



IMAGE:WIKIMEDIA

# Annual Report 2022



This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 773830





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



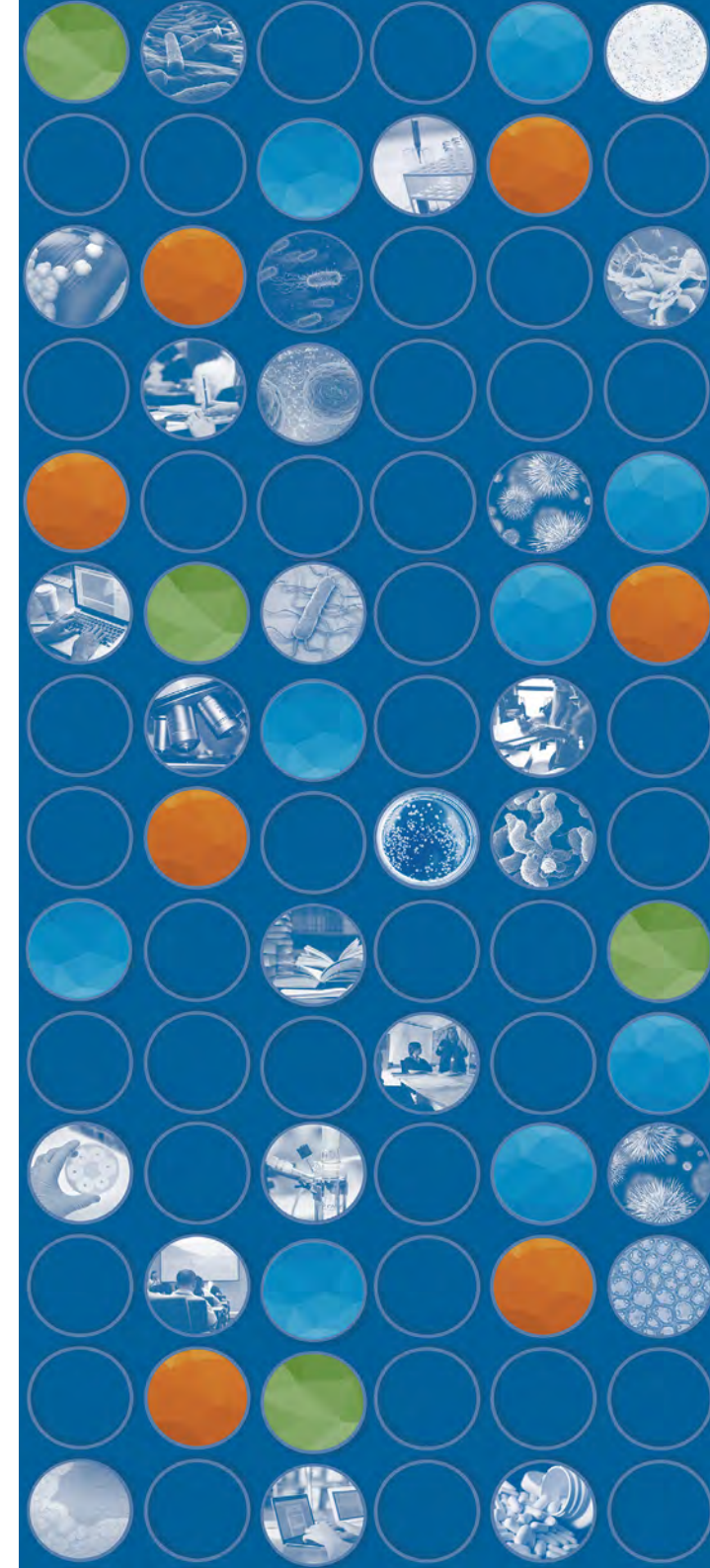
DISSEMINATION



EDUCATION

## CONTENTS

<b>Introduction</b>	<b>3</b>	ECO-HEN	49
<b>Vision</b>	<b>4</b>	LIN-RES	50
Our Objectives	4	HME-AMR	51
One Health EJP Governance	5	KENTUCKY	52
<b>Structure</b>	<b>6</b>	METAPRO	53
One Health EJP Structure	6	PEMbo	54
<b>Achievements</b>	<b>7</b>	MACE	55
Fifth Year Achievements	7	DESIRE	57
Coordination of the One Health EJP	8	UDoFRIC	58
Communicating Success	9	WILBR	59
Translation of Science to Policy	10	EnvDis	60
One Health EJP Scientific Outcomes	11	AptaTrich	61
CARE	14	VIMOGUT	62
OH-HARMONY-CAP	16	ToxSauQMRA	63
MATRIX	18	TRACE	65
COVRIN	20	Codes4strains	66
DISCOVER	22	SUSTAIN	67
BIOPIGEE	24	<b>Dissemination</b>	69
TOXOSOURCES	25	OHEJP Dissemination Activities	69
ADONIS	26	<b>Education</b>	75
BeONE	28	OHEJP Education and Training	75
FARMED	29		
FULL-FORCE	31		
WORLDCOM	32		
FED-AMR	34		
TELE-VIR	36		
MEmE	38		
PARADISE	39		
IDEMBRU	41		
OHEJP SIMEX PROJECT	43		
Publications 2021 - Ended Projects	44		
One Health EJP PhD Programme	48		





#### INTRODUCTION

## WHAT IS THE ONE HEALTH EJP?

The One Health European Joint Programme (OHEJP) is a landmark partnership between 43 public health, animal health and food organisations and the Med-Vet-Net Association, a European Network of Excellence for Zoonoses research. The OHEJP was launched in January 2018 and spans 22 countries across Europe.



#### VISION

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



#### STRUCTURE

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. These will improve the quality and compatibility of shared information for policy decision making.



#### ACHIEVEMENTS

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



#### OUTCOMES



#### DISSEMINATION



#### EDUCATION





## INTRODUCTION

# OUR OBJECTIVES

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

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



## VISION



## STRUCTURE



## ACHIEVEMENTS



## OUTCOMES



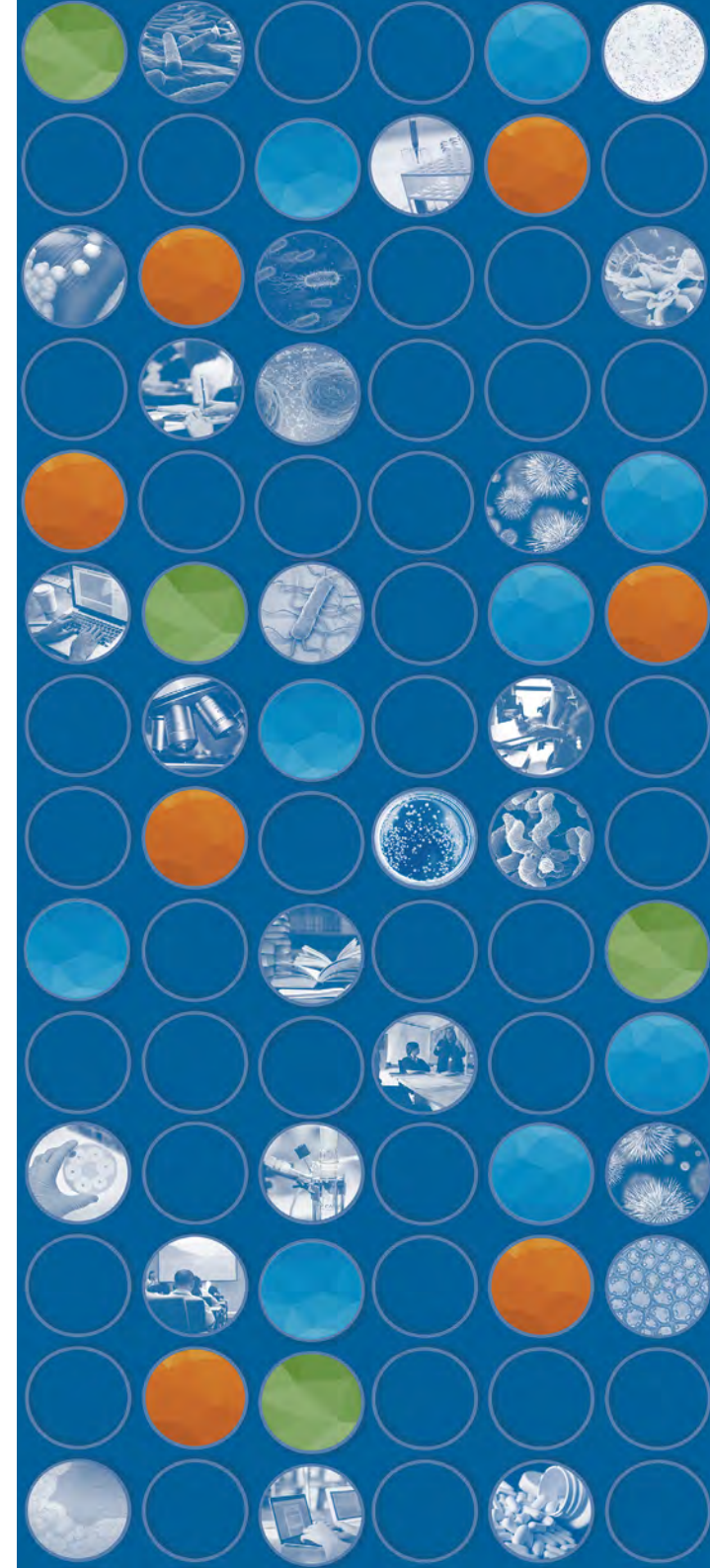
## DISSEMINATION



## EDUCATION

### 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 \(EFSA\)](#).
- To promote and develop One Health research in the European Unions by training, education and communication both nationally and internationally.





INTRODUCTION

## ONE HEALTH EJP GOVERNANCE

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

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

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

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

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

The Project Management Team consists of all the Work Package (WP) Leaders and Deputy Leaders.



VISION



STRUCTURE



ACHIEVEMENTS



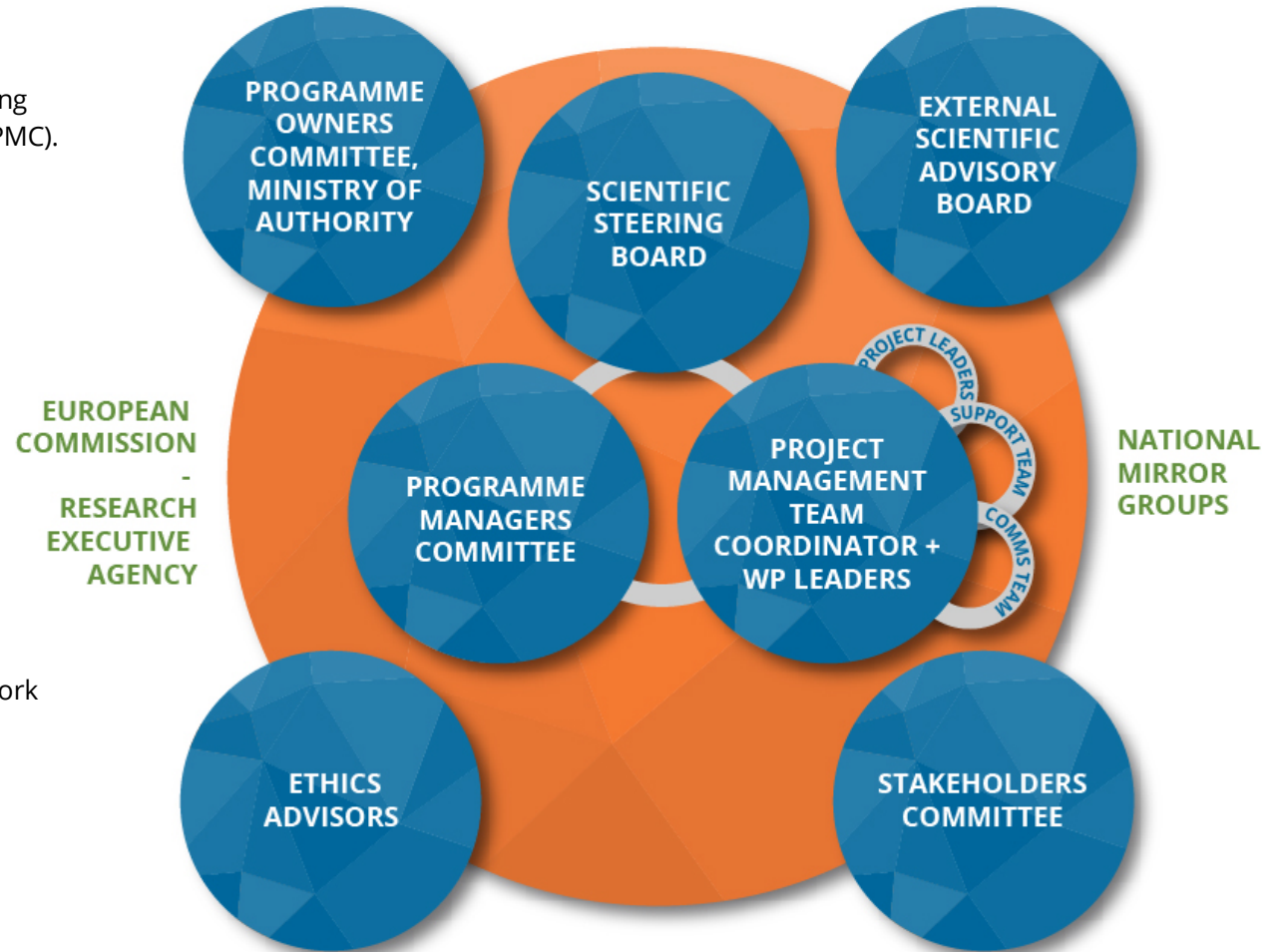
OUTCOMES



DISSEMINATION



EDUCATION





INTRODUCTION

## 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.



VISION

### Coordination - Work Package 1

WP1 enables the successful functioning of the OHEJP and maintains the environment where scientists can effectively and actively collaborate.



STRUCTURE

### 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.



ACHIEVEMENTS

### 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.



OUTCOMES

### 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.



DISSEMINATION

### Science to Policy Translation - Work Package 5

WP5 ensures best use of the outcomes of the OHEJP through dissemination activities and dialogue with OHEJP stakeholders.

### Education and Training - Work Package 6

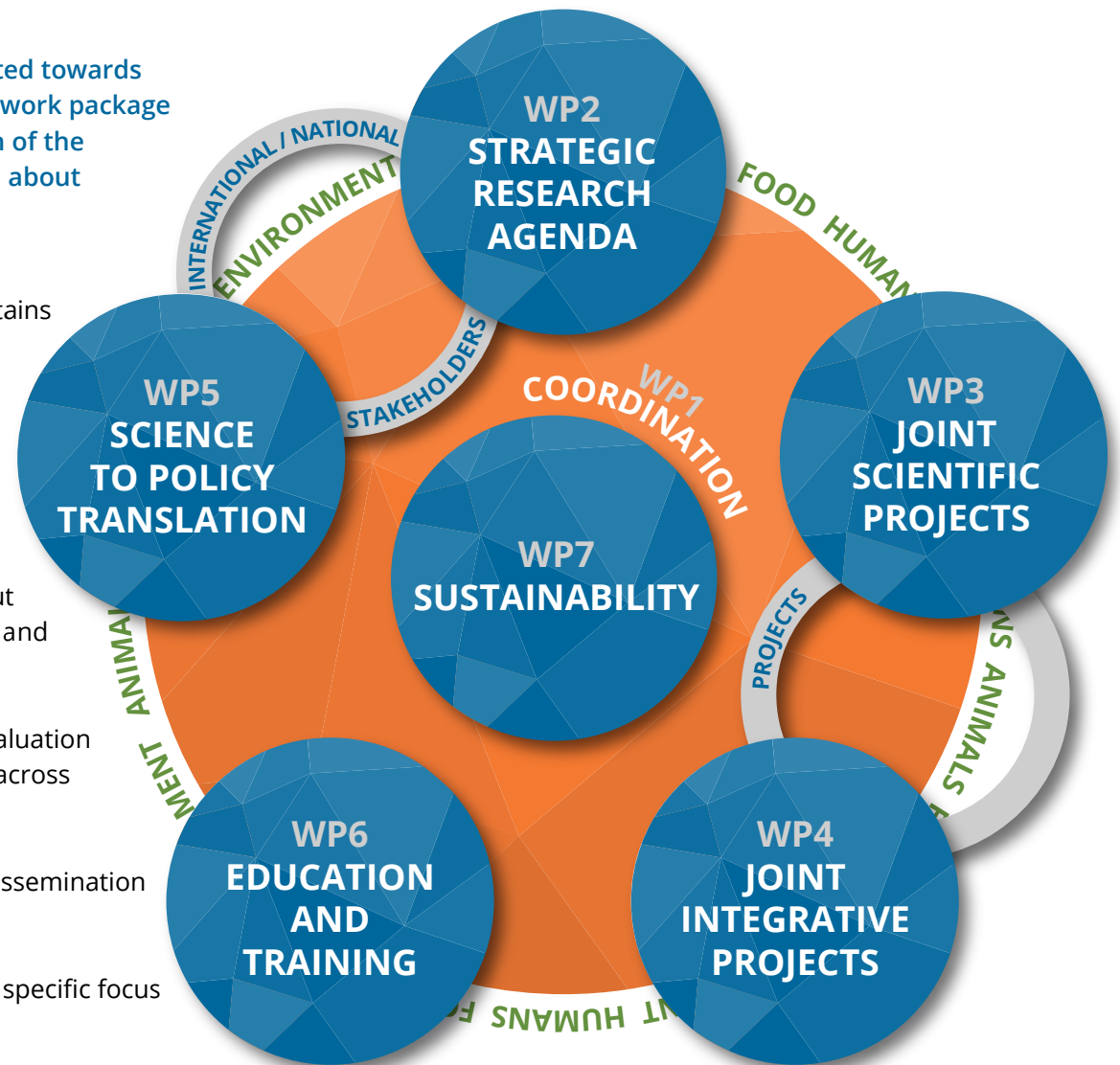
WP6 develops and delivers innovative training platforms with a specific focus on One Health.



EDUCATION

### Sustainability - Work Package 7

WP7 explores operational means to sustain long-term research and innovation beyond the duration of the OHEJP.





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

# What has the One Health EJP achieved in year five?



## INTRODUCTION

# COORDINATION OF THE ONE HEALTH EJP

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



## VISION



## STRUCTURE



## ACHIEVEMENTS



## OUTCOMES



## DISSEMINATION



## EDUCATION

- 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).
- Funding extensions that had been granted to JIPs and JRPs in response to the SARS-CoV-2 pandemic enabled research to be successfully completed for 16 projects in 2022.
- The additional JIP [COVRIN](#), that started in March 2021 was granted an extension for completion in March 2023.
- Continued coordination and communication to inform internal and external audiences of the OHEJP's role and the One Health approach to pandemic preparedness, following the SARS-CoV-2 pandemic.
- Within WP4, the subtask of the [One Health EJP SimEx](#) national exercises were delivered between May – September 2022. This provided the opportunity for partner institutes to participate in the conduction of a national-level One Health exercise held across 11 European countries.
- Ongoing support for the JIPs, JRPs, Education and Training activities and other OHEJP scientific events.
- Provided support for the Programme Managers Committee (PMC) and Programme Owners Committee (POC) joint meeting held at the Public Health Agency of Sweden (FoHM) in Stockholm and co-organised by the Swedish National Veterinary Institute (SVA).
- Consortium member institutes organised the events of the [Annual Scientific Meeting](#) (hybrid event in Orvieto, Italy, by [Istituto Superiore di Sanità](#)), [ASM Satellite Workshop](#) - Diagnostics workshop on mobile detection platforms for One Health diagnostics applications (online event by the [University of Surrey](#) and the [National University of Ireland](#)), [Final School](#) - Sustainability in One Health: how can it be achieved? (online event by the [University of Surrey](#)), [CPD Module](#) - Rapid diagnostics and harmonisation of diagnostic tests (hybrid event in Copenhagen, Denmark by [Statens Serum Institut](#) and [DTU Food](#)). Ten [Short Term Missions](#) were awarded, with them all completed by May 2023.
- Key OHEJP deliverables were managed and submitted on time to the REA.
- The Communications Team have continued to develop and enhance communications throughout the Consortium to stakeholders and external audiences. The OHEJP branded [website](#) and [newsletters](#), and social media platforms were used to inform audiences of all joint successes.







## COMMUNICATING SUCCESS

The Communications Team sits centrally in the OHEJP consortium in Coordination (WP1), delivering communications and supporting all OHEJP activities to ensure the OHEJP achieves its goals and fulfils its potential through effective dissemination.



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

- The OHEJP brand was strengthened by continuing to improve visibility across all platforms including meetings, workshops, Education and Training events, the OHEJP website and social media, which was highlighted by increased numbers of followers over the fifth year on Twitter and LinkedIn (each platform gaining over 1000 new followers). Additionally, communications to external organisations (e.g., EFSA and The One Health Commission) ensured that our activities were advertised in their newsletters to further widen OHEJP engagement.
- Supported all OHEJP events, which were successfully conducted as either hybrid or online events.
- Further developed communication and dissemination tools for Consortium members to support the dissemination of scientific outcomes and demonstrate impact.
- Supported OHEJP scientists in promoting the outputs, outcomes, and potential impacts of their research by disseminating this information on social media posts, internal and external newsletters, the website, and key documents.
- Created several interactive documents, including three project brochures for [IMPART](#), [TOX-Detect](#) and [LISTADAPT](#) and also [Short Term Mission](#) case studies for years 2019 to 2022, to showcase the OHEJP to scientific and non-scientific audiences across the globe.
- Created the [Cogwheel Workshops case study](#) to highlight the success of eight Cogwheel Workshops delivered in collaboration with other EU funded initiatives/projects from April 2018 – September 2021. Workshops focused on surveillance and prevention of antimicrobial resistance, infectious (animal and human) and foodborne diseases.
- Highlighted the work of OHEJP PhD students by creating social media and blog posts about their activities and providing information in newsletters, including the 3-minute thesis competition and links to their publications. The [#OHEJPphdlife](#) campaign continued in 2022 to showcase a day in the life of individual PhD students from the PhD projects [PEMbo](#), [METAPRO](#) and [EnvDis](#).
- Provided a presentation on 'Communication: more than just words' at the Final School in the session 'The importance of effective communication and collaboration'.
- Maintained the OHEJP Zenodo account to ensure that publications and deliverables are open access.





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

## 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 the Strategic Research and Innovation Agenda (WP7), which supports the sustainability of the Consortium.

The impact of the OHEJP's outcomes is maximised by targeted dissemination and dialogues with OHEJP Stakeholders (WP5).

### What were the key outcomes for year five?

- WP5 kept solid relations with Key EU stakeholders ECDC and EFSA, and other European and global stakeholders: European Environment Agency (EEA), European Medicines Agency (EMA), Food and Agriculture Organisation of the United Nations (FAO), the World Organisation for Animal Health (WOAH) and the World Health Organisation regional office for Europe (WHO-EURO). These stakeholders joined the bi-annual Stakeholders Committee meetings to discuss the impact and sustainability of the OHEJP.
- The public database [OHEJP Outcome Inventory](#) (OHOI), which documents and widely shares all outcomes from OHEJP projects, was regularly updated.
- The series of Dissemination Workshops targeted at policy and decision makers at the national level continued in year five, with two workshops held: [Improving One Health Preparedness to \(re\) Emerging Infectious Threats](#) and the [Joint SimEx/Dissemination Workshop – A One Health Simulation Exercise as a Roadmap for Future Foodborne Outbreak Preparedness](#). These workshops depict the practical use of OHEJP-developed solutions, and the benefits that they are having in Europe.
- Mutual support between stakeholders and OHEJP continued to be fruitful, as seen, for example, by representatives of the OHEJP being invited to join stakeholders' events and vice versa (e.g., RARA, ONE2022).
- WP5 supported the development of the Strategic Research and Innovation Agenda, in particular the identification of European One Health research and policy needs beyond the lifetime of the OHEJP.





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION



# One Health EJP Scientific Outcomes



## INTRODUCTION



## VISION



## STRUCTURE



## ACHIEVEMENTS



## OUTCOMES



## DISSEMINATION



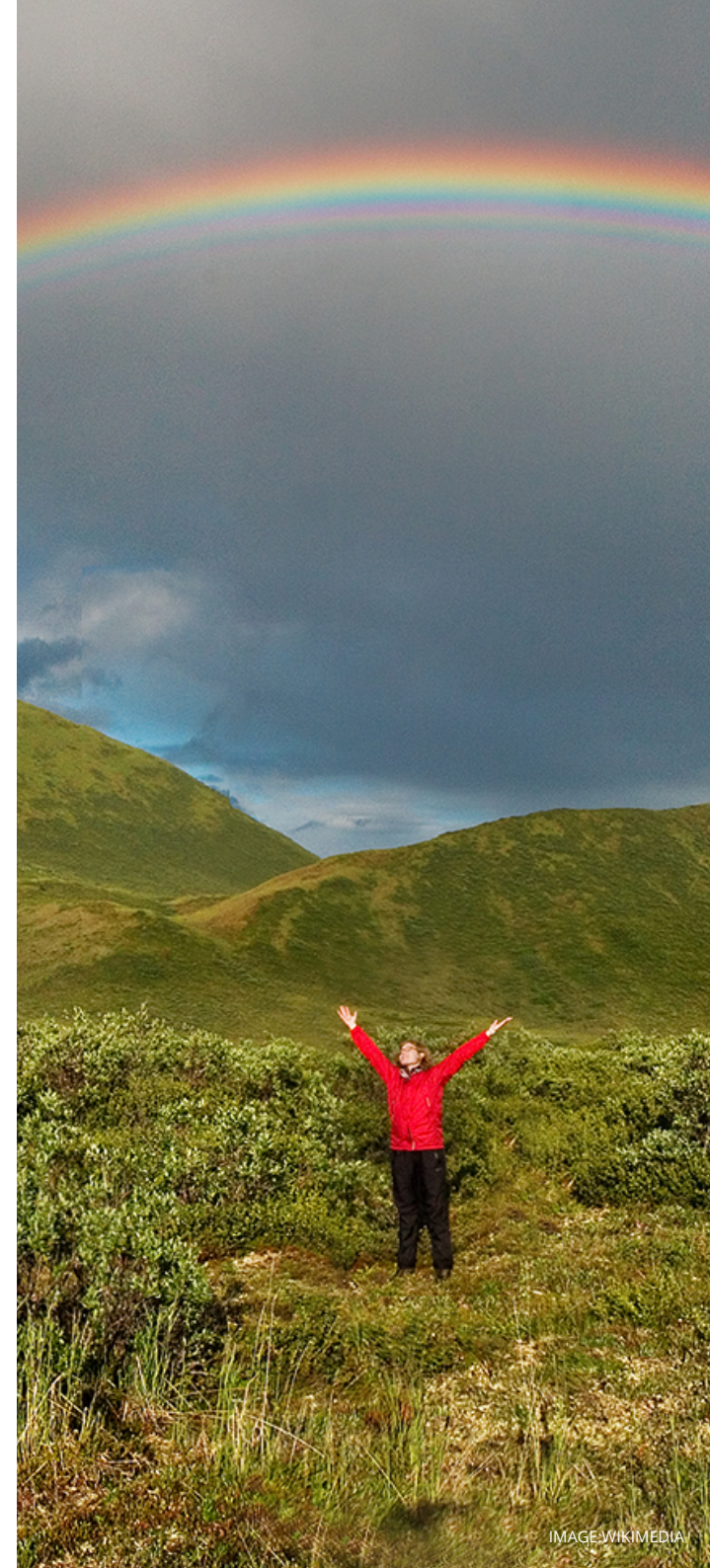
## EDUCATION

# ONE HEALTH EJP SCIENTIFIC OUTCOMES

To date, the One Health EJP has co-funded 6 JIPs, 24 JPRs and 17 PhD projects, and the OHEJP SimEx Project. In 2022, the Continuing Professional Development Module, Final School, Annual Scientific Meeting, and Annual Scientific Meeting Satellite Workshop and other workshops were organised. For 2022, ten Short Term Missions were also funded.

What were the key outcomes in the fifth year?

- The 6-month extensions granted to some projects enabled their successful completion despite the challenges due to the SARS-CoV-2 pandemic.
- The following JIPs were completed in 2022: CARE, OH-HARMONY-CAP and MATRIX in December 2022. The final JIP COVRIN received an extension to March 2023.
- The following JPRs were completed in December 2022: FARMED, WORLDCOM, FULL-FORCE, FED-AMR, TELE-Vir, IDEMBRU, MEmE, PARADISE, DiSCoVer, BIOPIGEE, TOXOSOURCES, ADONIS and BeONE.
- The OHEJP SimEx Project delivered a foodborne outbreak exercise scenario across 11 European countries employing the One Health approach. It led to intersectoral cooperation through the sharing of experiences, views, and perspectives to benefit outbreak preparedness.
- Established procedures continued to be followed to ensure that data and publications were open access.
- Over 80 peer-reviewed publications were published.
- The Fourth One Health EJP Annual Scientific Meeting was held in Italy and online, which brought together an international audience of 276 participants from 22 countries.
- One Health EJP PhD students' research was showcased in the third Three-Minute Thesis (3MT) competition when students gave their presentations in a hybrid format.
- OHEJP WP4 organised the 4th Thematic Integrative Meeting on 'Data sharing across disciplines' held online in February 2022.
- The series of Dissemination Workshops continued to showcase the impact of OHEJP outputs to national, European, and international decision makers and strategic planners. The second Dissemination Workshop on [Improving One Health Preparedness to \(re\) Emerging Infectious Threats](#) was held in March 2022. The joint OHEJP SimEx /Dissemination Workshop: [A One Health Simulation Exercise as a Roadmap for Future Foodborne Outbreak Preparedness](#) was co-organised by OHEJP SimEx Project and [Science to Policy Translation](#) (WP5) and held in





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



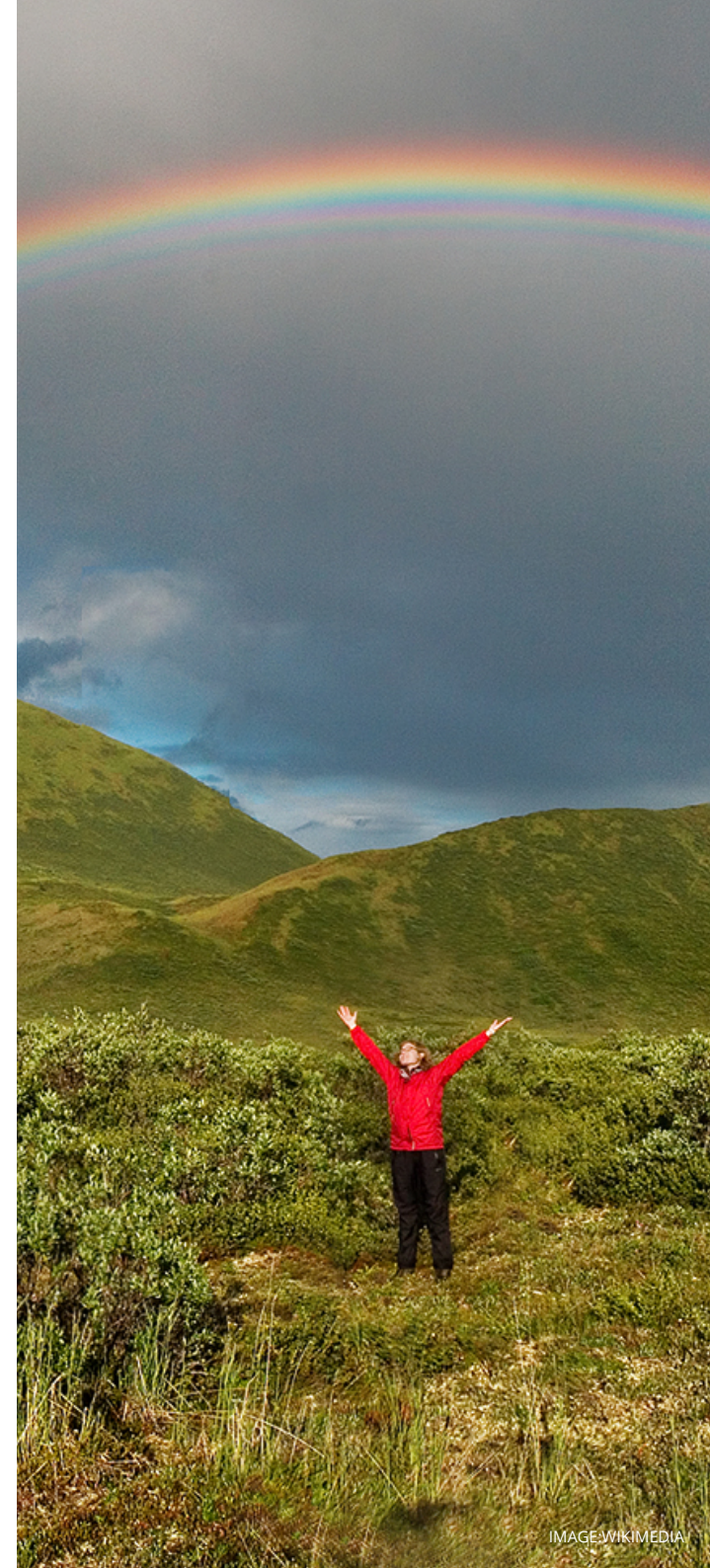
DISSEMINATION



EDUCATION

December 2022. This workshop focused on the experiences from conducting the OHEJP SimEx and its applications for using OHEJP solutions to improve outbreak preparedness.

- The MATRIX Webinar Series 2022 on Solutions for One Health Surveillance in Europe delivered seven webinars between May to December 2022. These presented the solutions developed by [JIP MATRIX](#) to support and advance the implementation of One Health Surveillance in European countries.
- [JRP BIOPIGEE](#) delivered two online workshops to wide audiences in September 2022. Workshops presented BIOPIGEE project outcomes on effective biosecurity measures at farms and slaughterhouses.





### CARE

#### INTRODUCTION

The CARE project aimed to develop new One Health concepts for external quality assurance (EQA) schemes for laboratories, reference materials (RM), and the quality and availability of demographic data through core objectives.



#### VISION

During the CARE project, 18 partner institutes from 14 European countries united to address the goals of the project. During 2022, the project focused further on the development of new proficiency testing (PT) schemes that could be used cross-sectorally to evaluate the capacity to manage foodborne problems from a One Health perspective. Furthermore, the project provided information on available and desirable RM, identifying sources of both strain and genomic data, including the field of antimicrobial resistance, provided by reference laboratories, national collections, and online repositories. Further emphasis was placed on ensuring the visibility of the reference material and associated data to contribute to the long-term needs of the European research community. Lastly, the CARE project further assessed the quality and availability of demographic data and focused on how to improve and make the data better by raising awareness of EU authorities collecting and organising demographic data.



#### STRUCTURE



#### ACHIEVEMENTS

Three PT/EGA trials were conducted throughout the project and provided individual feedback to all participants. A template was further developed for the planning, execution and evaluation of cross-sectoral PTs, the template covering all aspects of ISO/IEC 17043 conformity assessment. The template was robustly evaluated using these pilot studies, ensuring that it contained sufficient guidance on general considerations that should be taken before PT is executed.



#### OUTCOMES

Further analysis of the identified genomes for AMR, virulence, and other typing regimes continued throughout 2022. A web catalog dashboard was developed to ensure visibility and accessibility of these reference materials. Matrix-assisted laser desorption/ionisation-time-of-flight (MALDI-TOF) mass spectrometry (MS) reference spectra were produced for *Campylobacter*, *Yersinia*, and *Salmonella* strains in order to set up and share reference libraries. A sustainable development plan for the CARE collection was developed to ensure accessibility of reference material after the closure of the CARE project.

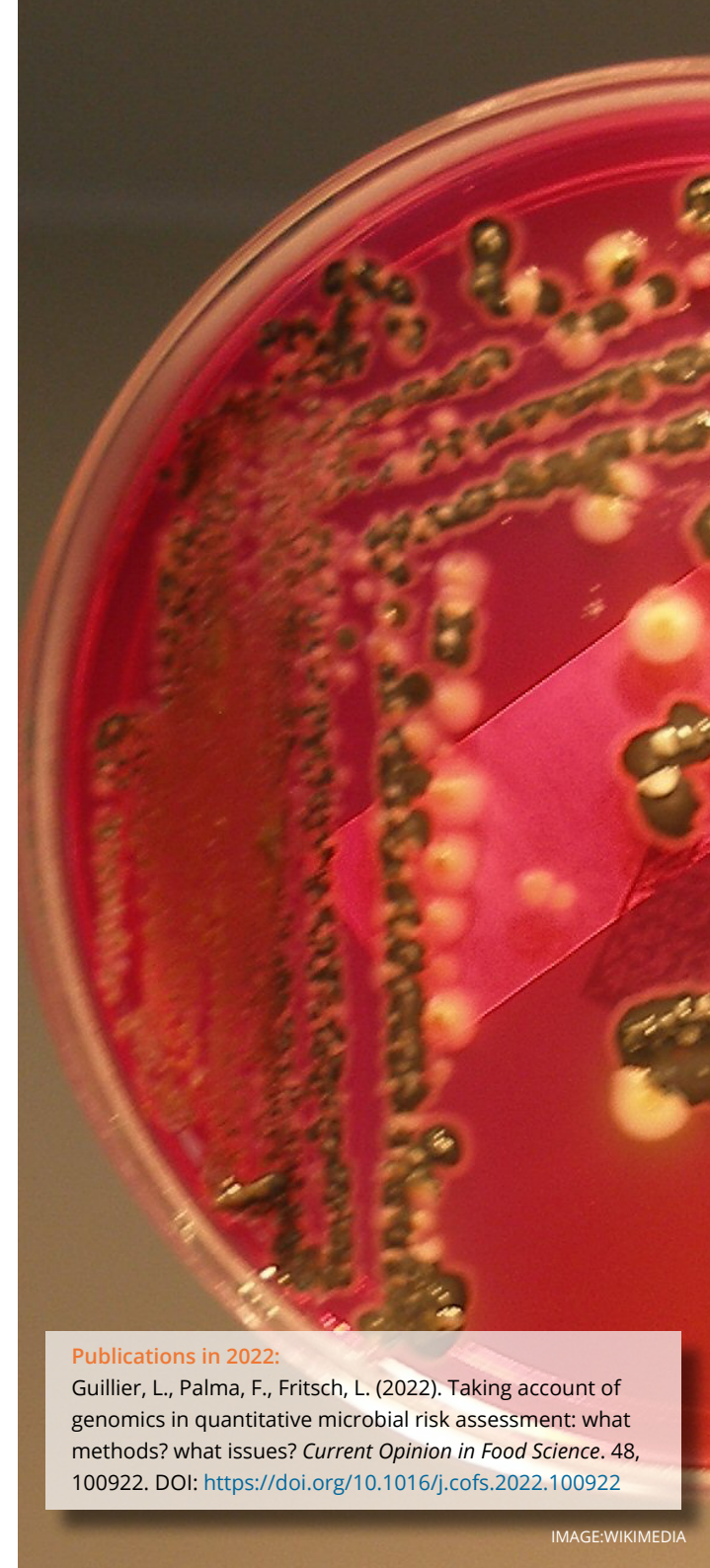


#### DISSEMINATION



#### EDUCATION

A risk assessment survey was conducted on relevant meta-data and data in relation to quantitative microbial risk assessments, and stakeholders assisted by increasing the accessibility of data, but also to avoid duplication. Thus, feeding into the quantitative microbial risk assessment analysis on data originating from the last five years and provided by 19 OHEJP members/ stakeholders including those from ORION and RADAR. Furthermore, a user guide for accessing relevant data for risk assessment as



#### Publications in 2022:

Guillier, L., Palma, F., Fritsch, L. (2022). Taking account of genomics in quantitative microbial risk assessment: what methods? what issues? *Current Opinion in Food Science*. 48, 100922. DOI: <https://doi.org/10.1016/j.cofs.2022.100922>

## JOINT INTEGRATIVE PROJECTS



### INTRODUCTION

a R software tool 'rStainSelect' to ease the process of selecting suitable reference strains based on exposure and risks. The user guide and R software contribute to and support the developed strategy by EU authorities to raise the awareness of relevant reference material microbial risk assessment analysis.



### VISION

In general, the CARE project has disseminated the acquired knowledge, developed useful tools, and built capacity both within and between countries. There is a broad application for these results and tools, with some already being used in practice, e.g., shiny Rrisk tool at BfR, the CIS in Italy and FCL Web internationally. The CARE project has promoted the importance of sustainable One Health approaches in risk analysis.



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## OH-HARMONY-CAP

The OH-HARMONY-CAP aimed to collect information on current capabilities, capacities, interoperability, and adaptability at both the National Reference Laboratory (NRL) and the primary diagnostic level across Europe by developing an in-depth OHLabCap survey. The quantitative description of current and best practices and the development of harmonised protocols identified the gaps and suggested future studies on how to best detect and characterise foodborne pathogens across One Health sectors (i.e., public health, veterinary & food/environment testing labs). The OH-HARMONY-CAP consortium consisted of 18 partner institutes from 14 European countries by the end of the project in 2022.

OH-HARMONY-CAP was a 3-year project and consisted of three core aims: i) the collection of information on the current capabilities, capacities, interoperability, and adaptability at both the NRL and primary diagnostic levels across Europe, by developing an in-depth OHLabCap survey; ii) examine the current and best practices within One health sectors in a 'sampling and testing', 'characterisation of isolates', and 'data management and harmonised reporting' including the identification of current knowledge gaps and the proposition of new studies and/or methods to fill these gaps; and iii) produce recommendations on how to harmonise the methodology for the detection and typing of pathogens.

The OHLabCap survey was developed and tested, for the first time, a One Health monitoring tool, which collected information on current capabilities, capacities, interoperability, and adaptability in EU/EEA countries across all One Health sectors: human clinical, food, feed, veterinary and environmental laboratories have been created. The tool focuses on selected priority parasites and bacteria, allowing major differences between diagnostic laboratories in human clinical and food/feed/veterinary sectors to be identified.

Current and best practices in 'sampling & testing', 'characterisation of isolates', and 'data management and harmonised reporting' for laboratories testing food, feed, environmental and human samples for Shiga toxin-producing *E. coli* (STEC), Enterotoxigenic *E. coli* (ETEC), *Cryptosporidium* and antimicrobial resistance (AMR) in *Salmonella* and *Campylobacter spp.* across the EU were surveyed using peer-reviewed papers, grey literature, and questionnaires. The latter was completed by European public health, veterinary, food testing, and National Reference Laboratories reached via the EURL network of NRLs, EFSA Zoonoses network, ECDC Food, and Waterborne Disease network, amongst others.

The proposed harmonisation procedures for the detection and typing of STEC and ETC were suggested. The evaluation of the chosen protocols included an examination of PCR primer choices. These examination





## JOINT INTEGRATIVE PROJECTS



### INTRODUCTION

points resulted in the development of alternative omni-primers for the detection of STEC and ETEC-defining genes as the chosen protocols did not reflect the current knowledge of new subtypes and variants of the genes. As such, four new subtypes of Shiga toxin genes were described and three of these have now been published as part of the OH-HARMONY-CAP projects harmonisation approach.



### VISION

The OH-HARMONY-CAP project made a significant impact by proposing procedures, that when implemented, will contribute to an improvement in the standardisation and harmonisation of protocols for the model organisms. The ranking of laboratory protocols also provided and suggested elements for consideration and choice of protocol that can be applied to other organisms and procedures in the One Health sectors. The development of new PCR primers for the detection of STEC and ETEC-related genes represents an update reflecting the current epidemiology and current knowledge of microbiology organisms that are forever changing. These so-called moving targets are an illustration that microbes constantly evolve and adapt, which in turn makes it increasingly important to update and share front-line detection and characterisation methodology.



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION



#### Publications in 2022:

Gill, A., Dussault, F., McMahon, T., Petronella, N., Wang, X., Cebelinski, E., Scheutz, F., Weedmark, K., Blais, B., & Carrillo, C. (2022). Characterisation of Atypical Shiga Toxin Gene Sequences and Description of Stx2j, a New Subtype. *Journal of Clinical Microbiology*. 60(3), e0222921. DOI: <https://doi.org/10.1128/jcm.02229-21>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## MATRIX

**MATRIX** aimed to advance the implementation of One Health Surveillance (OHS) in practice, by building on existing resources, adding value to them, and creating synergies among the animal health, public health, and food safety sectors. Learning from the experiences of the previous OHEJP joint integrative projects, **MATRIX** created solutions for European countries to support and advance the implementation of OHS, while looking at the entire OHS pathway and recognising the existence of different national realities. In 2022, **MATRIX** finalised the **MATRIX Solutions for One Health Surveillance** and applied the **MATRIX sustainability strategy**.

The **MATRIX Solutions for One Health Surveillance** are: i) **OH-EpiCap Tool** – an interactive tool to evaluate the capacities and capabilities for the OHS of a hazard of choice, identifying strengths and opportunities for improvement, as explained in the [OH-EpiCap tool flyer](#) and [OH-EpiCap tool user guide](#); ii) [Roadmap to develop national OHS](#) – a guideline that countries can use to develop OHS according to their needs and resources, expanding the work of the OHEJP COHESIVE project; iii) **Manual for OHS Dashboards** – an online dashboard inventory and practical manual to facilitate the design and implementation of OHS dashboards using open-source tools; it can be accessed through the [Dashboard Information Centre](#); iv) **Guidelines and checklists** – an interactive [guide to facilitate the development of multi-sectoral OHS frameworks](#) from existing animal health, public health and food safety surveillance systems; [best-practices to operationalise cross-sectoral collaborations](#) with a focus on data collection, data sharing, data analysis, and the dissemination of surveillance results; [guide to design, implement, and evaluate official controls within the food safety](#) using output-based standards. Hazard-specific tracks were also created to ensure that the **MATRIX Solutions for One Health Surveillance** were relevant to specific pathogens.

The hazards, chosen based on the operational priorities of **MATRIX** partner institutes and their One Health relevance, were *Listeria*, *Salmonella*, *Campylobacter* and emerging threats, including antimicrobial resistance (AMR).

The **MATRIX sustainability strategy**, built around the **MATRIX Solutions for One Health Surveillance** included 5 action points: i) to support **MATRIX** partners in making use of the **MATRIX Solutions for One Health Surveillance**; ii) to disseminate and pilot the **MATRIX Solutions for One Health Surveillance** among non-partners, notably by organising and delivering a series of seven webinars, the [Solutions for One Health Surveillance in Europe webinars](#); iii) to support the production of original peer-reviewed publications, by launching, jointly with CARE and OH-HARMONY-CAP, the thematic call titled '**One**





### INTRODUCTION

Health Surveillance in Practice: Experiences of Integration among Human Health, Animal Health, Environmental Health, and Food Safety Sectors' in the peer-reviewed journal *Frontiers in Public Health*: iv) to continue and enhance training and dissemination activities aiming to reach the broadest audience, and v) to continue the advancement and use of *The OHS Codex: The Knowledge Integration Platform* (developed by the OHEJP *ORION* project) – a framework to integrate various resources that help to implement OHS in all sectors and the *Food Safety Knowledge Exchange (FSKX) Format* – a format that supports the One Health community in sharing and re-using mathematical models as well as data analysis procedures.



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION



#### Publications in 2022:

Rodríguez, A., Iglesias, I., de la Torre, A. (2022). Prioritisation tool for targeting the monitoring of veterinary pharmaceuticals at national level: the case of Spain. *European Journal of Soil Sciences*. 73(4): e13268. DOI: <https://doi.org/10.1111/ejss.13268>

Swanson, D., Koren, C., Hopp, P., Jonsson, M.E., Rø, G. I., White, R. A., Grøneng, G. M. (2022). A One Health real-time surveillance system for nowcasting *Campylobacter* gastrointestinal illness outbreaks, Norway, week 30 2010 to week 11 2022. *Euro Surveillance*. 27(43): 2101121. Available at: <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2022.27.43.2101121>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## COVRIN

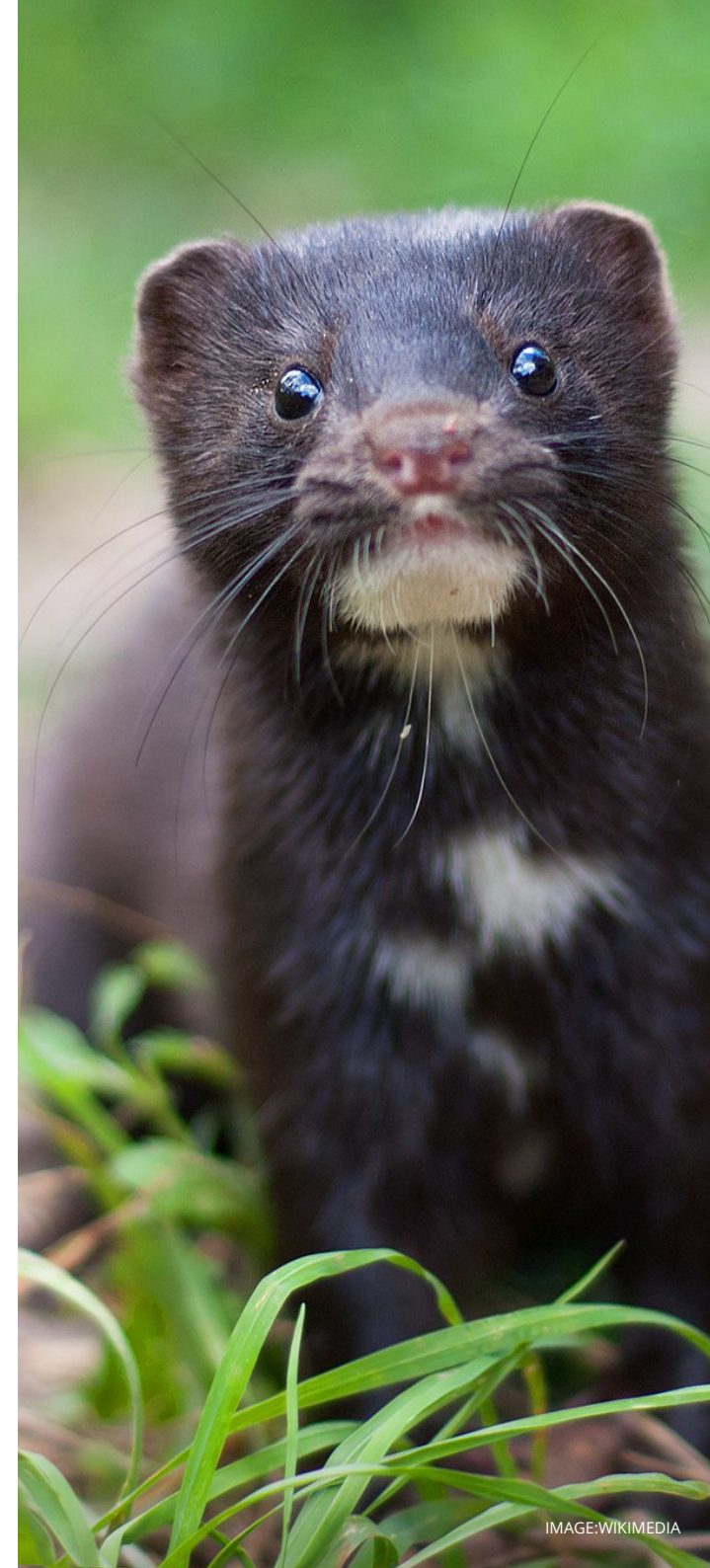
The COVRIN project aimed to reinforce collaboration and integration of research activities on SARS-CoV-2 and had two main operational objectives: i) to identify drivers for the emergence and spread of SARS-CoV-2; and ii) to generate data and build models for risk assessment of SARS-CoV-2. Activities to connect with stakeholders and avoid overlaps with other projects were a key focus and the project was split into four key research activities: i) research on the detection of SARS-CoV-2 in animal species and the environment; ii) research on SARS-CoV-2 molecular and biological characterisation; iii) SARS-CoV-2 surveillance and risk assessment, focused on the animal human interface; and iv) coronavirus preparedness.

For the key research area on the detection of SARS-CoV-2 in animal reservoirs and hosts, and the environment, SARS-CoV-2 genomes testing data, molecular assays and rapid antigen tests were further shared. Immunoassays methodologies were further shared and harmonised, and serological surveillance of SARS-CoV-2 was conducted in domestic and wildlife animals. Approaches in the tasks on the assessment of bioavailability were agreed and further developed.

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

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

Integrative activities on coronavirus preparedness, isolations of virus from different wildlife species were performed. An initial report on relevant sample types and hot spots for coronavirus sampling



## JOINT INTEGRATIVE PROJECTS



### INTRODUCTION

has started to evaluate the impacts on ecological factors and interventions. In 2022, more work was done on the study of virus – host interactions and on the identification of drivers of virus emergence through the evaluation of phylodynamic and cross-species interactions with a focus on zoonotic and reverse zoonotic aspects and adaptations. Generated data and research outcomes will be used in future national programmes and collaborative networks to develop control strategies, intervention strategies and prevention.



### VISION

COVRIN cooperated with nationally funded projects in partner countries, and international organisations like WHO, FAO, ECDC and EFSA. Links have been established with international research networks and projects like the UK Coronavirus Network, the PREZODE network and the ERRAZE project. COVRIN significant results have been reported to the national ministries and to ECDC and EFSA as appropriate. The aim is to make all COVRIN results available for the entire scientific community, primarily through peer reviewed publications, and to the general public.



### STRUCTURE



### ACHIEVEMENTS



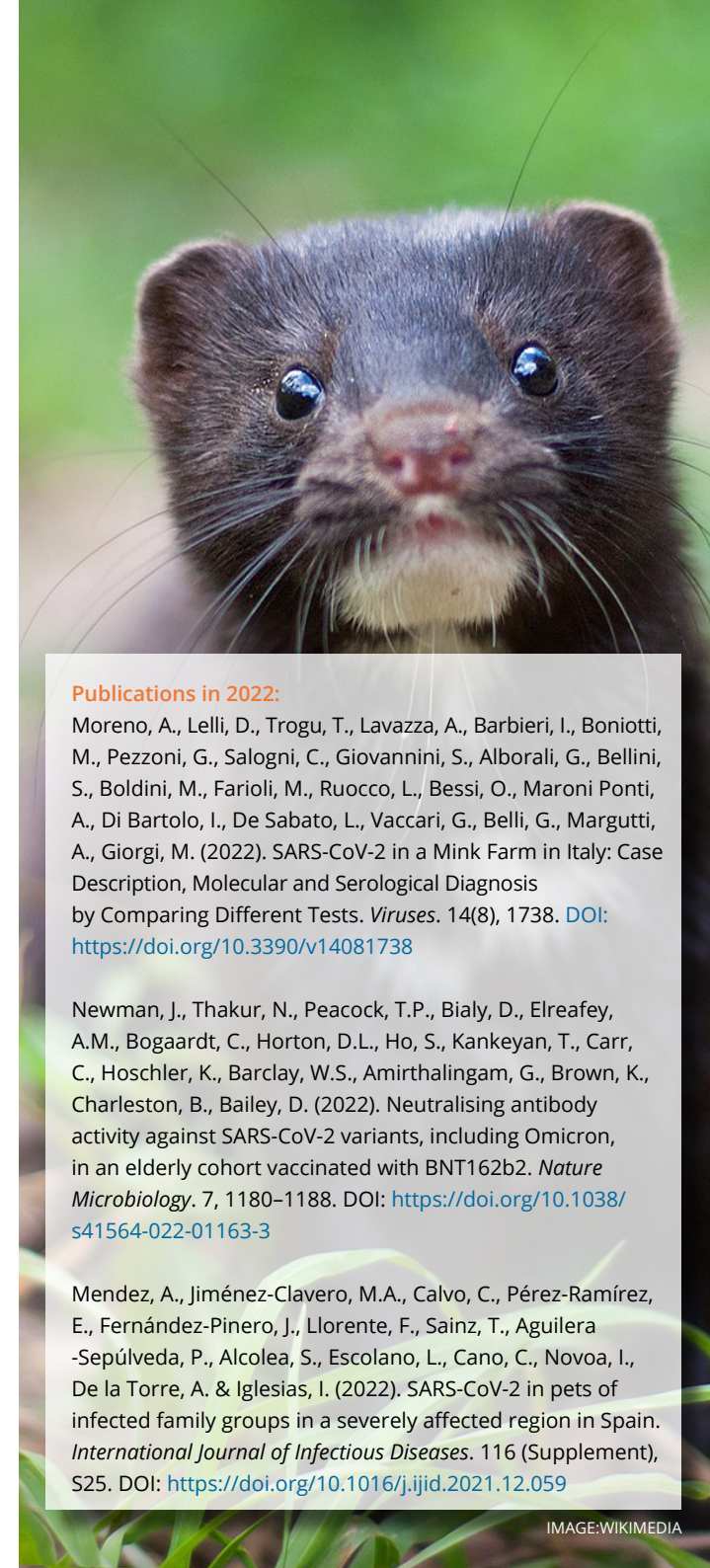
### OUTCOMES



### DISSEMINATION



### EDUCATION



#### Publications in 2022:

Moreno, A., Lelli, D., Trogu, T., Lavazza, A., Barbieri, I., Boniotti, M., Pezzoni, G., Salogni, C., Giovannini, S., Alborali, G., Bellini, S., Boldini, M., Farioli, M., Ruocco, L., Bessi, O., Maroni Ponti, A., Di Bartolo, I., De Sabato, L., Vaccari, G., Belli, G., Margutti, A., Giorgi, M. (2022). SARS-CoV-2 in a Mink Farm in Italy: Case Description, Molecular and Serological Diagnosis by Comparing Different Tests. *Viruses*. 14(8), 1738. DOI: <https://doi.org/10.3390/v14081738>

Newman, J., Thakur, N., Peacock, T.P., Bialy, D., Elreafey, A.M., Bogaardt, C., Horton, D.L., Ho, S., Kankeyan, T., Carr, C., Hoschler, K., Barclay, W.S., Amirthalingam, G., Brown, K., Charleston, B., Bailey, D. (2022). Neutralising antibody activity against SARS-CoV-2 variants, including Omicron, in an elderly cohort vaccinated with BNT162b2. *Nature Microbiology*. 7, 1180–1188. DOI: <https://doi.org/10.1038/s41564-022-01163-3>

Mendez, A., Jiménez-Clavero, M.A., Calvo, C., Pérez-Ramírez, E., Fernández-Pinero, J., Llorente, F., Sainz, T., Aguilera-Sepúlveda, P., Alcolea, S., Escolano, L., Cano, C., Novoa, I., De la Torre, A. & Iglesias, I. (2022). SARS-CoV-2 in pets of infected family groups in a severely affected region in Spain. *International Journal of Infectious Diseases*. 116 (Supplement), S25. DOI: <https://doi.org/10.1016/j.ijid.2021.12.059>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## DISCOVER

The DiSCoVeR project brought together researchers and experts from different disciplines (microbiology, bioinformatics and epidemiology) and sectors (veterinary science, food safety, public health, and environmental health) from 19 institutions in 13 European countries in a unique consortium to address the challenges of source attribution in an interdisciplinary manner. As there exists no gold standard for conducting source attribution, DiSCoVeR took a comprehensive approach applying several different methodologies and models in a comparative fashion.

The project started by mapping existing knowledge and data gaps and recommending new studies and/or methods needed to fill the identified gaps. This included mapping of available data by creating data inventories for DiSCoVeR target pathogens: *Salmonella*, *Campylobacter*, VTEC/STEC and ESBL *E. coli*. Comprehensive datasets included data from a broad range of reservoirs and sources, including those not traditionally part of the existing European or national monitoring and surveillance activities (e.g., pets, wildlife, and environmental sources). A substantial number of *Salmonella*, *Campylobacter*, and STEC isolates in the DiSCoVeR collection was sequenced and formed the basis of the whole genome sequencing-based source attribution approaches.

DiSCoVeR main outputs and outcomes include: i) a set of comprehensive and standardised datasets for *Salmonella* ( $n = 145,000$  isolates;  $s = 4,185$  sequences), *Campylobacter* ( $n = 5,361$  isolates/sequences), STEC ( $n = 7,552$  isolates;  $s = 3,418$ ), and ESBL ( $n = 10,674$  isolates) with the phenotypic and metadata made open accessible through Zenodo; and ii) the creation of a critical and systematic assessment of existing source attribution models and development of new phenotypic and genomic-based attribution models for foodborne pathogens and antimicrobial resistance. Furthermore, DiSCoVeR provided recommendations on the translation of results from source attribution models into action, by emphasising that *Salmonella* in pigs and pork and *Campylobacter* in broilers and chicken meat stand out as areas, where targeted future control could be implemented to reduce the burden of human infections significantly. While, making further recommendations following an evaluation of current surveillance and control activities by seeking a collaborative and synergistic approach to surveillance strategies within the environmental/ecosystem of One Health. Finally, the DiSCoVeR project has evaluated transfer of source attribution approaches as an opportunity to strengthen the institutional capacity building, by promoting increased network-collaboration and training within integrated surveillance, source attribution approaches, burden of disease methodologies, risk assessment, and system thinking.

### Publications in 2022:

de Freitas Costa, E., Hagens, T. J., Dame-Korevaar, A., Brouwer, M. S. M. & de Vos, C. J. (2022). Multidirectional dynamic model for the spread of extended-spectrum- $\beta$ -lactamase-producing *Escherichia coli* in the Netherlands. *Microbial Risk Analysis*. 22, 100230. DOI: <https://doi.org/10.1016/j.mran.2022.100230>

Chanamé Pinedo, L., Mughini-Gras, L., Franz, E., Hald, T., Pires, S. M. (2022). Sources and trends of human salmonellosis in Europe, 2015–2019: An analysis of outbreak data. *International Journal of Food Microbiology*. 379, 109850. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2022.109850>

Pista, A., Silveira, L., Ribeiro, S., Fontes, M., Castro, R., Coelho, A., Furtado, R., Lopes, T., Maia, C., Mixão, V., Borges, V., Sá, A., Soeiro, V., Correia, C.B., Gomes, J. P., Saraiva, M., Oleastro, M., Batista, R. (2022). Pathogenic *Escherichia coli*, *Salmonella spp.* and *Campylobacter spp.* in Two Natural Conservation Centers of Wildlife in Portugal: Genotypic and Phenotypic Characterisation. *Microorganisms*. 10(11), 2132. DOI: <https://doi.org/10.3390/microorganisms10112132>

Perestrelo, S., Correia Carreira, G., Valentin, L., Fischer, J., Pfeifer, Y., Werner, G., Schmiedel, J., Falgenhauer, L., Imirzalioglu, C., Chakraborty, T. & Käsbohrer, A. (2022). Comparison of approaches for source attribution of ESBL-producing *Escherichia coli* in Germany. *PLOS ONE*, 17(7), e0271317. DOI: <https://doi.org/10.1371/journal.pone.0271317>

Mughini-Gras, L., Benincà, E., McDonald, S. A., de Jong, A., Chardon, J., Evers, E., & Bonačić Marinović, A. A. (2022). A statistical modelling approach for source attribution meta-analysis of sporadic infection with foodborne pathogens. *Zoonoses and Public Health*, 00, 1– 12. DOI: <https://doi.org/10.1111/zph.12937>

## JOINT RESEARCH PROJECTS: FOODBORNE ZONOSSES (FBZ)



### INTRODUCTION

Dissemination of information was shared at a stakeholders' workshop in November 2022, which was used to present project results and engage stakeholders in discussions on the translation of results into public health action. Furthermore, DiSCoVeR published nine publications throughout 2022.



### VISION

The DiSCoVeR project made an impact and contributed to the risk management/decision making at stakeholder organisations such as ECDC, EFSA, EURLs and EC, and stakeholders in areas such as national food, veterinary and environmental authorities, farmers, and consumer organisations. The project has also created a secure space data sharing platform of [sciedata.dk](https://sciedata.dk) to share genomic sequences, with ongoing work to further expand this inventory.



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION





### INTRODUCTION

### BIOPIGEE

The **BIOPIGEE** project aimed to identify effective and cost-effective biosecurity measures to control the occurrence of potentially zoonotic hepatitis E virus (HEV) and *Salmonella* in European pig farming. Nineteen partner institutes from 12 countries collaborated to conduct comprehensive literature reviews, field and experimental studies, expert surveys, and risk modelling. The project aimed to develop tools to limit the pathogen load along the food chain, thus resulting in healthier animals and a safer food chain.



### VISION

The BIOPIGEE project main outputs and achievements include: i) The **BIOPIGEE Glossary** of pig farming and biosecurity terms for participants and stakeholders; ii) The BIOPIGEE Biosecurity Protocol designed to assess a farms biosecurity against *Salmonella* and HEV in pig farming; iii) **The BIOPIGEE Checklists** to be used by farmers and veterinarians to assess a farms biosecurity against *Salmonella* or HEV on an individual farm based on current scientific knowledge; iv) The BIOPIGEE Slaughterhouse Guidance Manual, which contains evidence of the effectiveness of biosecurity practices against *Salmonella* and HEV during the slaughterhouse processes; v) **The BIOPIGEE Cost-Effectiveness Support Tool** designed to estimate the benefit-cost ratios and net-benefits of the implementation of effective biosecurity measures against *Salmonella* or HEV for farms in selected countries; and vi) **The BIOPIGEE Information Material for Farming Schools**, developed to introduce good examples of biosecurity measures on pig farms in Europe.



### STRUCTURE



### ACHIEVEMENTS

Dissemination activities throughout 2022, included educational discussions, training activities and a Final Meeting. The BIOPIGEE Final Meeting was held in Giulianova, Italy and attracted 36 participants from 10 countries, and 16 institutes.



### OUTCOMES

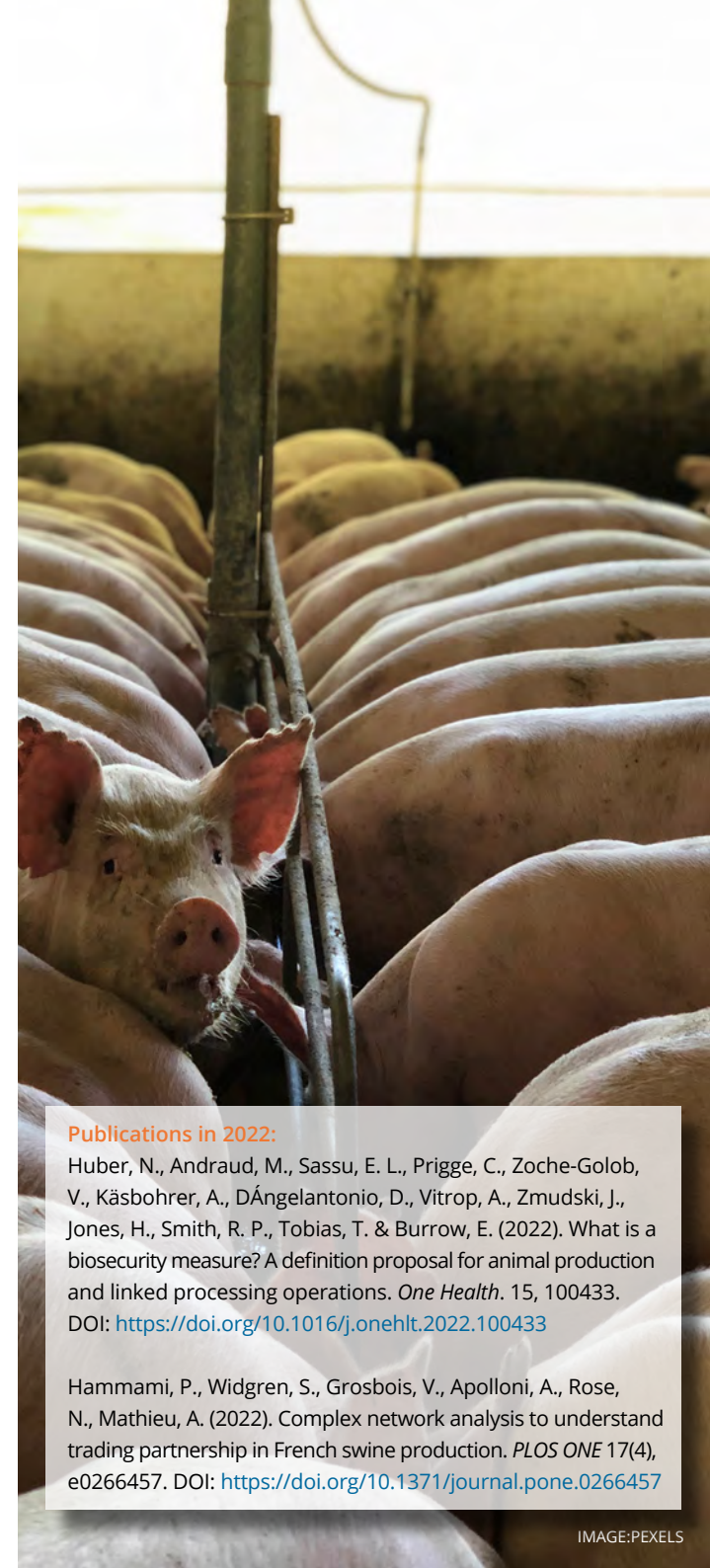
The BIOPIGEE project impacted on the animal health, public health, food safety sectors. Beneficiaries of these scientific outputs are farmers, veterinarians, consultants, slaughterhouses and educational institutions in European countries. In summary, BIOPIGEE has developed a robust risk-assessment strategy and biosecurity measures for the prevention of *Salmonella* and HEV infections in farms and slaughterhouses in Europe.



### DISSEMINATION



### EDUCATION



#### Publications in 2022:

Huber, N., Andraud, M., Sassu, E. L., Prigge, C., Zoche-Golob, V., Käsbohrer, A., D'Angelantonio, D., Vitrop, A., Zmudski, J., Jones, H., Smith, R. P., Tobias, T. & Burrow, E. (2022). What is a biosecurity measure? A definition proposal for animal production and linked processing operations. *One Health*. 15, 100433. DOI: <https://doi.org/10.1016/j.onehlt.2022.100433>

Hammami, P., Widgren, S., Grosbois, V., Apolloni, A., Rose, N., Mathieu, A. (2022). Complex network analysis to understand trading partnership in French swine production. *PLOS ONE* 17(4), e0266457. DOI: <https://doi.org/10.1371/journal.pone.0266457>





### INTRODUCTION

## TOXOSOURCES

The **TOXOSOURCES** project focused on the zoonotic parasite *Toxoplasma gondii* at the interface between humans, animals, food, and the environment. *Toxoplasma gondii* is a foodborne parasite that causes a high disease burden. The infection can be acquired by ingesting oocysts (environmental pathway) or tissue cysts (meat-borne pathway). The relative contributions of the two pathways to the infection and disease in humans remain unknown, partly due to a lack of appropriate methods. Environmental contamination with *T. gondii* oocysts is understudied and underestimated due to the lack of suitable harmonised sampling approaches and detection methods. TOXOSOURCES investigated the relative contributions of the different sources and transmission routes to *T. gondii* infections using multidisciplinary approaches and new methods to yield robust estimates that can inform risk managers and policymakers.



### VISION

The TOXOSOURCES consortium has collected data for a multicentre quantitative microbiological risk assessment for *T. gondii*. The data was sourced from a multicentre quantitative exposure survey and systematic literature reviews on *T. gondii* contamination of soil, water, fresh produce, and bivalve molluscs as well as on *T. gondii* prevalence in animals raised and hunted for human consumption as well as in cats, the definitive hosts of the parasite. An extensive literature review was performed to support the selection of the most suitable method to detect *T. gondii* oocysts in fresh produce, which led to the development, implementation, and validation of a Standard Operating Procedure, which is being applied in a multicentre survey on ready-to-eat salads.



### STRUCTURE



### ACHIEVEMENTS

Bioinformatic selection of promising antigens for a novel serology method was finalised and recombinant expression of selected proteins was conducted, as part of exploring serology for detecting *T. gondii* infections caused by oocysts. An unprecedented effort of whole genome sequencing of *T. gondii* isolates was used to identify polymorphic marker regions for the establishment of a new typing method to detect within-genotype variation and detect outbreaks as well as imported and recombinant strains.



### OUTCOMES

The main outcomes of TOXOSOURCES are: i) quantitative estimates of the contribution of the main sources and transmission routes of *T. gondii* infections across Europe, based on improved source attribution models; ii) new data filling the key knowledge gap about the role of increasingly popular but unstudied ready-to-eat (RTE) fresh produce; iii) a novel serological method that aims to specifically detect infections caused by oocysts; and iv) a novel typing method to improve preparedness to detect introduction of atypical *T. gondii* strains by import and to trace the infection sources in outbreaks.



### DISSEMINATION

The TOXOSOURCES consortium has actively disseminated its outcomes. All the results are integrated to contribute to developing efficient interventions at national, regional, European, and global levels. TOXOSOURCES will have immediate and long-term societal impact, and has potential, ability, and ambition to advance science.



### EDUCATION

### Publications in 2022:

Huertas-López, A., Contreras Rojo, M., Sukhumavasi, W., Martínez-Subiela, S., Álvarez-García, G., López-Ureña, N. M., Cerón, J. J., & Martínez-Carrasco, C. (2022). Comparative performance of five recombinant and chimeric antigens in a time-resolved fluorescence immunoassay for detection of *Toxoplasma gondii* infection in cats. *Veterinary Parasitology*. 304, 109703. DOI: <https://doi.org/10.1016/j.vetpar.2022.109703>

Calero-Bernal, R., Fernández-Escobar, M., Katzer, F., Su, C., & Ortega-Mora, L. M. (2022). Unifying Virulence Evaluation in *Toxoplasma gondii*: A Timely Task. *Frontiers in Cellular and Infection Microbiology*. 12, 868727. DOI: <https://doi.org/10.3389/fcimb.2022.868727>

Fernández-Escobar, M., Schares, G., Maksimov, P., Joeres, M., Ortega-Mora, L. M., & Calero-Bernal, R. (2022). *Toxoplasma gondii* Genotyping: A Closer Look Into Europe. *Frontiers in Cellular and Infection Microbiology*. 12, 842595. DOI: <https://doi.org/10.3389/fcimb.2022.842595>

Huertas-López, A., Sánchez-Sánchez, R., Diezma-Díaz, C., Álvarez-García, G., Martínez-Carrasco, C., Martínez-Subiela, S. & Cerón, J. J. (2022). Detection of anti-Neospora caninum antibodies in sheep's full-cream milk by a time-resolved fluorescence immunoassay. *Veterinary Parasitology*. 301, 109641. DOI: <https://doi.org/10.1016/j.vetpar.2021.109641>

Fernández-Escobar, M., Giorda, F., Mattioda, V., Audino, T., Di Nocera, F., Lucifora, G., Varelló, K., Grattarola, C., Ortega-Mora, L.M., Casalone, C., Calero-Bernal, R. (2022). *Toxoplasma gondii* Genetic Diversity in Mediterranean Dolphins. *Pathogens*. 11(8), 909. DOI: <https://doi.org/10.3390/pathogens11080909>

López Ureña NM., Chaudhry U., Calero Bernal R., Cano Alsua S., Messina D., Evangelista F, Betson M., Lalle M, Jokelainen P., Ortega Mora L.M., Álvarez García G. (2022). Contamination of Soil, Water, Fresh Produce, and Bivalve Mollusks with *Toxoplasma gondii* Oocysts: A Systematic Review. *Microorganisms*. 10(3), 517. DOI: <https://doi.org/10.3390/microorganisms10030517>

Kinnunen, P. M., Matomäki, A., Verkola, M., Heikinheimo, A., Vapalahti, O., Kallio-Kokko, H., Virtala, A. M., & Jokelainen, P. (2022). Veterinarians as a Risk Group for Zoonoses: Exposure, Knowledge and Protective Practices in Finland. *Safety and Health at Work*. 13(1), 78–85. DOI: <https://doi.org/10.1016/j.shaw.2021.10.008>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## ADONIS

The **ADONIS** project was formed to address the changing epidemiology of Salmonellosis, which is the second most common zoonotic pathogen in humans in the EU. Despite a significantly long-term decreasing incidence trend in humans this trend has now levelled off. The objective of the ADONIS project was to identify the determinants underlying the stagnation/reverse of the decreasing trends in the incidence of *Salmonella* Enteritidis (SE) in humans and poultry within member countries. The aim was to provide stakeholders and policy makers with anchor points to at least prevent continued stagnation or even reestablishment of *Salmonella* incidence through a One Health lens. The ADONIS project reviewed three areas, these were the primary production on poultry farms, the epidemiology of Salmonellosis, and the genomic level and accomplished a range of outcomes.

On the primary production it was concluded that there were some variations both in the way National Control Programmes (NCPs) were implemented as well as on the underlying poultry populations tested that could influence the performance of the surveillance activities. Despite the implementation of NCPs and their audit by the EU, ADONIS identified there were areas for improvement, including sampling, laboratory testing, reporting, restrictions, and measures taken along the food chain, as well as evaluations of NCPs progresses.

At the epidemiology level, it was shown that since 2015 the Netherlands and Belgium have had an upward trend in SE incidence. However, it was shown that the increase in the occurrence of potential outbreaks and invasive infections since 2015 could only partially explain the observed reduction trend. Furthermore, an analysis of outbreak data of the EU showed an increase in the number of outbreaks in the EU during 2015 - 2019 which was mainly caused by the increase in Eastern European related SE associated with eggs. It was concluded that neither changes in surveillance systems nor in the way Salmonellosis infects humans, nor the mechanisms by which humans become exposed are likely to explain the observed increases.

On the pathogen level, European SE was identified to be two separate major clades with little structure by collection date, source, or country of isolation. Genomic analysis revealed the wide-spread presence of plasmids carrying  $\beta$ -lactam resistance genes in humans, animals, and food. *In vivo* virulence assays showed an emergence in recent years of more virulent strains of SE in Europe. However, these more virulent strains appear randomly over genomic cluster and therefore cannot be responsible for the observed levelling off of in the Salmonellosis incidence.



### Publications in 2022:

Chanamé Pinedo, L., Mughini Gras, L., Franz, E., Hald, T., Pires, S. (2022). Sources and trends of human salmonellosis in Europe, 2015–2019: An analysis of outbreak data. *International Journal of Food Microbiology*. 379, 109850. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2022.109850>

Chanamé Pinedo, L., Franz, E., van den Beld, M., Van Goethem, N., Mattheus, W., Veldman, K., Bosch, T., Mughini-Gras, L., & Pijnacker, R. (2022). Changing epidemiology of *Salmonella* Enteritidis human infections in the Netherlands and Belgium, 2006 to 2019: a registry-based population study. *Euro Surveill*. 27(38), 2101174. DOI: <https://doi.org/10.2807/1560-7917.ES.2022.27.38.2101174>

Samper-Cativiela, C., Diéguez-Roda, B., Trigo da Roza, F., Ugarte-Ruiz, M., Elnekave, E., Lim, S., Hernández, M., Abad, D., Collado, S., Sáez, J. L., de Frutos, C., Agüero, M., Moreno, M. Á., Escudero, J. A., & Álvarez, J. (2022). Genomic characterisation of multidrug-resistant *Salmonella* serovar Kentucky ST198 isolated in poultry flocks in Spain (2011-2017). *Microbial Genomics*. 8(3), 000773. DOI: <https://doi.org/10.5281/zenodo.7082555>

## JOINT RESEARCH PROJECTS: FOODBORNE ZONOSSES (FBZ)



### INTRODUCTION

A multi-criteria decision analysis (MCDA) showed the rankings of the potential determinants and options for intervention for the stagnating SE trend in Europe pointed consistently to the level of poultry health and production. *Salmonella* control activities in poultry are harmonised across the EU for many years, but our results suggest that further improvements may be necessary for some countries.



### VISION

The ADNOIS project concluded with significant improvements in our understanding of Salmonellosis incidence and the underlying contributors to disease incidence by determining relevant anchor points. Further efforts should be undertaken to control *Salmonella* in the EU in order to restore a decrease in incidence.



### STRUCTURE

Dissemination activities throughout 2022 included three publications and a final meeting held in December 2022, in Pulawy, Poland.



### ACHIEVEMENTS



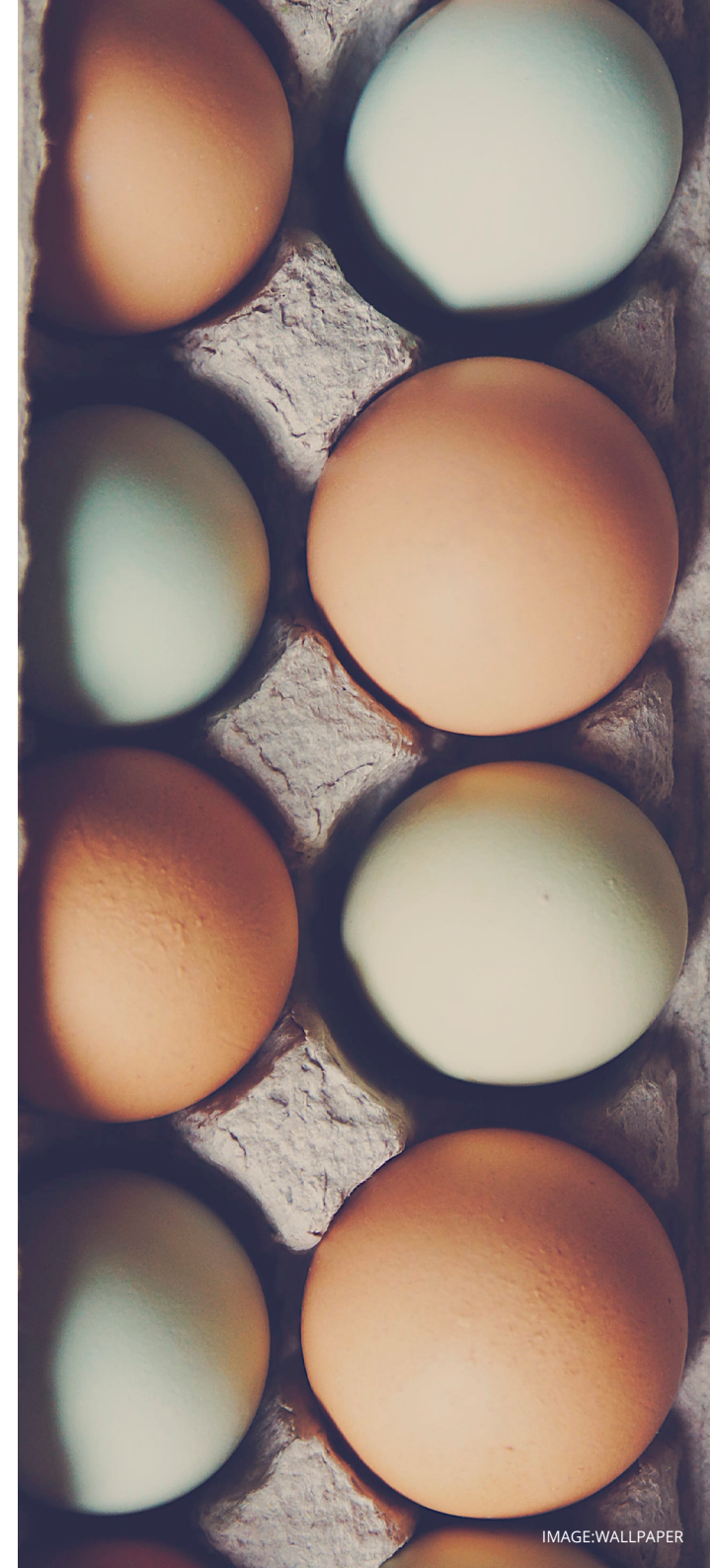
### OUTCOMES



### DISSEMINATION



### EDUCATION





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## BeOne

The BeONE project aimed to develop an integrated surveillance solutions in which molecular and epidemiological data for foodborne pathogens can be interactively analysed, visualised, and interpreted by experts across different sectors and disciplines. Surveillance of foodborne infections and outbreak distribution is primarily handled at the national or regional level. The cross-sectional and interdisciplinary datasets generated are complex, which requires the development of tools to facilitate data integration and analysis. The solutions developed by the BeONE project contribute to a sustainable and more efficient routine Food and Waterborne Disease surveillance, following a One Health approach.

In order to facilitate and enhance the connection between EU laboratories enrolled in the Food and Waterborne Disease (FWD) genomic surveillance, a digital structure for genomic and epidemiological data exchange was developed within BeONE. This structure relies on a harmonised data model that facilitates automated data exchange under legal and GDPR considerations, in agreement with the EFSA Data Collection Framework (DCF) and aims at cross-sector harmonisation with the ECDC. The BeONE data model is open to hold additional, non-harmonised information for further exploration. This structure promotes multi-country and intersectoral collaborations towards a One Health approach. To accommodate this structure, the BeONE Datahub has also been developed. This is a server-based web application that can work directly on the database of shared samples and metadata, and that launches **ReporTree**, a novel surveillance-oriented tool that strengthens the linkage between genomic and epidemiological data by rapidly identifying genetic clusters and generating surveillance-oriented reports for the derived clusters. In addition to its possible smooth integration in other surveillance-oriented workflows to enhance evidence-informed public-health decision making, **ReporTree** is also playing a central-role in the BeONE multi-country and inter-sectoral assessment of cluster congruence between the different genomic surveillance pipelines by facilitating and speeding-up the cluster detection and pipeline comparability. Furthermore, a BeONE algorithm to support outbreak detection was modelled and tested on *Listeria spp.*

In interconnection with the Datahub, epidata can be viewed and filtered in tables and geographically placed on a map and phylogenetic trees (with clustering data) can be visualised in the developed BeONE dashboard. Being provided as a standalone desktop tool and independent of a specific backend, the BeONE Dashboard can therefore be used to visualise data from multiple pipelines. The input format builds on the JSON format.

BeONE might open the possibility for different European laboratories to exchange data and interactively visualise the derived genetic clusters integrated with epidemiological and geographical data in the BeONE dashboard, in compliance with the 'lab-to-lab' data transfer agreements. Fully aligned with the One Health concept, BeONE may ultimately promote an enhanced interoperability at multi-country and intersectoral levels towards an evidence-informed public health policy- and decision-making.



### Publications in 2022:

Pista, A., Silveira, L., Ribeiro, S., Fontes, M., Castro, R., Coelho, A., Furtado, R., Lopes, T., Maia, C., Mixão, V., Borges, V., Sá, A., Soeiro, V., Correia, C.B., Gomes, J. P., Saraiva, M., Oleastro, M., Batista, R. (2022). Pathogenic *Escherichia coli*, *Salmonella spp.* and *Campylobacter spp.* in Two Natural Conservation Centers of Wildlife in Portugal: Genotypic and Phenotypic Characterisation. *Microorganisms*. 10(11), 2132. DOI: <https://doi.org/10.3390/microorganisms10112132>

Mixão, V., Pinto, M., Sobral, D., Di Pasquale, A., Gomes, J. P., and Borges, V. (2022). ReporTree: a surveillance-oriented tool to strengthen the linkage between pathogen genetic clusters and epidemiological data. *Research Square*. PPR552299. DOI: <https://doi.org/10.21203/rs.3.rs-1404655/v2>



### INTRODUCTION

### FARMED

Advances in sequencing technologies and bioinformatics have enabled easier detection of genetic material. The **FARMED** project allowed European institutes to assess the feasibility, and implementation, of long-read metagenome and resistome sequencing, to provide investigators with the early detection of pathogens to apply the most appropriate control measures.



### VISION

The FARMED project first identified and compared commercially available DNA extraction kits using simple and complex matrix samples (range covering the One Health spectrum), spiked with in-house or commercially available routinely identified bacterial species (mock communities). An important step in sequencing is the ability to extract DNA from samples, which is of suitable quality, quantity, and purity; however, the methods employed depend on the sample matrices. The feasibility of using long-read sequencing for species identification and antimicrobial resistance (AMR) detection in different sample matrices, in a diagnostic setting, was demonstrated. Hi-C sequencing was explored as a tool to define the context of AMR genes (e.g., linkage of plasmid to its host in metagenomics samples). Using a mock community, an assignment of AMR genes to species was possible with an accuracy, specificity, and sensitivity of >90%. However, the application of Hi-C sequencing is not yet standardised and far away from routine or field settings, both at wet lab and dry lab level.



### STRUCTURE

Since the inception of the FARMED project, the bioinformatics tool KMA (k-mer alignment) was further developed by one of the partners and thus was the preferred analysis tool. The tools KMA and ResFinder (<https://cge.food.dtu.dk/services/ResFinder/>) are ready-to-use to identify AMR genes from the sequencing data. Although KMA is a fast tool to process sequence data in real-time, the output required improvement to simplify and direct the user to the reliable results. For that purpose, FARMED developed a list of parameters, aiming to aid users in considering the confidence of presence of bacterial species within a sample.



### ACHIEVEMENTS

The FARMED project also worked on the implementation of on-site protocols for long-read metagenomic DNA sequencing, including isolation of DNA from sample matrices, long-read sequencing, and bioinformatics analysis, conducted outside of traditional laboratory setting. A review of the available literature identified several factors that were required and included four definitions of on-site sequencing with access to variable facilities such as internet connection, power for equipment and access to cold storage. Although this was not possible for all institutes, several were able to perform the required steps in the field/on-site or on-site simulation, with varying success. There are now kits available on the market enabling on-site DNA isolation which have been tested by the FARMED consortium with variable success. However, the current restricting barrier to accomplish full 'on-site' long-read metagenomics, including resistome analysis, is



### OUTCOMES



### DISSEMINATION



### EDUCATION



#### Publications in 2022:

D'aes, J., Fraiture, M-A., Bogaerts, B., De Keersmaecker, S. C. J., Roosens, N. H. C. J., Vanneste, K. (2022). Metagenomic Characterisation of Multiple Genetically Modified *Bacillus* Contaminations in Commercial Microbial Fermentation Products. *Life*. 12(12),1971. DOI: <https://doi.org/10.3390/life12121971>

## JOINT RESEARCH PROJECTS: ANTIMICROBIAL RESISTANCE (AMR)



### INTRODUCTION

the sequencing as this requires several hours to achieve the desired depth of sequencing and analysis steps, requiring sufficient computational power and time.



### VISION

FARMED has shown that long-read metagenomics is feasible for analysis of a variety of sample matrices, applicable to range of stakeholders, but still requires both wet-laboratory standardisation and bioinformatics automation to ensure confidence and accurate comparison of results between laboratories. For its full on-site application, some identified limitations should be addressed through further maturation of the technology.



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

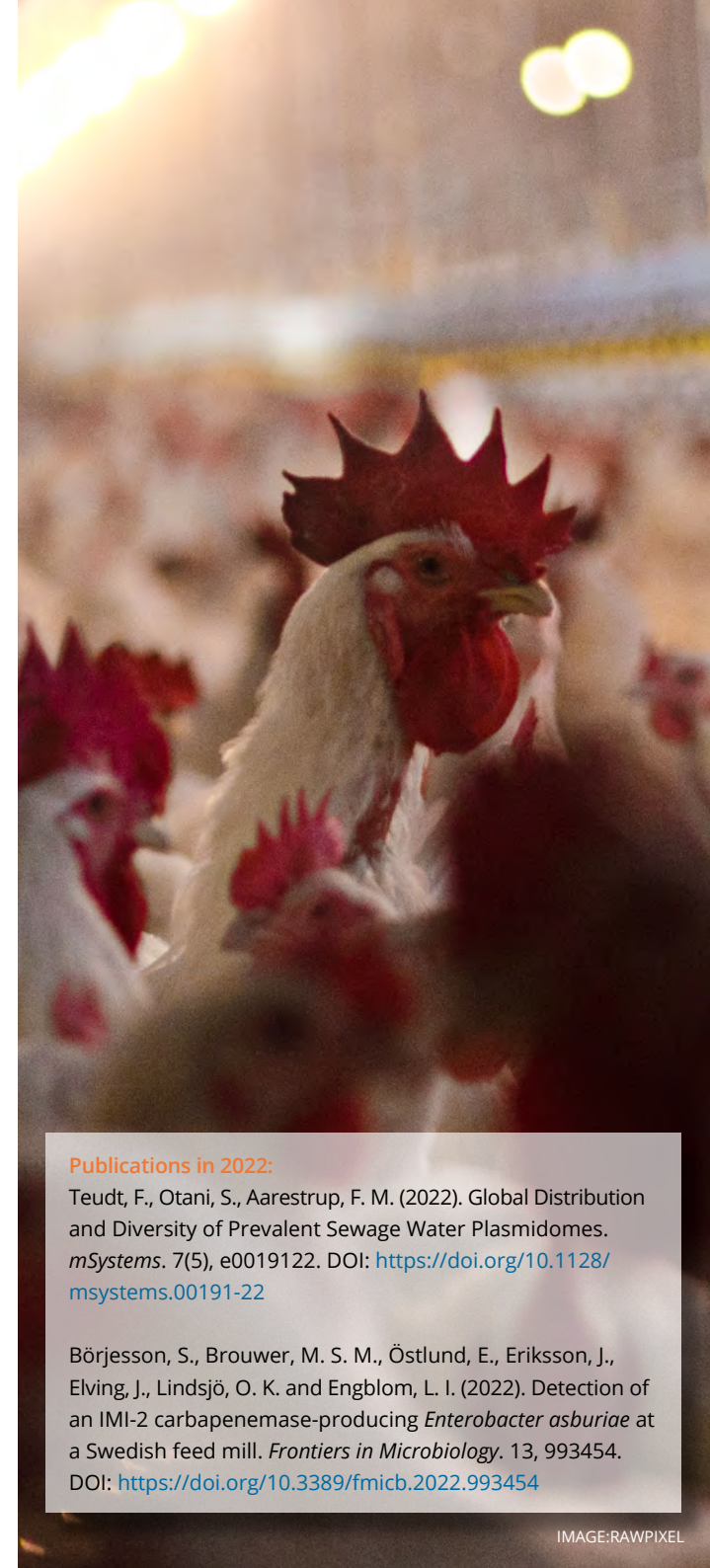
## FULL-FORCE

The **FULL-FORCE** project aimed to improve the surveillance of antimicrobial resistance (AMR) mediated by mobile genetic elements (MGEs), by introducing long-read sequencing methodology. It supplied 17 EU public health and veterinary partners with a technological toolbox and hands-on training in Single-Molecule Real-Time (SMRT) sequencing, enabling harmonisation of plasmid sequencing capacity. This knowledge was applied to six study cases and applications in metagenomics and AMR transmission models.

The FULL-FORCE project main outputs and achievements include: i) the successful harmonisation and delivery to the consortium of wet lab protocols on how to generate long-read sequencing data; ii) the construction and benchmarking of a common and open source bioinformatic pipeline, termed the **Full Force Plasmid Assembler** (FFPA) that allows harmonisation of bioinformatic skills needed for plasmid sequencing; and iii) the successful application of this methodology to six case studies. These case studies focused on: a) the identification of the *K. pneumoniae* plasmidome, in the context of carbapenem resistance; b) the detailed surveillance of the pESI megaplasmid, causing AMR in *Salmonella* Infantis throughout Europe, published in the following [research paper](#); c) the importance of IncZ plasmids in AMR horizontal transmission and the discovery of a new type on IncZ plasmids confined to bacteria isolated from sick patients; d) variations in plasmids in Extended Spectrum Beta-Lactamase (ESBL)-producing *E. coli* (AMR strains) isolated from horses; e) expanding the mining from metagenomics datasets; and f) transmission of AMR in isolates among broilers using two different types of transmission models that were able to describe the observed transmission dynamics within and between the production stages and quantitatively estimate the outcome of the interventions.

Thanks to its innovative methodologies, the FULL-FORCE project led to new discoveries in the field of AMR detection and surveillance. It was discovered the combination of both long- and short-read sequencing, termed hybrid sequencing, is the most reliable approach for AMR typing and risk assessment. Using this approach, the FULL-FORCE project uncovered; 1) the first evidence in Europe of an MDR, *bla*<sub>NDM-4</sub>-positive *Escherichia coli* isolated from a food-producing animal, harboured by a novel IncFII plasmid, published in a [research paper](#); and 2) a carbapenem-resistant isolate of *Enterobacter cloacae* complex that was also discovered and identified as *Enterobacter asburiae*, learn more by reading the research publication.

The FULL-FORCE project enabled the harmonisation of plasmid sequencing capacities across 17 public health and veterinary institutes and provided an unprecedented amount of open access data on fully sequenced bacterial strains, which can be used in follow-up studies. It also prepared public health institutes for an 'AMR surveillance 2.0' based on plasmid data, and by doing so, it paved the way for detailed surveillance of AMR transmission in the next decade.



### Publications in 2022:

Teudt, F., Otani, S., Aarestrup, F. M. (2022). Global Distribution and Diversity of Prevalent Sewage Water Plasmidomes. *mSystems*. 7(5), e0019122. DOI: <https://doi.org/10.1128/mSystems.00191-22>

Börjesson, S., Brouwer, M. S. M., Östlund, E., Eriksson, J., Elving, J., Lindsjö, O. K. and Engblom, L. I. (2022). Detection of an IMI-2 carbapenemase-producing *Enterobacter asburiae* at a Swedish feed mill. *Frontiers in Microbiology*. 13, 993454. DOI: <https://doi.org/10.3389/fmicb.2022.993454>



### INTRODUCTION

### WORLDCOM

The **WORLDCOM** project aimed to develop diagnostic tools linked with mobile referencing technology for detection of antimicrobial resistance (AMR) in zoonotic pathogens. Resulting technologies would enable rapid on-site detection of AMR in pathogens from animal and human populations, and the environment, facilitating early investigation of emerging resistances and the development of machine-learning algorithms for AMR detection and prediction.



### VISION

Extended-spectrum beta-lactamase (ESBL) and carbapenemase enzymes produced by *Enterobacteriaceae* confer resistance to beta-lactam antimicrobials presenting a major public health concern. A genomic approach was used to analyse publicly available sequences of relevant pathogens (*Escherichia coli*, *Klebsiella spp.*, *Salmonella spp.*, and *Acinetobacter spp.*) for circulating antibiotic resistance genes (ESBL, carbapenemase and mobile colistin resistance genes). Based on this information, targeted *de novo* sequencing and phenotypic characterisation of bacterial pathogens from human, animal and environmental origin were performed among partners, resulting in the generation of an in-house database for use in assay development. Biomarker genes associated with highly prevalent ESBLs, carbapenemases and colistin resistance were selected as AMR diagnostic targets. Loop-mediated isothermal amplification was selected as the *in vitro* amplification technology for detection of these targets due to its speed, specificity, and robustness, as well as its ease of application in the field, including on-farm and/or in remote settings.



### STRUCTURE



### ACHIEVEMENTS

Additionally, six AMR biomarkers were targeted using real-time LAMP assays. The assays were developed, optimised, and validated against a wide range of Gram-positive and Gram-negative bacterial isolates to confirm sensitivity and specificity using both fluorescent and colorimetric detection. The assays were further developed and implemented to successfully detect AMR biomarkers from environmental water and pig faecal samples within less than an hour. Additionally, a smartphone app has been developed to provide automated detection for LAMP diagnostic tests. The smartphone app automatically analyses captured images of colorimetric LAMP assays to facilitate field testing.



### OUTCOMES



### DISSEMINATION

Closely related ESBL biomarkers, preferentially associated with animal and human infection, were targeted with an internally controlled multiplex loop-primer endonuclease cleavage (LEC)-LAMP assay enabling differential point-mutation detection. Assay validation using: i) isolates from animal, human or environmental sources; and ii) DNA isolated directly from animal and environmental samples both demonstrated rapid detection (10 – 20 min), complete analytical specificity, and single-digit genome copy sensitivity. Development of a portable workstation incorporating the LEC-LAMP assay with a rapid



### EDUCATION

### Publications in 2022:

Higgins, O., Chueiri, A., O'Connor, L., Lahiff, S., Burke, L. P., Morris, D., Pfeifer, N. M., González Santamarina, B., Berens, C., Menge, C., Caniça, M., Manageiro, V., Kisand, V., Hassan, M. M., Gardner, B., van Vliet, A. H. M., La Ragione, R. M., Gonzalez-Zorn, B. & Smith, T. J. (2022). Portable Differential Detection of CTX-M ESBL Gene Variants, *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-15</sub>, from *Escherichia coli* Isolates and Animal Fecal Samples Using Loop-Primer Endonuclease Cleavage Loop-Mediated Isothermal Amplification. *Microbiology Spectrum*. e03316-22. Advance online publication. DOI: <https://doi.org/10.1128/spectrum.03316-22>

González-Santamarina, B., Weber, M., Menge C., Berens, C. (2022). Comparative Genomic Analysis of Antimicrobial Resistant *Escherichia coli* from South American Camelids in Central Germany. *Microorganisms*. 10(9), 1697. DOI: <https://doi.org/10.3390/microorganisms10091697>

Manageiro, V., Salgueiro, V., Rosado, T., Bandarra, N. M., Ferreira, E., Smith, T., Dias, E., Caniça, M. (2022). Genomic Analysis of a *mcr-9.1*-Harbouring IncHI2-ST1 Plasmid from *Enterobacter ludwigii* Isolated in Fish Farming. *Antibiotics*. 11(9), 1232. DOI: <https://doi.org/10.3390/antibiotics11091232>

Thomson, N. M., Gilroy, R., Getino, M., Foster-Nyarko, E., van Vliet, A, H, M., La Ragione, R. M., & Pallen, M. J. (2022). Remarkable genomic diversity among *Escherichia* isolates recovered from healthy chickens. *Peer J*. 10, e12935. DOI: <https://doi.org/10.7717/peerj.12935>

Tedersoo, T., Roasto, M., Mäesaar, M., Häkkinen, L., Kisand, K., Ivanova, M., Valli, M. H., & Meremäe, K. (2022). Antibiotic Resistance in *Campylobacter spp.* Isolated from Broiler Chicken Meat and Human Patients in Estonia. *Microorganisms*. 10(5), 1067. DOI: <https://doi.org/10.3390/microorganisms10051067>

González-Santamarina, B., Schnee, C., Koehler, H., Weber, M., Methner, U., Seyboldt, C., Berens, C. & Menge, C. (2022). Survey on shedding of selected pathogenic, zoonotic or antimicrobial resistant bacteria by South American camelids in Central Germany. *Berliner Und Münchener Tierärztliche Wochenschrift*. 135, 1–16. DOI: <https://doi.org/10.2376/1439-0299-2021-21>



## JOINT RESEARCH PROJECTS: ANTIMICROBIAL RESISTANCE (AMR)



### INTRODUCTION

DNA extraction protocol and validation for on-site application was completed with a research article relating to this work accepted for publication with *Microbiology Spectrum*. The WORLDCOM project developed and validated portable nucleic acid diagnostics applied to differential detection of prevalent ESBLs and carbapenemases, providing novel transferable diagnostic technology for improved epidemiological surveillance.



### VISION



### STRUCTURE



### ACHIEVEMENTS



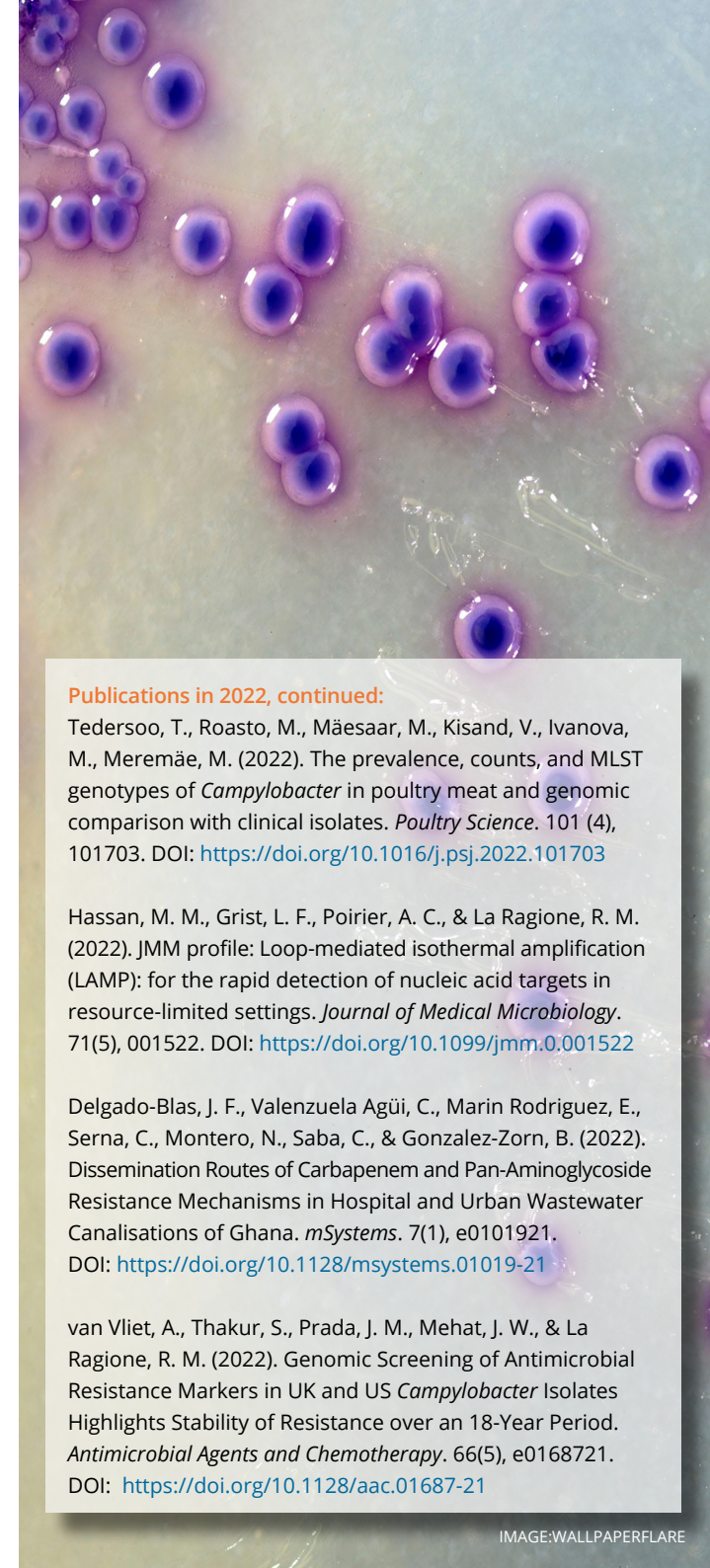
### OUTCOMES



### DISSEMINATION



### EDUCATION



#### Publications in 2022, continued:

Tedersoo, T., Roasto, M., Mäesaar, M., Kisand, V., Ivanova, M., Meremäe, M. (2022). The prevalence, counts, and MLST genotypes of *Campylobacter* in poultry meat and genomic comparison with clinical isolates. *Poultry Science*. 101 (4), 101703. DOI: <https://doi.org/10.1016/j.psj.2022.101703>

Hassan, M. M., Grist, L. F., Poirier, A. C., & La Ragione, R. M. (2022). JMM profile: Loop-mediated isothermal amplification (LAMP): for the rapid detection of nucleic acid targets in resource-limited settings. *Journal of Medical Microbiology*. 71(5), 001522. DOI: <https://doi.org/10.1099/jmm.0.001522>

Delgado-Blas, J. F., Valenzuela Agüi, C., Marin Rodriguez, E., Serna, C., Montero, N., Saba, C., & Gonzalez-Zorn, B. (2022). Dissemination Routes of Carbapenem and Pan-Aminoglycoside Resistance Mechanisms in Hospital and Urban Wastewater Canalizations of Ghana. *mSystems*. 7(1), e0101921. DOI: <https://doi.org/10.1128/msystems.01019-21>

van Vliet, A., Thakur, S., Prada, J. M., Mehat, J. W., & La Ragione, R. M. (2022). Genomic Screening of Antimicrobial Resistance Markers in UK and US *Campylobacter* Isolates Highlights Stability of Resistance over an 18-Year Period. *Antimicrobial Agents and Chemotherapy*. 66(5), e0168721. DOI: <https://doi.org/10.1128/aac.01687-21>



### INTRODUCTION

### FED-AMR

Bacterial transformation contributes to horizontal gene transfer (HGT) of antimicrobial resistance genes (ARGs), but there is a lack of empirical evidence on the impact of bacterial transformation in the environment. This is addressed through the **FED-AMR** project, which aimed to demonstrate the importance of extracellular free DNA (exDNA) as an environmental reservoir for ARGs. ARG concentrations, diversity, mobility, and bacterial biodiversity were determined in an annual longitudinal study that covered a crop growth season. Different fertilisation and land management techniques, and environmental compartments were investigated.



### VISION

In the final year of the FED-AMR project, a number of outputs and impacts were achieved to meet the scientific, societal, policy, and economic impact imparted on European countries as a result of antimicrobial resistance (AMR). The FED-AMR project consisted of six work packages that addressed the objectives of the joint research project.



### STRUCTURE

Firstly, the FED-AMR project aimed to quantify and assess microbial and AMR diversity using a gene enrichment approach at different stages of the food production chain during a 1-year crop growing season. The bacteria present within the environmental compartments of the food production chain were concluded to have the ability to take up exDNA and serve as receptors for ARG. Secondly, the role of *Clostridioides difficile* as a pathogen/ARG transfer platform over ecosystem boundaries and the genetic overlap between human and non-human zoonotic reservoirs was investigated. It was determined that at the farm-level, dominant clones in environmental compartments associated with pig production were identified, suggesting a transmission chain between compartments involving these animals is possible.



### ACHIEVEMENTS



### OUTCOMES

Thirdly, the selection pressures of pathogens within environmental ecosystems were elucidated, with the highest concentrations of antimicrobials in manure and faeces likely to select for ARGs. Fourthly, an *in vivo* pig gut model was created and optimised (pH, temperature, flow rates, and volumes) to represent the complex natural microbial community within the gut. The gut model demonstrated that pigs may have the ability to sufficiently grow and maintain *E. coli* J53 strain as a recipient of exDNA. Finally, a **protocol was created** for the systematic review of factors that influence the prevalence of AMR in the environment and the formulation and validation of a mathematical model for the dynamics of microbial communities.



### DISSEMINATION



### EDUCATION

Overall, the scientific impacts of the FED-AMR project include: i) advancements in high-throughput sequencing methods and analytical tools, allowing for the large scalability necessary to investigate

#### Publications in 2022:

Gardner, B., Betson, M., Cabal Rosel, A., Caniça, M., Chambers, M., Contadini, F. M., Gonzales Villetea, L. C. Hassan, M. M., La Ragione, R., De Menezes, A., Messina, D. Nichols, G., Olivenca, D. V., Phalkey, R., Prada, J. M., Ruppitsch, W., Santorelli, L. A., Selemetas, N., Tharmakulasingam, M., van Vliet, A. H. M., Wögerbauer, M., Deza-Cruz, I., Lo Iacono, G. (2022) Factors associated with the prevalence of antibiotic resistance in the environment from a One Health perspective: Protocol for a systematic evidence map. *Center for Open Science*. DOI: <https://doi.org/10.17605/OSF.IO/A8GV6>

Alves, F., Cano, M., Brondani, G., Nunes, A., & Oleastro, M. (2022). Airborne spores' dissemination of a swine associated *Clostridioides difficile* clone. *Anaerobe*. 78, 102651. DOI: <https://doi.org/10.1016/j.anaerobe.2022.102651>

Alves, F., Nunes, A., Castro, R., Sequeira, A., Moreira, O., Matias, R., Rodrigues, J. C., Silveira, L., Gomes, J. P., & Oleastro, M. (2022). Assessment of the Transmission Dynamics of *Clostridioides difficile* in a Farm Environment Reveals the Presence of a New Toxigenic Strain Connected to Swine Production. *Frontiers in Microbiology*. 13, 858310. DOI: <https://doi.org/10.3389/fmicb.2022.858310>

Cabal, A., Rab, G., Daza-Prieto, B., Stöger, A., Peischl, N., Chakeri, A., Mo, S.S., Bock, H., Fuchs, K., Sucher, J., Rathammer, K., Hasenberger, P., Stadtbauer, S., Caniça, M., Strauß, P., Allerberger, F., Wögerbauer, M., Ruppitsch, W. (2022). Characterising Antimicrobial Resistance in Clinically Relevant Bacteria Isolated at the Human/Animal/Environment Interface Using Whole-Genome Sequencing in Austria. *International Journal of Molecular Sciences*. 23(19), 11276. DOI: <https://doi.org/10.3390/ijms231911276>

## JOINT RESEARCH PROJECTS: ANTIMICROBIAL RESISTANCE (AMR)



### INTRODUCTION

bacterial communities by exploring and mapping AMR in exDNA through a One Health lens; ii) ARG monitoring in environmental compartments; and iii) using a One Health concept to identify environmental component such as farmers, pigs, wild animals, manure, air from pig barns, feeds, crops, soil, and water as potential reservoirs of AMR.



### VISION

While, the societal, policy-making and economic impacts included: i) producing impactful research that adds value to the European, national and internal level; ii) evaluating exDNA as a high-risk source of resistance determinants in agricultural soils and along the food/feed chain; iii) improving strategies to improve existing wastewater treatment plants; iv) evidencing the essential use of reliable and accurate surveillance to track the spread of specific ARGs geographically and over time, identifying new ARFs and supporting preventive measures against AMR pathogens; and lastly V) systematic evidence to inform future research, policy-relevant systematic review questions, and future funding strategies, concerning risk mitigation for antibiotic resistance emerging in the environment.



### STRUCTURE

In conclusion, the FED-AMR project has a societal, policy, and economic impact, as well as scientific impact through cross-sector communication of data contributing to the advancement of science. This project used the true concept of One Health, namely with an important environmental component (farmers, pigs, wild animals, manure, air from pig barns, feeds, crops, soil, water).



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## TELE-Vir

The SARS-CoV-2 pandemic and the reoccurrence of emerging virus pathogens highlighted the importance of rapid diagnostics and improved virus detection methods. The aim of the **TELE-Vir** project was to develop a fast point-of-evidence toolbox for identification and characterisation of emerging virus threats for human and/or domestic and wildlife animals. The TELE-Vir project combined 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.

More specifically, the TELE-Vir project developed a point of incidence (POI) toolbox, for metagenomic virus detection in the field that will contribute to the identification of known viruses, as well as of emerging virus threats. Metagenomic next generation sequencing (mNGS) has the ability to identify pathogens in a hypothesis-free manner compared to targeted strategies (Fomsgaard *et al.*, *J. Clin. Virol. Plus*, 2(4), 100120 (2022)). This is of advantage to conventional diagnostic tests, since different pathogens can produce similar symptoms, which makes it difficult to choose for a specific (targeted) test. The POI toolbox includes methods that can be transferred into a field setting to detect any kind of virus, in animals and humans, even in geographical areas without available laboratory infrastructure.

The project also established a POI protocol that includes sample pre-treatment, nucleic acids extraction, isothermal random amplification followed by non-targeted sequencing using the Oxford Nanopore Technology (ONT) system. It requires a minimum of laboratory equipment to be field employable and is relatively fast. This approach was supported by: i) improving and adapting the 'surveillance oriented' **INSaFLU-TELEVIR** platform to handle sequence data (ONT, Illumina and Ion Torrent) of any virus (from reads to quality control, mutations detection, consensus generation, classification, alignments, genotype-phenotype screening, phylogenetics, integrative phylogeographical and temporal analysis); and ii) by developing, benchmarking and implementing a novel pathogen detection module, from reads and quality control for the identification of both RNA and DNA viruses.

The POI toolbox was successfully tested in proof-of-concept studies by the TELEVIR members, for example under different conditions in the field in Norway, South France, and West Africa or by testing diverse sample material frozen and fresh material from *in vivo* laboratory studies to compare the POI protocol to well established detection methods. Sequencing data obtained from the partners performing the wet-lab activities were analysed with the INSaFLU-TELEVIR platform, as a complement to the multiple sample/virus tests performed during the tool development, benchmarking, and final refinement. A



## JOINT RESEARCH PROJECTS: EMERGING THREATS (ET)



### INTRODUCTION

broad range of RNA as well as DNA viruses could be detected in different sample materials, proving a good symbiosis between the developed POI protocol and the INSaFLU-TELEVIR platform, towards the deployment of a 'start-to-end' framework that can potentiate strengthened timely metagenomics virus detection and routine genomics surveillance. The POI toolbox will be made publicly available and will be disseminated across national and international parties.



### VISION

In summary, the TELEVIR POI toolbox allows for an easy, rapid and user friendly *in situ* identification with immediate characterisation and hence a fast response and improved decision-making during an outbreak or a potential outbreak of an emerging or re-emerging virus threat. This will aid in the overall outbreak preparedness in Europe.



### STRUCTURE

The dissemination of TELE-Vir research findings has included scientific publications, presentations at workshops, webinars, and conferences. The 2nd TELE-Vir consortium annual meeting was held in June 2022, together with a workshop where TELEVIR partners were trained to use the point-evidence toolbox.



### ACHIEVEMENTS



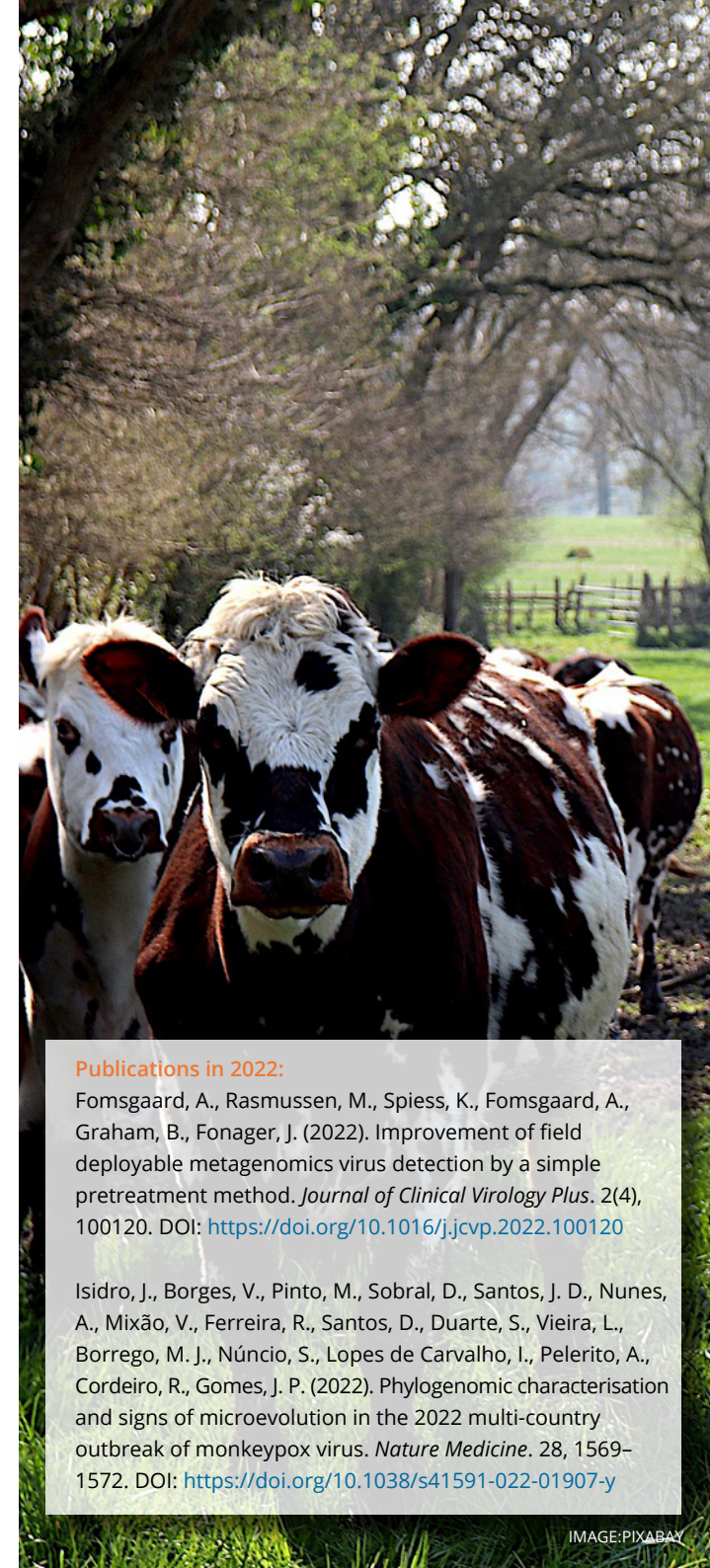
### OUTCOMES



### DISSEMINATION



### EDUCATION



#### Publications in 2022:

Fomsgaard, A., Rasmussen, M., Spiess, K., Fomsgaard, A., Graham, B., Fonager, J. (2022). Improvement of field deployable metagenomics virus detection by a simple pretreatment method. *Journal of Clinical Virology Plus*. 2(4), 100120. DOI: <https://doi.org/10.1016/j.jcvp.2022.100120>

Isidro, J., Borges, V., Pinto, M., Sobral, D., Santos, J. D., Nunes, A., Mixão, V., Ferreira, R., Santos, D., Duarte, S., Vieira, L., Borrego, M. J., Nuncio, S., Lopes de Carvalho, I., Pelerito, A., Cordeiro, R., Gomes, J. P. (2022). Phylogenomic characterisation and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. *Nature Medicine*. 28, 1569–1572. DOI: <https://doi.org/10.1038/s41591-022-01907-y>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## MEmE

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

The MEmE project main outputs and achievements include: i) the production of Standard Operating Procedures (SOPs) for the sampling of different matrices from naturally or experimentally infected definitive and intermediate animal hosts; ii) the validation of the parasitological (SSCT) and molecular diagnostic (multiplex- and MC-RT-PCRs) procedures to detect Em and Eg in different matrices along the food chain; iii) the development, validation and comparison of new molecular tools (comparison of DNA extraction and PCRs assays, novel probe-based qPCRs, PCR-RFLPs and multiplex PCR assays and NGS approach); iv) multicentre studies for the production of data relevant for epidemiological assessments: contamination of fresh vegetables for human consumption by Em/Eg; prevalence of Em/Eg in dog faeces; v) quantitative assessment on the impact of human CE and AE in Europe by means of systematic review approach; and vi) molecular and clinical epidemiology studies in selected geographical areas.

One of the main achievements of the MEmE project is the publication of a quantitative research article in *The Lancet Infectious Diseases*, as referenced in the publications list. This systematic review aims to decrease the limit of uncertainty on the impact of human CE in Europe.

MEmE project results were disseminated at different levels and to varied audiences (including the general public, scientific community, experts, health authorities and media).

The MEmE project impacted on animal health, public health, and food safety sectors. Beneficiaries of scientific outputs of MEmE are EU reference labs, international organisations, and all decision makers. In summary, MEmE has developed a set of molecular tools, epidemiological risk assessments and quantitative epidemiological models for the detection, surveillance, and control of *Echinococcus spp.* parasitic infectious diseases in Europe and beyond.

### Publications in 2021:

Casulli, A., Abela-Ridder, B., Petrone, D., Fabiani, M., Bobić, B., Carmena, D., Šoba, B., Zerem, E., Gargatè, M. J., Kuzmanovska, G., Calomfirescu, C., Rainova, I., Sotiraki, S., Lungu, V., Dezsényi, B., Herrador, Z., Karamon, J., Maksimov, P., Oksanen, A., Milion, L., Sviben, M., Shkjezi, R., Gjoni, V., Akshija, I., Saarma, U., Torgerson, P., Šnábel, V., Antolová, D., Muhovic, D., Besim, H., Chereau, F., Belhassen G. M., Chappuis, F., Gloor, S., Stoeckle, M., Müllhaupt, B., Manno, V., Santoro, A., Santolamazza, F. (2022). Unveiling the incidences and trends of the neglected zoonosis cystic echinococcosis in Europe: a systematic review from the MEmE project. *The Lancet Infectious Diseases*. 23(3), e95–e107. DOI: [https://doi.org/10.1016/S1473-3099\(22\)00638-7](https://doi.org/10.1016/S1473-3099(22)00638-7)

Tamarozzi, F., Kibona, T., de Glanville, W.A., Mappi, T., Adonikamu, E., Salewi, A., Misso, K., Maro, V., Casulli, A., Santoro, A., Santolamazza, F., Mmbaga, B. T. & Cleaveland, S. (2022). Cystic echinococcosis in northern Tanzania: a pilot study in Maasai livestock-keeping communities. *Parasites & Vectors*. 15(1), 396. DOI: <https://doi.org/10.1186/s13071-022-05518-x>

Casulli, A., Massolo, A., Saarma, U., Umhang, G., Santolamazza, F., Santoro, A. (2022). Species and genotypes belonging to *Echinococcus granulosus sensu lato* complex causing human cystic echinococcosis in Europe (2000–2021): a systematic review. *Parasites Vectors*. 15, 109. DOI: <https://doi.org/10.1186/s13071-022-05197-8>

Korhonen, P.K., Kinkar, L., Young, N.D. *et al.* (2022). Chromosome-scale *Echinococcus granulosus* (genotype G1) genome reveals the Eg95 gene family and conservation of the EG95-vaccine molecule. *Communications Biology*. 5, 199. DOI: <https://doi.org/10.1038/s42003-022-03125-1>

Issa, A.R., Arif, S.H., Mohammed, A.A., Santolamazza, F., Santoro, A., Mero, W.M.S., Casulli, A. (2022). Insights into Human Cystic Echinococcosis in the Kurdistan Region, Iraq: Characteristics and Molecular Identification of Cysts. *Pathogens*. 11(4), 408. DOI: <https://doi.org/10.3390/pathogens11040408>

Peruzzo, A., Mastrandrea, S., Fancellu, A., Bonelli, P., Muehlethaler, K., Masala, G., Santucci, C. (2022). Comparison and evaluation of analytic and diagnostic performances of four commercial kits for the detection of antibodies against *Echinococcus granulosus* and multilocularis in human sera. *Comparative Immunology, Microbiology and Infectious Diseases*. 86, 101816. DOI: <https://doi.org/10.1016/j.cimid.2022.101816>

Khan, H., Casulli, A., Harandi, M.F., Afzal, M.S., Saqib, M.A.N., Ahmed, H. A (2022). Retrospective Cohort Study on Human Cystic Echinococcosis in Khyber Pakhtunkhwa Province (Pakistan) Based on 16 Years of Hospital Discharge Records. *Pathogens*. 11, 194. DOI: <https://doi.org/10.3390/pathogens11020194>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## PARADISE

Foodborne parasites are major contributors to the global burden of gastrointestinal disease. In Europe, protozoa of the genera *Cryptosporidium* and *Giardia* are relevant, and have been associated with large outbreaks linked to contaminated water and food. Outbreak investigation and source attribution remain difficult, due to the lack of standardised typing methods. The PARADISE project successfully delivered informative genotyping schemes and innovative isolation strategies applicable to food and environmental matrices for the zoonotic pathogens *Cryptosporidium parvum* and *Giardia duodenalis*.

The PARADISE project has generated data providing the first large-scale analysis of the genomes of these organisms. The project also delivered improved strain-typing schemes for better investigations of outbreaks and understanding of the epidemiology (e.g., zoonotic transmission). Project work on enrichment strategies has delivered tools enabling cost-effective investigation of food and environmental matrices, leading to improved risk assessment.

The main outputs and achievements of the PARADISE project are summarised in more detail below.

A large sequencing effort resulted in the generation of about 130 whole genome sequences (WGS) from *C. parvum* (humans and ruminants), 50 WGS from *G. duodenalis* assemblage B (mostly from humans) and about 70 WGS from *G. duodenalis* assemblage A (humans and other mammals). Comparative genomic studies of this collection of European WGS are shedding light on the population structure, virulence, and evolution of these parasites, and providing insights for future functional studies. Ongoing collaborations with European Union Reference Laboratories for bacterial and viral pathogens are helping to foster the use of WGS for parasitic pathogens. The applicability of metagenomics for the detection of parasites in various biological, water and food samples, was critically evaluated by *in silico* and experimental studies. The analyses show that efficient detection can also be achieved in the presence of excess background material of eukaryotic origin (e.g., a vegetable matrix), but that confirmation of the results obtained by k-mer, or mapping procedure is essential to avoid false positives.

New multi-locus sequence typing (MLST) schemes for both *C. parvum* and *G. duodenalis* assemblage B have been designed and tested on hundreds of isolates from different hosts and geographic origin. The schemes demonstrated a high discriminatory power and represent valuable tools for outbreak investigations and epidemiological studies. Standard Operating Procedures (SOPs) are available and ring tests among consortium partners showed efficient implementation of the typing schemes.



## JOINT RESEARCH PROJECTS: EMERGING THREATS (ET)



Adoption of the two MLSTs by Reference Laboratories will address the specific need for robust molecular typing methods recently highlighted by EFSA.

### INTRODUCTION



### VISION

A post-DNA extraction capture system for *Cryptosporidium*, based on probes targeting the 18S rDNA and the gp60 genes, have been designed and extensively characterised, and demonstrated to have high sensitivity in interlaboratory studies. This technique will allow investigation of DNA extracts from samples from outbreaks of unknown aetiology.



### STRUCTURE

The pre-DNA enrichment strategies based on nanobody, and aptamer technologies culminated in the selection of nanobodies with high specificity towards *Giardia* cysts. These reagents could be used on matrices (e.g., food, water) contaminated with low number of parasites, whose detection is relevant for risk assessment.

In summary, PARADISE project has developed novel genotyping schemes and detection strategies for *Cryptosporidium* and *Giardia* parasites beneficial for outbreak investigation and risk assessment.



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION



#### Publications in 2021:

Klotz, C., Sannella, A.R., Weisz, F., Chaudhry, U., Sroka, J., Tůmová, P., Nohýnková, E., Ignatius, R., Aebischer, T., Betson, M., Troell, K. & Cacciò, S. (2022). Extensive testing of a multi-locus sequence typing scheme for *Giardia duodenalis* assemblage A confirms its good discriminatory power. *Parasites Vectors*. 15, 489. DOI: <https://doi.org/10.1186/s13071-022-05615-x>

Franceschelli, A., Bonadonna, L., Cacciò, S. M., Sannella, A. R., Cintori C., Gargiulo R., Coccia A. M., Paradiso R., Iaconelli M., Briancesco R. and Tripodi A. (2022). An outbreak of cryptosporidiosis associated with drinking water in north-eastern Italy, August 2019: microbiological and environmental investigations. *Eurosurveillance*. 27(35), 2200038. DOI: <https://doi.org/10.2807/1560-7917.ES.2022.27.35.2200038>

Chihi, A., O'Brien Andersen, L., Aoun, K., Bouratbine, A., & Stensvold, C. R. (2022). Amplicon-based next-generation sequencing of eukaryotic nuclear ribosomal genes (metabarcoding) for the detection of single-celled parasites in human faecal samples. *Parasite epidemiology and control*. 17, e00242. DOI: <https://doi.org/10.1016/j.parepi.2022.e00242>





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

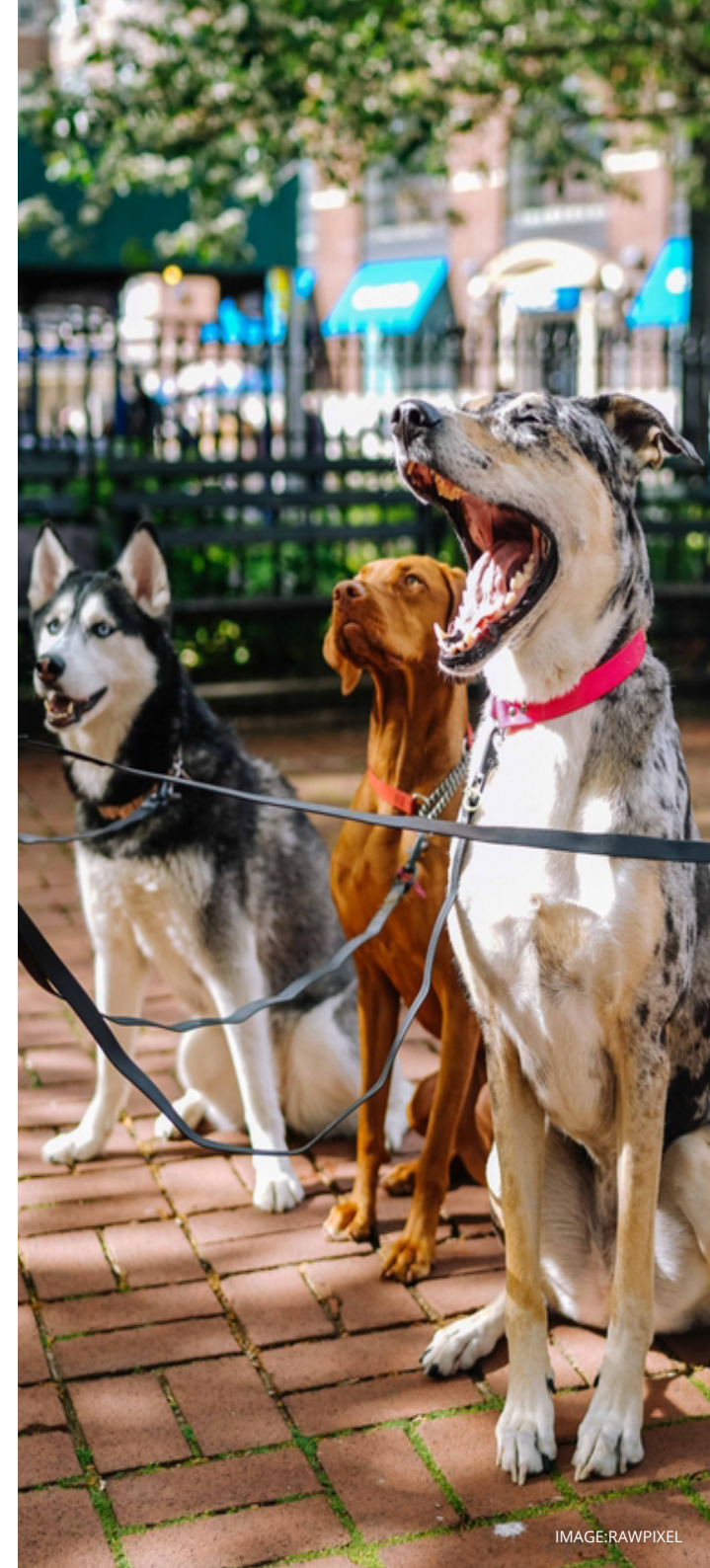
## IDEMBRU

The aims of the **IDEMBRU** project were to develop a toolkit for diagnostics, characterisation and assessment of the zoonotic potential of newly discovered *Brucella* strains, and identify unexplored wildlife and environmental reservoirs for core clade *Brucella* species. The core clade *Brucella spp.* are one of the most pathogenic bacteria described, which historically have been used for bioterrorism, while zoonotic potential of newly discovered species, until now, has not been analysed. Since IDEMBRU project members consisted of EU, and national reference and risk assessment laboratories, project results had direct impact on surveillance and management of brucellosis using the One Health concept.

IDEMBRU analysed sample collections from wildlife, environment, and humans to identify the presence of noncore clade *Brucella spp.* and new or emerging reservoirs for core clade species. This research prioritised genetic, phenotypic comparisons and development of *in vitro* model for characterisation of their zoonotic potential. These samples were processed and characterised at least by molecular or bacteriological methods. A panel of environmental strains of bacteria like *Brucella - Ochrobactrum spp.*, have been isolated, with genetic typing and phenotypic characterisation performed to compare with highly zoonotic *Brucella spp.*

During this project, newly emerging *Brucella canis*, from core type species spread quickly in different canine populations (adopted dogs in UK, breeding kennels in France and Italy), and three human cases were reported in Germany, the Netherlands and in the UK in 2022, as first occurrences in Europe. Since *B. canis* biology, pathogenicity, and host interactions many data are still missing, one part of the project was dedicated to this topic. To obtain *B. canis* strains, clinical canine and epidemiologically related samples were analysed in France, Italy, Portugal, and the UK. In Portugal, human blood, and tissue samples from selected professions at risk were analysed, with indirect and direct diagnostics compared. These results also compared the performance of diagnostic tools for *B. canis*. The network of human diagnostic laboratories was created to reinforce surveillance and create early warning systems for human brucellosis outbreaks, especially for the atypical cases and epidemiological sources.

DNA extraction methods from complex matrices (water, soil, and animal) and canine faeces were evaluated. The whole genome sequencing protocols were harmonised to obtain high quality short read libraries and are ready as part of the final toolkit. The environmental bacteria *Ochrobactrum spp.* has been added to the *Brucella* genus since several species are considered opportunistic pathogens and share more than 85% of genome with *Brucellae*. Isolated *Ochrobactrum spp.* strains are morphologically variable, which led to construction of specific primers for *Ochrobactrum spp.* to





### INTRODUCTION

identify both genera. A microfluidics PCR plate was designed that enables the diagnostic and typing of *Brucella*, *Brucella*-like and serologically cross-reacting bacteria to be performed, even from a few organisms for high volumes of samples/repetitions.



### VISION

Three bacteriological typing methods (motility, agglutination, and phage lysis) were examined in addition to conventional phenotyping differentiation methods. BIOLOG™ system that measures the production of NAD on various substrates was tested, as well as growth capacity on 40 different nutrient deprived media. Several potential sugars and specific media were found that can distinguish between core and non-core *Brucella* species. The existing MALDI-TOF spectral databases were extended with the novel *Brucella*, and several *Ochrobactrum* species. Unique biomarkers were found for every novel core, non-core *Brucella* spp. and *O. intermedium* and *O. anthropi*, which can be used for their identification and further immunogenic investigations. Several antibiotics' resistance was found to be very important for discrimination between core and non-core *Brucellae* including *Ochrobactrum* species. The *in vitro* cell infections pipeline was developed, for the pathogenicity classification. The gene expression by RNA-seq and cytokine profiling of infected cells with both pathogenic and benign *Brucella* species was analysed. IDEMBRU prepared three separate parts of the toolkit: for identification and characterisation of non-core *Brucella* isolates; for *B. canis* diagnostic and management of outbreaks; for diagnostic of core *Brucella* spp. in wildlife.



### STRUCTURE



### ACHIEVEMENTS

During this project, the Netherlands and the UK have recorded the first human *B. canis* cases in Europe. IDEMBRU collaborated with JIP COHESIVE to evaluate the presence, risks of *B. canis* infection in dogs and humans and determine its pathogenic characteristics. Identification of professional exposure groups (veterinary workers, veterinary students, kennel owners, diagnostic laboratories staff) led to sera testing on those individuals. The White Paper written on *B. canis* provides current knowledge and recommendations to stakeholders for further actions. An EREN communication was prepared for EFSA on the topic of *B. canis* epidemiology in Europe and current understanding of risks (EREN *B. canis* briefing note).



### OUTCOMES

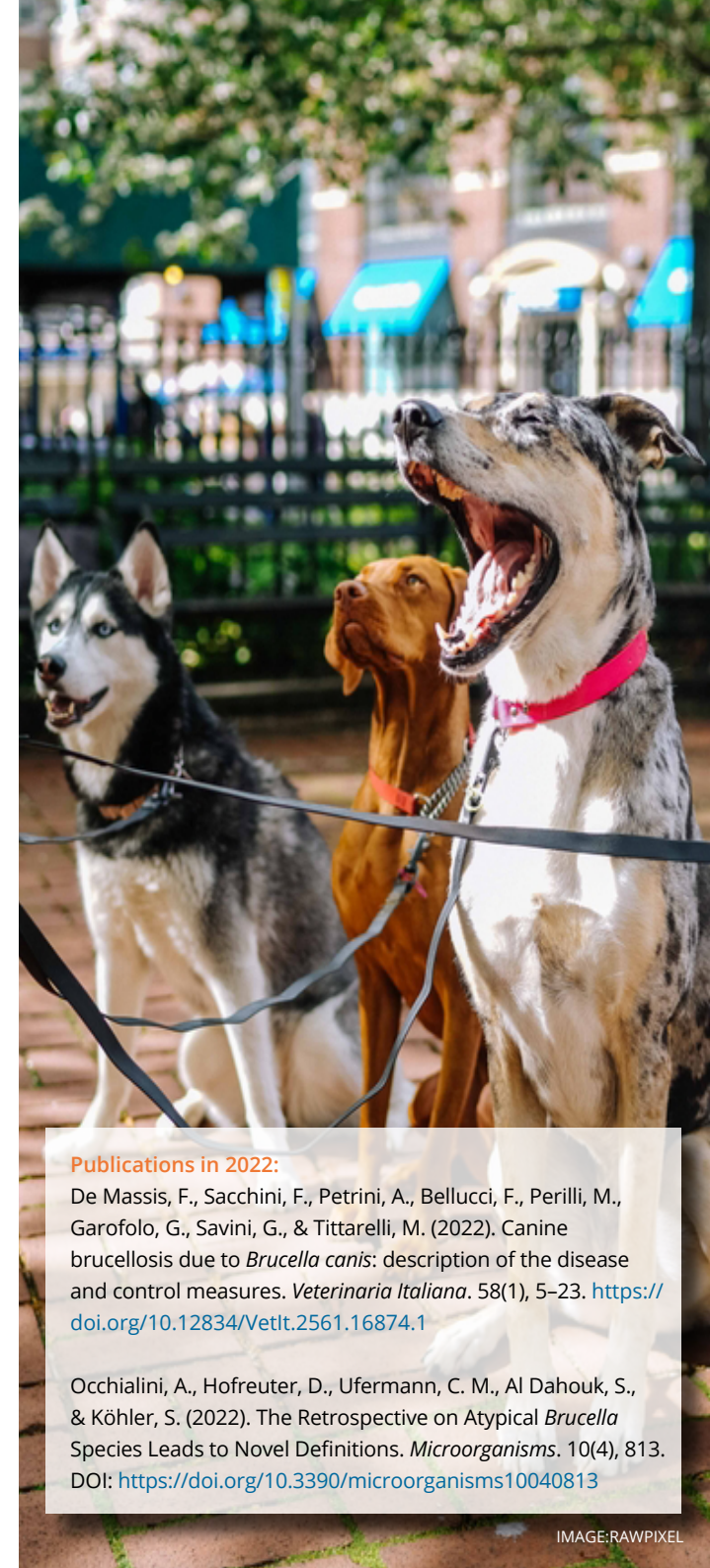


### DISSEMINATION

The results of IDEMBRU were presented at international Brucellosis symposiums, the One Health EJP ASM 2022, and stakeholders' meetings. IDEMBRU organised four awareness-raising seminars on *B. canis* for European veterinary professionals and diagnostic laboratories, with the support of EFSA.



### EDUCATION



#### Publications in 2022:

De Massis, F., Sacchini, F., Petrini, A., Bellucci, F., Perilli, M., Garofolo, G., Savini, G., & Tittarelli, M. (2022). Canine brucellosis due to *Brucella canis*: description of the disease and control measures. *Veterinaria Italiana*. 58(1), 5–23. <https://doi.org/10.12834/Vetit.2561.16874.1>

Occhialini, A., Hofreuter, D., Ufermann, C. M., Al Dahouk, S., & Köhler, S. (2022). The Retrospective on Atypical *Brucella* Species Leads to Novel Definitions. *Microorganisms*. 10(4), 813. DOI: <https://doi.org/10.3390/microorganisms10040813>



## INTRODUCTION

### OHEJP SIMEX PROJECT

The [OHEJP SimEx Project](#) was conceptualised under the One Health approach to address a foodborne zoonotic disease outbreak. The project overarching aim was to assess the communication and collaboration between public health, animal health and food safety authorities and laboratories within each participating country by providing a table-top exercise that covered a realistic foodborne outbreak at a national level. The OHEJP SimEx project was conducted between September 2021 and December 2022.



## VISION

During the summer of 2022, OHEJP SimEx Project Team successfully delivered a challenging foodborne outbreak exercise across 11 European countries (Denmark, Norway, Sweden, Finland, Estonia, Poland, France, Italy, Portugal, Belgium, and The Netherlands). The exercise focused on an outbreak caused by a foodborne pathogen affecting both human and pet food supplies. The exercise encouraged an intersectoral cooperation through the sharing of experiences, views, and perspectives. It highlighted the benefits of sharing relevant data and finding solutions to common issues across sectors. In addition, it showcased the importance of employing the One Health approach in an outbreak situation and indicated the areas requiring further development, improving each country's national preparedness for future One Health outbreaks.



## STRUCTURE



## ACHIEVEMENTS

On completion of the OHEJP SimEx Project, the dissemination workshop was co-organised with the Science to Policy Translation team (WP5) and held in December 2022. The workshop 'A One Health Simulation Exercise as a roadmap for future foodborne outbreak preparedness' was delivered online. It shared the main outcomes from the project based on the results of the countries' experiences and included good practices currently in place, whilst also identifying major gaps in outbreak preparedness. A report on this event [One Health EJP Report of the Joint SimEx/Dissemination Workshop: a One Health Simulation Exercise as a Roadmap for Future Foodborne Outbreak Preparedness](#) is available and all SimEx document and resources are accessible on the [OHEJP website](#).



## OUTCOMES



## DISSEMINATION



## EDUCATION



### Publications in 2022:

Alves F, Artursson K, Bloch J, Brisabois A, Imberechts H, Jokelainen P, La Ragione R, Lindblad M, Forss RL, Marston DA, Parvizi O, Tuominen L and Omazic A (2023) A multi-country One Health foodborne outbreak simulation exercise: cross-sectoral cooperation, data sharing and communication. *Front. Public Health.* 11:1121522. doi: [10.3389/fpubh.2023.1121522](https://doi.org/10.3389/fpubh.2023.1121522)



INTRODUCTION

**JIP ORION (ended June 2021)**

Foddai, A., Takeuchi-Storm, N., Høg, B. B., Kjeldgaard, J. S., Andersen, J. K., Ellis-Iversen, J. (2022). Assessing *Campylobacter* cross-contamination of Danish broiler flocks at slaughterhouses considering true flock prevalence estimates and ad-hoc sampling. *Microbial Risk Analysis*. 100214. DOI: <https://doi.org/10.1016/j.mran.2022.100214>



VISION

Günther, T., Kramer-Schadt, S., Fuhrmann, M. and Belik, V. (2022). Environmental factors associated with the prevalence of ESBL/AmpC-producing *Escherichia coli* in wild boar (*Sus scrofa*). *Frontiers of Veterinary Science*. 9:980554. DOI: <https://doi.org/10.3389/fvets.2022.980554>



STRUCTURE

Lopez de Abechucó, E., Dórea, F., Buschhardt, T., Scaccia, N., Günther, T., Foddai, A., Dups-Bergmann, J. and Filter, M. (2022). One Health Consensus Report Annotation Checklist (OH-CRAC): A cross-sector checklist to support harmonised annotation of surveillance data in reports. *Zoonoses and Public Health*, 69: 606–614. DOI: <https://doi.org/10.1111/zph.12947>



ACHIEVEMENTS

Foddai, A., Nauta, M. and Ellis-Iversen, J. (2022). Risk-based control of *Campylobacter spp.* in broiler farms and slaughtered flocks to mitigate risk of human campylobacteriosis – A One Health approach. *Microbial Risk Analysis*, 21: 100190. DOI: <https://doi.org/10.1016/j.mran.2021.100190>

**JRP NOVA (ended June 2021)**

Swanson, D., Koren, C., Hopp, P., Jonsson, M.E., Rø, G. I., White, R. A., Grøneng, G. M. (2022). A One Health real-time surveillance system for nowcasting *Campylobacter* gastrointestinal illness outbreaks, Norway, week 30 2010 to week 11 2022. *Euro Surveillance*. 27(43): 2101121. Available at: <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2022.27.43.2101121>



OUTCOMES

**JRP LISTADAPT (ended June 2021)**

Félix, B., Sevellec, Y., Palma, F. *et al.* (2022). A European-wide dataset to uncover adaptive traits of *Listeria monocytogenes* to diverse ecological niches. *Science Data*. 9, 190. DOI: <https://doi.org/10.1038/s41597-022-01278-6>



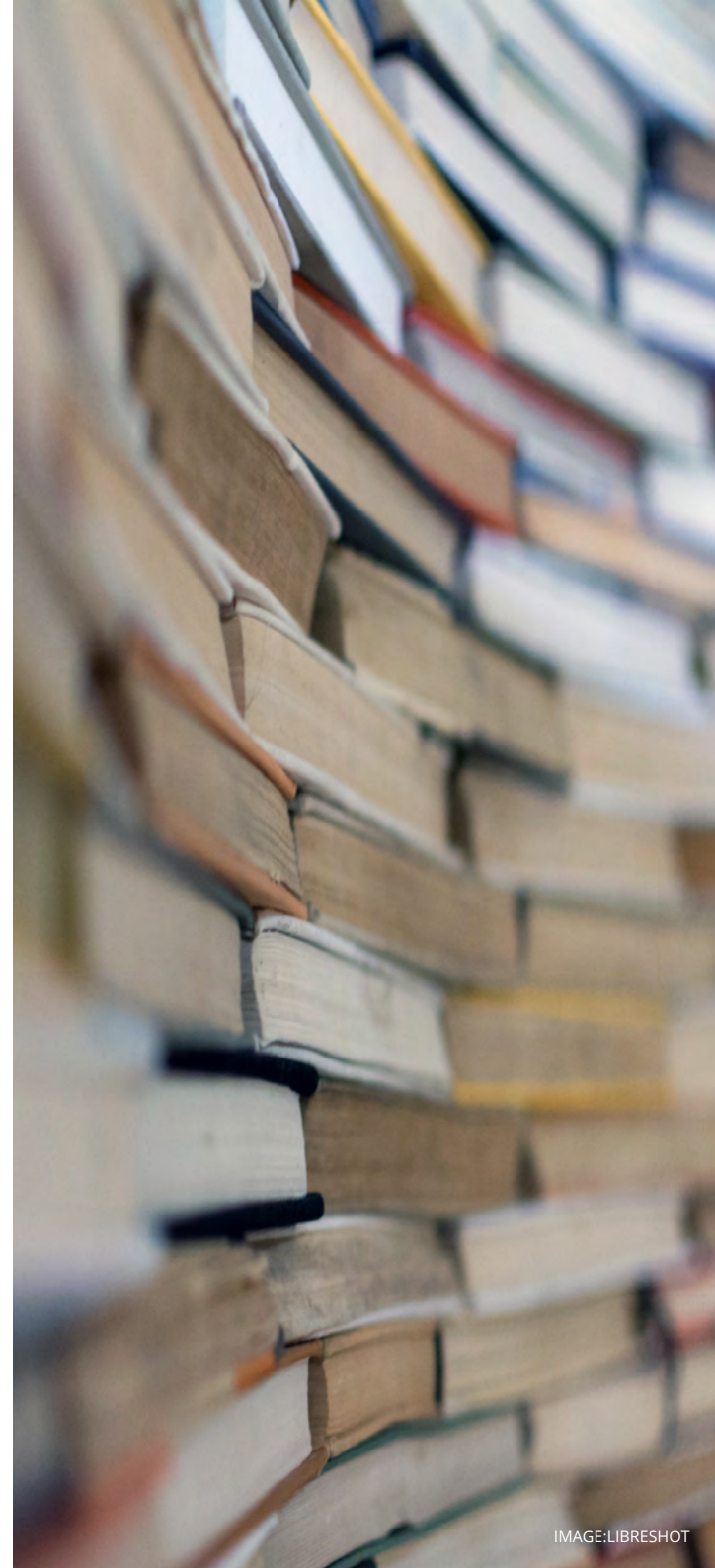
DISSEMINATION

Douarre, P. E., Sévellec, Y., Le Grandois, P., Soumet, C., Bridier, A., Roussel, S. (2022). FepR as a central genetic target in quaternary ammonium compounds (QAC)-adaptive process and cross-resistance to ciprofloxacin in *Listeria monocytogenes*. *Frontiers in Microbiology*. 13, 864576. DOI: <https://doi.org/10.3389/fmicb.2022.864576>



EDUCATION

Sévellec, Y., Ascencio, E., Douarre, P. E., Félix, B., Gal, L., Garmyn, D., Guillier, L., Piveteau, P., Roussel, S. (2022). *Listeria monocytogenes*: Investigation of fitness in soil does not support the relevance of ecotypes. *Frontiers in Microbiology*. 13, 917588. DOI: <https://doi.org/10.3389/fmicb.2022.917588>





INTRODUCTION

Palma, F., Radomski, N., Guérin, A., Sévellec, Y., Félix, B., Bridier, A., Soumet, C., Roussel, S., Guillier, L. (2022). Genomics elements located in the accessory repertoire drive the adaptation to biocides in *Listeria monocytogenes* strains from different ecological niches. *Food Microbiology*. 106, 103757. DOI: <https://doi.org/10.1016/j.fm.2021.103757>



VISION

### JRP MOMIR-PPC (ended June 2021)

Kempf, F., La Ragione, R., Chirullo, B., Schouler, C., & Velge, P. (2022). Super Shedding in Enteric Pathogens: A Review. *Microorganisms*. 10(11), 2101. DOI: <https://doi.org/10.3390/microorganisms10112101>



STRUCTURE

### JRP ARDIG (ended June 2021)

Nunez-Garcia, J., AbuOun, M., Storey, N., Brouwer, M. S., Delgado Blas, J. F., Mo, S. S., Ellaby, N., Veldman, K. T., Haenni, M., Châtre, P., Madec, J. Y., Hammerl, J. A., Serna, C., Getino, M., La Ragione, R., Naas, T., Telke, A. A., Glaser, P., Sunde, M., Gonzalez-Zorn, B., Ellington, M. J. & Anjum, M. F. (2022). Harmonisation of *in silico* next generation sequencing based methods for diagnostics and surveillance. *Scientific Reports*. 12, 14372. DOI: <https://doi.org/10.1038/s41598-022-16760-9>.



ACHIEVEMENTS

Getino, M., López-Díaz, M., Ellaby, N., Clark, J., Ellington, M. J., La Ragione, R. M. (2022). A Broad-Host-Range Plasmid Outbreak: Dynamics of IncL/M Plasmids Transferring Carbapenemase Genes. *Antibiotics*. 11(11), 1641. DOI: <https://doi.org/10.3390/antibiotics11111641>.



OUTCOMES

Storey, N., Cawthraw, S., Turner, O., Rambaldi, M., Lemma, F., Horton, R., Randall, L., Duggett, N. A., AbuOun, M., Martelli, F., & Anjum, M. F. (2022). Use of genomics to explore AMR persistence in an outdoor pig farm with low antimicrobial usage. *Microbial Genomics*. 8(3), 000782. DOI: <https://doi.org/10.1099/mgen.0.000782>.



DISSEMINATION

Thomson, N. M., Gilroy, R., Getino, M., Foster-Nyarko, E., van Vliet, A. H. M., La Ragione, R. M., & Pallen, M. J. (2022). Remarkable genomic diversity among *Escherichia* isolates recovered from healthy chickens. *Peer J*. 10, e12935. DOI: <https://doi.org/10.7717/peerj.12935>.

### JRP TOX-Detect (ended June 2021)

Kavanaugh, D. W., Glasset, B., Dervyn, R., Guérin, C., Plancade, S., Herbin, S., Brisabois, A., Nicolas, P., & Ramarao, N. (2022). New genetic biomarkers to differentiate non-pathogenic from clinically relevant *Bacillus cereus* strains. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 28(1), 137.e1–137.e8. DOI: <https://doi.org/10.1016/j.cmi.2021.05.035>



EDUCATION



INTRODUCTION

**PUBLICATIONS IMPART (ended Dec 2020)**

Perrin-Guyomard, A., Granier, S.A., Slettemeås, J.S., Anjum, M., Randall, L., AbuOun, M., Pauly, N., Irrgang, A., Hammerl, J.A., Kjeldgaard, J.S., Hammerum, A., Franco, A., Skarzyńska, M., Kamińska, E., Wasyl, D., Dierikx, C., Börjesson, S., Geurts, Y., Haenni, M. and Veldman, K. (2022). Multicentre evaluation of a selective isolation protocol for detection of *mcr*-positive *E. coli* and *Salmonella spp.* in food-producing animals and meat. *Letters in Applied Microbiology*. Early view. DOI: <https://doi.org/10.1111/lam.13717>



VISION

Dierikx C., Börjesson S., Perrin-Guyomard A., Haenni M., Norström M., Divon H. H., Ilag H. K., Granier S. A., Hammerum A., Sejer Kjeldgaard J., Pauly N., Randall L., Anjum M. F., Smialowska A., Franco A., Veldman K., Schau Slettemeås J. (2022). A European multicenter evaluation study to investigate the performance on commercially available selective agar plates for the detection of carbapenemase producing Enterobacteriaceae. *Journal of Microbiological Methods*. 193:106418 DOI: <https://doi.org/10.1016/j.mimet.2022.106418>



STRUCTURE

**PUBLICATIONS MEDVETKLEBS (ended Dec 2020)**

Hooban, B., Fitzhenry, K., O'Connor, L., Miliotis, G., Joyce, A., Chueiri, A., Farrell, M. L., DeLappe, N., Tuohy, A., Cormican, M., & Morris, D. (2022). A Longitudinal Survey of Antibiotic-Resistant Enterobacterales in the Irish Environment, 2019-2020. *Science of the Total Environment*. 828, 154488. Advance online publication. DOI: <https://doi.org/10.1016/j.scitotenv.2022.154488>



ACHIEVEMENTS

Rodrigues, C., Desai, S., Passet, V., Gajjar, D., & Brisse, S. (2022). Genomic evolution of the globally disseminated multidrug-resistant *Klebsiella pneumoniae* clonal group 147. *Microbial Genomics*. 8(1), 000737. DOI: <https://doi.org/10.1099/mgen.0.000737>



OUTCOMES

Rodrigues, C., Hauser, K., Cahill, N., Ligowska-Marzęta, M., Centorotola, G., Cornacchia, A., Garcia Fierro, R., Haenni, M., Nielsen, E. M., Piveteau, P., Barbier, E., Morris, D., Pomilio, F., & Brisse, S. (2022). High Prevalence of *Klebsiella pneumoniae* in European Food Products: a Multicentric Study Comparing Culture and Molecular Detection Methods. *Microbiology Spectrum*. 10(1), e0237621. DOI: <https://doi.org/10.1128/spectrum.02376-21>



DISSEMINATION

Garcia-Fierro, R., Drapeau, A., Dazas, M., Saras, E., Rodrigues, C., Brisse, S., Madec, J. Y., Haenni, M. (2022) Comparative phylogenomics of ESBL-, AmpC- and carbapenemase-producing *Klebsiella pneumoniae* originating from companion animals and humans. *Antimicrobial Chemotherapy*. doi: [10.1093/jac/dkac041](https://doi.org/10.1093/jac/dkac041)



EDUCATION

**PUBLICATIONS RADAR (ended Dec 2020)**

Kausrud, K., Skjerdal, T., Johannessen, G. S., Ilag, H. K., Norström, M. (2022). The Heat Is On: Modelling the Persistence of ESBL-Producing *E. coli* in Blue Mussels under Meal Preparation. *Foods*. 12(1), 14. DOI: <https://doi.org/10.3390/foods12010014>





### INTRODUCTION

McCarthy, C., Viel, A., Gavin, C., Sanders, P., Simons, R. R. L. (2022). Estimating the likelihood of ESBL-producing *E. coli* carriage in slaughter-aged pigs following bacterial introduction onto a farm: A multiscale risk assessment. *Microbial Risk Analysis*. 20, 100185. DOI: <https://doi.org/10.1016/j.mran.2021.100185>



### VISION

Godijk, N. G., Bootsma, M. C. J., Bonten, M. J. M. (2022). Transmission routes of antibiotic resistant bacteria: a systematic review. *BMC Infectious Disease*. 22(1), 482. DOI: <https://doi.org/10.1186/s12879-022-07360-z>



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION



## INTRODUCTION



## VISION



## STRUCTURE



## ACHIEVEMENTS



## OUTCOMES



## DISSEMINATION



## EDUCATION

## One Health EJP PhD Programme Progress

Between 2018 and 2022, 16 PhD projects were co-funded by the One Health EJP Education and Training activities as part of Work Package 6. The research focus on 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 through Work Package 7 Sustainability, which commenced in 2019 and focused 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 future generation of One Health scientific leaders 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 SARS-CoV-2 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 April 2022, where they presented their projects and research through presentation of a scientific poster and an oral presentation at the three-minute thesis (3MT) competition, to an audience of around 276 scientists and professionals across the globe.

To find more information about the PhD students, [click here](#).







### INTRODUCTION

#### ECO-HEN

The ECO-HEN project began in February 2019 and finished in October 2022. This PhD project studied the role of *Escherichia coli* from the intestinal microbiota of animals (from day-old chicks to laying hens) in a commercial laying hen farm in the spread of antimicrobial resistance (AMR).



### VISION

*E. coli* is able to survive on the food chain and could spread AMR genes across bacteria due to their ability for horizontal transfer of genetic platforms. 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 was to improve the knowledge on the transmission dynamics of AMR *E. coli* in commercial laying hens (egg production) and to determine to what extent the table egg production poses a public health risk through food and/or environmental contamination. To evaluate the risk of the spread of AMR to humans and the environment, genomic data will be shared across electronic platforms available for the greater community of clinical and environmental microbiologists for comparative analyses.



### STRUCTURE

The research focused on identifying *E. coli* resistance genes from animals and eggshells using classical and next-generation microbiology techniques. This work identified 687 isolates from commercial egg production farms, with 271 isolates characterised by whole genome sequencing (WGS). Of them, 217 non-repetitive isolates were retained for further analysis, 113 isolates harbouring at least one AMR gene. Up to 33 AMR genes were detected from these isolates (*tetA*, *bla*<sub>TEM1-B</sub>, *aadA1*, *sul2* and *strA/B* being the most frequent).



### ACHIEVEMENTS

In addition, the dynamics of AMR between different stages of egg production was explored by phylogenetic analysis from day-old chicks to pullets and laying hens. Based on phylogenetic distance, different clones were detected. This research identified 195 isolates where at least one of the 11 plasmid replicons was detected. Finally, 30 clones (comprising between two and eight isolates) were distinguished. The study detected different Russian dolls sets for AMR genes spread in this commercial layer farm and improved the understanding of the different roles played by integrons, MR regions, plasmids, and clones in the AMR flow. It was found that the most common models were plasmid clone sets involving both single and linked AMR genes and single patterns, when only plasmids harbouring AMR genes or clones were involved. In addition, events related to the gain or loss of AMR genes by plasmids or the gain or losses of AMR containing plasmids themselves by isolates were also recorded.



### OUTCOMES



### DISSEMINATION



### EDUCATION



#### Publications in 2022:

Aldea, I., Gibello, A., Hernández, M., & Moreno, M.A. (2022). Clonal and plasmid-mediated flow of ESBL/AmpC genes in *Escherichia coli* in a commercial laying hen farm. *Veterinary Microbiology*. 270, 109453. DOI: <https://doi.org/10.1016/j.vetmic.2022.109453>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## LIN-RES

Linezolid belongs to the oxazolidinone family of antimicrobials and is one of the last resort drugs used to fight infections in humans caused by multi-resistant Gram-positive bacteria such as *Streptococci*, *Staphylococci*, and *Enterococci*. This antimicrobial has been commercially available since 2000 and has not been licensed for use in animals. In 2008, the first instance of transferable resistance to Linezolid was observed in a US *Staphylococci* isolate removed from human infection cases. In China, since 2008, it emerged that Linezolid resistance has been recently detected in animal and human *Enterococci* and *Staphylococci* on both the American and European continents. To this end, the **LINE-RES** PhD project aimed to investigate the molecular basis, origin, transferability, and risk factors associated with the emergence in Gram-positive bacteria of human and animal origin in a one-health manner.

The research created a home-made liquid bead array based on the Ligase Chain Reaction (LCR; AMR-ARRAY), designed to target 53 antimicrobial gene markers and allow for the simultaneous detection of the most frequent genes and/or mutations associated with resistance to  $\beta$ -lactam, fluoroquinolone, colistin, macrolide and aminoglycoside families of antimicrobials in Enterobacteriaceae (*Escherichia coli*, *Shigella spp.*, and *Salmonella spp.*). A total of 648 *E. coli* isolates were selected, representing the AMR diversity observed in Enterobacteriaceae of Belgian origin, to assess the analytical scope of AMR-ARRAY.

The AMR-ARRAY was compared with susceptibility profiling, genetic resistance profiling, and whole genome sequencing data (WGS), respectively. First, the comparative analysis of the AMR phenotypic data found a weak concordance of 58.5% with the AmpC phenotype, while 100% concordance was observed for colistin resistance and the ESBL phenotype. Globally, AMR-ARRAY were 94.7% concordant with susceptibility profiles regardless of the origin of the isolates. Second, the AMR-ARRAY correctly detected all genes & single nucleotide polymorphisms (SNPs) responsible for ESBL or BL phenotypes and all, but a single SNP involved in resistance to quinolones. Lastly, a subset of 139 animal *E. coli* isolates that were sequenced through WGS, when assessed against AMR-ARRAY 697/702 determinants (genes/SNPs) expected from WGS analysis were detected by AMR-ARRAY.

In conclusion, the AMR-ARRAY tool is capable of being used: i) document susceptibility profiles by providing information on the genetic nature of the resistance; ii) study multiple resistance determinants in one large cohort using a 53 probed multiplex assay; and iii) as a screening tool to detect frequent resistance determinants and select which isolates should be sequenced in priority.





## INTRODUCTION



## VISION



## STRUCTURE



## ACHIEVEMENTS



## OUTCOMES



## DISSEMINATION



## EDUCATION

## HME-AMR

Selective pressures drive bacterial populations to evolve and may influence the dissemination of antimicrobial resistance genes (ARGs) in the gut microbiota of humans and animals, or in the environment. However, there is limited information on the impact of selective pressures on antimicrobial resistance (AMR) dissemination in the primary food production environment. Specifically, heavy metals can act as a selective pressure on AMR due to mechanisms which contribute to the co-selection of AMR and heavy metal resistance (HMR). Heavy metals are ubiquitous in the agricultural food environment; they are naturally present in the environment, or they can be released into it as a consequence of anthropogenic activities. In livestock production, for example, zinc and copper may be added to animal feed to promote health and growth. These heavy metals may not be fully absorbed from the animal gut and can then be excreted via faeces into the environment, affecting the heavy metal concentration present.

The HME-AMR PhD project was created to address knowledge gaps on the presence of zinc and its impact on AMR by investigating the presence of antimicrobial resistant bacteria (ARB) and assessing the bacterial resistome in zinc-containing environments. The project had four main objectives: i) comparison of the resistome in spinach and in zinc amended and un-amended soil used for horticultural crop production; ii) comparison of the resistome in low and high metal-containing soils used for dairy pasture; iii) comparison of the resistome in bovine milk filters from cows grazing on pastures with varying metal content; and iv) genotypic comparison of antimicrobial resistant Enterobacterales with human, environmental, and animal isolates from Irish and EU reference laboratories.

In 2022, the occurrence and characterisation of AMR Enterobacterales was explored in soil and spinach samples in the presence and absence of zinc, and in soil and bovine milk filters samples collected from dairy farms located in low and high zinc areas across Ireland. The identification of suitable regions and the recruitment of farms was facilitated through collaboration with Geological Survey Ireland and the Teagasc Advisory Service. These studies identified bacteria with diverse AMR profiles present in the environment and the studies are currently being drafted for submission for publication. The study to compare the phenotypic and genotypic profiles of these isolates with those from other One Health domains is ongoing.

Additional work will continue throughout 2023 and into 2024, with DNA from soil and milk filter samples undergoing metagenomic analysis. The analysis will predominantly focus on the detection of antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), and heavy metal resistance genes.





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## KENTUCKY

*Salmonella* Enterica serovar Kentucky (*S. Kentucky*) is a common causative agent of gastroenteritis in humans. It is one of the most notorious *Salmonella* serotypes, as it is strongly associated with antimicrobial resistance (AMR). Ciprofloxacin-resistant *S. Kentucky* (CIPR *S. Kentucky*) belongs to a single sequence type (ST198), acquired of a variant of the *Salmonella* genomic island 1 (SGI1) that confers resistance to first-line antimicrobials ( $\beta$ -lactams, aminoglycosides, sulphonamides, tetracyclines). The multi-drug resistant clone has since then also accumulated various substitution mutations in the quinolone resistance determining regions (QRDR) of DNA gyrase (*gyrA*) and DNA topoisomerase (*parC*), such that most strains carry three QRDR mutations, which together confer full resistance to ciprofloxacin. Phylogeographical analysis indicates that this clone first emerged in Egypt around the year 1890, before disseminating into Northern, Southern, and Western Africa, and then further to the Middle East, Asia, and Europe.

The **KENTUCKY** PhD project was designed to investigate: i) what explains the evolutionary successes of the multidrug resistant *S. Kentucky* ST198 clone; ii) what is the mechanism of the integration and potential further transfer of the extended spectrum beta lactamase (ESBL) gene in its chromosome; and iii) are there genetic determinants of different human-animal host ranges in epidemic *S. Kentucky* ST198 and ST152.

In 2022, the project completed the construction of a reported plasmid based on P1, the pMT *parS*/*ParB* partition system and the construction of a fluorescent recipient strain to visualise the transfer of pR27 in real-time. First, genetic engineering approaches were employed to increase the conjugation (i.e., transfer of genes) efficiency of pR27 to maximise the likelihood of observing the conjugation in real-time. The transposition unit ISECP1-*bla*<sub>CTX-M-14B</sub> with P1-*parS* and cloned it into pR27 yielding double *parS* labelled pR27 that can be tacked-in real-time using a fluorescent microscope. Second, the reporter systems enabled the study of plasmid transfer and their associated dynamics at the inter- and intracellular level using time-lapse fluorescent microscopy and microfluidics. Further optimisation is ongoing to track intracellular transposition dynamics in real-time.

Finally, the project will continue to investigate are probes required for candidate genes that are differentially regulated by the homologous H-NS copies and the possible effect of various environmental and/or biochemical cues on the conjugation frequencies.





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## METAPRO

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

The **METAPRO** PhD project was created to employ metagenomic approaches to study resistance determinants to plazomicin and next generation aminoglycosides in ecological niches from human, animal and environmental origins. The main aims of this project are: i) to evaluate the prevalence of next generation aminoglycosides resistance genes in different ecological setting; ii) to elucidate the bacterial and ecological reservoirs of these resistance determinants; iii) to predict the potential dissemination routes and describe the clones and mobile genetic elements that entail a major risk of aminoglycoside inefficacy; and iv) analyse the effect of the use of antibiotics in the promotion of selection of aminoglycoside resistance genes.

In 2022, continuing the metagenomic analysis carried out in 2021, pig faecal samples were analysed by conventional shotgun metagenomics and a targeted metagenomic methods, which revealed disparities in the detection of 16S rRNA methyltransferase. The targeted metagenomic approach not only helped to confirm the presence of these genes in an assembly approach but assisted in the understanding of real prevalence. Next, *npmA*, a low abundant methyltransferase with the widest range of aminoglycoside resistance was investigated, with samples containing *npmA* coming from humans, poultry and pig origins. These works confirmed previous findings regarding the global distribution of 16S rRNA methyltransferase, with 55% of samples containing *npmA* coming from China, compared to European countries like Germany representing 3.34% of samples.

Additional work to detect reservoirs of *npmA* was carried out, using fluorescence *in situ* hybridisation and fluorescence-assisted cell sorting, which has allowed individual cells carrying *npmA* to be able to precisely identify where it hides and guide future culture attempts of the reservoirs. The preliminary results with mock communities indicate that detection of a high proportion of labelled cells is possible. However, the use of environmental samples might entail more difficulties. Lastly, the effect of the use of apramycin in the selection of apramycin resistance genes to apramycin in poultry has shown that this antibiotic does not cause a major reconfiguration of the gut microbiome, but it increases the diversity of resistance genes. Further work will be carried out to determine the function of the other 16S rRNA methyltransferases found in this project.





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

### PEMbo

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* (*M. bovis*), is a zoonotic disease intimately and historically associated with cattle rearing and has a high socioeconomic impact. Although developing countries suffer significantly from bTB, this disease remains a major threat to developed countries. When cattle breeding was developed into an established industry, strong control strategies were initially established in Europe, and France was awarded the official bTB-free status from the EU. Despite considerable financial and social efforts, bTB continues to increase in incidence and persist at regional levels. The aim of the PEMbo project, a collaborative study between ANSES and INRA, is to better understand the complex biology of *M. bovis* through the study of the complete genomes of a large panel of isolates.

Ten complete genomes of *M. bovis* genotypes were sequenced using MinION (Nanopore) and Illumina technology from selected strains circulating in France. These complete genomes improved knowledge of genetic diversity of the *M. bovis* genomes. The copy number of an insertion sequence, IS6110, was found to be variable between the different complete genomes. This sequence plays an important role in genome plasticity (ability to adapt different forms depending on the environment) of the human pathogen *Mycobacterium tuberculosis* and was found in high numbers in representative genomes of highly prevalent genotypes, whereas *M. bovis* is considered to have one or very few copies.

The research also studied the abundance and location of IS6110 in a larger panel of *M. bovis* genomes representative of French diversity. These results confirmed that the most persistent genotypes were those with multiple copies of IS6110 and that two of the most common genotypes found in France in recent years have a very high copy number (>10). Further studies on 3 important genotypes, SB0120-DHV, SB0120-CO and a subgroup of Cluster A/F4 family, which are respectively found in Dordogne and Haute-Vienne, Côte d'Or and Atlantic Pyrenees (French regions), showed quite a high stability of the IS6110 number and its insertion site between host species over 6-to-17-year periods. Genetic changes did not appear to occur for host adaptation, as suggested for highly prevalent *M. tuberculosis*. These investigations may explain the differential phenotypical characteristics of different groups of *M. bovis*.

The impact of this research on the knowledge bases of *M. bovis* lineages circulating in France has been established by a lift of specific genetic characteristics for them. Such as IS6110, which seems to be correlated with the epidemiological success of certain genotypes. The new genomes will help to better understand the bTB transmission dynamics in multi-host systems and to implement more effective control measures to eradicate the disease in these areas.



#### Publications in 2022:

Charles, C., Conde, C., Biet, F., Boschirolì, M-L. & Michelet, L. (2022). IS6110 Copy Number in Multi-Host *Mycobacterium bovis* Strains Circulating in Bovine Tuberculosis Endemic French Regions. *Frontiers in Microbiology*. 9, 891902. DOI: <https://doi.org/10.3389/fmicb.2022.891902>



### INTRODUCTION

## MACE

Cystic echinococcosis (CE) is a zoonotic parasitic disease of significant public health concern worldwide, with over 5,000 new cases reported yearly in South America alone. The burden of this disease extends to encompass economic impacts, mostly felt in subsistence livestock keepers and other rural and peri-urban populations where other health competing interests persist. The lack of reporting of this disease is large and the evidence to inform efficient surveillance and control approaches remains scarce. Moreover, there is a wide range of surveillance and control measures that can be implemented across the different hosts, with varied applications across geographies.



### VISION

The **MACE** PhD project aims to inform the most efficient portfolio of surveillance options and interventions to control and eliminate CE, taking into account the various risks, disease control capacities, and risk preferences in geographies. The project is novel in the joint application of geostatistical analysis, mathematical modelling, and economic evaluation, in two countries, Argentina and Italy, with a distinct disease context while being endemic for CE infections.



### STRUCTURE

A spatial model was designed to investigate the spatial heterogeneity of CE infection in different livestock species across farms in Italy. The model allowed the identification of disease ‘hot spots’ at high spatial resolution between multiple Italian provinces, while also highlighting species-specific infection risk. The resulting maps of infection risk will be valuable for targeted intervention efforts and further surveillance programmes. This study was carried out in collaboration with Prof. Rinaldi the director of the WHO Collaborating Centre for Diagnosis of Intestinal Helminths and Protozoa.



### ACHIEVEMENTS

In 2022, an individual-based CE transmission model was developed and calibrated to Rio Negro, Argentina, a region with limited resources for tackling this disease. The model captures key aspects of the parasite life cycle and host interactions, allowing for the evaluation of intervention strategies (e.g., anthelmintic treatment, vaccinations), while also capturing individual-level heterogeneities. The results from the analysis carried out contributes to the generation of guidelines to reduce disease incidence, informing key stakeholders in making evidence-based decisions for disease management. The study was conducted in close collaboration with the Ministry of Health in Rio Negro.



### OUTCOMES

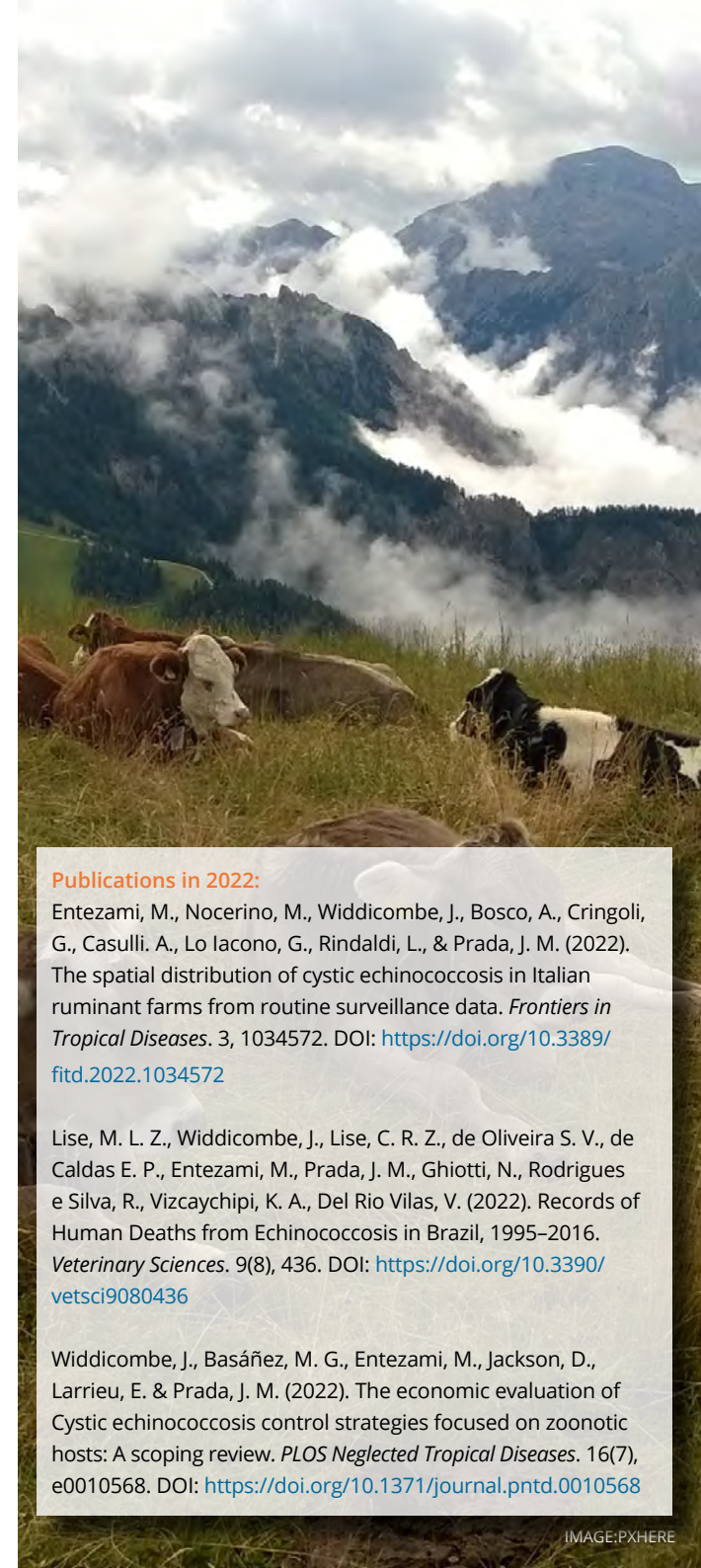
Furthermore, a socioeconomic study was carried out to determine the willingness of stakeholders to invest/disinvest in the sensitivity of infectious disease surveillance in a One Health context. Through a survey, different levels of surveillance sensitivity were evaluated to understand the priorities and financial constraints that will best inform a cost-effective surveillance strategy. The findings of this



### DISSEMINATION



### EDUCATION



#### Publications in 2022:

Entezami, M., Nocerino, M., Widdicombe, J., Bosco, A., Cringoli, G., Casulli, A., Lo Iacono, G., Rindaldi, L., & Prada, J. M. (2022). The spatial distribution of cystic echinococcosis in Italian ruminant farms from routine surveillance data. *Frontiers in Tropical Diseases*. 3, 1034572. DOI: <https://doi.org/10.3389/ftd.2022.1034572>

Lise, M. L. Z., Widdicombe, J., Lise, C. R. Z., de Oliveira S. V., de Caldas E. P., Entezami, M., Prada, J. M., Ghiotti, N., Rodrigues e Silva, R., Vizcaychipi, K. A., Del Rio Vilas, V. (2022). Records of Human Deaths from Echinococcosis in Brazil, 1995–2016. *Veterinary Sciences*. 9(8), 436. DOI: <https://doi.org/10.3390/vetsci9080436>

Widdicombe, J., Basáñez, M. G., Entezami, M., Jackson, D., Larrieu, E. & Prada, J. M. (2022). The economic evaluation of Cystic echinococcosis control strategies focused on zoonotic hosts: A scoping review. *PLOS Neglected Tropical Diseases*. 16(7), e0010568. DOI: <https://doi.org/10.1371/journal.pntd.0010568>



### INTRODUCTION

study will contribute to the development of a customised intervention plan that will balance the need for sensitivity and cost-effectiveness.



### VISION

The integrated approach, combining spatial analysis, transmission modelling, and social-economic elicitation, offers a comprehensive framework to understand the complex dynamics of CE transmission and control. The findings will be instrumental in guiding the development and implementation of an effective intervention and surveillance strategy, contributing to the reduction of the disease burden and economic impact of CE on affected communities.



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION







### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## DESIRE

Brown and black rats can carry a multitude of pathogens that are of public and veterinary health importance. Their potential to rapidly reach high population numbers could create an unpredictable situation of high pathogen transmission risk. Rat populations are affected by environmental changes. One of these changes that may influence rat populations is 'urban greening', which aims to improve biodiversity and living conditions in cities. However, this may have unintentional consequences. Insight is needed into the effects of urban greening, to mitigate the risk of transmission of pathogens of human or veterinary health risks.

To address these issues, the aim of the **DESIRE** PhD 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 into the surveillance of rat-borne zoonoses, and the effects of urban greening on rats and rat-borne zoonoses. The project will collaborate with national and international partners (WBVR and FLI).

In 2020 and 2021, to assess the effect of urban greening on rat populations and rat-borne zoonotic pathogens, data was collected in three cities in the Netherlands in locations with different percentages of greenness present. Greenness was measured by using the Normalised Difference Vegetation Index (NDVI). Captured rats were analysed for 18 different zoonotic pathogens using traditional serological and molecular techniques. Additionally, 16S rRNA gene sequencing was used on a subset of these samples and historical samples, to assess its potential as a test for this type of research with 16S rRNA gene sequencing, 14 potentially zoonotic bacterial pathogens (i.e., zoonotic *Leptospira spp.*, *Mycoplasma spp.*, and *Bartonella tribocorum*) were detected, including pathogens that were not included in the set of tests that were regularly used in our laboratory. These results indicated that 16S rRNA gene sequencing may have the potential to act as a pre-screening tool to gain an overview of potentially zoonotic bacterial pathogens circulating in wildlife. This may aid in prioritising research and funds and should give more insight into potentially zoonotic pathogens circulating in wildlife populations. Besides that, the effect of urban greening on rat populations was monitored using both rat trapping data and camera trapping data.

Lastly, a systematic literature review was performed to identify knowledge gaps in the surveillance of zoonotic pathogens in 10 common urban wildlife mammals: brown rats (*Rattus norvegicus*), house mice (*Mus musculus*), wood mice (*Apodemus sylvaticus*), common voles (*Microtus arvalis*), hedgehogs (*Erinaceus europaeus*), red squirrels (*Sciurus vulgaris*), rabbits (*Oryctolagus cuniculus*), moles (*Talpa europaea*), stone martens (*Martes foina*) and red foxes (*Vulpes vulpes*). All these individual studies will support the overarching goal of developing evidence-based surveillance interventions for emerging rat-borne zoonoses in changing environments.



#### Publications in 2022:

de Cock, M., Fonville, M., de Vries, A., Bossers, A., van den Bogert, B., Hakze-van der Honing, R., Koets, A., Sprong, H., van der Poel, W., & Maas, M. (2022). Screen the unforeseen: Microbiome profiling for detection of zoonotic pathogens in wild rats. *Transboundary and Emerging Diseases*. 00, 1– 15. DOI: <https://doi.org/10.1111/tbed.14759>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## 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 countries, antimicrobial resistance (AMR) monitoring in clinical *Campylobacter* isolates has reported an increasing prevalence of ciprofloxacin resistance. Ciprofloxacin is an antibiotic in the fluoroquinolone class, with the World Health Organisation classing it as critically important, therefore, an increase in resistance to ciprofloxacin is a major public health concern. The main source of campylobacteriosis in humans is considered to originate from poultry. The use of ciprofloxacin in broiler flocks have 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. A ban has been in place since 2006 in the EU, to prevent the routine use of feed supplemented with antibiotics, however, therapeutic use of fluoroquinolones in poultry remains an option for the industry.

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 fluoroquinolone resistance in UK and French broiler flocks. The project aims to examine the relationship between fluoroquinolone use in poultry and the development of resistance, assess fitness benefits/costs of acquired resistance and determine if any specific fluoroquinolone resistant variants found in poultry are more or less likely to persist and cause disease in humans. The data from this project would feed into the risk assessment for ongoing use of fluoroquinolone in poultry and consequent risks of fluoroquinolone resistance in clinical cases.

The UDoFRiC candidate spent time between January to September 2022 based at ANSES. Whilst at ANSES both *in vitro* and *in vivo* experiments to determine any fitness benefits/costs associated with fluoroquinolone resistance in *Campylobacter*. The *in vitro* work aimed to identify any fitness advantages at specific points in the production chain of chicken meat, namely, growth kinetics of the bacterium, in bird-to-bird transmission and ability to survive on food matrices. The *in vivo* experiments aimed to determine if fluoroquinolone resistance would influence the colonisation potential of *Campylobacter*. Furthermore, whole genome sequenced isolates taken from the *in vivo* experiments to determine any genetic differences in fluoroquinolone susceptible and resistant *Campylobacter* was carried out at APHA.





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## WILBR

The propagation and spread of microorganisms that develop antimicrobial resistance (AMR) is a global health emergency, 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 preparedness initiatives. However, there has been limited focus both in Europe and globally on the role of the environment as a propagator of 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 the 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 the identification and control of routes for the spread of AMR, as they often overwinter in countries or areas where there may be little information on resistance trends due to limited surveillance and diagnostic capacity, with the burden of AMR being 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 to the spread of AMR in the environment in general and on livestock farms in particular.

These works indicate that high-risk multidrug resistant bacterial clones can colonise wild birds and other host species. The presence of these clones in multiple epidemiological groups at the same time suggest that there is horizontal interspecies transmission of the isolates. The persistence of these bacterial clones over a sustained period of time in both wild birds and food-producing animals indicates repeated transmission events between these epidemiological groups. The persistence of AMR bacteria in the farm environment suggests that wild birds can play an important role in the dissemination of AMR bacteria even when the selective pressure of antibiotics are removed; evidencing that wild birds act as a reservoir for AMR bacteria.

To better understand the role of wild birds in the transmission of AMR bacteria, there needs to be more focus on spatial-temporal studies, involving the establishment of a GPS tracking system to study where populations of wild birds travel and their migration period in these geographies. Such a method would allow sampling to occur in these geographies to further determine the transmission of AMR bacteria. It could further elucidate whether the risk of onwards transmission of AMR bacteria from the farm environment to other wild birds, animals, and the environment is greater than the risk of wild birds acquiring AMR bacteria from other sources and introducing these to the farm environment.





### INTRODUCTION

#### EnvDis

Foodborne diseases remain a significant burden on morbidity, mortality, and healthcare costs worldwide. The problem is exacerbated by population growth and food insecurity. Furthermore, anthropogenic activities are constantly changing the environment; for example, climate change, land use, and socio-economic factors, are well known to drive disease occurrence and incidence. The environment can affect the abundance, survival and virulence of the pathogen and virulence, and host susceptibility to infection, including human behaviour. It is believed that the accelerating rate of the global temperature and environmental change may impact the distribution, frequency, and patterns of established diseases, including the emergence and re-emergence of new and old diseases.



### VISION

The EnvDis project aims to develop a model to assess the role of weather and climate in the incidence of diseases with an important seasonal component, contributing to increasing the knowledge. This will be achieved by using *Salmonella* incidence data, for which the literature is extensive.



### STRUCTURE

A statistical model was developed to estimate the incidence of salmonellosis in England and Wales conditional to different combinations of three simultaneous weather factors from fourteen available. The association was made from 17 years of historical salmonellosis cases reported to the UK Health Security Agency (UKHSA) alongside high-resolution spatial and temporal weather data. The incidence simulations based on weather data effectively replicated observed incidence patterns in England and Wales, validating the methodology and allowing to explore the effect of different weather combinations on the risk of contracting salmonellosis. A short-term mission in the RIVM (Netherlands) was carried out to evaluate the hypothesis that the effect of weather on salmonellosis cases is universal and not specific to a geography. To achieve this, historical epidemiological and meteorological data from the Netherlands were compared with the simulated cases based on the conditional incidence built on the English and Welsh data sets. The satisfactory performance of the model in the Netherlands suggests that a strong influence on weather that outgoes the reporting particularities of the location. Applications in additional countries would strengthen this hypothesis.



### ACHIEVEMENTS

Additional work was carried out, with the methodology being applied to the worst-case scenario of climate change projections in the UK to explore the state of salmonellosis in the near future, where no significant change in human behaviour or additional mitigation strategies are assumed against *Salmonella*. Preliminary results indicate an increase of at least 18% incidence rates throughout the year, with a rising peak in July projected by the year 2043. These findings should be interpreted with caution, given the uncertainty of climate projections and model limitations.



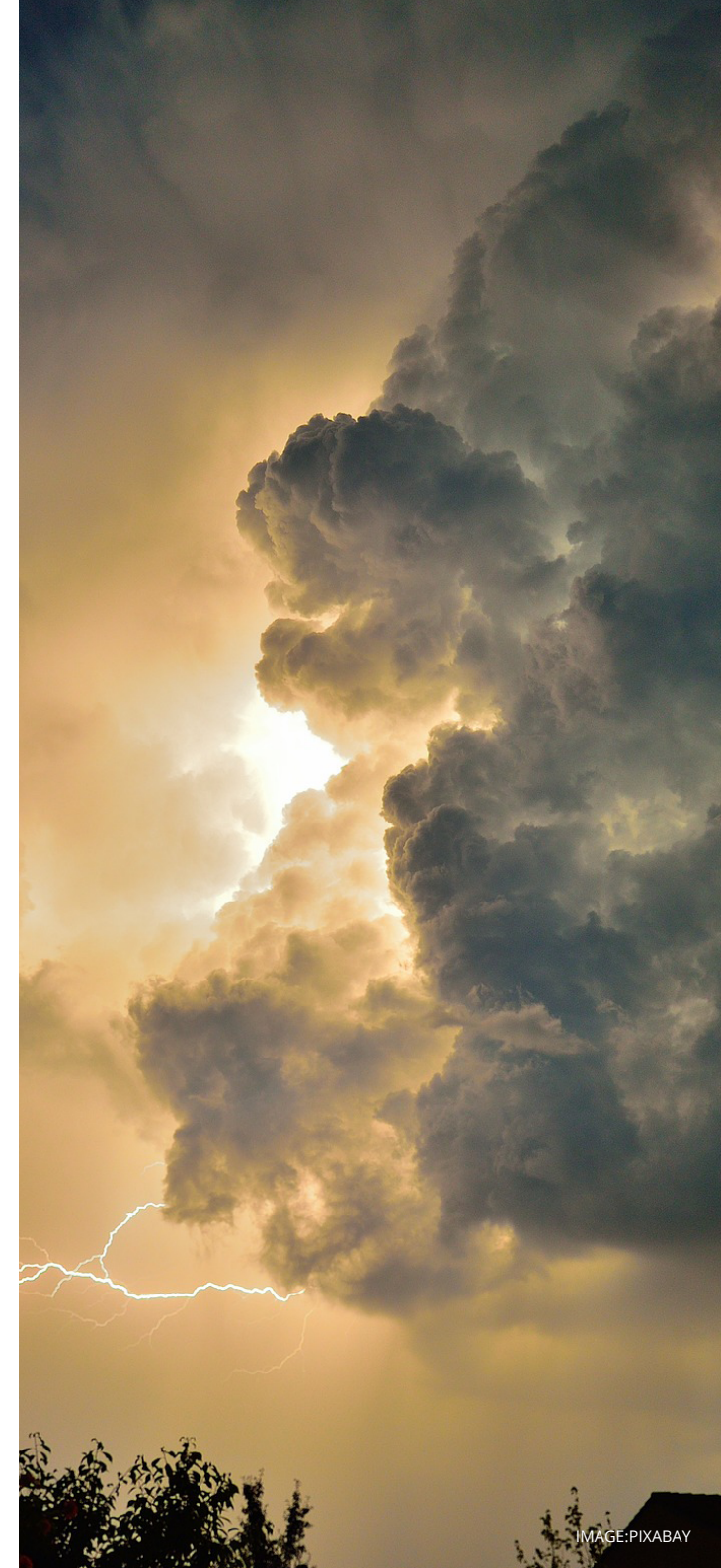
### OUTCOMES



### DISSEMINATION



### EDUCATION





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## 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.* Currently, *Trichinella* remains in the top three prioritised foodborne parasites in Europe and remains a major public health and economic concern at the international level. Due to the low prevalence of *Trichinella* in pigs, a test sensitivity bordering 100% is needed, as false positive samples would be tested with a second serological method. Such tests can only be performed by specialised laboratories, making testing logistics more complicated and expensive.

Therefore, new diagnostic methods with higher sensitivity and earlier detection are needed for the prevention, control, and detection of Trichinellosis. Such a method could involve the use of aptamers, synthetic nucleic acids that fold into unique 3D conformations capable of binding pathogen antigens with remarkable affinity and specificity. In addition to the ease of serological sampling, aptamers could be used to directly detect the presence of the pathogen at different stages of infection to improve monitoring in wildlife and domestic animals. Despite their fairly recent discovery, aptamers have already been used for the detection of parasites in fresh produce and other biological mediums by developing 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.

The PhD completed a range of its objectives in 2022, preliminary vaccination trials were carried out exploring the *T. spiralis* venom allergen-like immunogenic peptide (*TsVALp*). Blood samples were taken to monitor the levels of *TsVALp*-specific IgG and IgG2a by immunological methods. Preliminary results indicate that the naturally induced IgG could indeed recognise the use of the *TsVAL* peptide in the vaccine, supporting the previous proteomic identification of a *TsVAL*. Further investigation identified protective immunity results assessed by the number of ML recovered after 5 weeks correlated with ELISA results.

Further work was carried out to generate *Trichinella*-specific aptamer candidates by developing a novel whole-larva SELEX approach. In total, 16 rounds of SELEX were performed against the muscle larvae (ML) of *Trichinella*. Data produced from these works were qualitatively evaluated to determine aptamer binding to *T. spiralis* ML, the panel of aptamer sequences obtained from HTS and the bioinformatic analysis were subjected to confocal fluorescence microscopy assay. These methods allowed for the development and optimisation of a whole-larva SELEX method and confocal fluorescence microscopy confirmed the ability of most selected aptamers to bind the surface of *T. spiralis* ML.



#### Publications in 2022:

Le Dortz, L.L., Rouxel, C., Leroy, Q., Brosseau, N., Boulouis, H-J, Haddad, N., Lagrée, A-C., Deshuillers, P.L. (2022). Optimized Lambda Exonuclease Digestion or Purification Using Streptavidin-Coated Beads: Which One Is Best for Successful DNA Aptamer Selection? *Methods and Protocols*. 5(6), 89. DOI: <https://doi.org/10.3390/mps5060089>

### VIMOGUT



#### INTRODUCTION



#### VISION



#### STRUCTURE



#### ACHIEVEMENTS



#### OUTCOMES



#### DISSEMINATION



#### EDUCATION

Antimicrobial-resistance (AMR) is a major global health threat and public health concern. Many factors contribute to this, including the use of antimicrobials as growth promoters in livestock and the over-consumption of antimicrobials in humans and animals. It is essential to reduce the prevalence of AMR in livestock to diminish the probability of antibiotic-resistant bacteria passing through the food chain and to retain effective therapeutic effectiveness in the treatment of livestock. From all resistance mechanisms, extended-spectrum beta-lactamases (ESBL) are specifically relevant due to their ability to inactivate a large group of critically important antibiotics known as extended-spectrum cephalosporins, part of the  $\beta$ -lactam group. ESBLs have emerged globally in livestock and have been observed primarily in the Enterobacteriaceae family, especially in *Escherichia coli* strains. Despite efforts to reduce the burden of antimicrobial consumption and banning its use as growth promoters, a considerable proportion of ESBLs are observed in the gut microbiome of chickens. The **VIMOGUT** PhD project investigates the chicken gut microbiota development in relation to ESBL- *E. coli* colonisation during the chicken's lifespan

In 2022, the research continued with the development of a cost-efficient and animal-friendly tool, an *in vitro* chicken caecal model. This model is capable of reproducing the physiological conditions required to culture the main members of the chicken gut microbiota. The development of this model allows the PhD student to study the effects of microbiota-targeted intervention studies on the horizontal transmission of AMR genes. In addition, the *in vitro* gut model expands the possibilities of addressing additional microbiota-AMR related questions. The project further built upon this possibility; by optimising the protocol, evidencing continual improvement was required in setting up an *in vitro* cultured microbial community over time, ensuring experimental bias was reduced.

In 2022, the data from *in vivo* studies were published entitled 'Succession in the caecal microbiota of developing broilers colonised by extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli*'; which was submitted in 2021 as a manuscript. Further research is being carried out to determine whether similar patterns in the successional caecal microbiota dynamics are observed in slow-growing broilers naturally exposed to ESBL-*E. coli* colonisation.

Lastly, current research into 2023 is focused on applying these insights into a new *in vitro* system, which enables parallel operations of four bioreactors and comprises a more powerful and dedicated module for anaerobic experiments. Future work in the new *in vitro* system will be evaluated through two aims; i) the effect of media components on the maintenance of the *in vitro* caecal microbiota and ii) the effect of antibiotics and ESBL-*E. coli* on the *in vitro* microbiota.



#### Publications in 2022:

Cardenas-Rey, I., Bello Gonzalez, T.d.J., van der Goot, J., Ceccarelli, D., Bouwhuis, G., Schillemans, D., Jurburg, S., Veldman, K., De Visser, A., and Brouwer, M. (2022) Succession in the caecal microbiota of developing broilers colonised by extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli*. *Animal Microbiome*. 4(1), 51. DOI: <https://doi.org/10.1186/s42523-022-00199-4>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## ToxSauQMRA

*Toxoplasma gondii* (*T. gondii*) is an intracellular coccidian parasite capable of causing significant disease in the human population. Humans can act as intermediate hosts after being infected with *T. gondii* when handling soil, unwashed vegetables, cat litter, or tissue cysts in raw or undercooked meat. In France, in 2014 40.4 kg of pork was consumed, with  $\frac{3}{4}$  eaten in the form of sausage and salami products. Therefore, the health safety of these products is therefore a major issue. The **ToxSauQMRA** project aimed to answer the scientific question 'What is the attribution of the traditional raw pork products in human *T. gondii* infection?' based on three areas: i) a thorough investigation of the preferential sites for *T. gondii* in experimentally infected 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*; and iii) a quantitative microbiological risk assessment (QMRA) analysis to be conducted for the various raw pork products (e.g., dry sausage, dry ham, etc.).

In 2022, the project further explored various aspects to address the issues in pork production and reducing the spread of *toxoplasmosis* through pork products. These works were encapsulated into a comprehensive literature review of the most recent *T. gondii* in selected European animal populations, allowing for the creation of a Bayesian statistical model that estimates the age-specific seroprevalence for individual animal species, considering the effects of region, outdoor access, and sample matrix. The model and associated resources were made public to aid in the standardisation the conduct and reporting of future prevalence studies.

Furthermore, the potential effects of *T. gondii* genotype, stage of clinical response and tissue distribution in pigs were explored, providing insight into the epidemiology of *T. gondii* in pigs. The data found the highest prevalence of *T. gondii* load was observed in the uterus, while the highest prevalence of the parasite was observed in the shoulder muscles. The research expanded on its previous findings by investigating the distribution of *T. gondii* in pig tissues after experimental infection, highlighting the importance of considering infection routes and genotype in the development of preventive strategies against *T. gondii*.

Next, the inactivation potential of salt, nitrates, and nitrites on *T. gondii* in dry sausage and processed pork were assessed. It was observed that inactivation of the parasite in all tested recipes with salt concentrations above 2% after less than a day, occurred. Resulting from these works, an assessment of the French and European country-specific meat products including their composition and processing steps during production that may influence viability of potential *T. gondii* tissue cysts were carried out.





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

Finally, the necessary databases were updated for the development of novel heating and salting models applicable to meat products. The goal was to incorporate these models into the final quantitative microbial risk assessment (QMRA) model, which aims to estimate the relative contribution of different sources of *T. gondii* human infections. This novel QMRA model will provide an effective tool for decision-makers in the development of preventive strategies against the parasite.







### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## TRACE

Hepatitis E virus (HEV) is a zoonotic virus responsible for acute hepatitis E in humans. Worldwide 20 million HEV infections estimated annually. Genotypes 1 – 4 can infect humans and Genotypes 3 and 4 are zoonotic and responsible for HEV infections in developed countries. The main route of HEV 3 and 4 is by domestic and wild pigs (pork). Molecular analyses showed that HEV strains detected in humans and pigs in the same geography present high genetic similarity, this is indicating that swine are the main source of the incidence of HEV in humans in western countries. Since 2014, an increase in clinical cases of HEV has been observed in European countries, including the Netherlands. In the summer of 2017 – 18, a temporary drop in the incidence of HEV was observed, after which the incidence of HEV returned to ordinary levels. These events have remained unexplained to date.

The **TRACE** PhD project was created to explain the predominance of certain HEV variants and to explain differences in virulence factors in HEV strains detected in clinical patients, in the general population, and in domestic swine. Given the high genetic diversity of HEV, it is critical to obtain high-resolution genomic data (i.e., Whole Genome Sequences) to gain more understanding of its molecular epidemiology and possible variations in its adaptive traits.

The PhD student worked on a fast sample (pre)processing (enrichment) method to make Whole Genome Sequencing (WGS) possible, using specific primers, random primers, or specific probes to generate whole sequences of the HEV. These works are under review to be published as a manuscript in 2023. Next, the research using HEV WGS to construct a time-phylogeny of HEV based on core-genome single nucleotide polymorphisms (SNP) analysis. From this analysis evolutionary divergent time points can be inferred, coupled with geographical location date, allowing geographical origin to be determined. Lastly, genome-wide association procedures were used to identify genomic regions associated with HEV virulence and HEV infectivity by linking genomic data to available epidemiological data (i.e., population incidence of patients with acute hepatitis, clinical data, and veterinary data).

Additionally, these works will be built upon utilising bioinformatic pipelines to further understand the data in the context of HEV incidence and virulence in Europe into 2023.





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## Codes4strains

Whole Genome Sequencing (WGS) allows the identification and tracking of pathogenic strains, informing on infection control, diagnostics, and at times treatment strategies. To track strains globally as they spread between the environment, food, animals, and humans globally, universal strain nomenclatures are necessary. The core genome Multilocus Sequencing 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, Marakeby *et al.* (2014) introduced a new coding approach named Life Identification Number (LIN), which 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 the genome rather than to groups, they are stable.

The aim of the [Codes4strains](#) project was to develop a novel genome-based genotyping approach taking the beneficial parts of the two above classification approaches (i.e., combining the advantages of cgMLST with LIN code approach). Therefore, the aim was 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, such as the SNP address and multi-level single-linkage classification (MLSL). The important pathogen *Klebsiella pneumoniae* was used to develop, optimise, and evaluate this approach. The PhD project also encompassed studies of population-level studies of the agent of diphtheria, *Corynebacterium diphtheriae*.

In 2022, the PhD was completed, with the bioinformatic analyses of the population structure of antimicrobial resistance (AMR) in *C. diphtheriae* based on a large clinical isolate collection from France's metropolitan and overseas territories. A genome-wide association study (GWAS) was performed identifying a new penicillin resistance gene on a mobile element in *C. diphtheriae*. From these works, a bioinformatic genotyping tool, DIPHTOSCAN, was developed, to harmonise and facilitate the detection and genotyping of the main virulence factors and resistance genes of *C. diphtheriae*. Additionally, a strain nomenclature from assembled genomes was developed. The public availability of the tool (<https://gitlab.pasteur.fr/BEBP/diphotoscan>) and its ease of use will advance the genomic epidemiology of the agent of diphtheria, the clinical management of patients, and knowledge on the links between animal and human diphtheria cases.

The genomic taxonomy of *K. pneumoniae* strains developed based on LIN codes provides precise and stable strain nomenclatures. The proposed unified classification and naming of *K. pneumoniae* strains will facilitate advances on the biology of its sub-lineages across niches, time, and geographies, and will endow surveillance networks from different activity sectors, including food microbiology and veterinary and human medicine, with the capacity to efficiently monitor and control the emergence of sub-lineages of high public health relevance.



#### Publications in 2022:

Hennart, M., Guglielmini, J., Bridel, S., Maiden, M.C.J., Jolley, K.A., Criscuolo, A., Brisse, S. (2022). A Dual Barcoding Approach to Bacterial Strain Nomenclature: Genomic Taxonomy of *Klebsiella pneumoniae* Strains. *Molecular Biology and Evolution*. 39 (7), msac135. DOI: <https://doi.org/10.1093/molbev/msac135>



### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

## SUSTAIN

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

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

In 2022 the research was finalised, with the survey and interview studies providing insight into two One Health topics: Antimicrobial resistance (AMR) and the One Health networks. AMR is a public health threat as bacteria, viruses, and fungi continue to evolve resistance mechanism to first and final line treatments. AMR is a One Health topic as it affects the health of both humans and animals, by providing a challenge for medical treatments, while also effecting the environment directly and indirectly by facilitating the spread of AMR. Due to the complexity of AMR, it is crucial that stakeholders within different institutes and industries work together in combating the issue via AMR stewardship programmes and cross-sector networks. Encouraging the development of National Action Plans for AMR can help further assess the situation and address the challenges.

Within One Health networks, the environment sector is often neglected, albeit being one of the pillars of the human-animal-environment interface. Further, the engagement of actors from the social and political sciences is limited. However, the environment sector is a crucial part of the approach, as the environment can be a pathway, host, or barrier for diseases. On national levels, the different ways of working (e.g., methodological, or analytical approaches) can hinder collaboration and communication. Further, the political and social science sectors can complement One Health-related projects in providing tools to comprehend contexts, cultures, local circumstances, and to provide specific perspectives such



#### Publications in 2022:

Humboldt-Dachroeden, S. (2022). A governance and coordination perspective – Sweden's and Italy's approaches to implementing One Health. *SSM – Qualitative Research in Health*. 2, 100198. DOI: <https://doi.org/10.1016/j.ssm-qr.2022.100198>



### INTRODUCTION

as gender issues or knowledge from indigenous communities. Mandating the connection of environmental, social, and political experts and institutes can facilitate collaboration. Using clear strategies, mapping stakeholders, and translating knowledge across sectors will strengthen a One Health approach that includes all relevant actors and sectors.



### VISION

The results of this research will inform the operationalisation of the One Health approach within institutes. It can provide insight into challenges, but also opportunities that can be used to facilitate One Health institutionalisation, create networks, and bridge disciplinary silos.



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

# One Health EJP Dissemination Activities



INTRODUCTION

## 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 2022:



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

- OHEJP outcomes were regularly disseminated at key governance meetings, such as Scientific Steering Board meeting, Stakeholders Committee meeting, the Programme Management and Programme Owners Committee meeting.
- OHEJP WP4 organised the 4th Thematic Integrative Meeting on 'Data sharing across disciplines' held online in February 2022. The OHEJP was involved in several stakeholder activities, which included involvement in new initiatives, joining pre-existing initiatives, and providing other ad hoc support. For example, TOXOSOURCES project involvement in [DISH Cluster](#) – a European collaboration that aims to guide consumers towards a healthy and safe diet.
- The OHEJP contributed to several external international events, including the [PREZODE](#) initiative online event "Joining forces to escape the era of pandemic" in February 2022, two workshops at the [Geneva Health Forum](#) in May 2022 on [Time to rethink the science-policy interface? From COVID-19 lessons to strengthening One Health global policies](#) and [What training should we build for One Health / planetary health?](#), and two side events at the [ONE Conference 2022](#) in June 2022 on [Food Safety Knowledge Exchange \(FSKX\) Workshop](#) and [One Health EJP: Lessons learnt from a European multidisciplinary initiative](#).
- The [OHEJP SimEx Project](#) delivered a foodborne outbreak exercise scenario across 11 European countries employing the One Health approach. It led to intersectoral cooperation through the sharing of experiences, views, and perspectives.
- The series of Dissemination Workshops continued to showcase the impact of OHEJP outputs to national, European, and international decision makers and strategic planners. The second workshop was held in March 2022, which focused on One Health Preparedness and led to the [One Health EJP Report of the Dissemination Workshop on Improving One Health Preparedness to \(re\) Emerging Infectious Threats](#).
- The third Dissemination Workshop was organised in collaboration with OHEJP SimEx Project and Science to Policy Translation teams and held in December 2022. This workshop focused on the experiences from conducting the OHEJP SimEx and its applications for using OHEJP solutions to improve outbreak preparedness under the One Health approach. This event led to the [One Health EJP Report of the Joint SimEx/Dissemination Workshop: a One Health Simulation Exercise as a Roadmap for Future Foodborne Outbreak Preparedness](#).



ne HEALTHEJP

Save the Date!

One Health EJP side event at ONE 2022 conference:  
Lessons learnt from a European multidisciplinary initiative

25th MARCH 2022: agenda

10.00	Welcome from Work Package 5 Science to Policy Translation Anemarie Kibohrer, BPR, Mikko Jalavinen, SSP Introduction to One Health Preparedness Kitty Masson, RWM Aspect of Antimicrobial Resistance: AMR movement over ecosystem boundaries Warner Happpach, ACEF Aspect of zoonoses – a wide range of threats: Improved genotyping approach to support preparedness to detect new emerging strains of an endemic zoonotic parasite Geroen Schere, <a href="#">EFSA</a> Aspect of integration of approaches: Strengthening One Health collaboration in the area of risk analysis Kitty Masson, <a href="#">EFSA</a> , RWM Tools to support outbreak management and surveillance Marion Gottschald, <a href="#">EFSA</a> , BPR Integrated surveillance for foodborne disease Edo Frits, <a href="#">EFSA</a> , RWM
11.25	Break
11.30	Mapping existing surveillance chains at country level Francesco Cio, <a href="#">EFSA</a> How to assess capacity to perform One Health surveillance Viorica Hrisca, <a href="#">EFSA</a> , ANRS, Joaquin Prieto, <a href="#">EFSA</a> , University of Surrey Conclusion Guido Benedetti, SSP Discussion Chaired by Anemarie Kibohrer, BPR, Mikko Jalavinen, SSP European Climate And Health Observatory Aleksandra Kozmarczak, EMA
12.30	Close



INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES

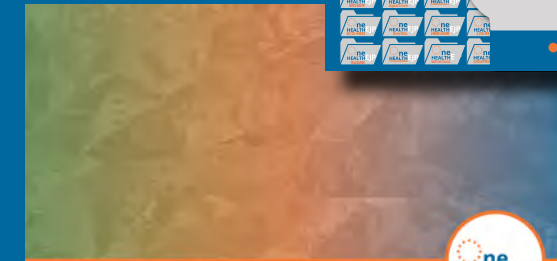


DISSEMINATION



EDUCATION

- The MATRIX Webinar Series 2022 on Solutions for One Health Surveillance in Europe delivered seven webinars between May to December 2022. These presented the solutions developed by [JIP MATRIX](#) to support and advance the implementation of One Health Surveillance in European countries.
- [JRP BIOPIGEE](#) delivered two online workshops to wide audiences in September 2022 that presented BIOPIGEE project outcomes on effective biosecurity measures at farms and slaughterhouses.
- Horizon – the EU Research and Innovation Magazine interviewed OHEJP Scientific Co-ordinator, Hein Imberechts, about antimicrobial resistance (AMR) in May 2022 and published the article [The end of superbugs starts with better animal welfare](#).
- [RIVM](#) interviewed [JIP COHESIVE](#) project leader Kitty Maassen in October 2022 and published the article [Reflecting on European One Health project COHESIVE](#).
- Targeted reports were created to ensure that key EU stakeholders are informed of the key scientific and integrative outcomes from the OHEJP.
- An additional [Cogwheel workshops case study](#) was produced in July 2022 and widely disseminated on OHEJP social media. It summarised the eight Cogwheel Workshops focused on surveillance and prevention of antimicrobial resistance, infectious (both animal and human) and foodborne diseases.
- The report on the [Analysis of outcomes and uptake of EJP's outputs by stakeholders](#) was produced to ensure that the OHEJP main scientific outputs, protocols, databases, and other strategic integration activities will be sustainable beyond the lifetime of the programme. A shortened version of this list was created for dissemination: [OHEJP Analysis of Outcomes](#).
- The [OHEJP Outcome Inventory](#) was regularly updated. It acts as a repository for all OHEJP outcomes (JIPs, JRPs and PhD projects) and is a hub for information for both national and international stakeholders and both internal and external audiences.
- The Data Management Plan continued to be implemented. An abundance of support has been provided to the Project Leaders of the OHEJP projects to ensure that data generated in their projects are FAIR (findable, accessible, interoperable, and reusable).
- The OHEJP [website](#) and social media platforms served as central platforms to share public events, news, research outcomes and key information to a global audience.
- The OHEJP issued frequent newsletters, which contained highlights and links to scientific and collaborative activities. The Consortium and external newsletters act as an important tool to disseminate news on a regular basis and are accessible on the [website](#).



Analysis of outcomes and uptake of One Health EJP outputs by stakeholders

February 2023



## DISSEMINATION



INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

- The OHEJP Communications Team worked with Project leaders to create three new project impact brochures for **IMPART**, **TOX-Detect** and **LISTADAPT**. These are a valuable dissemination tool to share key messages and outcomes from each project.
- The OHEJP Annual Scientific Meeting provided a central platform for communication and dissemination of the scientific outcomes from the Joint Research and Joint Integrative Projects, in addition to the PhD projects. This event also facilitated collaboration with One Health experts both internal and external to our Consortium.
- The establishment of excellent relationships with key EU and international stakeholders facilitates translation of science to policy, in addition to ensuring there is no duplication of research between major European organisations.
- The OHEJP disseminated important scientific results on a regular basis to the European Commission, the Research Executive Agency (REA), and other key European stakeholder members thus ensuring exchange of information and knowledge to maximise impact.
- Organisation of Education and Training activities to aid in training the next generation of One Health researchers.







INTRODUCTION

### The Fourth One Health EJP Annual Scientific Meeting

**Organising institutes:** Istituto Superiore di Sanità, Rome, Italy

**Location:** Hybrid event online and at The People's Palace in Orvieto, Italy

**Dates:** 11th-13th April 2022



VISION

The fourth [One Health EJP Annual Scientific Meeting](#) was a highly successful hybrid event that brought together 276 participants from across 22 countries joining in-person and online. The three-day conference highlighted the importance of an integrated, cross-sector approach to global health threats, which includes foodborne zoonoses, antimicrobial resistance and emerging threats, using the One Health approach. The scientific programme showcased the research developed by the One Health EJP projects that offers novel solutions to these threats. The wide variety of One Health research was shared in 30 oral presentations (including 6 keynote presentations), 99 poster presentations and 3 roundtable discussion sessions. A dedicated session was co-organised with the Med-Vet-Net Association on the topic of the sustainability of One Health activities between our organisations.



STRUCTURE

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



ACHIEVEMENTS

The Keynote speakers focused on One Health topics highly relevant to the recent global challenges impacting society, for human and animal populations. Prof. Tiago Correia from [NOVA University of Lisbon](#) (Portugal) discussed about a larger vision to protect public health. Dr. Elisabetta Suffredini from [Istituto Superiore di Sanità](#) (Italy) explained how to use wastewater for surveillance of foodborne and zoonotic infectious agents. Prof. Raina Plowright from [Montana State University](#) (USA) discussed pathogen spillover through the lens of emerging bat viruses. Dr. Jean-Yves Madec from ANSES (France) described antimicrobial resistance in relation to environmental issues. Prof. Bruno Gonzales Zorn from [Complutense University of Madrid](#) (Spain) discussed antimicrobial resistance in relation to COVID-19. Dr. Vittorio Fattori from the [Food and Agriculture Organisation](#) provided some global perspectives on food safety in respect to climate change and other emerging issues.



OUTCOMES

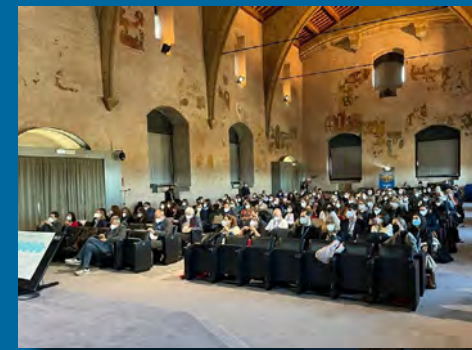
The social media presence for this event generated a great international response with interactions between scientists and stakeholders globally. News of our international meeting and the role of the One Health EJP in promoting the One Health approach was featured in the Italian publication of *Quotidiano Sanità* – the reference newsletter on public health and biomedical research in Italy. Read the article (in Italian) [here](#).



DISSEMINATION



EDUCATION





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

## Two One Health EJP side events at the ONE Conference 2022 in Brussels

### First event: Food Safety Knowledge Exchange (FSKX) Workshop

The workshop was successfully delivered on 20th June 2022 as a side event at this conference and funded by JIP MATRIX. Presenters from BfR, Statens Serum Institut, Anses, and Finnish Food Authority contributed to the event. The workshop demonstrated the importance of predictive models and data analysis pipelines with the harmonised FSKX format, to promote its adoption by One Health professionals and modellers. Participants performed hands-on exercises with an online model repository containing FSKX models in the RAKIP Model Repository. The wide range of participants from the German Federal Institute for Risk Assessment (BfR), French Agency for Food, Environmental and Occupational Health and Safety (Anses), SGS, National Cheng Kung University, Feed and Additives GmbH, Bogomolets National Medical University, University of Oslo, and European Food Safety Authority (EFSA), gained skills using these valuable modelling tools. Learn more about this well received workshop [here](#) and download the event [summary](#).

### Second event: One Health EJP - Lessons learnt from a European multidisciplinary initiative.

This high-level roundtable panel discussion was successfully held on 22nd June 2022 as a side event in hybrid format at this conference. It included eight speakers from key European organisations involved in public health, animal health and food safety sectors.

OHEJP Scientific Coordinator, Dr Hein Imberechts ([Sciensano](#)) provided a comprehensive overview of the One Health EJP. He explained that the Consortium's success was based on a multidisciplinary framework with cross-sector collaborations to facilitate One Health research and integrative activities. Dr Nadia Boisen ([Statens Serum Institut](#)) as Project Leader for JIP OH-HARMONY-CAP shared her experiences of coordinating multiple institutions to harmonise protocols for the detections of foodborne pathogens and AMR determinants across health sectors. Peter Sousa Hoejskov ([World Health Organisation Regional Office for Europe](#)), Dr Stef Bronzwaer ([European Food Safety Authority](#)) and Dr Ole Heuer ([European Centre for Disease Prevention and Control](#)) described each of their organisation's relationship with our Consortium to benefit One Health platforms and networks globally. These speakers and Prof. Thomas C. Mettenleiter ([Friedrich-Loeffler-Institut](#) and member of the Quadripartite One Health High-Level Expert Panel) also advised on increased involvement of the environment sector in One Health. Dr Arjen van de Giessen ([RIVM](#)) represented the [Med-Vet-Net Association](#) and he recommended strengthening One Health preparedness, based on lessons learnt from the COVID-19 pandemic. The importance of One Health EJP education and training opportunities were highlighted by Ingrid Cardenas Rey PhD student of [VIMOGUT](#) project based at [Wageningen University and Research](#). She explained how these activities had increased her skills and networks. This event was skilfully moderated by Dr Pikka Jokelainen ([Statens Serum Institut](#)).

A short video is available to watch on [YouTube](#). The full video of the event is on the [ONE2022 website](#).

[onehealth.ej.eu](https://onehealth.ej.eu) @OneHealthEJP ONE Health EJP





# Educating the next generation of One Health researchers



## EDUCATION AND TRAINING

The OHEJP Education and Training activities (WP6) developed and delivered innovative training platforms and materials with a specific focus on One Health.

INTRODUCTION



VISION

Our Education and Training activities uniquely brought together students, early-career researchers, and key experts with diverse expertise in the human, animal, and environmental health fields. These activities reinforced collaboration across multiple disciplines, and integration between the Consortium member institutes and stakeholders (and beyond), bringing these individuals together so that One Health knowledge and experiences can be shared, and collaborative engagements and future relationships can be formed for One Health activities.



STRUCTURE

## ONE HEALTH EJP FINAL (SUMMER) SCHOOL 2022

Our annual Summer Schools were an important component of the One Health EJP, as they provided training opportunities for the next generation of One Health scientists from across Europe and worldwide.



ACHIEVEMENTS

**Organising institutes:** University of Surrey (UK)  
**Theme:** Sustainability in One Health – how can it be achieved?  
**Location:** Online  
**Dates:** 5th to 7th December 2022



OUTCOMES

**Collaborative interactions:** A wide range of 30 scientists from 16 institutes across Europe, Australia, New Zealand, and Brazil, provided a comprehensive multidisciplinary expertise in veterinary and human medicine, environmental protection, disease surveillance and prevention, open research, scientific communication and One Health policies. The Chief Scientist of EFSA (one of the main OHEJP stakeholders) also enriched the programme by providing overviews of the challenges for the One Health governance in this post-pandemic era. Our expert speakers explained how the applications of their work can benefit people employed in public health, animal health and food safety sectors and in general for society. The collaborative interactions between the experts and delegates from countries across the globe provided opportunities for knowledge, skills, and competencies to be shared amongst individuals with different perspectives and experiences.



DISSEMINATION



EDUCATION





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

**Delegates:** Bringing together 246 delegates from 56 countries across the globe and 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. Participants attended from several countries within the EU but also from Asia, the Middle East, Africa, Australia, and the North, South and Central Americas. Delegates represented various career stages 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, engineering, chemistry, pharmaceuticals, toxicology, epidemiology, ecology, agriculture, economics, nutrition, and environmental, social, and biological sciences. The diversity in education background and expertise significantly enriched the experience of all those that participated and added value to the course.

**Programme:** Sustainability being at the core of the One Health approach, this final (summer) school programme aimed to illustrate sustainable approaches to One Health research, to demonstrate the importance of education and training activities for the future generations of One Health professionals, and the relevance of effective communication and open research for multisectoral collaborations; as well as to discuss how policies can shape the future of One Health and how research ideas into commercial products. The programme delivered insightful presentations from expert speakers about education and training opportunities, commercialisation, communication, sustainability, policy, and research projects applications, as well as lively question and answer discussions.

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

View the full report [here](#).





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

## 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 covered several themes in One Health and were targeted at Early Career Researchers who can apply the training they received in their future careers and in the training of future junior researchers

**Organising institutes:** Statens Serum Institut and DTU-Food, Denmark

**Theme:** Rapid diagnostics and harmonisation of diagnostic tests

**Location:** Online and at the Statens Serum Institut and DTU-Food, Copenhagen, Denmark

**Dates:** 2nd to 4th November 2022

**Collaborative interactions:** The module was delivered by leading European experts on diagnostics methods who discussed the role of diagnostics and related challenges from both microbiological and epidemiological perspectives. The module provided an opportunity to bring together experts and 32 participants from 14 countries, to share knowledge and experiences. The diversity in educational background, experience and countries significantly enhanced the quality of the module and the experience of all those that participated.

**Delegates:** This training event was attended by 9 in person participants and 23 online participants. Participants included early-career researchers and PhD students, up to senior scientists, from across our consortium partner network and beyond. Bringing together epidemiologists and microbiologists from across the health disciplines made this module cross- disciplinary and highlighted the role of diagnostics and related challenges from both microbiological and epidemiological perspectives, using a One Health approach. In total, 14 countries were represented by all attendees, which included those in Europe (Denmark, Italy, Iceland, the Netherlands, Poland, Portugal, Romania, Slovenia, Spain, Switzerland, UK and Ukraine) and some further afield (India and Turkey).

**Programme:** The CPD module was broken down into six sessions over three days; two hybrid sessions and four on-site sessions, at SSI and DTU Food. Several different teaching and learning methods were used in the module, conducted on-site and online, including hands-on exercises in the laboratory and at the desk, and focus group discussions. The module began with introductory lectures from the invited keynote speaker as well as several experts from the hosting OHEJP partner institutes and ended with a common restitution session. In the following four sessions, online participants worked



**one HEALTH EJP**

One Health EJP Continuing Professional Development Module: **Rapid diagnostics and harmonisation of diagnostic tests**  
COPENHAGEN, DENMARK

**WEDNESDAY 2nd NOVEMBER 2022**

09:00 **CEP LECTURE 1 - Hosted by DTU Food and SSI**  
Welcome and introduction to One Health EJP  
Keynote address: Caroline Eves and Rene Hendriksen  
Peter Carver-Saunders

10:00 **Break**

10:15 **One Health surveillance standardisation and harmonisation**  
Nadia Botos  
10:30 Pathogenic E. coli  
Franziska Schaeff

11:00 **Break**

11:05 **Whole Genome Sequencing and CGE Bioinformatics (online)**  
Jette Sajer Kjaergaard  
11:30 Disease surveillance and outbreak investigation  
Emily Ochoa-Winkel

12:00 **Break**

12:05 **SESSION 2 - Hosted by SSI Food**  
13:00 Introduction to E. coli Case Study  
Nadia Botos  
13:15 Lab work: Phenotypic and molecular typing and characterisation  
Susanne Jespersen, Nadia Botos and Rene Hendriksen

14:00 **Break**

14:05 Lab work: Phenotypic and molecular typing and characterisation  
Susanne Jespersen, Nadia Botos and Rene Hendriksen

15:00 **SESSION 3 - Hosted by DTU Food**  
15:00 Lab work: Interpretation and visualisation of results  
Susanne Jespersen, Nadia Botos and Rene Hendriksen

16:00 **Break**

16:45 **Epidemiology work: case definition and descriptive epidemiology**  
Emily Ochoa-Winkel and Louise Müller

17:45 **Break**

18:00 **Epidemiology work: patient interviews and hypothesis generation**  
Emily Ochoa-Winkel and Louise Müller

18:45 **Break & Reception**

@OneHealthEJP ONE Health EJP



INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

independently on a case study regarding an outbreak of cryptosporidiosis (*Cryptosporidium* parasite) among veterinary students; and in-person participants worked together in and out of the laboratory to carry out a case study of an *E. coli* (bacteria) outbreak. Participants were able to collaborate and work through various exercises and analyses together. The *E. coli* case study microbiology component was written by Susanne Jespersen, Sarah Marvig Johansson and Nadia Boisen (OHEJP JIP OH-Harmony-CAP) and the epidemiology component was written by Luise Müller and Emily Dibba White. The cryptosporidiosis case study was written by Guido Benedetti and utilized knowledge gathered by OHEJP JIPs MATRIX and OH-Harmony-CAP as well as JRP PARADISE.

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

View the full report [here](#).





INTRODUCTION

## ELEVEN ONE HEALTH EJP SHORT TERM MISSIONS IN 2022

Short Term Missions (STMs) were created as small travel grants with the aim of sharing scientific expertise, methodologies, equipment, and facilities to harmonise the existing approaches and methodologies within the large OHEJP network.



VISION

STMs have helped to drive OHEJP research forward in a collaborative and non-duplicative fashion. This has strengthened both the scientific capacity within the OHEJP and future prevention, preparedness, detection, and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors. Following the COVID-19 pandemic, several postponed STMs during pandemic took place in 2022, in addition to the ones funded through the 2022 call. Funding from previously cancelled STMs was re-allocated, through an additional call for STMs. A total of eleven STMs were successfully conducted in 2022.



STRUCTURE

### STM 1: Surveillance and source-attribution of AMR based on metagenomic analysis

**Theme:** Skills Development Missions

**Home Institute:** [National Institute for Agrarian and Veterinary Research \(INIAV\)](#), Portugal

**Mission Hosting Institute:** [Technical University of Denmark \(DTU Food\)](#), Denmark

**Duration of mission:** 2 weeks

**Background and aim of mission:** The aim of this STM was to learn novel approaches and models based on metagenomic data for surveillance and to infer source attribution of AMR determinants.

INIAV is the National Reference Laboratory for AMR in animals and food of animal origin and needs scientists with the skills to analyse and interpret metagenomics data. The STM gave the researcher the opportunity to improve their skills for analysing metagenomics data for the purpose of surveillance and source attribution of AMR. Ana was engaged in the whole process, from samples preparation to the final epidemiological data analyses, although the main focus was on the bioinformatic analyses and epidemiologically modelling. This mission will contribute to the development of skills in metagenomics, bioinformatics, and AMR source-attribution approaches, needed in the frame of the OHEJP consortium and in future collaborations. Use of data from omics technologies (WGS and metagenomics) for source attribution WGS and metagenomics-based surveillance allows the characterisation of antimicrobial resistance determinants, making it possible to identify the potential for their dissemination. The training will improve the participation of INIAV in the OHEJP DISCOVER project methods: assessment/improvement- related with source attribution of AMR based on metagenomics.

**STM 2:** Zoonotic pathogen detection in rats (funded through the 2021 STM call, postponed to 2022 due to COVID-19)

[onehealth.ej.eu](https://onehealth.ej.eu) @OneHealthEJP ONE Health EJP



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION







**Theme:** Emerging threats

**Home Institute:** National Institute of Public Health and the Environment (RIVM)

**Mission Hosting Institute:** Friedrich-Loeffler-Institut (FLI)

**Duration of mission:** 1 month

**Background and aim of mission:** During this the STM, Marieke de Cock, PhD student on the OHEJP PhD project DESIRE at the RIVM (in the Netherlands), went to the Friedrich-Loeffler Institut (FLI) in Germany to test rat and mouse samples (total n=670) on different zoonotic pathogens. The rat and mouse samples were collected during Marieke's fieldwork. The samples were tested for Cowpox virus, rat Hepatitis E virus (rat HEV), Lymphocytic choriomeningitis virus (LCMV) and Streptobacillus moniliformis. For the Cowpox virus testing two different tests were used: qPCR (on DNA extracted from nasal septum samples) and Immunofluorescence assays (IFA) using heart fluid on cowpox-infected cells. Both tests were performed in the Biological Safety Level 3 (BSL-3) laboratory. No Cowpox virus positive rats or mice were detected. For rat HEV, liver samples were tested with both conventional PCR and qPCR. In total, 15 rats were positive for rat HEV. These samples were further sequenced and analysed. Mouse liver samples were also tested for LCMV by conventional PCR and one positive house mouse was positive, which is an interesting result since LCMV has not been detected in wild house mice in the Netherlands for years. This sample will be further sequenced by WGS. The rat samples were tested for S. moniliformis by both qPCR and serology in collaboration with two German partner institutes. The serology validation tests were still ongoing at the end of the STM. Using serology on heart fluid, 33% of the samples were considered positive. However, using qPCR on DNA extracted from salivary glands, only seven positive samples were detected (<2%). All the pathogens that were tested will be included in the paper about zoonotic pathogen carriage in wild rats from urban areas and in additional pathogen-specific papers. Next to the valuable test results, this STM contributed to interesting discussions about rodent borne (zoonotic) pathogen research, increased contact between FLI and RIVM and led to new collaborations in sharing of data and writing of papers between FLI and RIVM.

INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

**STM 3:** Validation and exchange of modelling tools to assess the risk of human Salmonellosis based on environmental factors using multiple sources of data

**Theme:** One Health, skills development

**Home Institute:** University of Surrey, UK

**Mission Hosting Institute:** Dutch National Institute for Health and Environment (RIVM)

**Duration of mission:** 1 month

**Background and aim of mission:** The aim of this mission was to investigate whether the effect of weather on human salmonellosis cases is similar regardless of the country under study, using a novel statistical modelling approach developed in the University of Surrey, UK. The model was built on 30





### INTRODUCTION



### VISION



### STRUCTURE



### ACHIEVEMENTS



### OUTCOMES



### DISSEMINATION



### EDUCATION

years of daily epidemiological data from the UK Health Security Agency (UKHSA) and a high resolution spatio-temporal matching weather database from the Met Office. The model first estimates the probability to observe salmonellosis cases conditional on a given combination of weather factors. Based on this information together with local weather and demographic data, the model reproduces relatively well the empirical patterns from epidemiological surveillance data for England and Wales. It also points to maximum air temperature, relative humidity, and day length as one of the most relevant combinations that influence the incidence of the disease. During the STM, the model was applied to the Dutch setting, using 5 years of national surveillance data on salmonellosis cases reported to the Dutch National Institute of Public Health at a daily resolution and the weather variables of interest for the same period of time obtained from the open-resource Royal Netherlands Meteorological Institute (KNMI) website. To assess the universal component of the model to identify the weather-disease relationship regardless of geographical area, the probability of finding a salmonellosis case calculated for England and Wales were used. The model's results were then compared with salmonellosis disease records from The Netherlands. The preliminary results indicate that the model captures the magnitude and key seasonal patterns of the Dutch data. However, the model also results in some secondary peaks in the incidence in early spring not observed in the real data, perhaps related to differences in notification and health seeking behaviour. The STM has improved on the relationship between two existing One Health EJP partners and enhanced both current and future collaborations between the partner institutes. A joint publication of the collaborative results should be published soon and this STM should result into a unified programme of research integrating the two approaches over diverse geographic and socio-economics settings.

#### STM 4: Application of spatial models to identify new environmental surveillance indicators of *Salmonella* and *Campylobacter* in pig and poultry

**Theme:** One Health, skills development

**Home Institute:** The National Institute for Agricultural and Food Research and Technology (INIA), Spain

**Mission Hosting Institute:** National Institute for Public Health and the Environment (RIMV), The Netherlands

**Duration of mission:** 1 month

**Background and aim of mission:** The aim of this mission was to develop a procedure to model surveillance data of zoonotic diseases using machine learning methods. The model was tested on the Wild Boar Surveillance Plan of Andalucía (Spain) data, with the aim to determine the most important risk factors that shape wild boar diseases in Andalucía and study their interaction between each other. During this mission, the model validation was performed in two main steps. First, a single response variable was selected for the study: the *Salmonella* serology of hunted wild boars. In the first week of the STM, the data was organised in an appropriate way for training machine learning algorithms. Additionally, climate and wild boar distribution information from published maps were collected, to include them as

**SHORT TERM MISSION**  
Short Term Missions (STMs) are small travel grants with the aim of:  
• Harmonise the existing approaches and methodologies within the large OHEJP European network  
• Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the OHEJP  
• Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human/animal/environmental sectors.

**Application of spatial models to identify new environmental surveillance indicators of *Salmonella* and *Campylobacter* in pig and poultry**

**Theme:** One Health Missions - Skills development missions  
**Home Institute:** The National Institute for Agricultural and Food Research and Technology (INIA), Spain  
**Mission Hosting Institute:** National Institute for Public Health and the Environment (RIMV), The Netherlands  
**Duration of Mission:** 1 month

**“ I had the chance to meet a new research centre and to collaborate with and take advice from experts on the field. We obtained results that we could not have obtained working independently. I carried out an important advance in my research project and acquired new skills that we will use in future projects. I would like to highlight that thanks to my short-term mission I pushed my data analysis skills to an upper level.”**  
Anaïs Rodríguez  
The National Institute for Agricultural and Food Research and Technology Spain

The aim of this mission was to develop a procedure to model surveillance data of zoonotic diseases using machine learning methods. The model was tested on the Wild Boar Surveillance Plan of Andalucía (Spain) data, with the aim to determine the most important risk factors that shape wild boar diseases in Andalucía and study their interaction between each other.

During this mission, the model validation was performed in two main steps. First, a single response variable was selected for the study: the *Salmonella* serology of hunted wild boars. In the first week of the STM, the data was organised in an appropriate way for training machine learning algorithms. Additionally, climate and wild boar distribution information from published maps were collected, to include them as potential drivers of the pathogen. The results being conclusive, a modelling procedure valid for all the wild boar diseases in the dataset was designed. This includes the processing of the dataset before the modelling (pre-processing step), the model application itself and its validation, and the study of the model output. The best way to compensate the unbalance of the positives and negatives in the dataset was explored, to avoid reducing the sensitivity or specificity of the model. A script was prepared to provide several validation statistics based on the comparison between the predictions on the test data and the real positive and negative values. The variable importance was assessed on the training and test data, spatial dependence plots based on these data were created and detection of interactions between predictors on the test data was conducted. The developed procedure successfully determined the most important risk factors that shape wild boar diseases in Andalucía and studied their interaction between each other. Using the data from previous hunting seasons, it also allowed to identify areas with higher and lower probabilities of boar disease and to represent the predictors in a map.

The STM has improved on the relationship between two existing One Health EJP partners and enhanced both current and future collaborations between the partner institutes. Additionally, this procedure has been automated and can be easily applied to the data from the *Salmonella* and *Campylobacter* surveillance in the food chain by the Spanish Agency of Food Safety and Nutrition (AESAN) and the *Salmonella* and *Campylobacter* Spanish surveillance plan in poultry farm.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 717360.



INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

potential drivers of the pathogens. The results being conclusive, a modelling procedure valid for all the wild boar diseases in the dataset was designed. The developed procedure successfully determined the most important risk factors that shape wild boar diseases in Andalucía and studied their interaction between each other. Using the data from previous hunting seasons, it also allowed to identify areas with higher and lower probabilities of boar disease and to represent the predictions in a map. The STM has improved the relationship between two existing One Health EJP partners and enhanced both current and future collaborations between the partner institutes. Additionally, this procedure has been automatised and can be easily applied to the data from the *Salmonella* and *Campylobacter* surveillance in the food chain by the Spanish Agency of Food Security and Nutrition (AESAN) and the *Salmonella* and *Campylobacter* Spanish surveillance plan in poultry farm.

**STM 5:** Construction of dual labelled *E. coli* strains to study the effect of antibiotics and microbiota interventions on the horizontal transfer of ESBL genes in the *in vitro* chicken caecal microbiota

**Theme:** One Health Missions - Antimicrobial Resistance (AMR)

**Home Institute:** Wageningen Bioveterinary Research (WUR), The Netherlands

**Mission Hosting Institute:** University of Copenhagen, Denmark

**Duration of mission:** 2 months

**Background and aim of mission:** The aim of this mission was for the PhD student to learn bacterial cloning methods using fluorescent reporter proteins, to produce dual labelled *E. coli* strains. Dual labelling (fluorescent tagging of the chromosome and AMR-gene carrying plasmids) of bacteria is a powerful tool to study plasmid-mediated antimicrobial resistance among complex *in vitro* microbial communities simulated on *in vitro* gut systems like the chicken caeca. This STM enabled the PhD student to reach the objectives proposed in the OHEJP PhD project VIMOGUT, which studies the chicken gut microbiota and microbiota interventions to reduce horizontal transmission of Extended Spectrum  $\beta$ -Lactam (ESBL) genes. During this mission, five commensal ESBL *E. coli* strains that originated from chicken broiler caeca and belonged to the collection of The Dutch National Reference Lab were used for bacterial cloning experiments. All strains carried an ESBL ( $bla_{CTXM-1}$  and  $bla_{SHV-12}$ ) or AmpC  $\beta$ -lactamase ( $bla_{CMY-2}$ ) gene on plasmids highly prevalent in the broilers production and were susceptible to three antimicrobials. Two fluorescent reporter proteins were used for chromosome and plasmid tagging, namely, mCherry and Green fluorescent protein (GFP). The PhD student learned the bacterial cloning techniques needed for the completion of future *in vitro* experiments and developed and strengthened their lab skills. The STM opened significant cooperation channels between the AMR group at Wageningen Bioveterinary Research and the One Health Antimicrobial Resistance (OHAR) research group at the University of Copenhagen. The output of the work performed during the STM, and upcoming *in vitro* experiments are expected to be published as a collaborative research article in a scientific journal.

onehealth.ejp.eu @OneHealthEJP ONE Health EJP

**SHORT TERM MISSION**

Short Term Missions (STM) are small travel grants with the aim of:

- Sharing scientific expertise, methodologies, equipment and facilities to harmonise the existing approaches and methodologies within the large OHEJP European network
- Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the OHEJP
- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

**Construction of dual labelled *E. coli* strains to study the effect of antibiotics and microbiota interventions on the horizontal transfer of ESBL genes in the *in vitro* chicken caecal microbiota**

**Theme:** One Health Missions - Antimicrobial Resistance (AMR)  
**Home Institute:** Wageningen Bioveterinary Research (WUR), The Netherlands  
**Mission Hosting Institute:** University of Copenhagen, Denmark  
**Duration of Mission:** 2 months

**“A short term mission should be a must-do for all young researchers! It was a priceless experience that enriched my knowledge and skills and expanded my network. I built up and exercised my lab skills, critical thinking and cooperation in a multidisciplinary group. I was challenged daily with new knowledge and experimental work but also strongly supported by a wonderful support team!”**

**Signed/ Co-signed by:** Wageningen Bioveterinary Research, The Netherlands | One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773832.



## INTRODUCTION



## VISION



## STRUCTURE



## ACHIEVEMENTS



## OUTCOMES



## DISSEMINATION



## EDUCATION

### STM 6: Microbiological and molecular techniques for *Brucella* identification

**Theme:** One Health Missions

**Home Institute:** NDVRI, Bulgaria

**Mission Hosting Institute:** Anses, France

**Duration of mission:** 1 week

**Background and aim of mission:** The aim of this mission was for the three researchers to learn standardised microbiological and molecular techniques, including DNA extraction and real-time PCR, for the identification of *Brucella spp.* As Brucellosis is among the leading lab-acquired infections, learning effective biosafety/ biosecurity whilst working with the causative agent is paramount. This STM enabled the researchers to complete objectives in the OHEJP IDEMBRU project, which studies the zoonotic potential, virulence, and persistence markers in isolated strains of *Brucella spp.* During this mission, theoretical training was provided by the host institute on the critical steps for *Brucella* culture followed by principles of molecular approaches. Prior to the researchers undertaking any laboratory activities, practical demonstrations on bacterial isolation, culture and typing were provided, followed by demonstrations of molecular techniques including DNA extraction, real-time PCR protocols, gel electrophoresis and High-Resolution melting PCR. Each day, round table discussions provided additional insight into each step of the training. A final presentation on molecular epidemiology afforded further knowledge on applications, perspectives and interpretation of data. The researchers strengthened existing and developed new laboratory skills that will assist their future research. The STM provided valuable training, allowing for the isolation and identification of *Brucella spp.*; further experimentation will be performed on *Brucella spp.* strains, under rigorous biosafety standards, at the NDVRI laboratories in Bulgaria.

### STM 7: Tolerance of biofilm forming bacteria to disinfectants after repeated disinfectant exposure.

**Theme:** One Health Missions- Veterinary, Food, Medical and or Environmental research

**Home Institute:** Animal and Plant Health Agency (APHA), UK

**Mission Hosting Institute:** Norwegian Veterinary Institute (NVI), Norway

**Duration of mission:** 3 weeks

**Background and aim of mission:** The aim of this mission was to test methods for measuring disinfectants efficacy, after repeated use on bacteria that survived treatment due to the formation of biofilms. Methods for disinfectant efficacy testing against planktonic foodborne bacteria are well established, yet methods for efficacy of disinfectants for biofilms are still novel, particularly the effect of repeated exposure to disinfectants on the survivability of bacteria in biofilms. This STM aimed to promote harmonisation of current test methods and models used in biofilm and disinfectant testing. During this mission, biofilms of *Staphylococcus aureus* were exposed to different concentrations of Benzalkonium chloride disinfectant. Following neutralisation of the disinfectant, biofilm was removed via mechanical

**ne HEALTHEJP**

**SHORT TERM MISSION**

Short Term Missions (STMs) are small travel grants with the aim of:

- Sharing scientific expertise, methodologies, equipment and facilities to harmonise the existing approaches and methodologies within the large OHEJP European network.
- Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the OHEJP.
- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

**Microbiological and molecular techniques for *Brucella* identification**

**Theme:** One Health missions

**Home Institute:** NDVRI, Bulgaria

**Mission Hosting Institute:** ANSES, France

**Duration of Mission:** 1 week

**STM Researchers:** Albena Dimitrova Angelova, Gergana Masteva, Miloslava Vlastinska Miltova

**“The STM was an amazing experience and has provided newfound knowledge regarding the skill and requirements for microbiological manipulation and molecular diagnostics, especially when working with potentially infectious bacteria, such as *Brucella*. Disseminating this knowledge to other colleagues will further help and enhance everyday tasks in our laboratories”**

Albena Dimitrova Angelova, NDVRI, Bulgaria

The aim of this mission was for the three researchers to learn standardised microbiological and molecular techniques, including DNA extraction and real-time PCR, for the identification of *Brucella spp.* As Brucellosis is among the leading lab-acquired infections, learning effective biosafety/ biosecurity whilst working with the causative agent is paramount. This STM enabled the researchers to complete objectives in the OHEJP IDEMBRU project, which studies the zoonotic potential, virulence and persistence markers in isolated strains of *Brucella spp.*

During this mission, theoretical training was provided by the host institute on the critical steps for *Brucella* culture followed by principles of molecular approaches. Prior to the researchers undertaking any laboratory activities, practical demonstrations on bacterial isolation, culture and typing were provided, followed by demonstrations of molecular techniques including DNA extraction, real-time PCR protocols, gel electrophoresis and High-Resolution melting PCR. Each day, round table discussions provided additional insight into each step of the training. A final presentation on molecular epidemiology afforded further knowledge on applications, perspectives and interpretation of data. The researchers strengthened existing and developed new laboratory skills that will assist their future research.

The STM provided valuable training, allowing for the isolation and identification of *Brucella spp.*; further experimentation will be performed on *Brucella spp.* strains, under rigorous biosafety standards, at the NDVRI laboratories in Bulgaria.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773826

**ne HEALTHEJP**

**SHORT TERM MISSION**

Short Term Missions (STMs) are small travel grants with the aim of:

- Sharing scientific expertise, methodologies, equipment and facilities to harmonise the existing approaches and methodologies within the large OHEJP European network.
- Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the OHEJP.
- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

**Tolerance of biofilm forming bacteria to disinfectants after repeated disinfectant exposure**

**Theme:** One Health Missions- Veterinary, Food, Medical and or Environmental research

**Home Institute:** Animal and Plant Health Agency (APHA), UK

**Mission Hosting Institute:** Norwegian Veterinary Institute (NVI), Norway

**Duration of Mission:** 3 weeks

**“My personal and professional confidence grew during the STM. The information shared has been invaluable to my learning and development. I have gained experience in a wider range of methods used in biofilm and disinfectant research, as well as with a species of bacteria. We exchanged skills and to allow harmonisation of approaches. The methods and techniques learnt will be applied to ongoing and future projects back at my home institute...”**

Emilia Brock, APHA, UK

The aim of this mission was to test methods for measuring disinfectants efficacy, after repeated use on bacteria that survived treatment due to the formation of biofilms. Methods for disinfectant efficacy testing against planktonic foodborne bacteria are well established, yet methods for efficacy of disinfectants for biofilms are still novel, particularly the effect of repeated exposure to disinfectants on the survivability of bacteria in biofilms. This STM aimed to promote harmonisation of current test methods and models used in biofilm and disinfectant testing.

During this mission, biofilms of *Staphylococcus aureus* were exposed to different concentrations of Benzalkonium chloride disinfectant. Following neutralisation of the disinfectant, biofilm was removed via mechanical action. Persisting bacteria were enumerated and isolated to prepare next generation biofilms. These were then exposed to further disinfection using the same concentration to which they were previously exposed. This was repeated over nine consecutive experiments. The results have shown that increased tolerance to biofilms by *S. aureus* can occur. However, conflicting statistical results suggest the need for further investigation into the role of biofilm producing bacteria in the dissemination of biofilm resistance and factors affecting the variability in biofilm behaviour.

The STM has improved on the relationship between two existing One Health EJP partners and enhanced both current and future collaborations between the partner institutes. The STM has supported collaborative research in the current project (OHEJP2) through improvements of working arrangements and understanding of the used methods.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773826



INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

action. Persisting bacteria were enumerated and isolated to prepare next-generation biofilms. These were then exposed to further disinfection using the same concentration to which they were previously exposed. This was repeated over nine consecutive experiments. The results have shown that increased tolerance to biocides by biofilm forming bacteria can occur. However, conflicting statistical results suggest the need for further investigation. Future studies should expand on the data collected in this study, to help develop knowledge on the role of biofilm producing bacteria in the dissemination of biocide resistance and factors affecting the variability in biofilm behaviour. The STM has enhanced both current and future collaborations between the partner institutes. The STM has supported collaborative research in the current project (BIOPIGEE) through improvements of working arrangements and understanding of the used methods.

### STM 8: Metagenomics tools for the study of SARS-COV-2 in animals

**Theme:** One Health, Skills Development Missions

**Home Institute:** INIA, Spain

**Mission Hosting Institute:** INSA, Portugal

**Duration of mission:** 2 weeks

**Background and aim of mission:** The aim of this mission was to expand the researcher's training to a higher and more practical level by learning the application of metagenomics and completing sequencing within a One Health context. These tools are essential for modern surveillance and diagnostics of infectious diseases and are key to shed light on the epidemiology of novel viruses. During the mission, Carlos had the opportunity to learn how to use the INSaFLUTELE-VIR suite: an easy-to-use open web-based bioinformatics suite that was designed for the genomic surveillance of human seasonal influenza and SARS-CoV-2, and recently adapted to monkey pox virus (now renamed Mpox). This platform was proven to be an excellent surveillance tool during the SARS-CoV-2 pandemic and a new module has been designed by researchers in the mission hosting institute to simplify metagenomic analyses and identify new viruses. Carlos also learned about the ReporTree tool, a surveillance-oriented tool to strengthen the linkage between pathogen genetic clusters and epidemiological data and was trained on the use of nanopore sequencing with MinION. The MinION was used during the mission for the complete sequencing of SARS-CoV-2, the diagnostics of MPox virus and sequencing of a poliovirus. The 'Young Researcher Day' and 'INSA Day' took place during the mission, showcasing scientific and cultural activities. It allowed Carlos to learn about the different research lines carried out in the three INSA headquarters in Portugal and to expand his professional network. The mission provided Carlos the training to diagnose and characterise future SARS-CoV-2 cases in animals using metagenomics, which is key to determine whether a virus has changed/mutated during a spill over event. One of the outputs of the project will be the adaptation of modern metagenomics and bioinformatics tools in the COVRIN project. The





mission also strengthened further the collaboration between the institutes, with a new collaboration established to research on hepatitis E virus in wildlife.

## INTRODUCTION



## VISION



## STRUCTURE



## ACHIEVEMENTS



## OUTCOMES



## DISSEMINATION



## EDUCATION

### STM 9: Genotypic characterisation of antimicrobial susceptibility and isolation of *Aeromonas* and *Vibrio* phages from water samples

**Theme:** One Health, Skills Development Missions

**Home Institute:** Anses, France

**Mission Hosting Institute:** BfR, Germany

**Duration of mission:** 4 weeks

**Background and aim of mission:** The objective of the mission was to share technical knowledge on the detection and characterisation of *Aeromonas* and *Vibrio* genus and to establish a collaboration between the institutes, to study of the dissemination of antibiotic resistance in aquatic environments. During this mission, Sandrine and Laetitia were trained on phages' cultivation, enumeration, and conservation methods. They now have in hands the detailed protocols, as well as phages and competent bacteria cultures, provided by the BfR team. They will be able to put the methods into practice in Anses. Sandrine and Laetitia travelled to BfR with culture media for the detection of *Aeromonas spp.*, from water and fish samples. Water samples have been collected nearby the BfR laboratory and the German team performed the entire handling: filtration, cultivation, and presumptive identification. The partner teams established common protocols for the study of the *Aeromonas* and *Vibrio* genera and initiated the sharing of the Maldi-ToF databases, for the improvement of the species identification. The two institutes are also now both involved in a research group, which aims to improve the methodology of antimicrobial susceptibility testing in aquatic bacteria and determine Epidemiological cut off values (Ecoff). At the end of this STM, two research project proposals were elaborated. This short-term mission strengthened the emerging collaboration between the two teams from the One Health EJP consortium. It has also led to a reflection on the use of phages to improve and reduce the use of antibiotics in aquaculture, a farming sector from which a One Health approach is absolutely needed.

### STM 10: Stable Intra-specific genomic bacterial classification systems

**Theme:** One Health Missions

**Home Institute:** Norwegian Veterinary Institute (NVI), Norway

**Mission Hosting Institute:** Institut Pasteur, France

**Duration of mission:** 4 days

**Background and aim of mission:** The aim of this mission was for the researcher to gain knowledge on the usage and design of a stable classification system and nomenclature approach for bacterial strains and to understand advantages and limitations of its use in genomic epidemiology. Previous isolate

**ne HEALTHEJP**

**SHORT TERM MISSION**

Short Term Missions (STMs) are small travel grants with the aim of:

- Sharing scientific expertise, methodologies, equipment and facilities to harmonise the existing approaches and methodologies within the large CH2EP European network
- Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the CH2EP
- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human/animal/environmental sectors.

**Genotypic characterisation of antimicrobial susceptibility and isolation of *Aeromonas* and *Vibrio* phages from water samples**

**Theme:** One Health, Skills Development Missions

**Home Institute:** ANSES, France

**Mission Hosting Institute:** BfR, Germany

**Duration of Mission:** 4 weeks

**“ This mission was a great opportunity to discuss the dissemination of antibiotic resistance in the aquatic environment in connection with aquaculture activities. In addition to the improvement of the technical skills of both teams, the diversity of our fields of study (water, fish farming vs food) opened up perspectives for new collaborations, using a One Health approach.”**

Sandrine Sarin and Laetitia Le Dervenec, ANSES, France

The objective of the mission was to share technical knowledge on the detection and characterisation of *Aeromonas* and *Vibrio* genus and to establish a collaboration between the institutes, to study of the dissemination of antibiotic resistance in aquatic environments.

During this mission, Sandrine and Laetitia were trained on phages' cultivation, enumeration and conservation methods. They now have in hands the detailed protocols, as well as phages and competent bacteria cultures, provided by the BfR team. They will be able to put the methods into practice in Anses. Sandrine and Laetitia travelled to BfR with culture media for the detection of *Aeromonas* sp., from water and fish samples. Water samples have been collected nearby the BfR laboratory and the German team performed the entire handling: filtration, cultivation and presumptive identification. The partner teams established common protocols for the study of the *Aeromonas* and *Vibrio* genera and initiated the sharing of the Maldi-ToF databases, for the improvement of the species identification of bacteria from the *Aeromonas* and *Vibrio* genera. The two institutes are also now both involved in a research group, which aims to improve the methodology of antimicrobial susceptibility testing in aquatic bacteria and determine Epidemiological cut off values (Ecoff). Meetings and video conferences with French and Canadian researchers took place, to discuss research ideas. At the end of this STM, two research project proposals were elaborated.

This short-term mission strengthened the emerging collaboration between the two teams from the One Health EJP consortium. It has also led to a reflection on the use of phages to improve and reduce the use of antibiotics in aquaculture, a farming sector from which a One Health approach is absolutely needed.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 735800.

**ne HEALTHEJP**

**SHORT TERM MISSION**

Short Term Missions (STMs) are small travel grants with the aim of:

- Sharing scientific expertise, methodologies, equipment and facilities to harmonise the existing approaches and methodologies within the large CH2EP European network
- Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the CH2EP
- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human/animal/environmental sectors.

**Stable Intra-specific genomic bacterial classification systems**

**Theme:** One Health

**Home Institute:** Norwegian Veterinary Institute (NVI), Norway

**Mission Hosting Institute:** Institut Pasteur, France

**Duration of Mission:** 5 days

**“ The STM allowed me to understand and practice applying the newly developed qPCR bacterial nomenclature system. I now have a better understanding on how the classification system can be used for genomic epidemiology of zoonotic and infectious diseases and able to disseminate this knowledge to other scientists. This will strengthen the activities in One Health to address existing and emerging threats of foodborne and zoonotic agents as well as animal pathogens.”**

Ein Zeyi Rindbeck, NVI, Norway

The aim of this mission was for the researcher to gain knowledge on the usage and design of a stable classification system and nomenclature approach for bacterial strains and to understand advantages and limitations of its use in genomic epidemiology.

Previous isolate nomenclature/classification systems including serotyping and MLST typing do not always satisfy two major criteria: long-term stability and congruence with phylogenetic relationships, which are essential for routine epidemiological surveillance. During this mission, theoretical training was provided by the host institute, to gain deep understanding and practical experience of a novel classification system and nomenclature approach for bacterial strains (qPCR codes). Training also allowed further understanding of the advantages and limitations of its use in genomic epidemiology.

The STM provided valuable training, allowed knowledge transfer and strengthened the collaboration between One Health partners. Further funding will be sought to continue this valuable collaboration in order to further develop and expand the nomenclature system for additional bacterial pathogens.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 735800.



INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

nomenclature/classification systems including serotyping and MLST typing do not always satisfy two major criteria: long-term stability and congruence with phylogenetic relationships, which are essential for routine epidemiological surveillance. During this mission, theoretical training was provided by the host institute, to gain deep understanding and practical experience of a novel classification system and nomenclature approach for bacterial strains (cgLIN codes). Training also allowed further understanding of the advantages and limitations of its use in genomic epidemiology. The STM provided valuable training, allowed knowledge transfer, and strengthened the collaboration between One Health partners. Further funding will be sought to continue this valuable collaboration, to further develop and expand the nomenclature system for additional bacterial pathogens.

**STM 11:** Application of 'Single-Cell Genomics' for the study of the bacterial reservoirs of plazomicin resistance determinants

**Home Institute:** [Universidad Complutense de Madrid, Spain](#)

**Mission Hosting Institute:** [Karlsruhe Institute of Technology, Germany](#)

**Duration of mission:** 3 months

**Background and aim of mission:** The aim of this mission was the development of a single cell workflow, for the detection of low abundant reservoirs of next-generation aminoglycoside resistance mechanisms.

The main objective was to label single cells from complex environments with fluorescence markers specific for the genes of interest. This Short-Term Mission was framed within the OHEJP PhD METAPRO project and was expected to produce results complementing the ones already produced with the metagenomic analyses. Three different labelled probes were designed for the detection of the aminoglycoside resistance gene *npmA*, a gene that confers high level of resistance to aminoglycosides. To test the probes, the resistance gene was introduced in two plasmids with different copy numbers to use as a positive control, and the same plasmids without the resistance gene were used as a negative control. All these control plasmids were introduced in *E. coli* cells and a classical fluorescence *in situ* hybridisation (FISH) protocol was performed with the three probes independently, to check their labelling efficiency. Two out of the three probes designed showed promising results to be used in environmental samples. Samples have been taken to evaluate the potential sorting of *npmA* positive cells and is planned to be performed in the upcoming months. This Short-Term Mission has opened a new collaboration channel between two research groups with different scopes for the study of antimicrobial resistance with a One Health approach. The collaboration between the partner institutes is expected to last longer than the extend of the mission and we expect to produce interesting results than could potentially be published as a research article in a scientific journal.

For further details, read the [D6.20: Report on Short Term Missions 2022 \(Y5\)](#).





INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

### The One Health EJP Annual Scientific Meeting Satellite Workshop

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

**Organising institute:** University of Surrey, UK with involvement from the National University of Ireland, Ireland

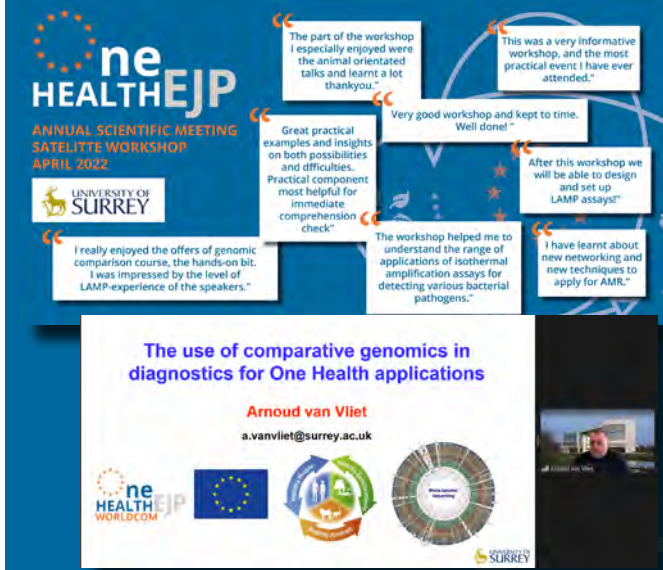
**Theme:** Diagnostics workshop on mobile detection platforms for One Health diagnostics applications

**Location:** Online

**Dates:** 14th April 2022

**Collaborative interactions:** The workshop focused on innovative rapid diagnostic technologies and mobile detection platforms for One Health applications. The main objectives were to: i) discuss factors influencing the design of One Health diagnostics assays, ii) reflect on current standard diagnostics in public and animal health, iii) showcase the application, advantages, and challenges of two key mobile diagnostics techniques: loop-mediated isothermal amplification (LAMP) and loop-primer endonuclease cleavage LAMP (LEC-LAMP). LAMP is a rapid, sensitive, and specific nucleic acid amplification technology and LEC-LAMP is a recently developed modification of LAMP that enables multiplex real-time pathogen detection with single-base specificity for point-mutation detection. The workshop facilitated training and discussion in the area of rapid and innovative nucleic acid diagnostic assay development for pathogen detection and antimicrobial resistance identification in both humans and animals. There were opportunities for ECRs and PGRs to discuss solutions for improving current One Health diagnostics between themselves and with experts and to foster new collaborations.

**Delegates:** A total of 99 delegates across all OHEJP partner institutes and countries, with some representation from the industry sector, registered to attend – including PhD students, research fellows (experienced and unexperienced in molecular diagnostics) and group leaders that had interest in molecular diagnostics and the application to One Health. Three VIP places were taken by stakeholders. Bringing together people from across the health disciplines made this module truly cross-disciplinary







INTRODUCTION



VISION



STRUCTURE



ACHIEVEMENTS



OUTCOMES



DISSEMINATION



EDUCATION

and highlighted the possible applications of diagnostics solutions to the different domains of One Health. The delegates' educational backgrounds were diverse across the One Health fields and included biological sciences, veterinary medicine, food safety, social sciences, environmental science, economics, toxicology, and public health, which helped to facilitate the sharing of One Health knowledge and expertise.

**Programme:** The online workshop was split into two sessions. In the morning session, seven expert speakers from the University of Surrey and the University of Galway discussed the design, implementation, and applications of rapid molecular diagnostics for the detection of zoonotic pathogens and antimicrobial resistance in public health, animal, and environmental samples. The afternoon session gave the opportunity for the delegates to interact with the experts in a dedicated panel discussion and provided them with the opportunity for a hands-on *in silico* interactive session on the design of diagnostic assays using LAMP and LEC-LAMP methods.

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

View the full report [here](#).





# ONE HEALTH

PREVENT • DETECT • RESPOND

[onehealthjp.eu](http://onehealthjp.eu)

 @OneHealthEJP  ONE Health EJP