possible to help advance the use of modern surveillance principles across Europe, in addition to having cost-saving impacts on how surveillance of existing and emerging zoonotic diseases is being conducted across Europe.

Studies of potential barriers and opportunities for surveillance across the food chain were ongoing in the third year, preparations to use the best methodologies to collect these data were carefully considered and interviews with disease surveillance experts is still ongoing. Additionally, a large dataset of food purchase data has been obtained, which will enable analyses of simulated outbreaks. An electronic web-based module, in which consumers can give consent for their purchase data to be used has also been prepared. Research related to food purchase data at an institute level for investigation into hospital foodborne outbreaks is ongoing and includes a literature review manuscript and a questionnaire study. Development of improved tools for food risk mapping are ongoing and are being integrated into the FoodChain-Lab, a state-of-the-art tool for tracing foodborne disease outbreaks.

Improvement of surveillance systems is being investigated with the aim of improving outbreak detection in humans. For example, veterinary and public health institutes in Norway have come together to integrate veterinary data, such as incidence of *Campylobacter* in poultry, and environmental data, such as rainfall and temperature, to current public health surveillance systems for human gastrointestinal outbreaks. These data have then been used by the SVA to develop a surveillance system that processes these data to calculate the value of evidence for outbreaks on a weekly basis. Partners at ANSES are also working to develop detection algorithms that can process data from seven animal, food and human databases from multiple time series simultaneously, in order to predict human gastrointestinal outbreaks. Other algorithms that are being developed as part of the NOVA project include using machine learning to correlate *Salmonella* infection data with environmental drivers, with the aim of applying these algorithms to different scenarios to identify environmental risk factors for *Salmonella* outbreaks and the incidence of antimicrobial resistance (AMR).

Research with different transmission models to investigate potential disease spread and compare surveillance strategies is in the final stages. Additionally, the coding of a simulation model to utilise metagenomics on samples from very large pools of data has been developed and aims to improve the ability to measure the occurrence of AMR in animal production at a national level. A model for estimating the cost-effectiveness of retail sampling in the prevention of human disease outbreaks has also been produced.

Publications in 2020:

Rosendal, T., Widgren, S., Ståhl, K., Frössling, J. (2020). Modelling spread and surveillance of Mycobacterium avium subsp. paratuberculosis in the Swedish cattle trade network. *Preventative Veterinary Medicine*. 183, 105152. DOI: https://doi.org/10.1016/j.prevetmed.2020.105152.

Apenteng, OO., Arnold, ME., Vigre, H. (2020). Using stochastic dynamic modelling to estimate the sensitivity of current and alternative surveillance program of *Salmonella* in conventional broiler production. *Scientific Reports*, 10, pp. 19441. DOI: https://doi.org/10.1038/s41598-020-76514-3.

Bosch, J., Iglesias, I., Martínez, M., de la Torre, A. (2020). Climatic and topographic tolerance limits of wild boar in Eurasia: implications for their expansion. *Geography, Environment, Sustainability*, 13(1), pp.107-114. DOI: https://doi.org/10.24057/2071-9388-2019-52.

IMAGE:PXFUEL

LISTADAPT- year 3

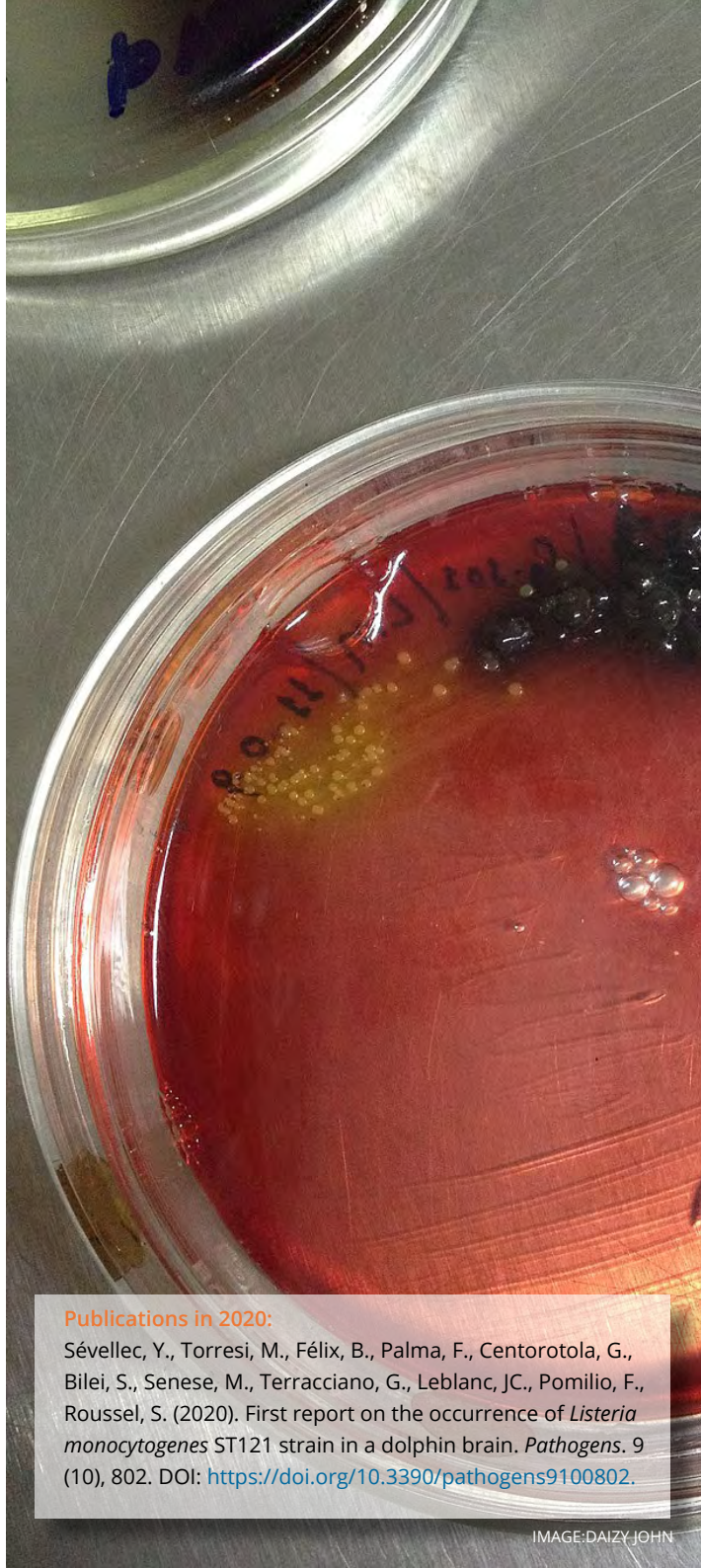
The LISTADAPT project aims to decipher the molecular mechanisms of adaptation seen in *Listeria monocytogenes* to its various ecological niches by comparing both phenotypic and genotypic data from a large, balanced set of strains from human clinical cases, animals, food and environments in several European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

With 21 partner institutes from public health, veterinary health, food and environment laboratories, the LISTADAPT project complied a dataset of 1575 *L. monocytogenes* strains and their genomes. These strains were collected from 20 European countries which ensured dataset was representative of the large number of clonal complexes occurring worldwide, and also covered many diverse habitats which was balanced between ecological niches and geographical regions. The aim of this dataset is to contribute to the improved understanding of *L. monocytogenes* and improve surveillance, therefore it was important to make all of this information available to the scientific community (it has been submitted to the European Nucleotide Archive (ENA)).

Of the 1575 strains collected, a subset of 200 were selected from 34 clonal complexes with a balance between three ecological niches: environment, animal and food. Phenotypic tests were performed on these strains to investigate the strain's ability to survive in soil and subsequently the strains were categorised into three groups depending on survival. Genotypic analysis was then performed by way of Genome Wide Association Studies (GWAS) to investigate genetic factors that may contribute to improved survival, of which there were many. A more in-depth investigation on strains from the same niche or clonal complex identified genomic variations in various transcriptional regulators, stress genes and (pro)phage related genes. The 200 strains in the main library showed the same growth kinetics at pH 7 while there was more variation at pH 5.4. The deviating strains belonged to different clonal complex groups and niches. An in-depth analysis is therefore needed to explore which DNA sequences that are related to the deviating growth kinetics.

Antimicrobial susceptibility testing also played a significant role in the LISADAPT project with 11 antibiotics and 4 biocides being tested against the panel of *L. monocytogenes* isolates used in this project. Overall, results revealed that strains isolated from food had overall higher minimum inhibitory concentrations (MICs) for the following biocides: quaternary ammonia compounds and peracetic acid compared to strains isolated from animal or the environment. Conversely, no significant differences were observed for MIC of antibiotics from strains from different niches.

Interestingly, repeated exposure to quaternary ammonia compounds recurrently led to a decrease



Publications in 2020:

Sévellec, Y., Torresi, M., Félix, B., Palma, F., Centorotola, G., Bilei, S., Senese, M., Terracciano, G., Leblanc, JC., Pomilio, F., Roussel, S. (2020). First report on the occurrence of *Listeria monocytogenes* ST121 strain in a dolphin brain. *Pathogens*. 9 (10), 802. DOI: https://doi.org/10.3390/pathogens9100802.

IMAGE:DAIZY JOHN

of susceptibility toward ciprofloxacin, a fluoroquinolone antibiotic, largely used in human and veterinary medicine and considered as a critically important antimicrobial. Additionally, these lower levels of susceptibility to ciprofloxacin remained stable in most strains even after subculture without biocide selection pressure, suggesting an adaptation involving modifications at the genetic level. Genomic analysis suggested that the accessory genome was associated with biocide tolerance, often linked to prophages and mobile genetic elements, thus demonstrating the adaptability of *L. monocytogenes* and the need to further characterise and understand these important organisms.

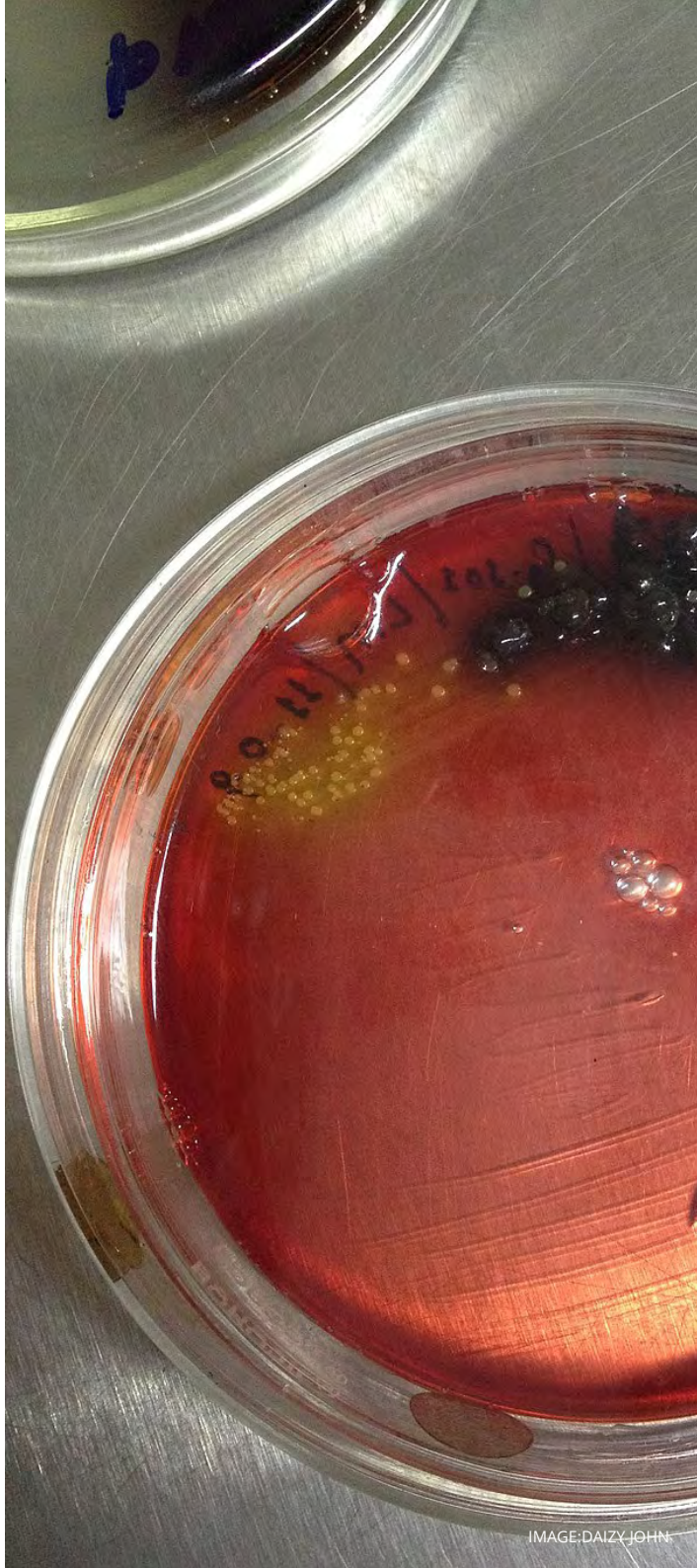


IMAGE: DAIZY JOHN

METASTAVA - ended in December 2020

The METASTAVA project investigated the use of metagenomic high throughput sequencing methods for unbiased pathogen detection in public veterinary, medical and food laboratories. This project focussed on providing guidelines for informed metagenomic experimental design for diagnostic purposes. This included standardisation of methods for generating and analysing data, providing quality control metrics, tools and interlaboratory assessments, and assessing the analytical properties of metagenomics for pathogens and samples relevant to the One Health EJP.

Standardisation of methods for metagenomic data generation and analysis were completed in the third year of the project. Investigation of publicly available next generation sequencing raw data indicated that metagenomic sequencing data on pathogen/sample combinations relevant to the METASTAVA projects lacked sufficient sample and methodological metadata, thus highlighting the need to focus on harmonised datasets within this project. A general guidance document was created for the informed implementation of metagenomic methods for diagnostics. This document aimed to inform scientists and stakeholders on an abundance of published information about past and ongoing efforts in the field of metagenomics, in addition to documenting validation and quality control guidelines.

Extensive investigations were carried out to ensure that metagenomic approaches could be applied to and used in diagnostic settings. For example, proficiency testing evaluated the interlaboratory reproducibility and robustness of methods for data generation and analysis of swine faecal samples naturally infected with several porcine astrovirus species. Laboratories were provided samples and used methods that had be standardised earlier on in the METASTAVA project.

METASTAVA protocols, expertise and resources were applied across the different partners and research included investigation of the virome in farms where neonatal piglet diarrhoea was problematic, in addition to the characterisation of SARS-CoV-2 during the COVID-19 pandemic and characterisation of *Norovirus* variants. METASTAVA protocols were validated for characterisation for animal coronaviruses, in addition to investigating animal samples yielding false positive test results using the antigen detection lateral flow device for rapid detection of SARS-CoV-2.

The analytical properties of metagenomics for model pathogens and standardised procedures defined earlier in the project were investigated for detection of hepatitis E virus, Norovirus, animal zoonotic pox virus, Shiga toxin producing *E. coli* and antibiotic resistance genes. These investigations showed that different approaches are needed for different pathogen/sample matrix combinations and that metagenomic approaches may also need to be coupled with targeted sequencing and sample enrichment prior to metagenomic sequencing depending on the diagnostic or scientific question at hand.

Publications in 2020:

Oude Munnink, BB., Nieuwenhuijse, DF., Stein, M., O'Toole, A., Haverkate, M., Mollers, M.,Kamga, SK., Schapendonk, C., Pronk, M., Lexmond, P., van der Linden, A., Bestebroer, T., Chestakova, I., Overmars, RJ., van Nieuwkoop, S., Molenkamp, R., van der Eijk, AA,. Geurtsvan Kessel, C., Vennema, H., Meijer, A., Rambaut, A., van Dissel, J., Sikkema, RS., Timen, A., Koopmans, M. (2020). Rapid SARS-CoV-2 whole-genome sequencing and analysis for informed public health decision-making in the Netherlands. *Nature Medicine*. 26, p 1405–1410. DOI: https://doi.org/10.1038/s41591-020-0997-y.

Van Borm, S., Fu, Q., Winand, R., Vanneste, K., Hakhverdyan, M., Höper, D., Vandenbussche, F. (2020) Evaluation of a commercial exogenous internal process control for diagnostic RNA virus metagenomics from different animal clinical samples. *Journal of Virological Methods*, 283. DOI: https://doi.org/10.1016/j.jviromet.2020.113916.

Izquierdo Lara, RW., Elsinga, G., Heijnen, L., Oude Munnink, BB., Schapendonk, CME., Nieuwenhuijse, D., Kon, M., Lu, L., Aarestrup, FM., Lycett, S., Medema, G., Koopmans, MPG., Miranda de Graaf, M. (2020) Monitoring SARS-CoV-2 circulation and diversity through community wastewater sequencing. *medRxiv* DOI: https://doi.org/10.1101/2020.09.21.20198838

Van Borm S, Vanneste K, Fu Q, Maes D, Schoos A, Vallaey E, Vandenbussche F. (2020). Increased viral read counts and metagenomic full genome characterisation of porcine astrovirus 4 and Posavirus 1 in sows in a swine farm with unexplained neonatal piglet diarrhea. *Virus Genes*. 56(6):696-704. DOI: https://doi.org/10.1007/s11262-020-01791-z

IMAGE:FLICKR

AIRSAMPLE - ended December 2020

The AIRSAMPLE project aimed to develop and validate air sampling as a low-cost method for detection of *Campylobacter* in broiler production; air sampling was investigated as a method to replace analysis of faecal droppings and boot swabbing.

The research in this project moved through four phases:
Harmonisation -> Implementation -> Evaluation -> Validation.

In the third and final year of the project, the validation phase was the key focus, in addition to ensuring the methods used in this project were sustainable. In order to do this, the project focussed on producing publications, standard operating procedures and guidelines for the use of air sampling methods.

An online video to educate the broiler industry was created and can be found here. Furthermore, the air sampling guidelines have been converted into user guidelines for wider communications to the public and stakeholders and can be found here. This research and subsequent communications have been disseminated to EU authorities and the European reference laboratory for *Campylobacter* (EURL- *Campylobacter*), and are predicted to make significant impact as the results showed that air sampling, when coupled with real-time PCR can improve detection of *Campylobacter* in commercial settings across Europe. The benefit of a European-wide approach to validation of these air sampling methods was that it showed that even for low-prevalence countries such as Norway, air sampling and real-time PCR were as effective as culture.

The studies for the AIRSAMPLE project demonstrated that the likelihood of detecting *Campylobacter* using air sampling and real-time PCR quadrupled compared to more traditional swab and culture methods. As a result, air sampling could be especially useful for farmers when assessing the cleanliness of their poultry houses before introducing new chicks for production.

Publications in 2020:

Hoorfar, J., Koláčková, I., Johannessen, GS., Garofolo, G., Marotta, F., Wieczorek, K., Osek, J., Torp, M., Spilsberg, B., Sekse, C., Thornval, NR., Karpíšková, R. (2020). A multi -center proposal for a fast screening tool in biosecured chicken flocks. Foodborne *Campylobacter*. *Applied and Environmental Microbiology*, 86 (20) e01051-20. DOI: https://doi.org/10.1128/AEM.01051-20.

Johannessen, GS., Garofolo, G., Di Serafino, G., Koláčková, I., Karpíšková, R., Wieczorek, K., Osek, J., Christensen, J., Torp, M., Hoorfar, J. (2020) *Campylobacter* in chicken- Critical parameters for international, multi-centre evaluation of air sampling and detection method. *Food Microbiology*, 90, 1-6. DOI: https://doi.org/10.1016/j.fm.2020.103455.

Wieczorek, K., Wołkowicz, T., Osek, J. (2020) MLST-based genetic relatedness of *Campylobacter jejuni* isolated from chickens and humans in Poland. *PLOS ONE*, 15(1), e0226238. DOI: https://doi.org/10.1371/journal.pone.0226238.

Wieczorek, K., Bocian, L., Osek, J. (2020) Prevalence and antimicrobial resistance of *Campylobacter* isolated from carcasses of chickens slaughtered in Poland – a retrospective study. *Food Control,* 112, 107159. DOI: https://doi.org/10.1016/j.foodcont.2020.107159.

Marotta, F., Janowicz, A., Di Marcantonio, L., Ercole, C., Di Donato, G., Garofolo, G., Di Giannatale, E. (2020) Molecular characterisation and antimicrobial susceptibility of *C. jejuni* isolates from Italian wild bird populations. *Pathogens*, 9(4), 304. DOI: https://doi.org/10.3390/pathogens9040304.

IMAGE:KUBO MICHAL

MoMIR - year 3

The MoMIR project aims to develop new approaches to predict, identify and prevent the emergence of animal and human super-shedders based on immune responses and gut microbiota composition. In order to achieve this, the key focuses of the project were to define predictive markers that signal the risk of animals and humans becoming *Salmonella* super-shedders, to identify immune and microbiota biomarkers to detect super and low-shedders, to identify measures to prevent and control *Salmonella* super-shedders and to develop mathematical models for risk management.

Studies to identify predictive biomarkers for *Salmonella* super-shedders have been undertaken and allowed partners to identify biomarkers based on the composition of the chicken gut microbiota. However, further studies to identify these markers and immune biomarkers have been delayed due to the COVID-19 pandemic. A number of joint publications have been accepted or are in preparation to disseminate these results. Early investigation into other biomarkers which may indicate super or low *Salmonella* shedders in both chicken and pigs have suggested that the number of immune cells in the blood cannot be used to distinguish super vs low shedder animals. Additionally, the virulence of the *Salmonella* strain did not correlate to super or low shedders. Recruitment of participants in the human aspect of the project is now ongoing.

To investigate possible mitigation strategies for the control of *Salmonella* super-shedders, probiotics were investigated. Numerous probiotic strains were isolated and characterised by partner institutes, and two poultry derived strains and two porcine derived strains were selected for further investigation. The COVID-19 pandemic has delayed some of the *in vivo* studies, however the initial results indicate that the pre and probiotics are efficacious at reducing *Salmonella* shedding in pigs and chickens. Once they are complete, over 3000 samples will be sent for metagenomic studies to determine how pre- and probiotic interventions and *Salmonella* status influence the gut microbiome. Studies to determine the influence of commensal bacteria on *Salmonella* shedding status and immune responses have been completed and the data are under analysis.

Studies have revealed that a combination of probiotics were, in part, able to protect chickens from *Salmonella* colonisation. Additionally, chickens fed with fermented defatted 'alperujo' (a by-product of olive oil extraction), were also, in part, protected from *Salmonella* colonisation.

A first version of a mathematical model of the interplay between the gut microbiota, the pathogen and the host's immune response has been developed.

Finally, a draft inventory of relevant intervention strategies against *Salmonella* in laying hens has been developed and the cost effectiveness of probiotic intervention strategies has been calculated.

Publications in 2020:

van Wagenberg, CPA., van Horne, P.L.M., van Asseldonk, M.A.P.M. (2020). Cost-effectiveness analysis of using probiotics, prebiotics, or synbiotics to control *Campylobacter* in broilers. *Poultry Science*. 99 (8), pp. 4077-4084. DOI: https://doi.org/10.1016/j.psj.2020.05.003.

Labarthe, S., Laroche, B., Nguyen, T. N. T., Polizzi, B., Patout, F., Ribot, M., Stegmaier, T. (2020). A Multi-Scale Epidemic Model of *Salmonella* infection with Heterogeneous Shedding. ESAIM: *Proceedings and Surveys*, 67, p. 261-284. DOI: https://doi.org/10.1051/proc/202067015.

Rebollada-Merino, A., Ugarte-Ruiz, M., Hernández, M., Miguela-Villoldo, P., Abad, D., Rodríguez-Lázaro, D., de Juan, L., Domínguez, L., Rodríguez-Bertos, A. (2020). Reduction of *Salmonella* Typhimurium cecal colonisation and improvement of intestinal health in broilers supplemented with fermented defatted 'alperujo', an olive oil by-product. *Animals*. 10(10), 1931. DOI: https://doi.org/10.3390/ani10101931.

Rebollada-Merino, A., Ugarte-Ruiz, M., Hernández, M., Miguela-Villoldo, P., Abad, D., Cuesta-Álvaro, P., Rodríguez-Lázaro, D., de Juan, L., Domínguez, L., Rodríguez-Bertos, A. (2020). Dietary supplementation with fermented defatted "alperujo" induces modifications of the intestinal mucosa and cecal microbiota of broiler chickens. *Poultry Science*. DOI: https://doi.org/10.1016/j.psj.2020.07.015.

Kempf, F., Menanteau, P., Rychlik, I., Kubasová, T., Trotereau, J., Virlogeux-Payant, I., Schaeffer, S., Schouler, C., Drumo, R., Guitton, E., Velge, P. (2020) Gut microbiota composition before infection determines the *Salmonella* super- and low-shedder phenotypes in chicken. *Microbial Biotechnology*. 13 (5), 1611– 1630. DOI: https://doi.org/10.1111/1751-7915.13621.

IMAGE:PIXABAY

MedVetKlebs - ended December 2020

The MedVetKlebs project aimed to enhance *Klebsiella pneumoniae* research and surveillance by developing and harmonising study methods and by investigating its ecology and transmission across sources. The need for this research is increasingly important due to the emergence of multidrug resistance *K. pneumoniae* strains, which are an increasing concern for public health, animal health, environmental health and its ability to contaminate food.

The MedVetKlebs project developed protocols for *Klebsiella* isolation from various sources including food, water and human and animal faeces. Molecular detection methods were also developed using a highly sensitive qPCR method known as the ZKIR assay, this assay detects *K. pneumoniae* and its closely related species from soil, food, faeces, and other complex matrices. During the project, novel taxonomic classifications of four new *Klebsiella* species and subspecies were identified, in addition to novel biomarkers and laboratory identification tools such as MALDI-TOF mass spectrometry. Protocols developed during the projects were widely disseminated through publications and the open platform: protocols.io. Further methodological developments such as development of a combined qPCR method for detecting and differentiating medically important *Klebsiella* species and creating a publicly available MALDI-TOF *Klebsiella* identification website will be finalised shortly.

Using protocols harmonised in the MedVetKlebs project, nearly 4000 samples were collected from different sources including food, the environment and healthy humans and animals. These samples were investigated for *Klebsiella* contamination and it was found that among the food samples tested, chicken meat and ready to eat salads had the highest *K. pneumoniae* recovery rate: 30% and 20%, respectively. In the environment, all sources tested had a high prevalence of *K. pneumoniae*. As a result of this broad testing, more specific sources of potential relevance for One Health related transmission were investigated. This included chicken meat, ready to eat salads, onions, seawater, soil, and human carriage.

Whole genome sequencing of *K. pneumoniae* isolated from these identified sources was performed and there were found to be a very high level of genetic diversity between strains, but importantly, a low prevalence of antimicrobial resistance and virulence genes. Targeted metagenomic approaches are currently being used to explore the diversity and prevalence of *K. pneumoniae* in the human gut.

Finally, mathematical modelling was explored to simulate the diversification and mutation rate of a *K. pneumoniae* lineage which contaminates food. This research will be used to define clusters of related *K. pneumoniae* strains that impact the food chain. This model can be generalised to all foodborne or environmental bacterial species and will help with defining short term transmission of pathogens to humans and animals.

Publications in 2020:

Chiarelli, A., Cabanel, N., Rosinski-Chupin, I., Zongo PD., Naas. T., Bonnin, RA., Glaser, P. (2020). Diversity of mucoid to non-mucoid switch among carbapenemase-producing *Klebsiella pneumoniae*. *BMC Microbiology*, 20, 325. DOI: https://doi.org/10.1186/s12866-020-02007-y.

Barbier, E., Rodrigues, C., Depret, G., Passet, V., Gal, L., Piveteau, P., Brisse, S. (2020). The ZKIR Assay, a Real-Time PCR Method for the Detection of *Klebsiella pneumoniae* and Closely Related Species in Environmental Samples. *Applied and Environmental Microbiology,* 86(7), pp. e02711-19. DOI: https://doi.org/10.1128/AEM.02711-19.

Huynh, BT., Passet, V., Rakotondrasoa, A.,. Diallo, T., Kerleguer, A., Hennart, M., Lauzanne, A., Herindrainy, P., Seck, A., Bercion, R., Borand, L., Pardos de la Gandara, M., Delarocque-Astagneau, E., Guillemot, D., Vray, M., Garin, B., Collard, JM., Rodrigues, C., Brisse, S. (2020). *Klebsiella pneumoniae* carriage in low-income countries: antimicrobial resistance, genomic diversity and risk factors. *Gut Microbes*, 11(5), pp. 1287-1299. DOI: https://doi.org/10.1080/19490976.2020.1748257.

Loncaric, I., Rosel, AC., Szostak, MP., Licka, TF., Allerberger, F., Ruppitsch, W., Spergser, J. Broad-Spectrum Cephalosporin -Resistant *Klebsiella* spp. Isolated from Diseased Horses in Austria (2020). *Animals*, 10(2), pp. 332. DOI: https://doi.org/10.3390/ani10020332.

Rebollada-Merino A., Bárcena C., Ugarte-Ruiz M., Porras-González N., Mayoral-Alegre F., Tome-Sánchez I., Domínguez L. and Rodríguez-Bertos A. (2020). Effects on Intestinal Mucosal Morphology, Productive Parameters and Microbiota Composition after Supplementation with Fermented Defatted Alperujo (FDA) in Laying Hens. *Antibiotics*, 8(4), pp. 215. DOI: https://doi.org/10.3390/antibiotics8040215.

IMAGE:CHIARA MARRACCINI

DISCOVER - year 1

The DISCOVER project aims to address the challenges of source attribution using an interdisciplinary One Health approach. As there is no gold standard for source attribution, this project will take a comprehensive approach by applying several different methodologies and models in a comparative fashion. The project aims to map existing knowledge and recommend new studies and methods that may be required to fill knowledge gaps.

The research into mapping existing knowledge gaps has begun and has used input from all partners in the DISCOVER project. A systematic literature search was completed with defined search criteria to search for literature relevant to the source attribution of *Salmonella*, *Campylobacter* and *E. coli*, in addition to searching for antimicrobial resistance in these bacterial species. Using the data collected from these literature searches, knowledge maps were created which showed the number of publications that were found for each method of source attribution for each bacterial species.

Mapping of existing data for *Salmonella*, *Campylobacter* and *E. coli* and associated antimicrobial resistance profiles was completed. An inventory of information was compiled with the aim of providing an overview of strains and their genome sequences to pinpoint areas with limited data availability. The work to further expand this inventory is ongoing.

Data has been collected from each of the partner institutes regarding *Salmonella*, *Campylobacter* and *E. coli* and antimicrobial resistance. Discussions have taken place to determine how these datasets could be improved throughout the project and to determine which source attribution approaches will be applied, further developed and compared during the project.

IMAGE:PIXABAY

BIOPIGEE - year 1

The BIOPIGEE project aims to identify cost-effective biosecurity best practices which will reduce the occurrence of *Salmonella* and hepatitis E virus in European pig production. The project will develop tools to limit pathogen load along the food chain, thus resulting in healthier animals and a safer food chain. This research will create a benchmark of biosecurity practices throughout different stages of pig production in several European countries.

A biosecurity questionnaire for European pig farms was developed to assess the relevance of control measures for the prevalence of *Salmonella* and hepatitis E virus. Software was used to conduct the survey and to translate the questionnaire into the different languages of the participating countries. Protocols for farm recruitment, sampling and laboratory testing were designed, and farm recruitment is currently in progress, with 61 farm visits completed by the end of the first year of the project. Additionally, existing biosecurity protocols for slaughterhouses across all partner countries have been collated.

Discussions are in progress to select a panel of *Salmonella* strains to compare methods for disinfectant effectivity testing, and to define harmonised methods for biofilm assays. Furthermore, work is ongoing to further develop a harmonised hepatitis E virus infectivity assay.

An online catalogue of effective biosecurity measures against *Salmonella* and hepatitis E virus was created. A systematic literature review on the efficacy of biosecurity methods was conducted between the BIOPIGEE partners. Literature was screened, assessed and information was extracted and compiled for data to be prepared for meta-analyses. An expert panel of scientists was built to rate the biosecurity measures during development of the biosecurity questionnaire. This panel has been expanded with practicing veterinarians, advisors and inspectors in order to also identify factors that will be used to create the benchmark of biosecurity practices later in the project.

Additionally, a BIOPIGEE flyer was created to promote the project.

IMAGE:NEIL THEASBY

TOXOSOURCES - year 1

The TOXOSOURCES project aims to answer the research question: what are the relative contributions of the different sources of *Toxoplasma gondii* infection? The project addresses this question using several multidisciplinary approaches and novel and improved methods to yield robust estimates that can inform risk managers and policy makers. *Toxoplasma gondii* is a highly prioritised foodborne pathogen that causes a high disease burden. The relative contributions of the different transmissible stages, sources and transmission pathways to the infection and disease in humans remain unknown, partly due to lack of appropriate methods, and as a consequence, systematic control of this zoonotic foodborne pathogen is lacking in Europe and globally.

In the first year, the TOXOSOURCES project started a collection of data and the building of a quantitative microbiological risk assessment model for *T. gondii*. The quantitative microbiological risk assessment model will cover both meatborne and environmental exposure, and will be applied in a multi-country study. A questionnaire was developed for collecting food consumption data from the selection of countries across Europe. For the first time in Europe, a systematic literature review on *T. gondii* prevalence covering animal species raised and hunted for human consumption as well as cats, which are the definitive hosts of the parasite, was performed.

An extensive literature review was performed, and practices and experiences were surveyed to support the selection of the most suitable molecular method to detect *T. gondii* oocysts in fresh produce, which will be applied in a multicentre study. Bioinformatic selection of promising protein candidates for novel serology method was finalised and recombinant expression of selected proteins was started, as part of exploring serology for detecting *T. gondii* infections caused by oocysts. An unprecedented effort of Whole Genome Sequencing of *T. gondii* isolates and DNAs was used to identify polymorphic marker regions, which will pave the way for a new typing method to be developed for detecting within-genotype variation.

Publications:

Klein, S., Stern, D., Seeber, F. Expression of in vivo biotinylated recombinant antigens SAG1 and SAG2A from *Toxoplasma gondii* for improved seroepidemiological bead-based multiplex assays. (2020). *BMC Biotechnology*. 20, 53. DOI: https://doi.org/10.1186/s12896-020-00646-7.

Fernández-Escobar, M., Calero-Bernal, R., Benavides, J., Regidor-Cerrillo, J., Guerrero-Molina, M. C., Gutiérrez-Expósito, D., Collantes-Fernández, E., Ortega-Mora, L. M. Isolation and genetic characterization of *Toxoplasma gondii* in Spanish sheep flocks. (2020). *Parasites and Vectors*. DOI: https://doi.org/10.1186/s13071-020-04275-z.

Fabian, B. T., Hedar, F., Koethe, M., Bangoura, B., Maksimov, P., Conraths, F. J., Villena, I., Aubert, D., Seeber, F., Schares, G. Fluorescent bead-based serological detection of *Toxoplasma gondii* infection in chickens. (2020). *Parasites and Vectors*. DOI: https://doi.org/10.1186/s13071-020-04244-6.

IMAGE:PIXABAY

ADONIS - year 1

The ADONIS project aims to identify determinants responsible for the increasing incidence of *Salmonella enteritidis* in humans and poultry in the EU. A cross- sectorial One Health approach will be used to investigate factors that may be related to increasing incidence in primary poultry production, epidemiology and exposure to *S*. Enteritidis and also investigating the pathogen itself.

In the first year, the ADONIS project focussed on preparation for data gathering and data analysis. This included gathering audit reports from *Salmonella* National Control Programmes, preparing a survey to collect data on the key information from the *Salmonella* National Control Programmes in laying hens at the country level, and the design of a study protocol for primary production in-field investigations. Currently, 232 recommendations were identified from 38 public audit reports from 24 countries and the surveys prepared are currently being shared among veterinary agencies in the partner countries of the ADONIS project.

Countries were selected for evaluation of human surveillance systems and detailed epidemiological trend analysis for *Salmonella*. A document was prepared which highlighted the trends of each country, from which countries were selected depending on the identified trends in *Salmonella*: the UK and Spain were selected as countries with an increasing trend, the Netherlands and Belgium were selected as countries with a stable trend and Norway was selected as a country with a decreasing trend.

Additionally, preparations for an inventory of available *Salmonella* sequence data and a pilot genome wide association study began. This research will investigate DNA markers for specific *Salmonella* phenotypes and determinants possibly associated with the increasing trend in *Salmonella* incidence and the possible interventions.

IMAGE:PIXABAY

BeOne - year 1

The BeOne project aims to develop an integrated surveillance dashboard in which molecular and epidemiologically data for foodborne pathogens can be analysed, visualised and interpreted interactively by experts across different disciplines and sectors. Surveillance of foodborne infections and outbreak detection is primarily handled at the national or regional level and often spans different sectors and disciplines. This means that data are increasingly complex and not interoperable, therefore, highlighting the need for tools that can allow the integration of various types of data and facilitate their analysis and interpretation.

In the first year of the project, it was decided that the BeOne project would build upon the work achieved in the One Health EJP ORION project. This work began with discussing the advantages and disadvantages of centralised vs decentralised approaches to surveillance and investigating the currently available surveillance platforms with regard to software, data management and analysis pipelines.

A survey was conducted between the BeOne project partners to define their whole genome sequence metadata contribution and to create harmonised guidelines for whole genome sequence data and metadata collection. A database system was decided within the project, this system can be built upon; for example, compatible data structures can be built to capture the complexity of intersectoral surveillance data, while maintaining focus on the usability of the data.

The BeOne project aims to link genomics and epidemiology by building knowledge and algorithms for outbreak detection. In the first year, a summary of the existing knowledge on epidemiology of targeted pathogens was created. After which, the BeOne project outlined detection issues and outlined a conceptual model for the biological and epidemiological factors impacting outbreak detection. This model aims to serve as a first step in developing and evaluating new algorithms for the detection of pathogens and outbreaks.

The project aims to ensure that its findings are sustainable and therefore the base code created during the project is available on Github, with full support and documentation.

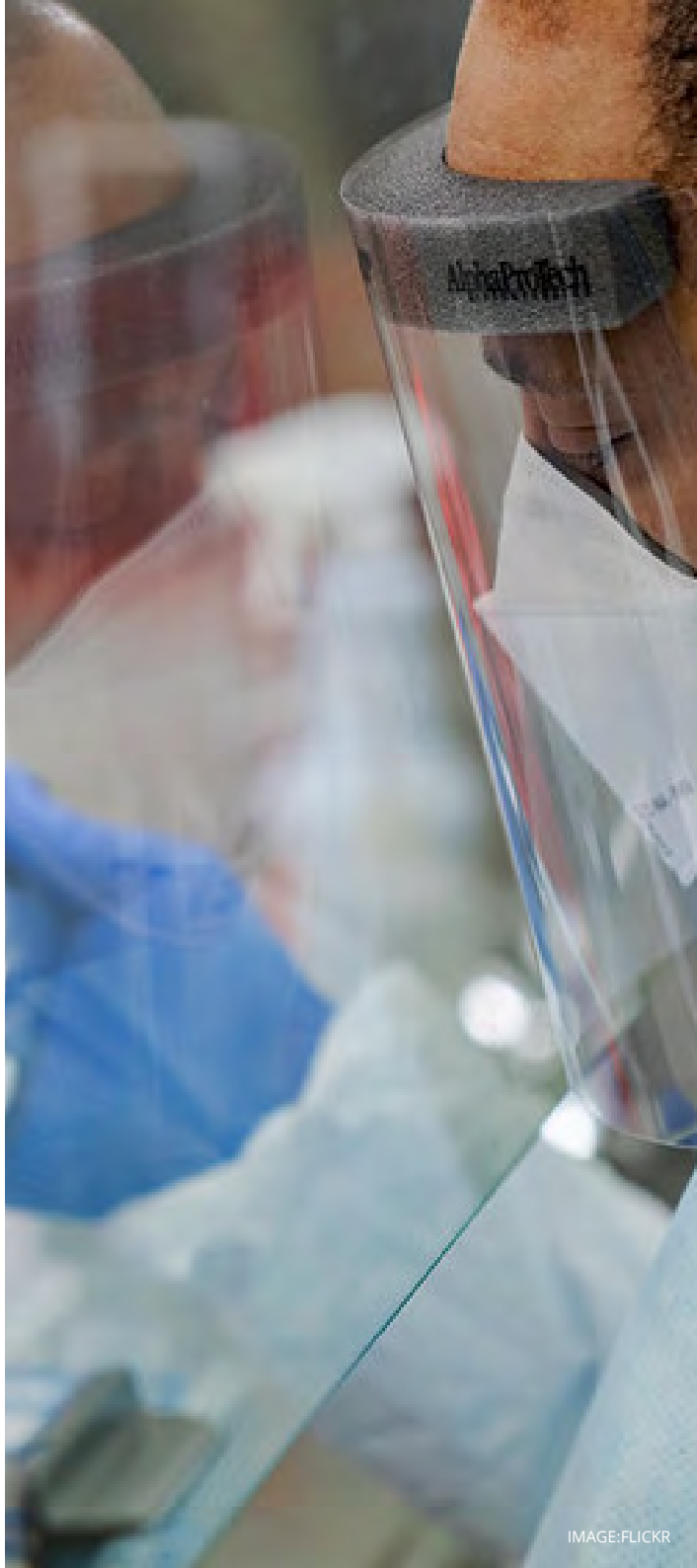


IMAGE:FLICKR

IMPART - ended December 2020

The IMPART project aims to develop and harmonise laboratory methods for the detection of antimicrobial resistance. The optimisation and harmonisation of laboratory methods for detection of resistant bacteria is essential for antimicrobial resistance surveillance in food producing animal pathogens and determining the risk of transmission of resistance from animals to humans via food and the environment.

The results of the final ring trial investigating standardised laboratory methods for detection of colistin-resistant *Enterobacteriaceae* using a combination of commercial selective media and PCR were collated and sent to all participants. Results showed that PCR detection of *mcr*- genes showed 100% specificity and one of the three media tested demonstrated higher sensitivity for selecting colistin-resistant, *mcr*-positive strains. Additionally, it showed that screening for colistin-resistant strains was more successful from caecal samples, compared the meat samples under experimental conditions.

The final ring trial for detection of carbapenemase-producing *Enterobacteriaceae* was also completed, and results were distributed to participants near the end of the third year. The main outcome indicated that some of the commercially available selective agar media are not sensitive or selective enough to detect bacteria expressing low levels of carbapenemase production. Some bacteria harbouring specific genes that circulate in European livestock populations may be missed using protocols currently set by the EU. Therefore, more studies are required to test additional culture techniques.

The production and collection of minimum inhibitory concentrations (MICs) from over 2800 bacterial strains from 17 different species for 34 different antimicrobials was completed and uploaded to the EUCAST website. To date, new epidemiological cut off values have been set for *Staphylococcus pseudintermedius*, *S. hyicus*, *Pasteurella multocida* and *Mannheimia haemolytica* (https://mic.eucast.org/).

A disk diffusion method as an alternative method for antimicrobial susceptibility testing of *Clostridioides difficile* was optimised and validated using a collection of 527 well-characterised *C. difficile* strains. A robust protocol for the disk diffusion was established and inhibition zone diameter distributions were determined for eight antimicrobials using the entire strain collection. A ring trial to finalise the validation of this method was completed in the third year. Overall the optimised method proved to be reliable for most of the tested antimicrobials and highly reproducible.

Publications in 2020

Pauly, N., Hammerl, JA., Grobbel, M., Käsbohrer, A., Tenhagen, BA., Malorny, B., Schwarz, S., Meemken, D., Irrgang, A. (2020). Identification of a blaVIM-1-Carrying IncA/C2 Multi-resistance Plasmid in an *Escherichia coli* Isolate Recovered from the German Food Chain. *Microorganisms*. 9, 29. DOI: https://doi.org/10.3390/microorganisms9010029.

Pauly, N., Hammerl, JA., Schwarz, S., Grobbel, M., Meemken, D., Malorny, B., Tenhagen, BA., Käsbohrer, A., Irrgang, A. (2020). Co-occurrence of the blaVIM-1 and blaSHV-12 genes on an IncHI2 plasmid of an *Escherichia coli* isolate recovered from German livestock. *Journal of Antimicrobial Chemotherapy*. 76(2), 531–533. DOI: https://doi.org/10.1093/jac/dkaa436.

Diaconu, EL., Carfora, V., Alba, P., Di Matteo, P., Stravino, F., Buccella, C., Dell'Aira, E., Onorati, R., Sorbara, L., Battisti, A., Franco, A. (2020). Novel IncFII plasmid harbouring blaNDM-4 in a carbapenem-resistant *Escherichia coli* of pig origin, Italy. *Journal of Antimicrobial Chemotherapy*. 75(12), 3475–3479. DOI: https://doi.org/10.1093/jac/dkaa374.

Irrgang, A., Tausch SH., Pauly, N., Grobbel, M., Kaesbohrer, A,. Hammerl, JA. (2020). First Detection of GES-5-Producing *Escherichia coli* from Livestock—An Increasing Diversity of Carbapenemases Recognized from German Pig Production. *Microorganisms*. 8 (10), 159. DOI: https://doi.org/10.3390/microorganisms8101593.

Pauly, N., Hammerl, JA., Grobbel, M., Tenhagen, BA., Käsbohrer, A., Bisenius, S., Fuchs, J., Horlacher, S., Lingstädt, H., Mauermann, U., Mitro, S., Müller, M., Rohrmann, S., Schiffmann, AP., Stührenberg, B., Zimmermann, P., Schwarz, S., Meemken, D., Irrgang, A. (2020). ChromID® CARBA Agar Fails to Detect Carbapenem-Resistant Enterobacteriaceae With Slightly Reduced Susceptibility to Carbapenems. *Frontiers in Microbiology*. 11, 1678. DOI: https://doi.org/10.3390/microorganisms9010029.

Irrgang, A., Pauly, N., Tenhagen, BA., Grobbel, M., Kaesbohrer, A., Hammerl, JA. (2020). Spill-Over from Public Health? First Detection of an OXA-48-Producing *Escherichia coli* in a German Pig Farm. *Microorganisms*. 8(6), 855. DOI: https://doi.org/10.3390/microorganisms8060855.

IMAGE:FLICKR

ARDIG - final year

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.

Antimicrobial resistance and antimicrobial usage data was obtained from healthy and diseased animals from different partners and countries across Europe. Preliminary analysis of these data has shown that due to the difference in methodology and interpretation criteria, direct comparisons of minimum inhibitory concentrations (MIC) and antibiotic disk diffusions data will be difficult- hence the importance of improving the interoperability of data. Due to these difficulties, the ARDIG project has explored statistical methods to set epidemiological cut-off (ECOFF) values for each antimicrobial. By using a harmonised method to calculate the ECOFF value, a more 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.

Both public health and animal health partners collected clinically important *E. coli* strains from farm and hospital studies over 12 months. Many partners have characterised *E. coli* isolated through national surveillance programmes. These became part of a whole genome sequencing project 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. In addition to the genotypic studies, AMR phenotypes were investigated for these strains using the EFSA panel of antimicrobials with the aim of comparing AMR genotype to corresponding phenotype.

The *E. coli* strains collected were characterised to investigate AMR genes, plasmids and mobile genetic elements using whole genome sequencing as well as other molecular techniques. Phylogenetic analysis was performed on datasets, which helped to identify transmission events or epidemiological links between isolates of particular sequence types or clones of *E. coli* collected from different hospital or farm settings. AMR pipeline comparison work was also carried out to assist in the harmonisation of *in silico* AMR gene prediction. Analysis of all the phenotypic and genotypic data is in progress.

Publications in 2020

Brouwer, MSM., Goodman, RN., Kant, A., Mevius, D., Newire, E., Roberts, AP., Veldman, KT. (2020). Mobile colistin resistance gene mcr-1 detected on an IncI1 plasmid in *Escherichia coli* from meat. *Journal of Global Antimicrobial Resistance*. 23, 145-148. DOI: https://doi.org/10.1016/j.jgar.2020.08.018.

Duggett, N., AbuOun, M., Randall, L., Horton, R., Lemma, F., Rogers, J., Crook, D., Teale, C., Anjum, MF. (2020). The importance of using whole genome sequencing and extended spectrum beta-lactamase selective media when monitoring antimicrobial resistance. *Scientific Reports*. 10, 19880. DOI: https://doi.org/10.1038/s41598-020-76877-7.

Massot, M., Haenni, M., Nguyen, TT., Madec, JY., Mentré, F., Denamur, E. (2020). Temporal dynamics of the fecal microbiota in veal calves in a 6-month field trial. *Animal Microbiome*. 2(32). DOI: https://doi.org/10.1186/s42523-020-00052-6.

Rodriguez-Rubio, L., Serna, C., Ares-Arroyo, M., Matamoros, BR., Delgado-Blas, JF., Montero, N., Bernabe-Balas, C., Wedel, EF., Mendez, IS., Muniesa, M., Gonzalez-Zorn, B. (2020). Extensive antimicrobial resistance mobilization via multicopy plasmid encapsidation mediated by temperate phages. *Journal of Antimicrobial Chemotherapy*, 75 (11), pp. 3173–3180. DOI: https://doi.org/10.1093/jac/dkaa311.

Patiño-Navarrete, R., Rosinski-Chupin, I., Cabanel, N., Gauthier, L., Takissian, J., Madec, JY., Hamze, M., Bonnin, RA., Naas, T., Glaser, P. (2020). Stepwise evolution and convergent recombination underlie the global dissemination of carbapenemase-producing *Escherichia coli*. *Genome Medicine*, 12(10). DOI: https://doi.org/10.1186/s13073-019-0699-6.

IMAGE:PIXABAY

RADAR - ended December 2020

The RaDAR project aims to improve and harmonise modelling methods for better understanding of risks, sources and disease burden by developing risk assessment models to quantify the on-farm development of antimicrobial resistance (AMR) and the subsequent spread to humans. AMR threatens the effective prevention and treatment of an ever-increasing range of infections and is a global threat to public, animal and environmental health. Assessment of the importance of different transmission routes and quantifying public health effects (i.e. disease burden) associated with AMR represent major knowledge gaps.

The RaDAR project contributed significantly to these identified knowledge gaps by producing and harmonising modelling methods and frameworks specifically for AMR-related problems using a cross-discipline approach across all pillars of One Health.

A large-scale database of AMR plasmids from a range of different bacterial species and sources was curated, which gave an overview of plasmid classification and diversity. The novel resource aims to help researchers to understand the genetic plasticity and transmission route of plasmids, which are crucial in the fight against antimicrobial resistant pathogens. The database is available here.

Harmonising risk assessment tools was also an important component of the RaDAR project. Infrastructure for exchanging and annotating risk assessment models was developed in an exchangeable and reproducible file format called FSK, which means that this infrastructure can be used across all pillars of One Health, by risk assessors worldwide. Additionally, state of the art AMR risk assessment models for different food chains were produced using a generic framework. Using generic frameworks may be cruder, but allows for combining risks in different categories, thus may help to create a more complete picture of AMR issues. Machine learning methods were also applied to AMR risk to identify risk factors from data with many variables.

A mathematical model was designed to estimate the excess disease burden of AMR, this model considers the mortality and morbidity associated with AMR which is over and above the mortality and morbidity associated with the same, antibiotic sensitive infection. A One Health source attribution model was also produced which can estimate the contribution of reservoirs of AMR and transmission routes. Finally, metagenomic approaches were applied to AMR surveillance, in general, these data correlated at both the phenotypic and genotypic level.

Publications in 2020

Douarre, P E, Mallet, L, Radomski, N, Felten, A, Mistou, M. (2020). Analysis of COMPASS, a New Comprehensive Plasmid Database Revealed Prevalence of Multireplicon and Extensive Diversity of IncF Plasmids. *Frontiers in Microbiology*, 11, pp. 1-15. DOI: https://doi.org/10.3389/fmicb.2020.00483.

IMAGE:FLICKR

FARMED - year 1

Antimicrobial resistance (AMR) threatens global health and 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 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 metagenome sequencing using the MinION from Oxford Nanopore Technology (ONT), for use as a diagnostic tool to detect pathogens and AMR or virulence genes.

To begin this work, in the first year of the project, a survey of the FARMED project consortium was used to identify the different methods and experiences of each of the institutes. This included experience with both short- and long-read sequencing technologies, and highlighted experience with different matrices from a variety of human, animal, environmental sources, DNA extraction methods and bioinformatics tools used to detect bacterial species and/or AMR. Based on the survey results, sample matrices were selected, these included simple matrices such as water and saliva and complex matrices such as human/animal faeces, feed additives and boot swabs. To test the effectiveness of the different laboratory-based DNA extraction methods used by each institute, a defined microbial community will be used to inoculate a simple (water) and complex (animal faeces) matrix. Work is underway to compare the output from each of the partner institutes, DNA extraction methods, and sequencing technology, to identify and optimise a metagenome sequencing workflow. The bioinformatics analysis tool, KMA (DTU), is being used and outputs optimised to characterise the metagenome as well as identify the bacterial species included in the defined microbial community.

The FARMED project has undertaken a literature review of commonly used metagenome DNA extractions methods, considered the key requirements for onsite metagenome DNA extraction and long read sequencing and summary of equipment with the potential for onsite DNA extraction and sequencing (planned to be trialled in during the project).

As much of the FARMED project is laboratory-based work, the COVID-19 pandemic has severely affected progress.

IMAGE:FLICKR

FULL-FORCE - year 1

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 case studies and applications in metagenomic and AMR transmission models. This technology allows real-time sequencing of long sequence reads, providing additional information of where the read should be placed in the genome. Using this state-of-the-art technology, public health and veterinary laboratories will have the ability to use full-length sequencing and gain detailed insights into mobile genetic elements (MGEs) which carry antimicrobial resistance and virulence genes within, and across species.

Currently, European monitoring systems for antimicrobial resistance falls short in distinguishing clonal from horizontal transmission of AMR genes and the FULL-FORCE project aims to improve this technology.

Progress of this project has been significantly hindered by the COVID-19 pandemic, and therefore many of the activities have been delayed.

Despite these delays, the FULL-FORCE project made progress on many aspects of the project. A final consensus protocol for SMRT sequencing was agreed and distributed between partners, alongside instructions for a proficiency test. The SMRT sequencing workshop was initially postponed as it was designed to be an onsite event, however, was rescheduled as a virtual event in September 2020. During this workshop the FULL-FORCE Plasmid Assembler was created and is currently undergoing refinement.

The focus of the FULL-FORCE AMR genome studies was determined in the first year to investigate mobile genetic element evolution across data sets shared from other One Health EJP projects, other EU funded projects and National Surveillance programmes. Furthermore, the project has made progress on developing, evaluating and creating a database of mobile genetic elements from single isolates and metagenomic databases. This database currently contains approximately 4450 mobile genetic element sequences from approximately 1050 different bacterial species, and several types of mobile genetic element have been identified. Finally, the design of a transmission spread model of plasmid AMR transmission has begun.

Publications in 2020:

Diaconu, EL., Carfora, V., Alba, P., Di Matteo, P., Stravino, F., Buccella, C., Dell'Aira, E., Onorati, R., Sorbara, L., Battisti, A., Franco, A. (2020). Novel IncFII plasmid harbouring blaNDM-4 in a carbapenem-resistant *Escherichia coli* of pig origin, Italy. *Journal of Antimicrobial Chemotherapy*. 75(12), 3475–3479. DOI: https://doi.org/10.1093/jac/dkaa374.

IMAGE:PIXABAY

WORLDCOM - year 1

The WORLDCOM project aims to develop on-site diagnostic tools, linked to mobile referencing technology, for the detection of antimicrobial resistance (AMR) genes in *E. coli*, *Salmonella* and *Campylobacter* in agricultural and environmental settings. These rapid on-site detection tools will be applicable to all pillars of One Health and have the potential to detect the presence of AMR in various environments at earlier stages than is currently possible. Furthermore, with the development of machine learning algorithms, will enhance assay utility and enable predictions of AMR in the environment to be made, thus improving surveillance and possible intervention.

All *E. coli*, *Salmonella*, *Klebsiella* and *Acinetobacter* genomes were extracted from the NCBI Pathogens database. From this database of genomes, all types and sub-types of Extended Spectrum β-Lactamases (ESBLs, enzymes that break down β-lactam ring containing compounds), carbapenemases and plasmid-mediated colistin resistance genes were analysed for their frequency among *E. coli*, *Salmonella*, *Klebsiella* and *Acinetobacter*. High frequency resistance gene subtypes were highlighted for further sequence analysis to illustrate geographic distribution and geography specified single nucleotide polymorphisms (SNPs). Results can be found here.

Whole genome sequencing has been undertaken on a number of strains known to harbour antimicrobial resistance genes. This includes:

- *De novo* sequencing and genome assembly of *E. coli* strains.
- 88 *Campylobacter* strains underwent whole genome sequencing, assembly and MLST typing.
- Aminoglycoside and β-lactam resistance genes encoded by Enterobacteria from companion animals were analysed, which led to the identification of new epidemic plasmids that will be studied by the WORLCOM project.
- 50 plazmocin resistant Enterobacteria isolated from animals, sewage, wastewater, humans and food were sequenced.
- 30 *E. coli* strains, resistant to 3rd and 4th generation cephalosporins, were isolated from swine and alpaca hosts, characterised with respect to phylogeny, antimicrobial resistance patterns and plasmid profile, and whole genome sequenced.
- *E. coli* strains with the CTX-M gene were whole genome sequenced from environmental samples.
- *E. coli* strains resistant to 3rd and 4th generation cephalosporins and/or carbapenems and/or colistin from human and animal origin were also sequenced by WGS.

IMAGE:PIXABAY

The development of Loop-mediated Isothermal Amplification (LAMP) assays has made significant progress in the first year of the project. Development and validation of assays to detect CTX-M resistance genes in *E. coli* strains has been ongoing and protocols have been shared with partners within the project for further testing and validation using veterinary samples. LAMP assays to detect colistin, carbapenem and β- lactamase resistance genes have been developed and validated and can detect certain resistance genes in 3-5 minutes.

Furthermore, good progress has been made on the development and evaluation of rapid DNA extraction protocols for use in the field, including detection of AMR genes in water samples. LAMP assays targeting AMR genes coupled with optimised sample-preparation methods will be crucial for on-site detection of AMR in different settings.

IMAGE:PIXABAY

FED-AMR - year 1

The FED-AMR project aims to investigate the relevance of extracellular free DNA in transferring antimicrobial resistance (AMR) via horizontal antimicrobial resistance gene transfer on free extracellular DNA over ecosystem boundaries relative to bacterial conjugation. Free extracellular DNA is abundant in natural environments and is sufficiently stable to constitute an important reservoir for AMR genes. AMR gene concentrations, diversity, variability, mobility and bacterial biodiversity will be determined in an annual longitudinal study covering a crop's growing period.

Different fertilisation and land management techniques will be investigated, in addition to different environmental compartments, for example: pig farm > manure > soil > crop/food/feed > ground surface water > pig farm. Movement of AMR genes across these different environments will be inferred by sequence comparisons and linkages between human and non-human reservoirs of AMR will be investigated. Zoonotic vs anthropogenic antimicrobial resistance transmission over ecosystem boundaries will be investigated with *C. difficile* as a model organism. The prevailing selection pressure in each tested habitat during the longitudinal study is determined by quantifying antimicrobials, herbicides and trace elements in the tested compartments. Environmental conditions which may induce or inhibit the expression of competence genes which are necessary to enable the uptake of free extracellular DNA by bacteria will be identified in soil microcosms and in a pig gut model. Experimental data obtained during the project will be used to feed and tune probabilistic modelling of the emergence of AMR in target bacteria and to delineate the relative contribution of transformation and conjugation to antimicrobial resistance gene acquisition in soil environments. Mechanistic models will address key questions regarding the spatio-temporal changes observed in microbial communities and shall support the identification of critical control points for intervention to reduce the spread of AMR from environmental sources.

In the first year, the longitudinal study to investigate different crop growing techniques began, with 11 different environmental compartments currently being studied. This study aims to analyse microbial biodiversity and AMR genes across food/feed chains, evaluate the relevance of free extracellular DNA on horizontal gene transfer across ecosystem boundaries, identify points for intervention to reduce the spread of AMR genes, compare geographical differences in trends in AMR and antimicrobials in natural environments and focus on multidrug and emerging resistances.

Overall, the FED-AMR project has been able to coordinate all the different sampling campaigns across four EU regions and generate novel sampling protocols for all the compartments included in the

IMAGE: PIXABAY

longitudinal study. Bacterial culture protocols, in addition to protocols for analysis of antimicrobials and herbicides in environmental samples have been created. Furthermore, guidelines for sample distribution, transportation and conservation have been established. Protocols for extracellular DNA extraction in the various different compartments (e.g. soil, water, manure, faeces and crops) were compiled, harmonised and finalised across partner institutes. One of the main developments in the first year of the project was the consensual replacement of the qPCRs by a target enrichment approach, which will significantly help to address the aims of the project. In addition, the first preliminary results from all aspects of the project have already been obtained.

IMAGE: PIXABAY

TOX-detect - year 3

The TOX-Detect project aims to contribute to increased consumer health protection by filling the critical knowledge gaps in the detection of bacterial toxins and characterising foodborne toxigenic bacteria.

The TOX-Detect project compiled a strain collection of *S. aureus*, *B. cereus* and *C. perfringens* and exchange of these strains between the partner institutes is planned. Culture conditions for *B. cereus* and *C. perfringens* were optimised and defined in standard operating procedures which have been shared between partner institutes. Additionally, methods for cytotoxicity testing and High Content Analysis tests were developed. High Content Analysis assays will investigate apoptosis, DNA Damage, mitochondrial membrane potential and the pro-inflammatory responses to these bacteria and their toxins.

RNA extraction protocols were developed and optimised in various culture conditions for *B. cereus* and *C. perfringens*. These protocols ensured that sufficient RNA was extracted to develop qRT-PCR assays and to send for RNA depletion and sequencing. Bioinformatic analysis was performed including differential gene expression analyses. For both organisms, the methods have been developed and validated, and the pipeline for data analysis is functional. These results will allow correlation between gene expression and strain patterns to be studied.

Analytical development and analyses of *Staphylococcus* enterotoxins M, N & O is underway. A global method based on an "on filter" digestion was finalised and the selection of peptides for further analysis has been done based on the theoretical protein sequence.

Maldi-Tof library and methods to detect toxins *B. cereus* and *C. perfringens* have been developed and shared with the partners of the TOX-Detect project in order for the methods to be shared and inter-laboratory testing to be performed for validation of standard operating procedures.

Publications in 2020:

Lima, DB., Dupré, M., Santos, MDM., Carvalho, PC., Chamot-Rooke, J., (2020). Development, validation and application of an LC-MS/MS method for SEN quantification. *Journal of the American Society of Mass Spectrometry.* Accepted.

IMAGE:FLICKR

TELE-Vir - year 1

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 animals and wildlife. 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 protocols for field analysis. These tools are being designed such that they only require minimal laboratory equipment and are being designed to be compatible with MinION sequencing technology.

Moreover, the project aims to combine and integrate in the point-of-care toolbox phenotypic and epidemiological data to aid risk assessment and management. The toolbox will be made available to other interested national and international stakeholders and shared with established networks.

The TELE-Vir project used coronaviruses and influenza A virus as model viruses for the proof-of-principle studies. A literature review was performed to identify coronavirus phenotypes of relevance to tropism, emergence, and clinical disease, and any data available for their prediction based on genotype. A summary was prepared for circulation to partner institutes and reference laboratories for expert opinion, and a similar exercise is underway for influenza A virus.

Many of the TELE-Vir partners have been involved in SARS-CoV-2 diagnostics and due to the shortage of reagents for nucleic acid extraction, the partners have been forced to develop alternative methods of extraction. This work aligns with the aim of developing a field-based protocol for MinION sequencing using a minimum of laboratory equipment (the point-of-care toolbox). In addition, surveillance programs for SARS-CoV-2 are based on sequencing of the virus, which has resulted in an upgraded version of the INSaFLU software.

A survey was created and circulated to TELE-Vir partners, other One Health EJP partner institutes and associated virologists, to obtain the views of virologists (potential end users of the TELE-Vir toolbox) on coronavirus phenotype prediction and variant monitoring activities that they would like to see in a genomic surveillance toolbox; and to obtain test datasets for further development of the toolkit. Analysis of these responses is ongoing.

Overall, the COVID-19 crisis has had a positive impact on the TELE-Vir project and many of the experiences and problems encountered during the crisis have been used or translated to further develop the TELE-Vir point-of-care toolbox, which aims to help control future outbreaks of new emerging viruses at national, regional, European and even global levels.

Publications:

Fomsgaard, AS., Rosenstierne, MW. (2020). An alternative workflow for molecular detection of SARS-CoV-2 – escape from the NA extraction kit-shortage, Copenhagen, Denmark, March 2020. *Eurosurveillance*. 25(14), pp: 2000398. DOI: https://doi.org/10.2807/1560-7917.ES.2020.25.14.2000398.

IMAGE:PIXABAY

MEmE - year 1

The MEmE project is a multicentre collaborative international project which aims to fill relevant research gaps highlighted by international agencies (EFSA, ECDC, WHO) for the detection and control of cystic and alveolar echinococcosis.

The MEmE project focusses on the standardisation, harmonisation and validation of existing parasitological and molecular methods, in addition to the development and comparative assessment of innovative molecular tools and biomarkers to detect *Echinococcus multilocularis* and *Echinococcus granulosus s.l.* along the food chain. Production of epidemiological data on the presence of *Echinococcus multilocularis* and *Echinococcus granulosus s.l.* eggs in the food chain focuses on vegetables for human consumption as well as canine faeces in selected endemic countries. The MEmE project includes integrative activities to harmonise procedures and to improve detection of *Echinococcus multilocularis* and *Echinococcus granulosus s.l.*

During its first year, MEmE produced standard operating procedures (SOPs) for sampling different matrices for detection of *Echinococcus* spp. The collection of samples started in the field. Parasite sample collection from red foxes, arctic foxes, dogs, pigs, sheep and intermediate hosts is ongoing.

Furthermore, organisation for the collection of faecal samples from dogs for an epidemiological study began. Participants were sent information and collection kits for owners and veterinary practises in rural areas of highly endemic regions. Currently, three hundred and eighty faecal samples from dogs were collected in this way. Additionally, hundreds of dog faecal samples from the environment were collected as a potential matrix for validation.

The target group for dogs included in the epidemiological study are those originating from highly endemic areas (high prevalence of *E. multilocularis* in foxes as a proxy), or from regions with relatively high prevalence of *E. granulosus* in sheep. A questionnaire was compiled and sent to owners and vets, with questions concerning data which will be used in epidemiological analysis.

An additional multicentre study for the detection of *Echinococcus* eggs in fresh vegetables (lettuces) for human consumption is ongoing. Protocols for washing, filtration and molecular identification of *Echinococcus* were established and sampling will start soon.

Another core activity conducted during the first year of the MEmE project was the validation of established parasitological and novel molecular diagnostic procedures. This work aims to detect

Publications:

Bonelli, P., Dei Giudici, S., Peruzzu, A., Mura, L., Santucciu, C., Maestrale, C., Masala, G. (2021). Identification of *Echinococcus granulosus* Genotypes G1 and G3 by SNPs Genotyping Assays. *Pathogens*. 10, 125. DOI: https://doi.org/10.3390/pathogens10020125.

Skrzypek, K., Karamon, J., Samorek-Pieróg, M., Dąbrowska, J., Kochanowski, M., Sroka, J., Bilska-Zając, E., Cencek Tomasz. (2020). Comparison of Two DNA Extraction Methods and Two PCRs for Detection of *Echinococcus multilocularis* in the Stool Samples of Naturally Infected Red Foxes. *Animals*. 10, 2381. DOI: https://doi.org/10.3390/ani10122381.

Santucciu, C., Bonelli, P., Peruzzu, A., Fancellu, A., Marras, V., Carta, A., Mastrandrea, S., Bagella, G., Piseddu, T., Profili, S., Porcu, A., Masala, G. (2020). Cystic Echinococcosis: Clinical, Immunological, and Biomolecular Evaluation of Patients from Sardinia (Italy). *Pathogens*. 9(11), 907. DOI: https://doi.org/10.3390/pathogens9110907.

Santolamazza, F., Santoro, A., Possenti, A., Cacciò, S M., Casulli, A. (2020). A validated method to identify *Echinococcus granulosus sensu lato* at species level. *Infection, Genetics and Evolution*. 85, 104575. DOI: https://doi.org/10.1016/j.meegid.2020.104575.

Bonelli , P., Loi, F., Cancedda, MG., Peruzzu, A., Antuofermo, E., Pintore, E., Piseddu, T., Garippa, G., Masalam G. (2020). Bayesian Analysis of Three Methods for Diagnosis of Cystic Echinococcosis in Sheep. *Pathogens.* 9(10), 796. DOI: https://doi.org/10.3390/pathogens9100796.

Maksimov, P., Bergmann, H., Wassermann, M., Romig, T., Gottstein, B., Casulli, A., Conraths, FJ. (2020). Species Detection within the *Echinococcus granulosus sensu lato* Complex by Novel Probe-Based Real-Time PCRs. *Pathogens,* 9(10), 791. DOI: https://doi.org/10.3390/pathogens9100791.

M'rad, S., Oudni-M'rad, M., Bastid, V., Bournez, L., Mosbahi, S., Nouri, A., Babba, H., Grenouillet, F., Boué, F., Umhang, G. (2020). Microsatellite Investigations of Multiple *Echinococcus granulosus* Sensu Stricto Cysts in Single Hosts Reveal Different Patterns of Infection Events between Livestock and Humans. *Pathogens*, 9(6), pp. 444. DOI: https://doi.org/10.3390/pathogens9060444.

Casulli, A, (2020), Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis, *The Lancet*, 8 (4):PE470-E471. DOI: https://doi.org/10.1016/S2214-109X(20)30066-8.

IMAGE:FLICKR

E .multilocularis and E. *granulosus s.l.* genotype/species in different matrices along the food chain.

A key aim of the MEmE project is to generate new innovative tools for rapid detection, differential diagnosis, and tracking of infection, both at large and small-scale settings. Work has begun towards the development and validation of the following new tools: new molecular markers for *Echinococcus* species from rapid diagnostics to source attribution; new multiplex qPCR for detection and discrimination of *E. multilocularis* and *E. granulosus s.l* and *E. granulosus s.l* genotypes; sequencing using Region-Specific Extraction (RSE) and Next Generation Sequencing for the detection of *E. multilocularis* and *E. granulosus s.l* in complex samples.

IMAGE:FLICKR

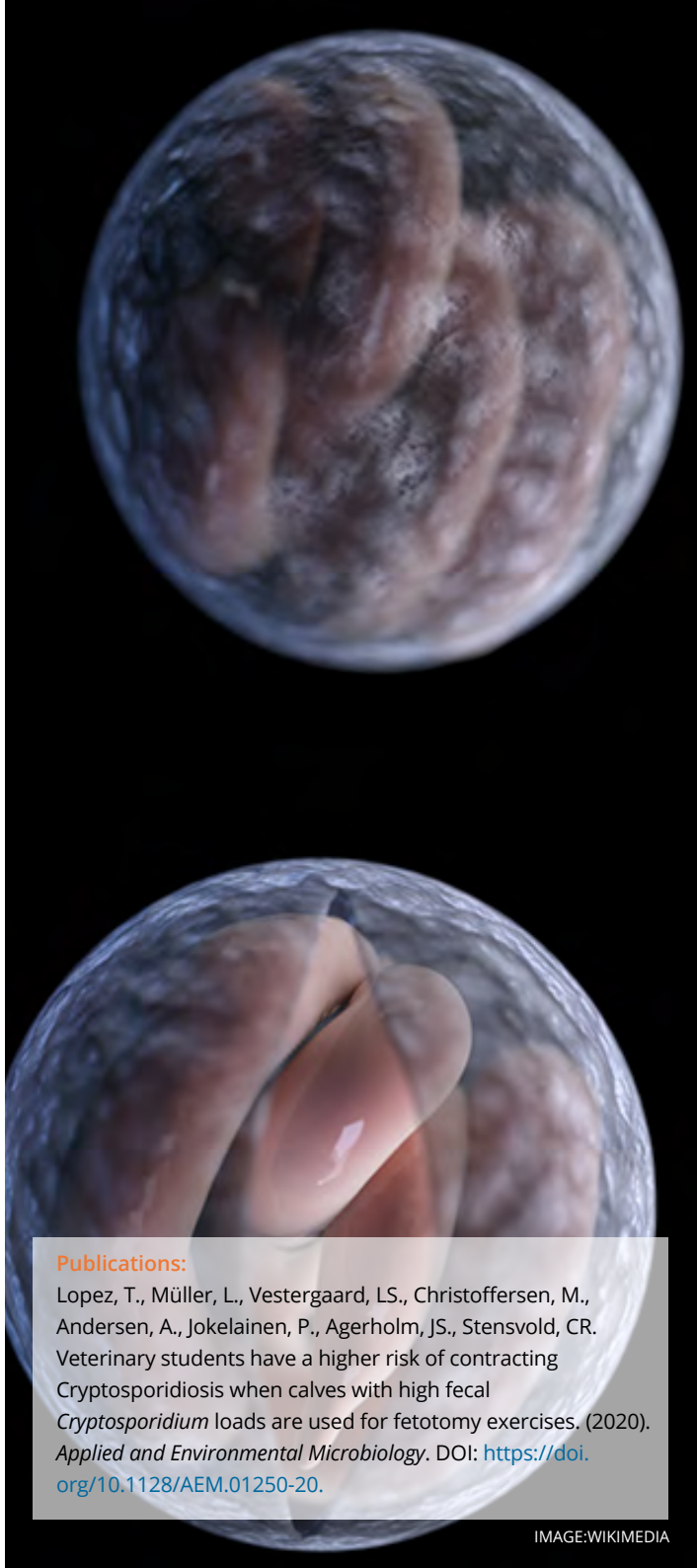
PARADISE - year 1

The PARADISE project aims to deliver informative typing schemes and innovative detection strategies applicable to food matrices for both *Cryptosporidium* and *Giardia,* two foodborne parasites that are major contributors to the global burden of gastrointestinal disease. Using Next Generation Sequencing technologies (genomics and metagenomics), the project will generate much needed data that will enrich the understanding of the epidemiology and genomics of these organisms and provide the basis on which improved strain-typing schemes will be developed and rigorously tested. In parallel, strategies to enrich for the target pathogens in different matrices will also be developed and tested.

During the first year of the project, many partners in the PARADISE project collected samples, which allowed the project to sequence 24 novel *Giardia duodenalis* and 44 novel *Cryptosporidium parvum* genomes. Based on genome wide analysis of the available genomes, a first selection of *C. parvum* and *G. duodenalis* assemblage B variable genomic regions was achieved. This resulted in the identification of genomic regions suitable for inclusion in novel typing schemes. An inventory of the parasite samples (genomic DNA, faeces, and other relevant matrices) available at each partner institute was compiled; samples will now be used to test the identified markers.

In silico metagenomics was performed to confirm the presence of parasite sequences in public metagenomes generated from various matrices. In addition, the amplicon-based sequencing approach was extensively tested using new primers for the detection of flagellates (including *Giardia*). Reference material (parasite cysts) for spiking experiments was also produced, and experiments have been planned to evaluate the limit of detection, the specificity and sensitivity, which are essential parameters to understand the applicability of amplicon-based and shotgun metagenomics as a platform for foodborne parasite detection.

As per the enrichment strategies, the work on nanobodies has progressed and potential binders have been obtained using whole cyst and oocyst antigens as prey. Two capture systems for DNA fishing were designed for *Cryptosporidium* and one for *Giardia*. High specificity and sensitivity demonstrated for *Cryptosporidium*.



Publications:

Lopez, T., Müller, L., Vestergaard, LS., Christoffersen, M., Andersen, A., Jokelainen, P., Agerholm, JS., Stensvold, CR. Veterinary students have a higher risk of contracting Cryptosporidiosis when calves with high fecal *Cryptosporidium* loads are used for fetotomy exercises. (2020). *Applied and Environmental Microbiology*. DOI: https://doi.org/10.1128/AEM.01250-20.

IMAGE:WIKIMEDIA

IDEMBRU - year 1

The IDEMBRU project aims to create a toolkit for the identification of emerging *Brucella* species and their reservoirs, this will ensure the rapid detection, identification and characterisation of *Brucella.* The project will investigate the detection and investigation of these pathogens from different sources and locations, phenotypically and genotypically characterise emerging *Brucella* species, understand the virulence and zoonotic potential of these isolates and develop a toolkit for the integration of data across Europe and a guide to characterisation of *Brucella.*

In the first year, the IDEMBRU project created a common database for all samples collected and treated before and during the project. In 2021, each partner will include their sample collection of atypical *Brucella* spp. originating from multiple animal species and environment. As further animal and environmental sampling had to be postponed due to the COVID-19 pandemic, it was decided to use the existing collections from previous projects in partner institutions, to generate initial data on which sampling strategy can be based.

A sampling and analytical strategy list of new targets, according to previous epidemiological information from the different partners was finalised. Three environments from which *Brucella* has been isolated will be targeted: forest, freshwater habitats and coastal regions; animal species to target and number of samples will be defined by each partner. An epidemiological questionnaire and associated standard operating procedures for sample collection and identification were prepared for sample collection to ensure harmonisation of approaches.

One key aim for IDEMBRU is to develop a diagnostic toolkit for emerging *Brucella* in humans. A survey will be created for different networks to ask how they would diagnose emerging *Brucella* and how they would differentiate classical from atypic species cases. To further understand infection in humans a study will be conducted to test potential antigens in humans. Testing thus far has highlighted the need to design new serological tests more adapted to atypical and emerging *Brucella* species.

IDEMBRU partner institutes have contributed to a survey describing DNA extraction protocols currently applied to relevant samples their laboratories; harmonisation and optimisation of these methods is ongoing.

Research into standardised protocols which will be used for phenotypic and genotypic characterisation of novel emerging *Brucella* spp., is ongoing and will be adapted, as necessary during the project work.

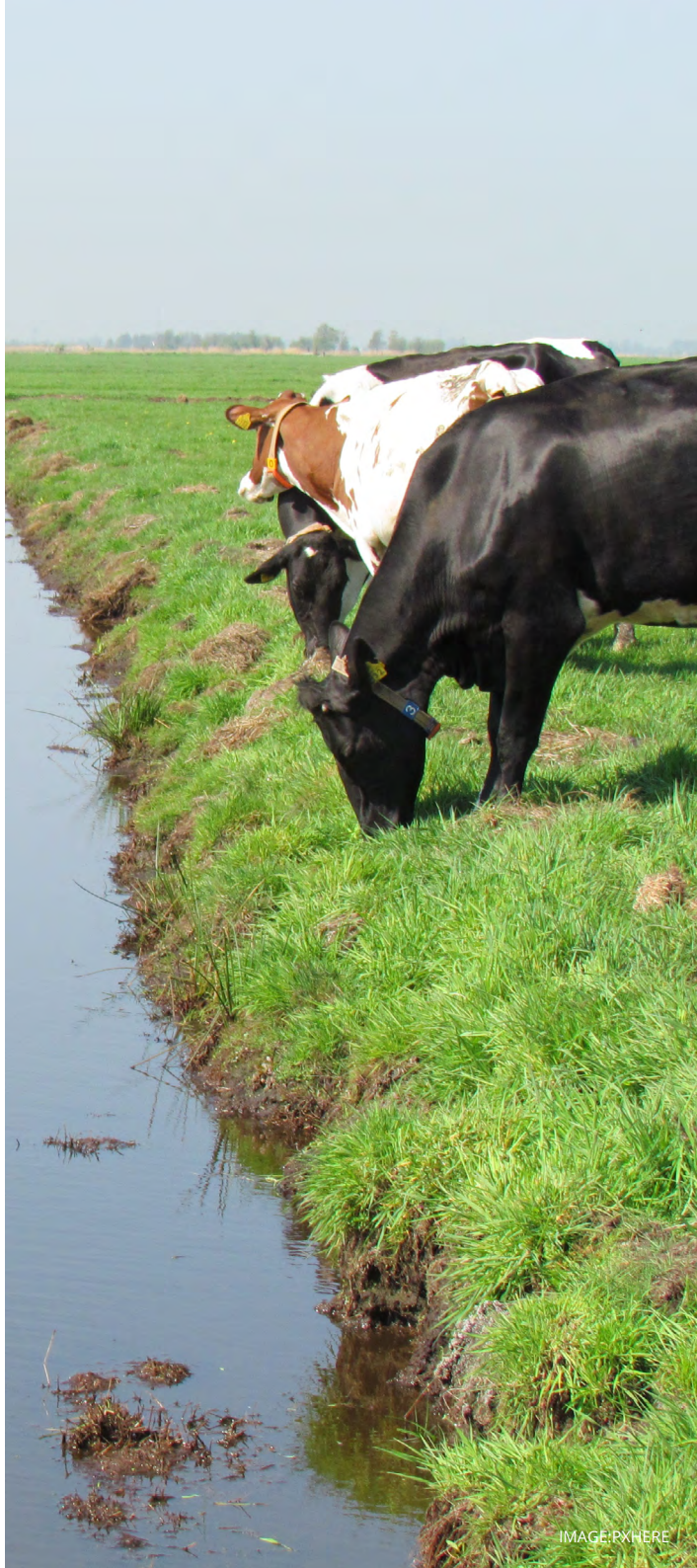


IMAGE: PXHERE

One Health EJP PhD Programme Progress

Between 2018 and 2019, 16 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 May 2020, where they presented their projects and research through presentation of their scientific poster and an oral presentation at the three-minute thesis (3MT) competition, to an audience of over 750 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.

IMAGE:PXHERE

ECO-HEN

The ECO-HEN project commenced in February 2019, and the project studies *Escherichia coli* (*E. coli*), which contributes to antimicrobial resistance (AMR) spread by clonal strains being able to survive on the food chain and by its ability for horizontal transfer of genetic platforms (such as plasmids and integrons) containing AMR genes across bacteria. 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 this animal production poses a public health risk through food and/or environment contamination.

The knowledge obtained from this PhD will reveal the extent to which the table egg production system represents a risk for spread of AMR to humans and the environment as 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 2020 significant progress was made on the reconstruction of plasmids which spread AMR genes from animal isolates to eggshell isolates. The objective of this work was 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.

Analysing relationships between AMR bacteria of laying hens and eggs was a key aim in 2020 and the research focused on the in deep analysis of the surroundings of the trimethoprim resistance gene *dfrA36*, which was identified as the gene responsible for trimethoprim resistance in some isolates of *E. coli* obtained from day-old chicks. In particular, the large plasmid harbouring this gene is under analysis.

In addition, the in deep characterisation of the isolates obtained in selective media (with cefotaxime and with ciprofloxacin, respectively) is also in progress. The aim is to check if the absence of AMR use along the rearing and production periods affects their persistence on the farm throughout the production cycle, as well as the characterisation of possible mobile genetic elements for these AMR determinants.

IMAGE PIXABAY

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 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. The following tasks for this project were successfully completed in 2020:

- Sampling and collection of bacteria
- Next-Generation Sequencing (NGS) resistance analysis of all isolates
- NGS subtyping of the strains and associated host specificity
- Investigation of the genetic organisation of the contigs carrying LIN-RES genes and incompatibility groups

From early 2019 to early 2020, 1325 faeces samples (from cattle, pigs or poultry) and 148 nasal swabs samples (from pigs) were collected in Belgium for the official monitoring of antimicrobial resistance and were analysed on blood agar supplemented with linezolid to select resistant strains (a process called selective monitoring).

In 2020, 40 nasal swab samples and 392 faecal samples collected were screened for linezolid resistant bacteria and analysed by mass spectrometry (MALDI-TOF). The 155 linezolid resistant isolates were all sequenced by whole genome sequencing (WGS), assembled and analysed. A core genome Multilocus Sequence Typing (cgMLST) analysis was conducted to study the relatedness of these isolates and compare them with published sequences of linezolid-resistant isolates. Three different resistance genes, *cfr*, *optrA* and *poxtA*, were found in this collection as well as mutations in the 23SrRNA gene conferring resistance to linezolid. The project will focus on the NGS analysis of all isolates and the investigation of putative risk factors associated with the numerous positive farms from which these isolates came from.

The next task in this project has started, which is to conduct laboratory experiments to demonstrate transferability of linezolid resistance genes and estimate transfer rates. The establishment and testing of the protocol and a first conjugation experiment was performed to assess the transferability of linezolid resistance genes.

HME-AMR

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.

There has been a substantial delay to the commencement of this project due to recruitment issues, followed by the COVID-19 pandemic. Prior to the pandemic, there were significant challenges during the recruitment process which led to two further recruitment rounds. In March 2020, a suitable candidate was identified and offered he position, but they were unable to procure a study visa for Ireland by October 2020.

The position was advertised once again, and a candidate has now accepted the position and has commenced work in February 2021. Tighter sampling schedules will be employed to address project delays to date. Furthermore, Dr Burgess and Dr Morris have spoken with Geological Survey Ireland (GSI) regarding the identification of suitable sampling sites to ensure the project sampling campaign started at the same time the student started the position and are also collaborating with a nationally funded project to procure control samples in areas of high zinc application. The project has also been highlighted in internal workshops within Teagasc to encourage cross programme linkages.

IMAGE: PIXABAY

KENTUCKY

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 (CIP^R *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 (β-lactams, aminoglycosides, sulphonamides, tetracyclines).

In addition to CIP^R, *S.* Kentucky is able to gain additional antibiotic resistance determinants through the acquisition of locally circulating plasmid-borne genes, such as ESBLs, AmpC and/or carbapenemase. Most recently, the situation has worsened, as ECDC launched an Urgent Inquiry (UI-464) on a CIP^R *S.* Kentucky ST198 strain carrying a chromosomally integrated $bla_{CTX\text{-}M\text{-}14b}$ gene encoding for cephalosporin resistance. The insertion event was traced back to Malta, but the strain has already spread to Belgium, UK, The Netherlands and five other EU countries. To date, this clone is only reported in humans, as opposed to (for example) the Cip^S *S.* Kentucky ST152 clone widely found in poultry in the USA but rarely reported in humans.

The KENTUCKY PhD project will 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.

The PhD student was recruited in January 2020. In the first year of study, the project focused on the genetic environment of the integrated $bla_{CTX\text{-}M\text{-}14b}$ gene. By hybrid sequencing, the entire genomes of four clinical *S.* Kentucky strains with this genotype were reconstituted. The ISEcp1B transposase, which is part of the IS1380 family, was detected in this region adjacent to the ESBL gene and was hypothesised to catalyse the transfer. This finding was investigated further by initiating an *in silico* database mining to correlate insertion sequences with AMR genes among clinical *S. enterica*, *Klebsiella pneumoniae* and *Escherichia coli* isolates. The results are expected by April 2021.

Meanwhile, the transfer of the resistance gene from plasmid to chromosome using *in vivo* transposition dynamics via time-lapse microscopy is being investigated. The aim is to track and quantify the (inducers of) genetic hopping using fluorescence microscopy. In 2021, the influence of serotype, species and antibiotics and other stressors on the chromosomal transfer will be explored, and therefore will be able to perform a risk assessment on this dangerous genotype.

IMAGE:PIXABAY

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 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 methyltranferases by bacteria results in complete resistance to plazomicin.

The PhD candidate was recruited in March 2020, at the start of the COVID-19 pandemic. The student is based at a lab in the Complutense University of Madrid, which became part of a network to diagnose COVID-19 in elderly homes in Madrid at the start of the PhD. The PhD student was actively involved in this activity and due to all the restrictions applied in Spain, no progress could be made in his PhD project until June 2020. In addition, the national COVID-19 restrictions added further difficulties in advancing the research between June and September 2020, specifically in relation to the task of establishing the potential sampling points in Spain.

Therefore, the plans for the PhD project were adapted, and the team decided to start the analysis with samples that were collected from previous projects that the lab was involved in. Preliminary bioinformatic analyses have been made with metagenomic sequences belonging to different pig farms across Spain and several acquired 16S rRNA methyltransferases have been detected, including some of low worldwide prevalence when using a read-based approach. The assembly-based analysis did not yield concluding results since the samples were only sequenced via Illumina (short reads). A DNA re-extraction and sequencing of the same faecal samples via both short and long-read technologies is foreseen in January 2021.

In addition, in the period of November to December 2020, contact was established with a few potential sampling points to start sampling as soon as possible in the beginning of 2021. There are still several ecological niches that were intended to be sampled missing at this stage, however it is hoped that in early 2021, the situation will allow suitable sampling spots to be found.

IMAGE:PIXABAY

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, two French One Health EJP partners, is to better understand the complex biology of *M. bovis* through the study of the complete genomes of a large panel of isolates.

The first task, culture, cloning and extraction of *M. bovis* strains of work package 1 (WP1) of this project (Establishment of reference sequences from the main French clonal groups) was completed in 2020. Ten representative *M. bovis* genotypes were selected from strains circulating in France. These were selected and cultured, which took between 3-6 months given that field *M. bovis* isolates are very slow growers. The DNA from these isolates was extracted for long-read sequencing. As long-read sequencing requires a high DNA quantity and quality, the extraction process was optimised so that appropriate concentrations of high-quality DNA were obtained. DNA genotypes were sent to for quality checking and DNA purification protocol validation. This methodology has been validated for the first strains tested, and the quality control of the remaining DNA samples is underway.

Once the DNA quality control is completed the following tasks will begin: the second task which is sequencing and *de novo* assembly, and the first task, sequencing of supplementary strains, will start.

The progress of the second task, Genomic markers analysis, is ahead of schedule. Genomic analyses on previously available genomes were performed. The first steps in bioinformatics focused on the presence and distribution of an insertion sequence, IS*6110*, in the genomes of *M. bovis.* IS*6110* is a very useful genetic marker of the *Mycobacterium tuberculosis* complex employed for TB direct detection by PCR and for genotyping. The bioinformatics scripts were adapted to find the number and localisation of IS*6110* in the bacterial genomes using contigs from reads of Illumina sequencing. A wide diversity in the copy number and the localisation of this sequence in the different genetic families of *M. bovis* in France was highlighted. These multicopy strains were grouped on specific nodes. These genotypes are mostly found in French bTB endemic regions and another study shows the strong stability of IS6110 number and genomic location in these groups over time. At present, *in silico* analyses is being performed to determine if these insertions can lead to phenotypic behaviours, which could explain a better fitness or transmissibility and thus a potential epidemiological success.

IMAGE:PIXABAY

MACE

Cystic echinococcosis is a zoonotic parasite disease of significant public health concern in many parts of the world, with over 5000 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).

In January 2020, the PhD student was recruited, and work commenced. The following tasks were progressed in this project:

- An elicitation questionnaire was developed to capture the attitude to investment into disease surveillance sensitivity by stakeholders. This is in planning to be conducted in a representative sample audience of stakeholders.
- A spatio-temporal model was developed to estimate the risk and prevalence of cystic echinococcosis in dogs across all regions of Uruguay (excluding Montevideo). This framework will be extended and adapted to support current planning of a screening programme across the country.
- A review of cystic echinococcosis surveillance and control methods, as well as historical mathematical modelling approaches used, has been drafted in preparation for a report due 15 months after the start of the PhD (Confirmation report).

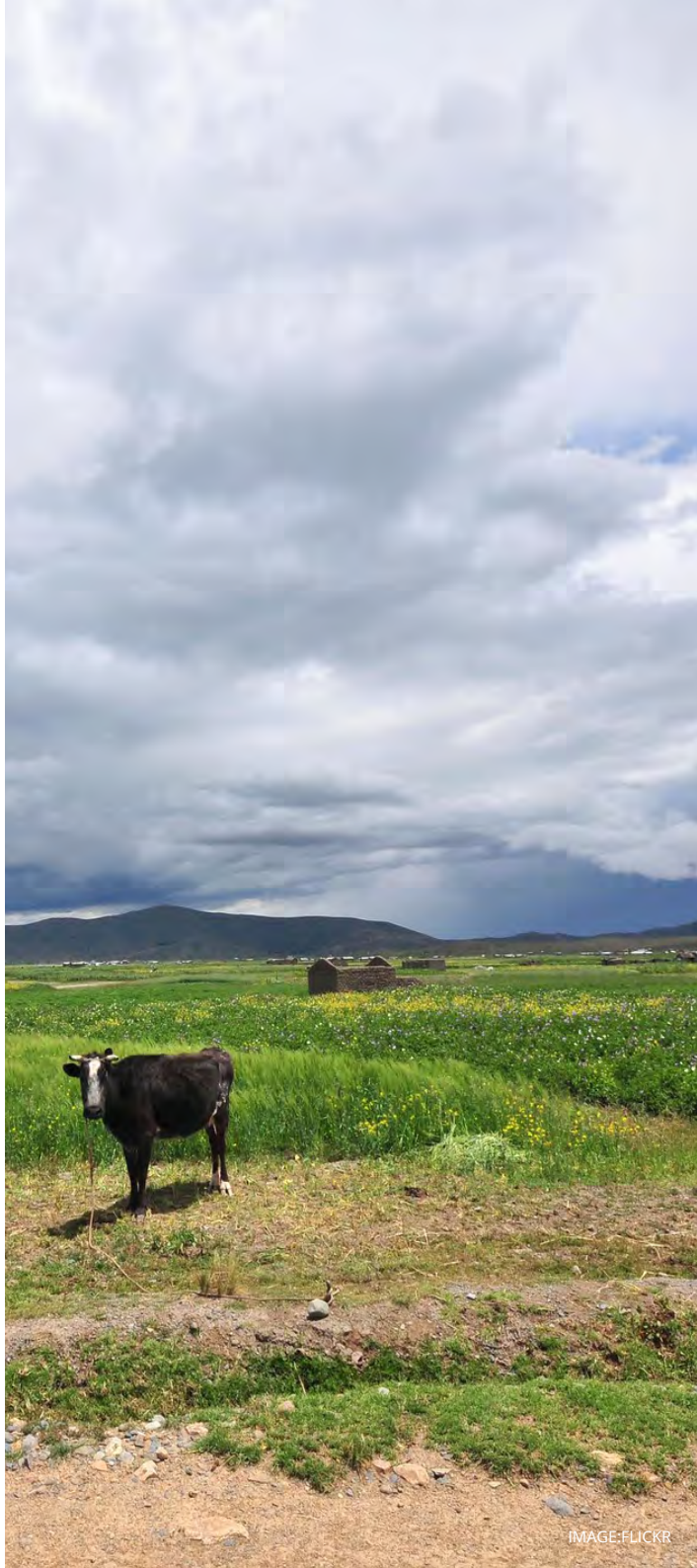


IMAGE:FLICKR

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.

The focus for the PhD candidate in the first year was on the field study, comprising of several tasks. The work started with interviews of various stakeholders, including municipal employees who oversee urban ecology and/or pest management. This resulted in a better problem definition and helped the design of the field study. The field study commenced in May and ran till October 2020, leaving little time for other activities. It comprised of two cities, one of which, unfortunately did not result in many captured rats. Therefore, to ensure enough power to perform the proposed analyses, another city will be added to the project in 2021. After collection of the samples the first set of diagnostic analyses were performed for zoonotic pathogens. However, this was disrupted shortly afterwards due to lab restrictions at the RIVM due to COVID-19.

Furthermore, the PhD candidate has started on the second task, which is Next-Generation Sequencing analysis of the bacterial pathobiome, using rat samples from the biobank. It was decided to extend this with the analysis of the viral pathobiome, which will be done in the first half of 2021.

IMAGE:FLICKR

UDoFRIC

Since 2005, *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 *Campylobacter* isolates has reported increasingly high levels of ciprofloxacin resistance. Ciprofloxacin is an antibiotic of the fluroquinolone class and has been declared as a major public health concern by the World Health Organisation. *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 *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 fluroquinolones in poultry remains an option.

The UDoFRIC project aims to exploit the archives of *Campylobacter* and associated information from surveillance and research across the food-chain to investigate temporal trends in the development and diversity of fluroquinolone resistance in UK and French broiler flocks. The project will examine the relationship between fluroquinolone use in poultry and development of resistance, assess fitness benefits/costs of acquired resistance and determine if any specific fluroquinolone 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 fluroquinolone in poultry and consequent risks of fluroquinolone resistance in clinical cases.

The PhD student was recruited in March 2020 and has been making progress in the project's objectives. There has been advancement in the writing of a literature review focusing on the background and previous research conducted on fluroquinolone resistance in *Campylobacter*.

Data analysis has been carried out on a subset of data available to this project, obtained by previous UK national research and surveillance studies in poultry. The project has access to six distinct datasets over a 24-year period. *Campylobacter* samples were obtained by sampling either caecal contents of broilers at slaughter or from broiler carcasses after processing.

There has been progress with analysing the trends of fluroquinolone resistance over time and production factors associated with fluroquinolone resistance (e.g. bird age, farming method, bird weight). Work has also been conducted identifying *Campylobacter* lineages and aims to determine if there is a link between MLST or whole genome sequence information and fluroquinolone resistance.

IMAGE:FLICKR

A collection of historic isolates and information relating to them has also been collated and used to identify gaps in the data collected.

To create uniformity in the information available in each dataset for comparison, work has begun on historic isolates. These historic isolates have been recovered from the *Campylobacter* national reference laboratory archives, their species classification has been confirmed and the DNA of these isolates has been extracted and sequenced ready for further analysis.

IMAGE:FLICKR

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. Although, initial plans were made for desk, laboratory and farm-based work to be performed in the first year of the PhD, due to the COVID-19 pandemic, only desk and lab-based tasks have commenced.

In 2020, a literature review was undertaken on the role of wild birds in spread and persistence of 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 environment in persistence and dissemination of AMR; the role of AMR surveillance; and evaluation of different methodologies for identifying AMR by phenotype and genotype, in bacteria.

Some historical *E. coli* isolated from gull faeces, collected from an outdoor pig farm, during a longitudinal study in the One Health EJP JRP project ARDIG are being utilised in this PhD project because of the delays to farm based work caused by COVID-19. Over 200 previously unused isolates have undergone whole genome sequencing, and downstream bioinformatics is currently taking place. An outdoor pig farm, known to have wild birds persistently present on farm, had been recruited for a longitudinal

IMAGE:WIKIMEDIA

study, but due to COVID-19 restrictions we were unable to undertake any farm visit to collect environmental samples, faecal samples from pigs, and caecal samples from corvids present on farm. Although this farm is now unlikely to be sampled during the course this project, an opportunity to examine the spread of AMR in wild bird populations across the UK for a 12-month period through the APHA's wild bird scanning surveillance programme is currently being pursued.

IMAGE:WIKIMEDIA

EnvDis

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.

The literature review carried out by the PhD student at the start of 2020 showed that salmonellosis continues to be the second most reported foodborne disease in humans in Europe, with a seasonal pattern of incidence focused on the warmest weeks of the year. Amongst the food sources of infection, eggs and chicken meat were identified as relevant sources of contamination.

As a first step in the project, an equation was developed linking the growth of *Salmonella* on eggs and chicken meat to temperature. Assuming that the probability for a human getting infected is proportional to the temperature-dependent number of bacteria, a simplistic predictive model was created using the high-resolution meteorological records for the past 20 years provided by the Met Office. To validate the model, the predictions were compared to the incidence of salmonellosis in England and Wales provided by PHE for the same period. Both curves have the same pattern albeit some minor differences, suggesting that the three parameters considered (eggs, chicken and temperature) play a big role on the seasonality of salmonellosis.

For this work, different exploratory charts were created to find further trends and causality, such as a geographical effect by comparing different areas of England and Wales, to hypothesise different contribution in the risk factor associated with eggs versus chicken, and to create a model to investigate the effect of an average increasing of temperatures in the incidence of the disease.

IMAGE:PIXABAY

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.

In January to December 2020, two protocols for *Trichinella spiralis*-specific aptamers selection were planned. The first one is against whole muscle larvae, the infective stage of this nematode. The second one is against protein(s) considered as potential biomarker(s) from the excretory or secretory whole muscle larvae products. The PhD student was trained at McGill institute for the Systematic Evolution of Ligands by Enrichment methods (SELEX) methods. For both protocols, much work has been accomplished in producing single-stranded DNA sequences from double-stranded DNA PCR products, an essential step in the successful isolation of target specific aptamers. Furthermore, the protocols to adapt the SELEX method to *T. spiralis* whole muscle larvae have been established and optimised at ANSES.

IMAGE:FLICKR

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. By screening these samples for the presence of ESBL *E. coli*, the significance of the reduced diversity of early colonised chickens will be determined. The *in vitro* chicken gut model has been set up to test strategies for the reduction of ESBL *E. coli* and compare these with published data from *in vivo* studies. When the model can efficiently reproduce these *in vivo* studies, it can be used for further study of new ESBL *E. coli* colonisation prevention strategies.

A dataset was generated of the microbiome of broiler chickens on a single farm which complements a preliminary dataset from the same flock to generate additional statistical power for the analysis. The datasets were merged and analysed. The outcome of the analysis is currently prepared in a manuscript for publication. Preparations to start sampling new broiler chicken farms in the Netherlands will commence shortly, and additional farms will be visited in 2021 to collect data from broiler flocks (if it is possible during the COVID-19 pandemic). This research aims to confirm if the differences in microbiome composition that were measured in the first dataset can be found in additional flocks.

Setup of an *in vitro* model of the broiler caecum was designed and completed using a continuous culture fermenter system. The model was seeded with caecal content from broiler chickens to create a stable microbial community. This model will be used to test the effects of feed interventions and to study the effect of substances on the transfer of AMR encoding plasmids between bacteria in the established microbial community. Initial runs have been performed to test the effects of two phytochemicals for which microbiome analysis is currently carried out.

IMAGE:FLICKR

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 key activities, results, and achievements in 2020 are the following:

1. Successful experimental infection of pigs with oocysts and tissue cysts.
2. Successful collection of meat samples.
3. Complete artificial digestion of meat samples from "oocysts" pig group.
4. Manufacturing of 168 dry sausages and their analysis by bioassays.
5. Manufacturing of long-salting dry ham and the analysis by bioassays.

IMAGE:FLICKR

TRACE

Hepatitis E virus is a zoonotic virus responsible of acute hepatitis E in human 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 in the Netherlands, a temporal drop was observed in acute hepatitis E virus 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 virus variants and attempt to identify 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) in order to gain more understanding on its molecular epidemiology and possible variations in its adaptive traits.

The PhD student was recruited in January 2020, and the timing of the COVID-19 crisis did impact the progress of the PhD. However, some progress was made on establishing optimisation of sample (pre) processing to make whole genome sequencing (WGS) possible. Different methods were used, and DNA depletion treatments were explored. The messenger RNA of 18S (host) and 16S (bacterial) origin was successfully removed. The hepatitis E virus whole genome sequences were successfully generated using above enrichment methods and specific primers. Work is currently ongoing to analyse the sensitivity. 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 BIOPIGEE project).

IMAGE:HIPPO PX

Codes4strains

Whole genome sequencing allows the tracking of pathogenic strains and informs infection control, diagnostics 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. The two important pathogens *Klebsiella pneumoniae* and *E.coli* are being used to develop and evaluate our approach.

In 2020, the Codes4strains project developed procedures (bioinformatics and algorithmic) to for a cgMLST-based LIN code system. cgMLST profiles from genomes were generated using defined schemes, resulting in the cgMLST schemes being finalised. For *K. pneumoniae*, 629 gene loci were defined for the cgMLST scheme from a previously published data. *For E. coli*, the cgMLST scheme from EnteroBase was utilised, which included 2513 gene loci.

As a second task, the development of the cgMLST-based LIN code algorithm was defined and a bioinformatics implementation was developed in Python. A set of bioinformatics tools and metrics were used to define thresholds, considering the phylogenetic structure of the species, and with the view to maximise its usefulness in population biology and epidemiology. For *K. pneumoniae,* a large dataset of 7060 high-quality genomes was used to define the overall structure and diversity of the population and to finalise a cgLINcode system comprising 11 thresholds. For *E.coli*, a genome dataset that was previously published by Public Health England (PHE) was utilised. This allowed a database of cgLINcodes aligned with other approaches to be created. Finally, a comparison between the different classification systems (HierCC, cgLINcodes and SNP address) was carried out for *E.coli*. For *K. pneumoniae*, only cgLINcodes and multi-level Single Linkage clustering (equivalent to HierCC) were compared, as the SNP database required for the SNPaddress approach remains to be constructed.

Publications:

Hennart, M., Panunzi, LG., Rodrigues, C., Gaday, Q., Baines, SL., Barros-Pinkelnig, M., Carmi-Leroy, A., Dazas, M., Wehenkel, AM., Didelot, X., Toubiana, J., Badell, E., Brisse, S. (2020). Population genomics and antimicrobial resistance in *Corynebacterium diphtheriae. Genome Medicine*. 12, p 1-18. DOI: https://doi.org/10.1186/s13073-020-00805-7.

SUSTAIN

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 first step of this project was a literature search to inform subsequent studies. Hereafter, quantitative studies of databases and of a survey addressed to institutions working on One Health topics will follow. A qualitative study will be conducted which will include interviews and observations. The studies will inform different aspects, such as the current state of One Health institutionalisation in the EU as well as on a national level, and the One Health networks' interactions and relationships.

In 2020, data gathering has started as planned for the PhD project. This included conducting interviews with experts from National Public Health, Veterinary, Food and Environment agencies in Sweden. Initially, it was planned to travel to Sweden to conduct the interviews in person. Due to the COVID-19 pandemic, the interviews were conducted online via videoconferencing. A one month trip to the Istituto Superiore di Sanità (ISS) in Italy was completed in autumn 2020, during this trip the PhD student was integrated into the everyday work of the institute and was able to conduct interviews with experts working in different departments (public health, veterinary health, environment, nutrition, and food) at ISS.

Most of the data from interviews with experts have been gathered, but a few interviews are left to be conducted with Italian experts, especially those working in the veterinary (Istituto Zooprofilattico Sperimentale) and environmental institutes (Istituto Superiore per la Protezione e la Ricerca Ambientale). Data from the interviews in Sweden was analysed in NVivo and a first-authored article was prepared for publication, submitted, and accepted in December 2020. The data analysis from the Italian interviews began in November 2020 and will be ongoing in 2021. The process will be completed when all interviews have been conducted and analysed. Thereafter, a publication will be prepared.

Additionally, preparation of a survey to address ministries, EU institutions and high-level members of staff in public health, veterinary health, environment and food agencies, has started and is planned for dissemination in spring 2021. This survey will aim to collect data about the understanding of One Health and how One Health is put into practice.

Publications:

Sarah Humboldt-Dachroeden, S., Olivier, R., Frid-Nielsen, SS. (2020). The state of One Health research across disciplines and sectors – a bibliometric analysis. *One Health,* 10, pp. 100146. DOI: 10.1016/j.onehlt.2020.100146.

IMAGE:LIBRESHOT

One Health EJP Dissemination Activities

IMAGE:PIXABAY

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 2020:

- 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.
- Two targeted reports were created to ensure that key EU Stakeholders are informed of the key scientific and integrative outcomes from the OHEJP.
- To highlight the responsiveness and timeliness of the OHEJP, two additional reports were created including "Links between COVID-19 related needs of stakeholders and One Health EJP activities" and "One Health EJP Thematic Report on AMR".
- The OHEJP Outcome Inventory was updated and acts as a repository for all OHEJP outcomes and is a hub for information for both national and international stakeholders and both internal and external audiences.
- The Data Management Plan was further developed, and an abundance of support has been provided to the Project Leaders of OHEJP projects to ensure that data generated in their projects are FAIR (findable, accessible, interoperable and reusable).
- Case studies for several OHEJP project were created and disseminated to a global audience to raise awareness of results and their impact.
- 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 activities, in addition to the OHEJP 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.

IMAGE:PIXABAY

- 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 and maximising impact.
- Close relationships were established with other EU projects such as EU- JAMRAI, JPIAMR and AVANT with the aim of exchanging scientific and technical expertise in the scientific community.
- Organisation of Education and Training activities to aid in training the next generation of One Health researchers.
- A Dissemination Information Pack was created to support consortium members with dissemination of their outcomes.
- Attending global conferences such as the World One Health Congress in November 2020 extended the reach to global audiences and provided a platform to demonstrate the impact that the OHEJP has in One Health research.

IMAGE:PIXABAY

The second One Health EJP Annual Scientific Meeting

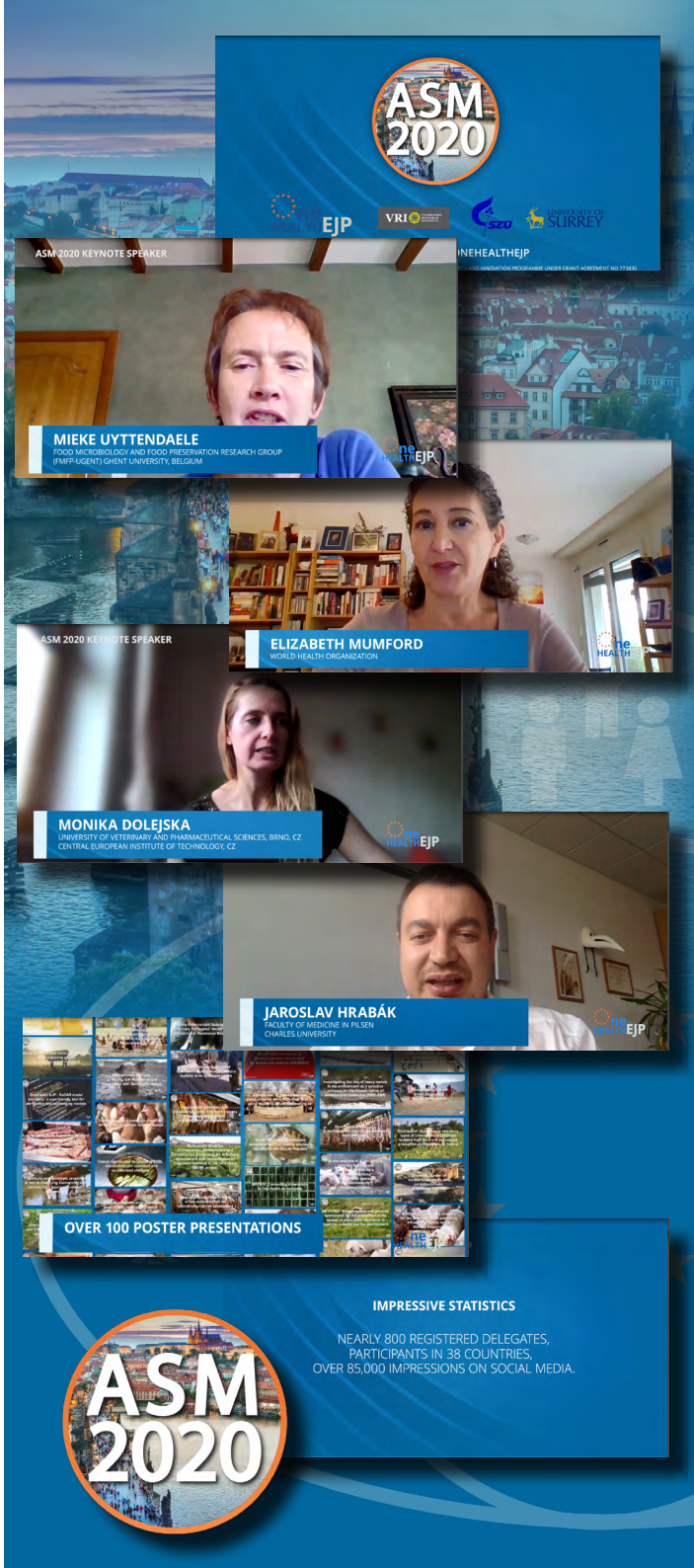
Organising institutes: the National Institute of Public Health and the Veterinary Research Institute, (Czech Republic) in collaboration with University of Surrey (UK)
Location: Online
Dates: 27th-29th May 2020

Streamed live to over 750 participants worldwide, the second One Health EJP Annual Scientific Meeting was a huge success. The event showcased a wide variety of One Health research with 5 Keynote speakers, 48 oral presentations and 110 poster presentations, focussing on the themes of foodborne zoonoses, antimicrobial resistance and emerging threats.

The second One Health EJP Annual Scientific Meeting included the first 3 Minute Thesis competition of the One Health EJP PhD students. This event gave each of the 17 PhD students the opportunity to showcase their work to a global audience.

The Keynote speakers brought a wealth of experience from across the globe and included Stef Bronzwaer from EFSA, Jaroslav Hrabák from University Hospital Pilsen, Czech Republic, Monika Dolejská from VFU Brno, Czech Republic, Mieke Uyttendaele from Ghent University, Belgium and Elizabeth Mumford from the WHO.

The event also included a significant social media presence with excellent interaction with stakeholders and scientists worldwide.



Educating the next generation of One Health researchers

IMAGE:PXHERE

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 in these health fields, 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 2020, as global travel was significantly impacted by the COVID-19 pandemic, WP6 pivoted to deliver our training events online until safe travel resumes to providing unique opportunities for training in One Health and encouraging collaboration and exchange of knowledge and experiences amongst scientists across the globe.

ONE HEALTH EJP SUMMER SCHOOL 2020

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: Wageningen Bioveterinary Research (the Netherlands) in collaboration with Wageningen Institute of Animal Sciences, Netherlands Centre for One Health, Agreenium (France), INRA (France,) and University of Surrey (UK).
Theme: Global One Health- From Research to Practice
Location: Online
Dates: 17th-28th August 2020

Collaborative interactions: The programme was delivered by leading EU and international experts in public health, animal health and environmental health who face today's challenges to implement One Health strategies in the different health fields. The collaborative interactions between these experts and 35 delegates from 22 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, Germany, France, Spain, and Romania, but also from countries beyond the EU such as Ghana, India, Ukraine, Mexico, USA, China, Canada and many more. This diversity significantly enriched the experience of all those that participated and added value to the course.

Delegates and lecturers had the opportunity to network and socialise with each other at the virtual social events arranged on the first and last day of the summer school.

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 veterinary medicine, human medicine, biological sciences, infectious diseases, disease surveillance, microbiology, mathematical modelling, economics, social sciences, sustainability sciences, public health, and virology.

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 learn how to operationalise a global One Health approach to improve the health of people, animals and plants within a sustainable ecosystem. The programme delivered an introduction to global One Health basics, prediction approaches, analyses of integrated disease surveillance, outcomes research, risk management, and decision quality. Delegates had opportunities to present their own experiences and ideas in the global One Health field which were subsequently discussed with the key experts delivering the course.

To read more about this event, 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 you gain as you work. CPD includes formal or informal learning beyond any initial qualification or training you have undertaken. The OHEJP's CPD modules cover several themes in One Heath 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: National Institute of Public Health and the Environment, RIVM (the Netherlands)
Theme: Outbreak Preparedness
Location: Online
Dates: 16th and 17th November 2020

Collaborative interactions: The module consisted of sessions delivered by leading European experts in the fields of national outbreak preparedness, risk analysis, risk communication and response in the One Health domains of human health, animal health and environmental health. The module provided an opportunity to bring experts and 20 delegates from our consortium partner institutes, stakeholders and OHEJP alumni together to share knowledge and experiences from countries such as France, the Netherlands, Italy, Norway, Switzerland, Sweden, Poland, UK, Germany and more. 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 PhD students and Early Career Researchers from across our consortium partner network, each with some experience in a related health field. Participants from our stakeholders EFSA, also participated. Bringing together people from across the health disciplines made this module truly cross- disciplinary and highlighted the advantages of a One Health approach. The delegate's educational backgrounds were diverse across the One Health fields and included biological sciences, veterinary medicine, social sciences, and public health, which helped to facilitate the sharing of One Health knowledge and expertise.

Programme: The module delivered a platform to share experiences, knowledge and lessons learned from past outbreaks. Outbreaks involving new or re-emerging zoonoses can occur anytime and everywhere, something that the entire world has experienced with the COVID-19 pandemic. Strengthening the human-veterinary collaboration is essential to prevent, detect and respond to zoonotic threats. Yet, implementation and operationalisation of the One Health concept often remains

a challenge. In addition, countries differ globally in their approaches to One Health issues. Sharing best practices and experiences of One Health approaches stood at the centre of this module, providing a learning platform for knowledge integration, and strengthening of One Health collaboration across Europe. The outbreak preparedness module consisted of lectures and interactive working group sessions, and a simulation exercise where participants were able to learn several technical skills.

To read more about this event, view the programme and blog post on our website click here.
View the full report here.

ONE HEALTH EJP COMMUNICATION AND MEDIA WORKSHOP

Communication of science is one of the most challenging skills to acquire and yet is crucial to realising the impact of research. A comprehensive communication and media workshop was organised in 2020, to develop the communication skills of the young researchers to equip them to drive the research forward and generating maximum impact for their target audiences.

Organising institutes: Bulgarian Food Safety Agency, BFSA (Bulgaria) in collaboration with University of Surrey (UK).
Theme: How science achievements reach people and contribute to a better life
Location: Online
Dates: 5th and 6th October 2020

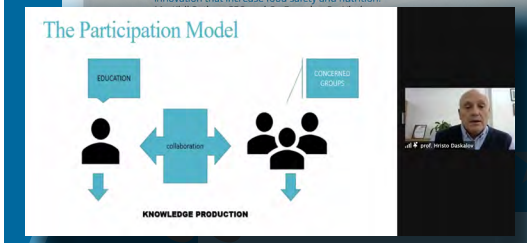
Collaborative interactions: The training programme was delivered by communication experts in public health with a specific focus on food safety and risk assessment, the OHEJP Communications Team, and included training by a former TV News broadcaster, presenter and reporter who is an expert on media performance and media interviews. This programme brought together the experiences of our key experts and 35 delegates from 18 countries across the globe on the challenges of communicating scientific research to defined target audiences, and an assessment of the risk communication practices and roles of public and private media in this process in different countries.

Delegates attended from across the globe, including Sweden, France, Spain, the UK, Nigeria, India, Greece, USA, Nepal. In addition, our stakeholders, World Organisation for Animal Health (OIE) also participated in this event. This diversity in experience and background significantly enhanced the experience of all those that participated.

Some delegates from this workshop also attended other OHEJP training events, which further contributes to building a future consortium to support the sustainability of the OHEJP.

Delegates: Delegates represented various career stages, including bachelor's students, master's students, PhD students, early career post-doctoral researchers and Doctor of Human (MD) and Veterinary medicine (DVM). They also represented all pillars of One Health: veterinary medicine, human medicine, biological sciences, environmental sciences, pharmaceutical sciences, public health, infectious diseases, microbiology, mathematics, social sciences, economics, and communications. Bringing together people from across disciplines made this workshop truly cross-disciplinary and highlighted the advantages of a One Health approach.

"I really enjoy the breakout workshop where we have learnt from our experiences and created a presentation that reflects our different contexts."
Gerome Sambou, Ministry of livestock and animal production of Senagal, Senegal



A really well organized, info
and interactive workshop
very well and friendly supe
and motivated for my futur
Jennie Fischer, BfR, Germ

Programme: The comprehensive workshop programme provided delegates with the opportunity to learn from experts about risk communication, EU communication campaigns, and the coordination of communication activities and strategies related to public health and food safety risks. Delegates also learned techniques and tools to communicate their research more effectively through the creation of a communication strategy, brand awareness and the use of social media. To ensure the workshop was interactive despite being online, delegates participated in group-work presentation exercises, and had the opportunity to attend and participate in exemplary 'live' public media mock interviews on their topical research area, teaching them how to answer the questions of journalists and how to deliver their key messages effectively.

To read more about this event, view the programme and blog post on our website click here.
View the full report here.

ONE HEALTH EJP FUNDED 5 SHORT TERM MISSIONS IN 2020

The onset of the pandemic affected the success of these missions considerably due to the disruption to travel plans. Two of five STMs took place before travel was disrupted in March 2020. Of the remaining three STMs, although one mission was cancelled, the remaining two missions and training have been postponed until when safe travel resumes.

STM 1: Study of the interactions between STEC and human gut microbiota in the ARtificial COLon (ARCOL) model
Theme: Skills Development Missions
Home Institute: Istituto Superiore di Sanità (ISS), Italy
Mission Hosting Institute: Université d'Auvergne Clermont, France
Duration of mission: 5 weeks

Background and Aim of mission: Shiga toxin-producing *E. coli* (STEC) are zoonotic pathogens, that may cause severe symptoms in humans, including uncomplicated diarrhoea, haemorrhagic colitis, up to the life-threatening haemolytic uraemic syndrome. The different clinical demonstrations appear to be linked to several factors, counting the age of the patient, the host immune status and the interaction of the infecting strains with the intestinal microbiota. Indeed, bacterial species inhabiting the human intestine can interfere with STEC in colonising the gastrointestinal tract. On the other hand, commensal bacteria may influence the severity of disease and amplify the production and release of the Shiga toxin by offering a susceptible bacterial population for the Stx-phage amplification.

The aim of this study was to investigate the interactions between STEC and human intestinal microbiota, using the ARtificial COLon model (ARCOL), which simulates the human large intestine functionality. In detail, the aim of the STM was to perform *in vitro* infection experiments with STEC in presence of a normal human intestinal microbiota using this innovative model to understand the role played by the human intestinal microflora upon STEC infection, and to investigate changes occurring in the gut microbiota composition in presence of infecting STEC strains.

For further details, read the full report here.

STM 2: Cross-domain and cross-country collaboration to develop multivariate syndromic surveillance
Theme: Skills Development Missions
Home Institute: Swedish National Veterinary Institute (SVA), Sweden
Mission Hosting Institute: Norwegian Institute of Public Health (NIPH), Norway
Duration of mission: 2 weeks

Aim and background of mission: The aim of the STM was collaboration and knowledge exchange between Norway and Sweden in the development of One Health systems for multivariate syndromic surveillance of veterinary and public health data combined. Two separate systems are in development in Sweden at the SVA and Norway at the NIPH. The Swedish system is explanatory, aiming to combine several data sources to explain outbreaks and improve the accuracy of detection. Norway, on the other hand, is developing a predictive system, which aims to use some source(s) of data to predict the outcome of others. As a test case, both systems are being evaluated on the surveillance and outbreak detection of *Campylobacter* in humans, using data from public health, broiler chicken and weather.

For further details, read the full report here.

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