Joint PMC POC Meeting

Poster Session

Folkhälsomyndigheten

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JRP06
NOVA

Novel approaches for design and evaluation of cost-effective surveillance across the food chain

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1. SVA, Sweden • 2. SSI, Denmark • 3. Sciensano, Belgium • 4. AUTH, Greece • 5. Anses, France • 6. INIA, Spain • 7. DTU-FOOD, Denmark

In this research project (JRP06), more than 65 project participants from 18 public health and veterinary institutes in 10 countries collaborated over a project period of 3½ years. NOVA has addressed improvement of disease surveillance through a large number of separate projects, organised into five main themes, as reflected in its overall work package structure:

Basic aspects and issues in connection with performing One Health surveillance

Use of electronic traces of purchase of food for surveillance and outbreak investigations

One Health developments of syndromic surveillance methods

Use and development of spatial risk mapping

Modelling the cost efficiency of surveillance programs

OUTCOME HIGHLIGHT 1: METHODS FOR MODERN OUTPUT-BASED SURVEILLANCE

- Modelling of **disease spread and surveillance in the same model**
- Several spread scenarios investigated
- Alternative sampling strategies compared
- Traditional measures of surveillance sensitivity complemented by measures of **time to detection**, and outbreak size at time of detection
- National adaptation to new Animal Health Law: Output from this modelling has been important as a **basis for discussion and decision** with the Board of Agriculture, animal health organisations and other stakeholders.
- Continuing work on this modelling framework in other research projects, including second round of OHEJP projects (FullForce and Biopigee).

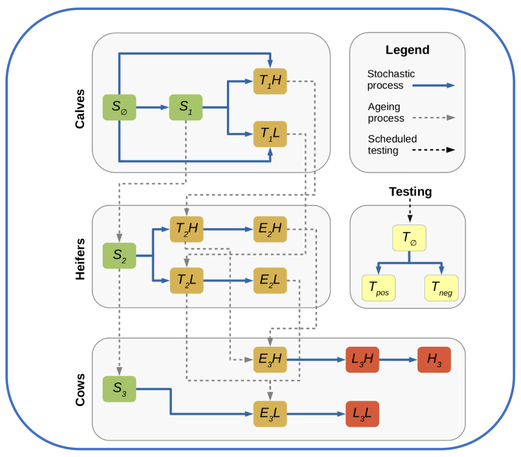


Fig 1 Compartments representing diagnostic testing (yellow) included in spread model

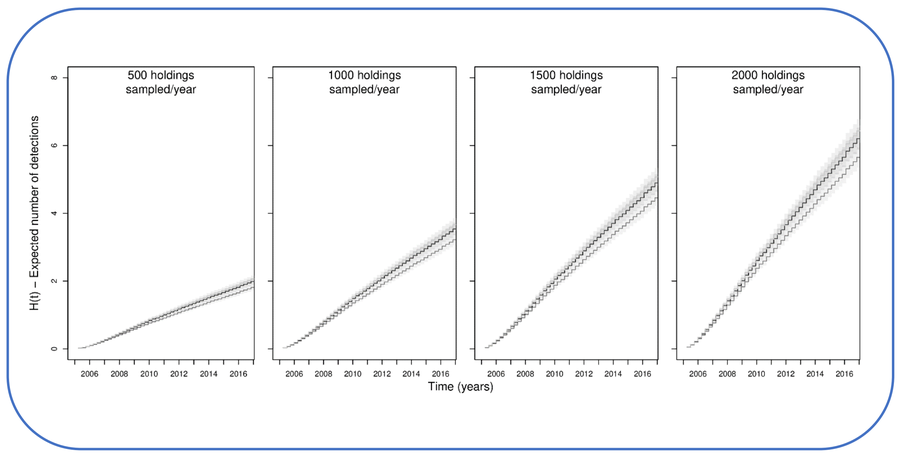


Fig 2 Time to detection of outbreak based on four surveillance strategies with different number of tested holdings


Photo: B. Ekberg/SVA

OUTCOME HIGHLIGHT 2: DATA AVAILABILITY

Missing data and non-digital data → Modern digital data systems → Computational power and ability to handle large datasets → Legal restrictions in data sharing

- Data availability and access investigated in several WPs, from different perspectives.
- **General difficulty to share data**, also between authorities in the same MS
- Imbalance in data formats and data access

IMPACT SUMMARY

- Understanding and improving access to data → more information, lower cost, more cost-effective surveillance
- General models and open code → use in MS with similar data
- Support for outcome-based surveillance
- Outcomes used in ongoing surveillance and several research projects
- New network, increased contact across disciplines → improved collaboration


Photo: F. Dórea/SVA

This poster is part of the European Joint Programme One Health EJP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

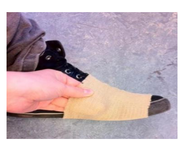
AIR-SAMPLE

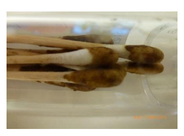
A low-cost screening tool in bio-secured broiler production

Gro S. Johannessen
Norwegian Veterinary Institute

BACKGROUND AND AIM

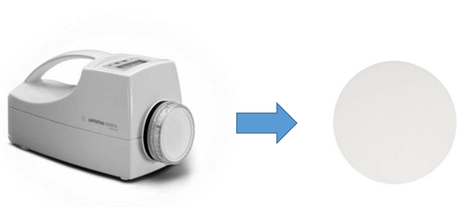
- Monitoring of *Campylobacter* in poultry production is mandatory
- Current methods: boot swabs, fecal droppings



Modernization of sampling is desirable

Sampling of ambient air in broiler houses has previously been sucessfully tested for screening of *Campylobacter*.



In AIR-SAMPLE: further testing, harmonization and evaluation of protocols in five countries across Europe has been carried out.

RESULTS IN BRIEF

Testing of air sampling was carried out over two seasons in five European countries.

- A pilot study (year 1) indicated suitability to detect *Campylobacter* in biosecured broiler production.
- A multi-centre evaluation (year 2) of harmonized protocols indicated that air sampling in combination with real-time PCR can produce fast and reliable results.
- The use of metagenomic (shot-gun) analyses of air samples to detect *Campylobacter* was tested.
- A SOP, guidelines and a video demonstration were produced.

Publications:

Johannessen et al. 2020. *Campylobacter* in chicken – critical parameters for international, multicentre evaluation of air sampling and detection methods. doi:10.1016/j.fm.2020.103455

Hoorfar et al. 2020. A Multicenter proposal for a fast tool to screen biosecure chicken flock for the foodborne pathogen *Campylobacter.* doi: 10.1128/AEM.01051-20

Video demonstration: https://youtu.be/LMD03UAAPUw

OUTCOMES AND IMPACT

Harmonized and multi-center evaluated protocols for collecting air samples in broiler houses, DNA extraction from filters and real-time PCR for *Campylobacter* detection.

Fast and sensitive method that increased the likelyhood of detecting *Campylobacter* in infected flocks.
Guidelines, SOPs, instructions videos, scientific publications and presentations have been developed.

➡ Air sampling can be implemented as an alternative to other methods for sampling for screening of *Campylobacter* in broiler houses.
The use of air samples could be expanded to include more pathogen targets, thus being a simpe tool for sampling of relevant organisms. Such further use needs testing.

Use of metagenomic (shot gun) analyses of air samples to detect *Campylobacter*

Identified and tested a DNA extraction protocol that can be used for both real-time PCR and metagenomic studies
Carried out a pilot study using inoculated samples to see if we were able to identify *Campylobacter* using shot-gun metagenomics

➡ A step towards using metagenomics for detection of pathogenic bacteria or other microorganisms in food production environment
that can be used for multiple purposes. Area for further research that requires cooperation across many fields.

Impact

➡ Early detection of *Campylobacter* and potentially other microorganisms that may be harmful to humans and animals enables measures to be put in place to ensure the safety of the product, animal and public health.
A step towards using metagenomics for the detection of foodborne pathogens, with its potential of use for multiple purposes.

Aknowledgement: Prof. Jeffrey Hoorfar (retired), DTU, initiator and coordinator of the project until December 2020.
Project partners from DTU, IZSAM, PiWet, VUVeL and NVI.

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DiSCoVeR – Discovering the sources of *Salmonella*, *Campylobacter*, VTEC and antimicrobial Resistance

OBJECTIVES

1. To provide a hub for sharing genomic, other microbiological and epidemiological data to perform source attribution analyses for *Salmonella*, *Campylobacter*, Shiga toxin-producing *E. coli* (STEC), and antimicrobial resistance (AMR) across Europe.
2. To critically assess existing source attribution models and to develop novel or improve current source attribution models suited to the data of the project or to be collected in the foreseeable future in human, animal, food and environmental samples.to trace the origins of *Salmonella*, *Campylobacter*, STEC and AMR at different levels of the reservoir-to-exposure continuum.
3. To produce quantitative estimates for the sources of *Salmonella*, *Campylobacter*, STEC and AMR that also account for the environment, non-livestock reservoirs, multi-directionality of transmission, and geographical differences.
4. To evaluate existing data for source attribution, the applicability of existing and novel methods, and the interpretability of results to fill gaps in knowledge, data and methodologies.
5. To liaise with decision makers and other stakeholders in order to evaluate how source attribution analyses and approaches can reinforce current and future control policies for *Salmonella*, *Campylobacter*, STEC and AMR, as well as integrated surveillance programmes.

CONSORTIUM

DiSCoVeR brings together experts from different disciplines (microbiology, bioinformatics and epidemiology) and sectors (veterinary science, food safety, public and environmental health) from 19 institutes in 13 European countries (Fig. 1) to address source attribution in an interdisciplinary manner. The DiSCoVeR consortium is led by the DTU and RIVM.

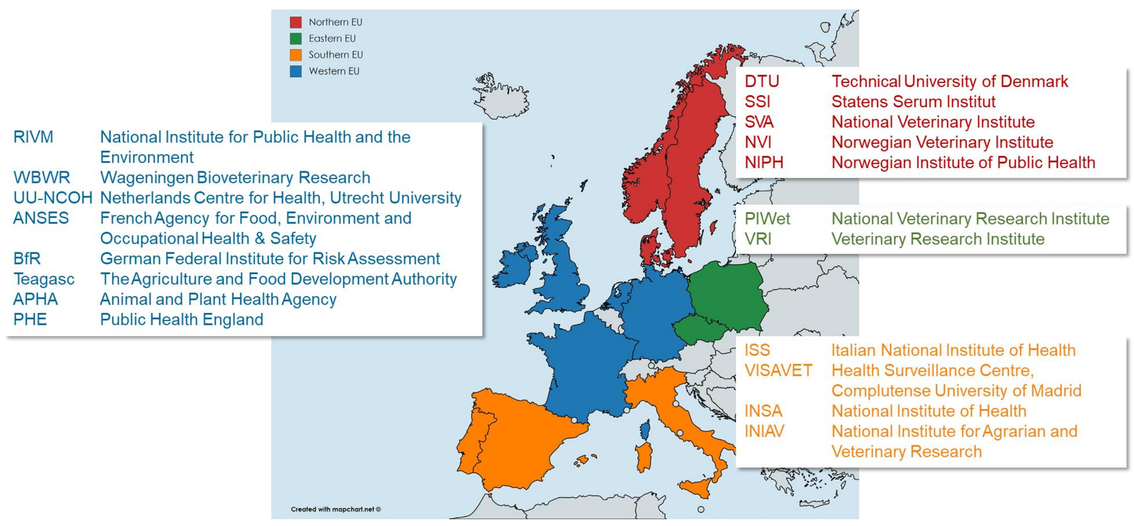


Fig. 1. Composition of the DiSCoVeR consortium.

STRUCTURE

As shown in Fig.2, DiSCoVeR is structured in 5 interconnected work packages (WPs) encompassing project coordination (WP1), data collection (WP2), methodological assessment (WP3), estimate generation (WP4) and science-to-policy translation (WP5).

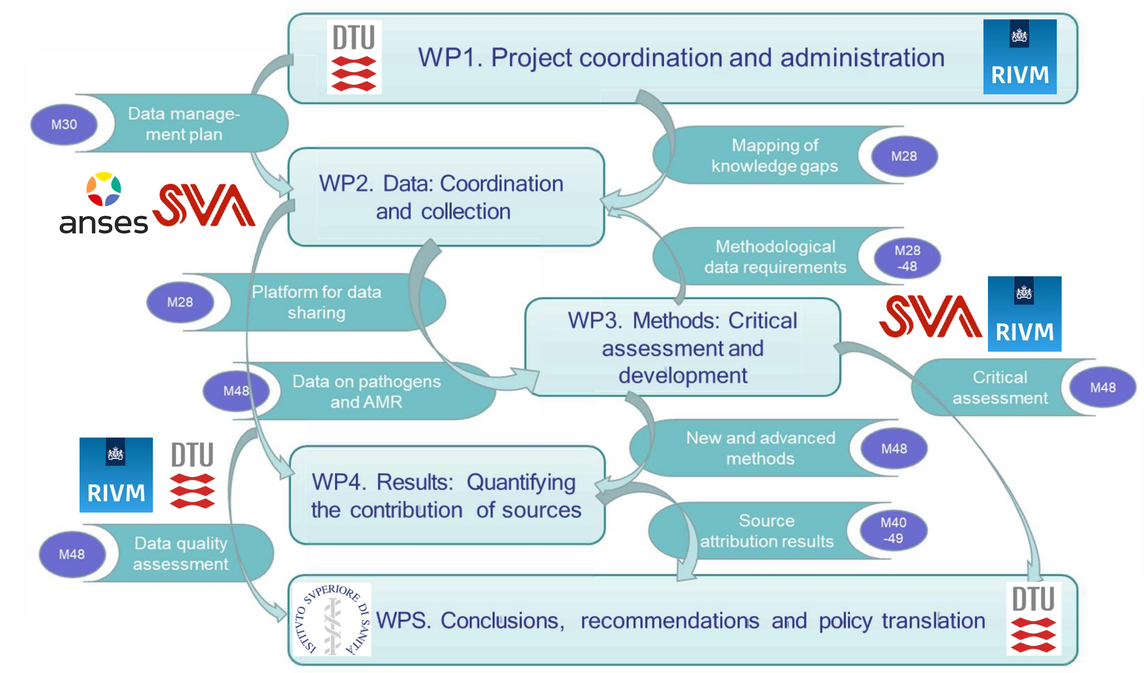


Fig. 2. Interrelationships and leadership of the different WPs of DiSCoVeR. .

OUTCOMES

Five different analytical approaches are applied to the three target pathogens and AMR (Table 1).

Table 1. Analytical approaches used in DiSCoVeR.

	Subtyping (phenotype)	Subtyping (genotype)	Outbreak data	Case-control study	Comparative exposure assessment
Salmonella	Serotyping (frequency-matching)	cgMLST (population genetics, machine-learning)	EFSA data	Meta-analysis literature	Dogs and cats
Campylobacter	-	cgMLST, Kmer, SNP	-	Meta-analysis literature	Dogs and cats
VTEC	Serotyping + stx, eae… (frequency-matching)	cgMLST, pangenome (population genetics, machine-learning)	-	-	Dogs and cats
AMR	-	ESBL genotypes (frequency-matching, dynamic modelling)	-	-	Dogs and cats

- Results are presented per pathogen, method, data type and country, to identify country differences for pathogen-data-approach combinations that may reflect differences in epidemiology. Multi-country analyses are also performed.
- Particular attention is given to environmental and non-livestock (pets and wildlife) sources besides the 'traditional' livestock/food sources, although data for these sources generally are scarce.
- Existing and novel methods are improved and applied in parallel, notably the use of machine-learning methods like random forest using the increasingly available genomic data from WGS surveillance. See, as an example, the outcomes for *Salmonella* Typhimurium in Fig. 3 and comparable results obtained with other methods (Fig. 4).

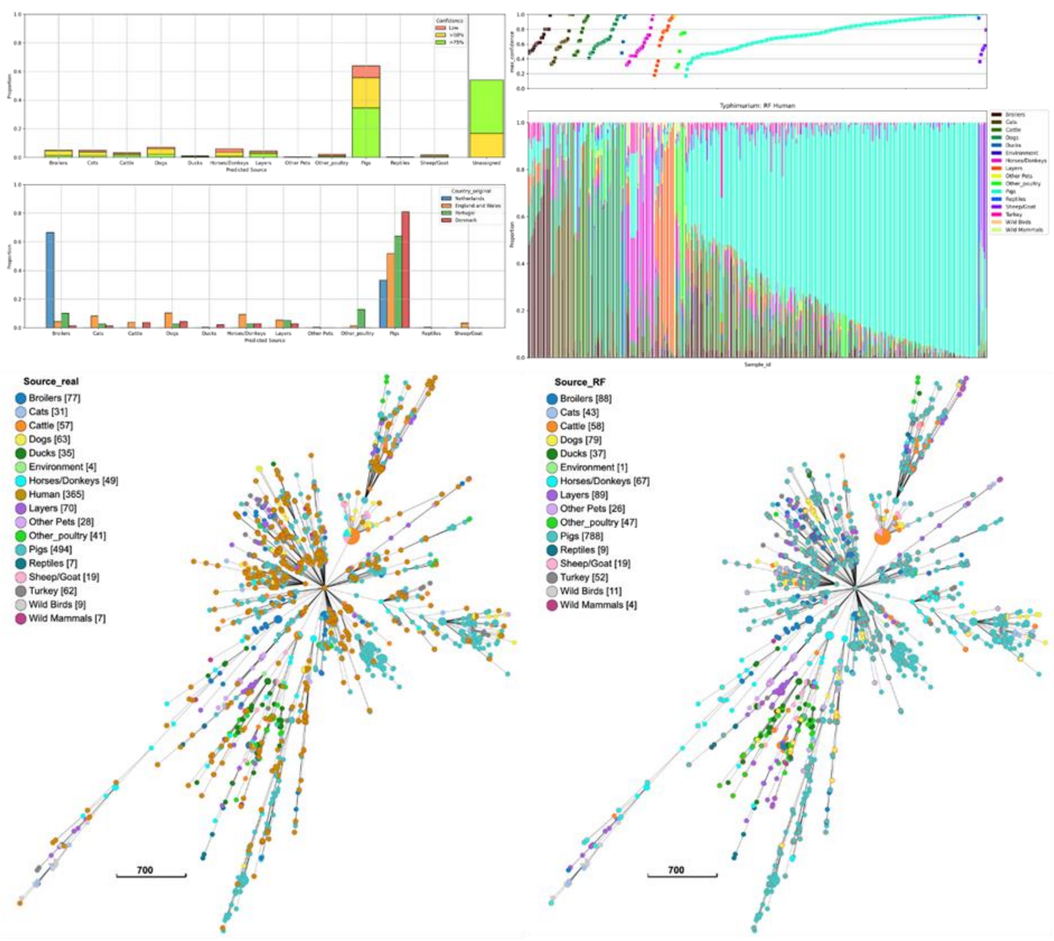
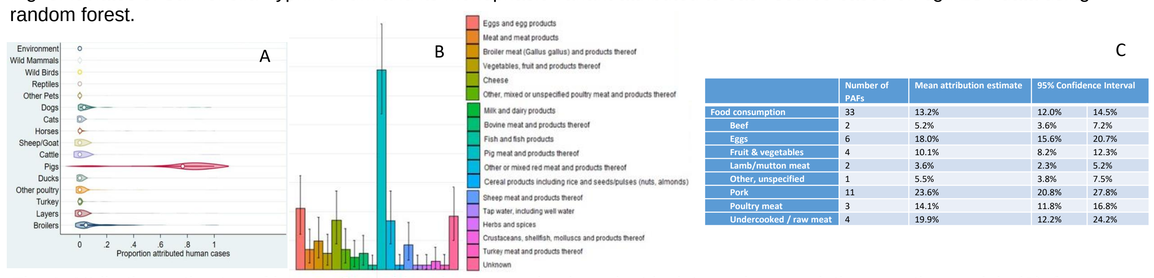


Fig. 3. Isolates of *Salmonella* Typhimurium and its monophasic variant attributed to each source based on cgMLST data using random forest.



	Number of PAFs	Mean attribution estimate	95% Confidence Interval	
Food consumption	33	13.2%	12.0%	14.5%
Beef	2	5.2%	3.6%	7.2%
Eggs	6	18.0%	15.6%	20.7%
Fruit & vegetables	4	10.1%	8.2%	12.3%
Lamb/mutton meat	2	3.6%	2.3%	5.2%
Other, unspecified	1	5.5%	3.8%	7.5%
Pork	11	23.6%	20.8%	27.8%
Poultry meat	3	14.1%	11.8%	16.8%
Undercooked / raw meat	4	19.9%	12.2%	24.2%

Fig. 4. Attribution estimates of human *S.* Typhimurium/monophasic variant isolates using population genetics models (A), for *Salmonella* spp. analysis of outbreak data (B), and meta-analysis of case-control studies (C).

IMPACT

- DiSCoVeR has identified and filled important knowledge, methodological and data gaps about source attribution of top-priority zoonotic pathogens in the EU and AMR through systematic collection and analysis of existing and new data and by applying existing, modified and novel approaches.
- Results allow for recommendations on how source attribution estimates can be translated into policy to support the surveillance, control and prevention of foodborne infections in Europe.

This poster is part of the European Joint Programme One Health EJP.
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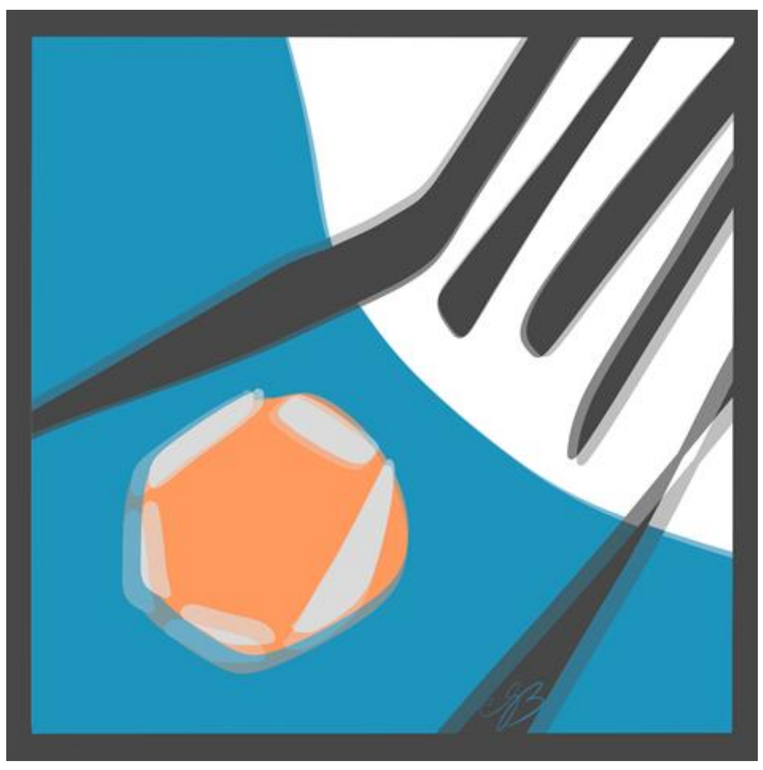
TOXOSOURCES
Toxoplasma gondii sources quantified

Pikka Jokelainen (SSI, Denmark), Joke van der Giessen (RIVM, the Netherlands),
Marieke Opsteegh (RIVM, the Netherlands), Sara Monteiro Pires (DTU-FOOD, Denmark),
Marco Lalle (ISS, Italy), Anne Mayer-Scholl (BfR, Germany),
Furio Spano (ISS, Italy), Frank Seeber (RKI, Germany),
Gereon Schares (FLI, Germany), Simone Cacciò (ISS, Italy),
and other TOXOSOURCES Consortium members

Toxoplasma gondii is a zoonotic parasite that causes a high disease burden.

MAIN GAP ADDRESSED

Relative importance of the main transmission pathways, via the environment (oocyst-borne) or via meat (tissue-cyst-borne), has been a major issue and only answered with expert elicitations. *(EFSA 2018)*



Contact: PIJO@ssi.dk
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https://onehealthejp.eu/jrp-toxosources/

Methods

- Harmonized approaches enabling comparable data
- Better preparedness to detect and investigate outbreaks and emerging strains
- New and improved methods
  - Improved quantitative microbial risk assessment (QMRA)
  - Standard operating procedure and interlaboratory validation of detection of oocyst contamination in leafy green vegetables
  - Serology to detect infections caused by oocysts explored
  - Harmonizing current state-of-the-art genotyping: ring trials
  - Novel typing method to detect within-genotype variation
- Methods already applied in several laboratories across Europe

Science

- Major contributions to our understanding regarding sources and transmission pathways, epidemiology and molecular epidemiology, and performance of methods
  - Several literature reviews, high number of scientific publications
  - New data from multicenter exposure survey and QMRA
  - New data on role of ready-to-eat fresh produce from large multicenter study
  - Unique data on performance of a selection of antigens for stage-specific serology
  - Whole genome sequences of a high number of *Toxoplasma gondii* strains
  - Quantitative estimates of main sources of *Toxoplasma gondii* infections

Consortium

- Multidisciplinary and cross-sectoral One Health collaborations
- 21 One Health EJP partners plus external collaborators
- Participation of early-career colleagues, career advancement
- Networking, including across projects within and beyond One Health EJP
- Active dissemination, including collaborations with social sciences and policy platforms, and using social media #TOXOSOURCES

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Understanding the changing epidemiology of *Salmonella* (the ADONIS project)

Project leader: Eelco Franz (RIVM)
Work package leaders: Marianne Chemaly (ANSES), Roan Pijnacker (RIVM), Eva Litrup (SSI), Lapo Mughini Gras (RIVM)

Background

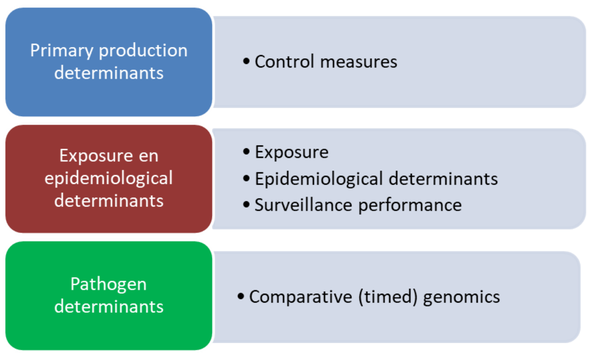
ECDC has revealed the decrease in Salmonella incidence in the EU has levelled off.

ADONIS will identify determinants underlying the stagnation/reversal of the decreasing trend in Salmonella Enteritidis incidence in humans and poultry in the EU

Deliver stakeholders and policy makers with anchor points to at least prevent a continued stagnation or even an re-establish a decreasing trend in Salmonella incidence in humans and poultry.

Approach

The project approach is to investigate possible determinants at the level of primary production (WP2), epidemiology (WP3) and the pathogen (WP4); a multi-criteria- decision analysis (MCDA) is performed in WP5 (Fig. 1).



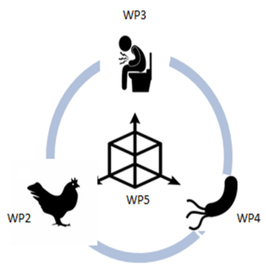


Fig. 1. project overview

Primary production

- Comparison of National Control Plans (NCPs) in poultry in ADONIS partners revealed a common framework that was nevertheless differed in certain aspects in terms of the predominant production systems and implementation details
- Factors associated with increased odds of *Salmonella* detection in poultry are very heterogenous depending on the study, but sampler (Competent authority vs. Food Business Operator) is strongly associated with an increased probability of detection.
- Analysis of NCP data from one MS found that the risk of detection has remained in similar levels in the last years

Epidemiology

- **Outbreaks (EU)**: source attribution based on EU outbreak data showed poultry/eggs are still the main sources; Reported Salmonella outbreaks have increased significantly in occurrence from 2015 to 2019, particularly outbreaks caused by SE in Eastern European countries.

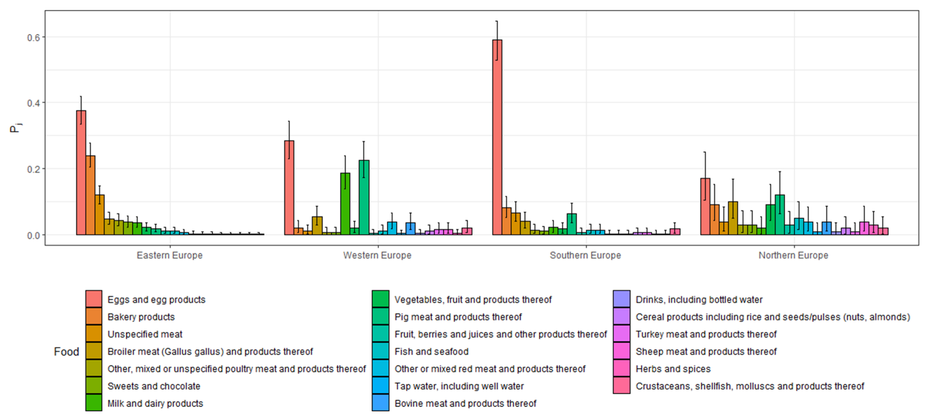


Fig. 2. Proportions of human salmonellosis outbreaks attributed to simple and unknown food-sources by European region with their respective 95% uncertainty intervals, 2015-2019

- **Incidence (NL/BE):** S. Enteritidis incidence decreased significantly in both countries until 2015, followed by an increasing trend, which was particularly pronounced in the Netherlands. Potential SE outbreaks in both countries and invasive infections in the Netherlands also increased after 2015.

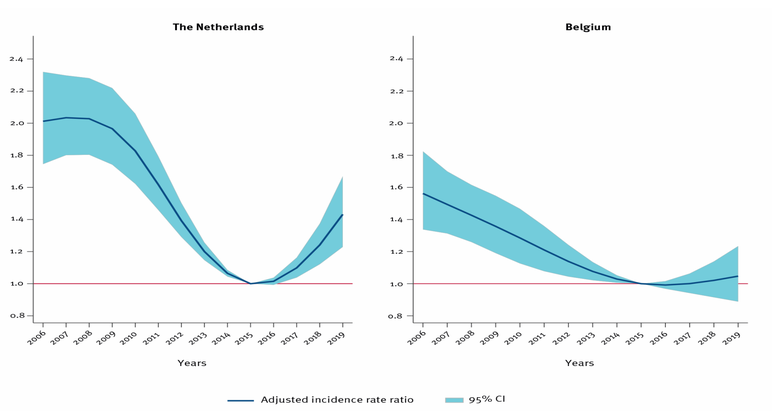


Fig. 3. Changes over time in the incidence of *Salmonella* Enteritidis (SE) human infection, with 2006 as reference year, in the Netherlands and Belgium, 2006–2019

Pathogen

- **AMR:** with decreasing antimicrobial use the resistance among *Salmonella* shows a decreasing trend; except third-generation cephalosporins and gentamycin (mainly driven by S. Infantis, and S. Kentucky).
- **MGE**: Prophage specific for strains in old clade is undergoing mutant creation (removal of prophage) and phenotypic experimentsy.
- **Geo-pyhologeny**: the population of S. Enteritidis is dominated by ST11 and characterized by relatively low geographical/niche structuring.
- **Phylodynamics**: the presence of two large, relatively distant clades within the dominant ST instructs us towards an approach centered on distinct phylodynamics analyses.

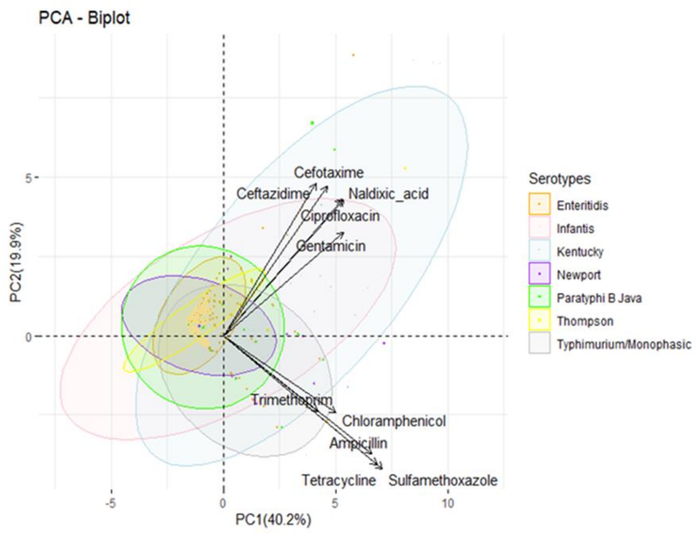


Fig. 4. Principal component plot of antimicrobial resistance by Salmonella serotype.

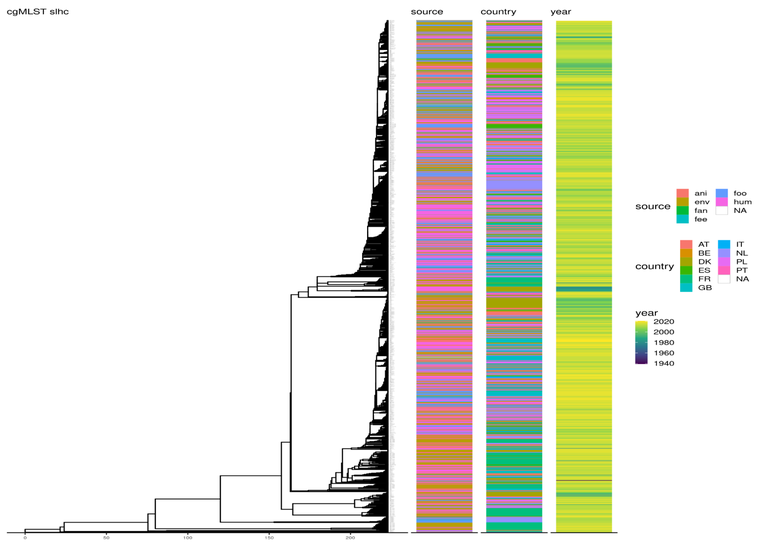


Fig. 5. Single linkage phylogenetic tree nnotaed to country, source and year.

Multi-criteria decision analysis

- The alternatives for the determinants and options for intervention that ranked the highest were consistently those related to the level of poultry health and production.

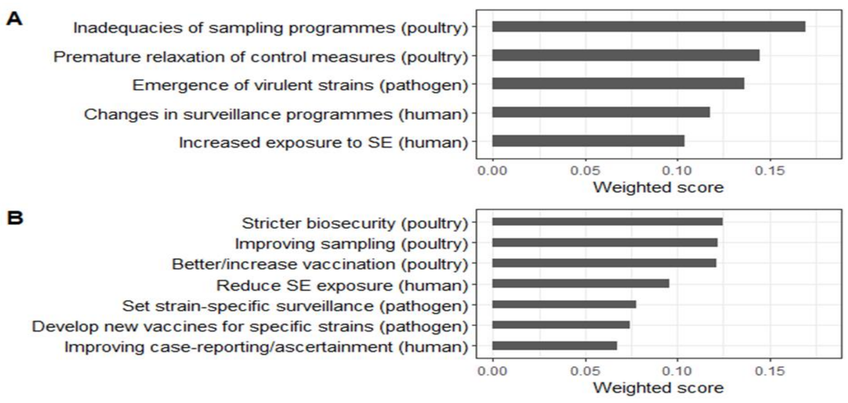


Fig. 7. Ranking of the determinants (A) and options for intervention (B) of the stagnating trend.

Policy impact

- We observed a no longer decreasing trend in Salmonellosis, increased number of outbreaks and more severe infections
- Relaxation of sampling and control measures at poultry primary production was perceived as most important determinant
- Public health and veterinary institutes at national and supra-national level should prioritize salmonellosis control in terms of improved monitoring and surveillance as well as adequate source finding with outbreaks

This poster is part of the European Joint Programme One Health EJP.
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BeONE: Building Integrative Tools for One Health Surveillance

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Finn Gruwier Larsen[4], Katrine Joensen[4], Kristoffer Kiil[4], Sofie Holtsmark Nielsen[4] and BeONE Consortium

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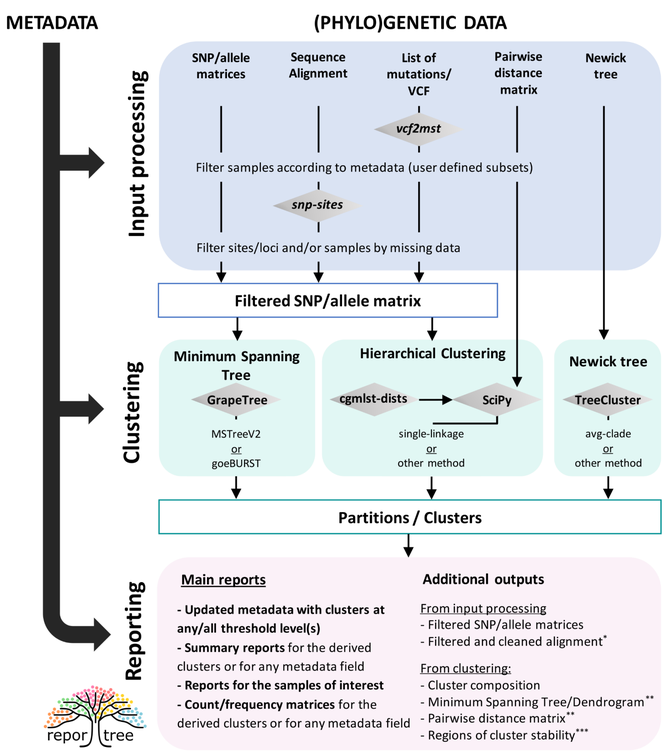
Enhancing evidence-informed public-health decision-making

Output: A surveillance-oriented tool to strengthen the linkage between genetic clusters and epidemiological data

Available at GitHub:

- Rapid identification of genetic clusters at any (or all) distance thresholds (e.g., high resolution thresholds used for outbreak detection or stable threshold ranges for nomenclature design)
- Generate surveillance-oriented reports based on the available metadata, such as timespan, geography or vaccination/clinical status



- Can be smoothly implemented in routine surveillance, with negligible computational and time costs
- Contributes to a sustainable and efficient public health genomics-informed pathogen surveillance

Collaboration with:

Outcomes

✓ *Key-role in routine surveillance and outbreak investigation of bacterial and viral pathogens in Portugal*

✓ *Integration in INSaFLU-TELEVIR platform*

✓ *Integration in the COHESIVE platform*

ReporTree is an automated and flexible pipeline, applicable to multiple pathogens, with a concept aligned with "One Health" perspectives

Connecting European genomic surveillance laboratories

Output: Digital structure for the exchange of genomic and epidemiological data

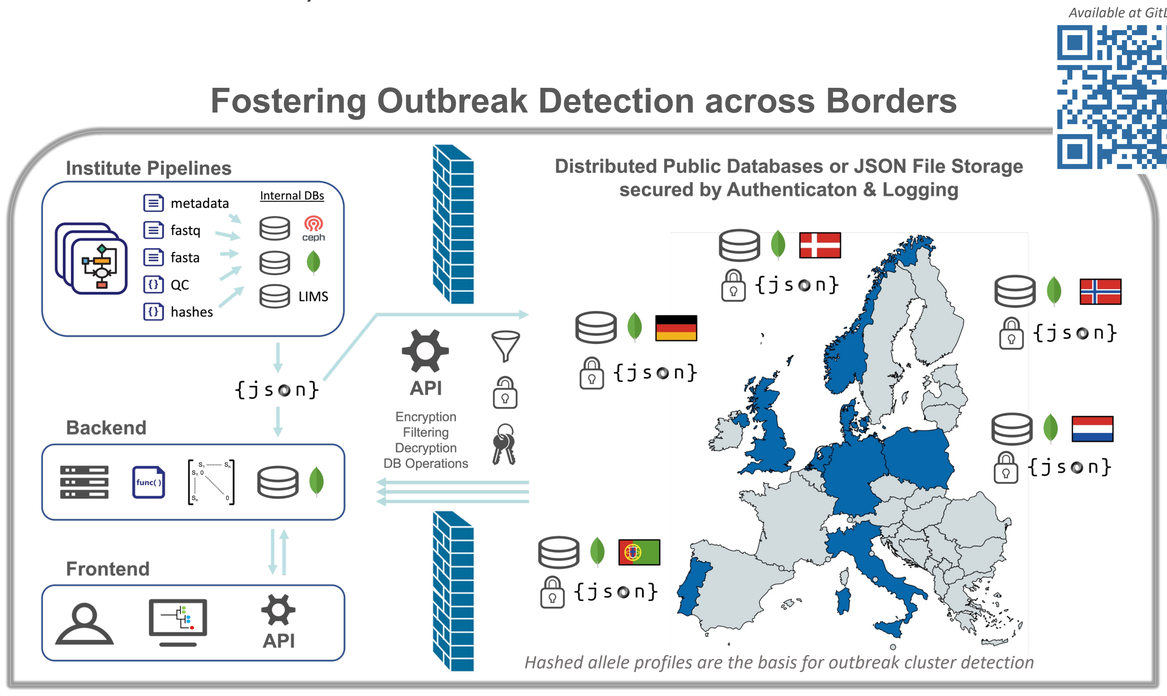
- Structure of the epidemiological data shared between laboratories/countries utilizes controlled vocabulary based on the EFSA Standard Sample Description
- Data is imported into a publicly accessible MongoDB and may be filtered and/or encrypted concurrently in an information hierarchy-aware manner

Outcomes

✓ *Pilot tested in Germany*

✓ *Simulation of multilateral data exchange within BeONE*

Available at GitLab:



With this harmonized data model and data aggregation in a non-relational database, bi- or multilateral automated data exchange under legal and GDPR considerations becomes feasible

Promoting comparability and innovation in One Health surveillance

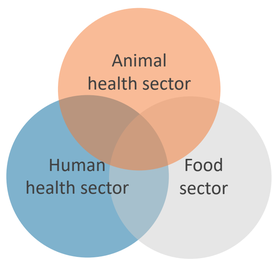
Output: Multi-country and inter-sectoral assessment of cluster congruence between different genomic surveillance pipelines

- Comparison of different typing methods used in the different countries/sectors, covering the levels of resolution needed for long-term routine surveillance and outbreak detection/investigation

✓ *Thresholds at several levels (for different "epidemiological" goals) that can be the basis for efficient nomenclature systems*

	Campylobacter jejuni	*Escherichia coli*	*Listeria monocytogenes*	*Salmonella enterica*
Denmark	■ ■	■ ■	■ ■	■ ■
The Netherlands	■	■	■	■
Germany	■	■	■	■
Portugal	■	■	■	■
United Kingdom				□
Germany	□	□	□	□
Italy	■ □	■ □	■ □	■ □
Poland	■	■	■	■
Germany	■ □	■ □	■ □	■ □

■ Assembly-based □ Read-based ■ Commercial software

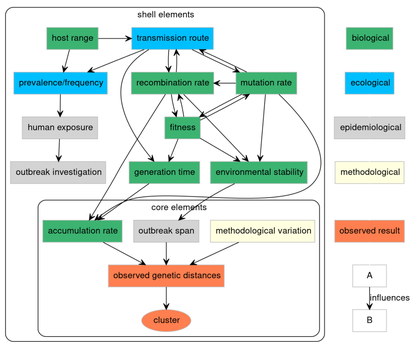


Comparability between different pipelines, promoting a multi-country and intersectoral One Health approach

Output: Conceptual model of genetic clustering

- Address the outbreak detection problem and outline a conceptual model that underlines the complex relations between the biological and ecological factors at play in the evolution of common foodborne pathogens

✓ *This model will be refined upon emergence of new data on this innovative topic*



First step in developing and evaluating new algorithms for cluster definition/detection towards a transdisciplinary One Health approach

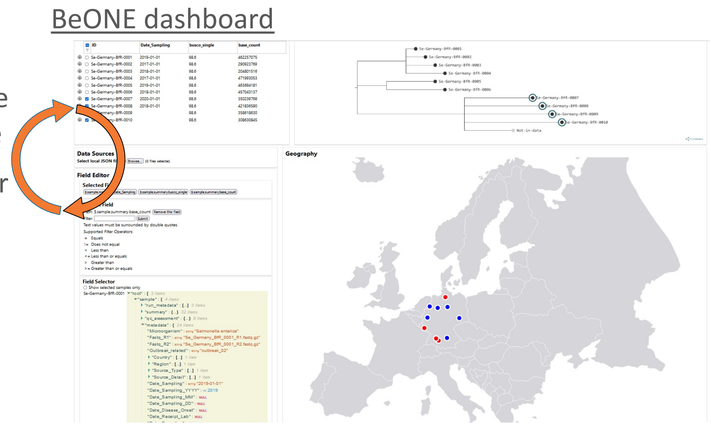
Integrating different surveillance-oriented solutions

Output: BeONE datahub and dashboard

BeONE datahub

- Web application connected to the BeONE MongoDB sample database
- Integration of ReporTree for cluster identification and reporting

BeONE dashboard



Available at GitHub:

Integrative web application for the visualization of genetic clusters integrated with epidemiological and geographical data

ADDITIONAL OUTPUS

Diverse datasets of four important foodborne pathogens

Public datasets comprising genome assemblies and allelic profiles of *Listeria monocytogenes* (3,300 isolates), *Salmonella enterica* (2,766 isolates), *Escherichia coli* (2,307 isolates) and *Campylobacter jejuni* (3,686 isolates). Part of these isolates were anonymously shared within the BeONE Consortium. The remaining ones were carefully selected from public databases to ensure a wide genetic diversity in the dataset. Useful asset for future surveillance and research works.

Available at Zenodo:

One Health Sequencing for Surveillance Handbook

Online handbook covering the main technical and practical aspects associated with the application of WGS for foodborne diseases surveillance. By following a simple and straightforward writing and structure, it can be easily understandable by laboratory staff starting in the field, thus helping national and local labs to build capacity and competence on the use of NGS methods for surveillance purposes.

Collaboration with:

IMPACT

BeONE tools and outputs are expected to i) contribute to the capacitation of EU laboratories to carry out routine surveillance integrating both genomic and epidemiological data, ii) launch new research lines to improve food and waterborne diseases (FWD) genomic epidemiology, and iii) facilitate data sharing and comparability among EU countries, international organizations, and/or other stakeholders involved in FWD prevention and control. 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 to decrease the FWD burden.

This poster is part of the European Joint Programme One Health EJP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830

Main publications

Mixão V et al. (2022) ReporTree: a surveillance-oriented tool to strengthen the linkage between pathogen genetic clusters and epidemiological data. *Research Square*. doi: 10.21203/rs.3.rs-1404655/v2

Deneke C et al. (2021) Species-Specific Quality Control, Assembly and Contamination Detection in Microbial Isolate Sequences with AQUAMIS. *Genes*. 12(5):644. doi: 10.3390/genes12050644

Deneke C et al. (2021) Decentralized Investigation of Bacterial Outbreaks Based on Hashed cgMLST. *Front Microbiol*. 12:649517. 10.3389/fmicb.2021.649517

BIOPIGEE

Biosecurity practices in pig farming across Europe

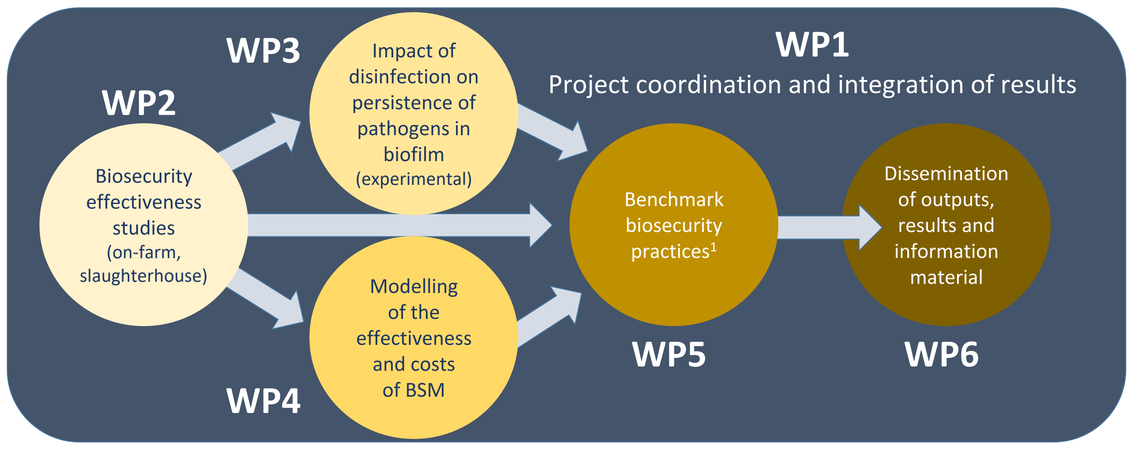
E. Burow (WP1, BfR), R. Smith (WP2, APHA), W. v.d. Poel (WP3, WBVR), R. Simons (WP4, APHA), V. Zoche-Golob (WP5, BfR), M. Sjölund (WP6, SVA), BIOPIGEE Consortium

Project background

Salmonella (SAL) and hepatitis E virus (HEV) are considered zoonotic pathogens which rarely lead to clinical signs in pigs but can cause severe diseases in humans. Since pigs can serve as reservoirs, the relevance of biosecurity measures (BSM; defined by Huber et al. 2022) for controlling transmission of SAL and HEV into and within pig farming was studied.

Objective: Identification of effective and cost-efficient biosecurity measures along the pig production chain to prevent SAL and HEV from entering the food supply chain.

Briefly, work packages including literature, expert panel, field and experimental studies as well as modelling were arranged and finally informed a package disseminating the findings.



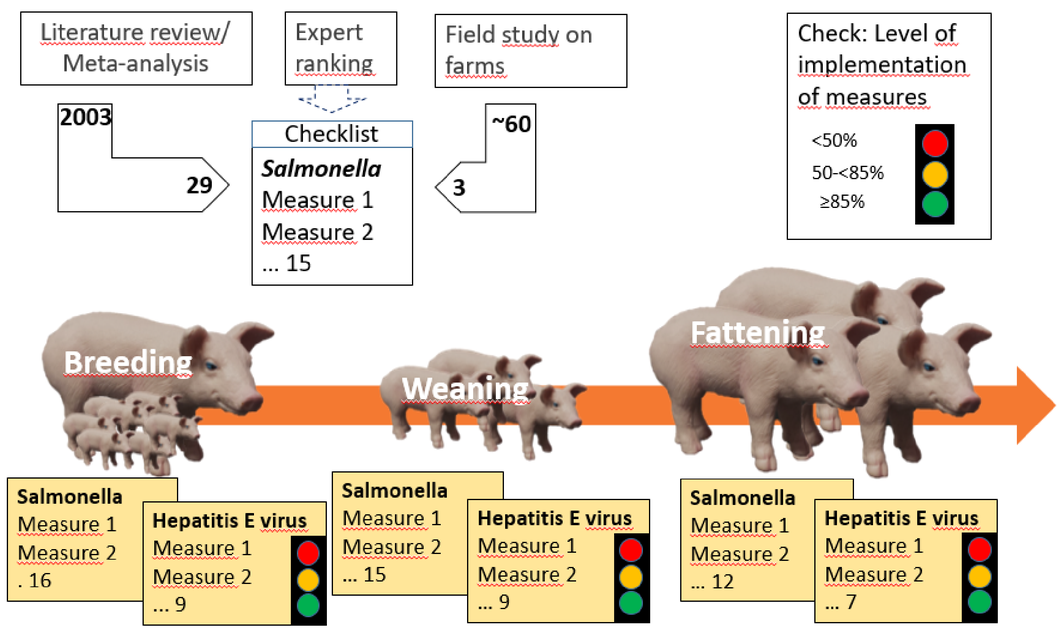
[1]WP5: Definition biosecurity measure, literature review, expert survey, checklist

Key output: On-farm checklists

Checklists were developed (WP5.5) which include BSM

- proven to be effective for pig farms based on extractions from peer-reviewed papers (WP5.2) and BIOPIGEE field studies (WP2.2)
- meet the definition by Huber et al. 2022 (WP5.0).
- ordered based on expert opinion (highest ranked measures at the top of lists, WP5.4).

A traffic light system indicates implementation rate (WP5.5).

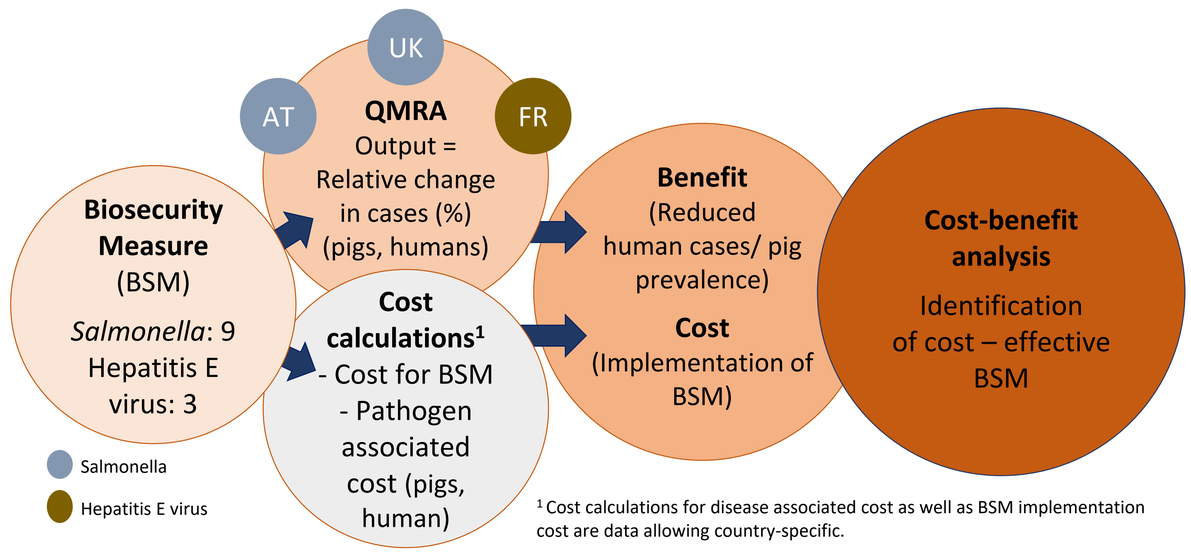


Key output: Guidance document for abattoirs

A guidance document was produced (WP2.3), listing 23 quantitatively effective BSMs to control SAL and *E. coli* occurrence in slaughterhouses, identified from 51 relevant research papers & reports.

Steps in slaughter process	Number of effective BSMs/publications
Lairage hygiene and management	4/10
Scalding methods & conditions	2/8
Singeing methods & conditions	1/3
Polishing	3/5
Evisceration hygiene & technics	3/7
Carcass splitting	2/3
Decontamination of carcasses	4/8
Chilling of carcasses	4/4

Key output: Cost-benefit of biosecurity measures



[1] Cost calculations for disease associated cost as well as BSM implementation cost are data allowing country-specific.

The impact on the number of human cases by as effective identified BSM (WP5.2) was evaluated using established quantitative microbial risk assessment models (QMRA) (WP4.2). In a cost-benefit analysis (WP4.4) the cost (€) of the applied BSM in 100% of farms/slaughter pigs within a country was compared against the resulting benefit (€) (i.e. reduced number of human cases, reduced prevalence in pig population) for the year 2019. The results of only 3 BSM for SAL showed a BCR >1 for the selected countries. Due to data gaps, only benefits resulting from a reduced number of human cases could be calculated for HEV. All evaluated BSM for HEV had a BCR <1.

Economic assessment

BCR = Total benefit/Total cost

Results for a 100% application rate of the BSM on farms/in slaughter pigs to reduce Salmonella.

Biosecurity measure	Austria	United Kingdom
SALMONELLA	Benefit Cost Ratio	
Acid in feed	1.49	1.50
Acid in water	1.12	1.91
Anal plugging	1.42	1.42
Disinfection	<1	1.02

Project outcomes and impact

Outcomes

- Guidance for farmers, veterinarians, consultants, slaughterhouses in European countries
- Exchange with expert panel, stakeholders and head organizations at various workshops and other dissemination events was organized across partner countries
- Illustrative flyers and web content to inform stakeholders, incl. collaborating farm education centers and other projects is produced and offered in partner countries

Impact

Societal and policy

- **Reliable** guidance on **cost-efficient** measures
- **Reduction** of SAL & HEV **spread**, and of animal and human **diseases**
- Basis for **monitoring** & **regulation** in a One Health context

Scientific

Applied support tools can be developed further

- **Integration** of new evidence on cost-effective measures
- **Adaptation** to cover also **other pathogens**, **livestock species**, **production systems**
- **Definition** of **biosecurity measures** as fundamental basis for targeted implementation

Acknowledgements

Contribution: A. Viltrop (slaughterhouse study, Estonian University of Life Sciences), C. Bester (cost-benefit study, Vetmeduni Vienna)

Reference

N. Huber et al. 2022 https://doi.org/10.1016/j.onehlt.2022.100433

This poster is part of the One Health European Joint Programme.

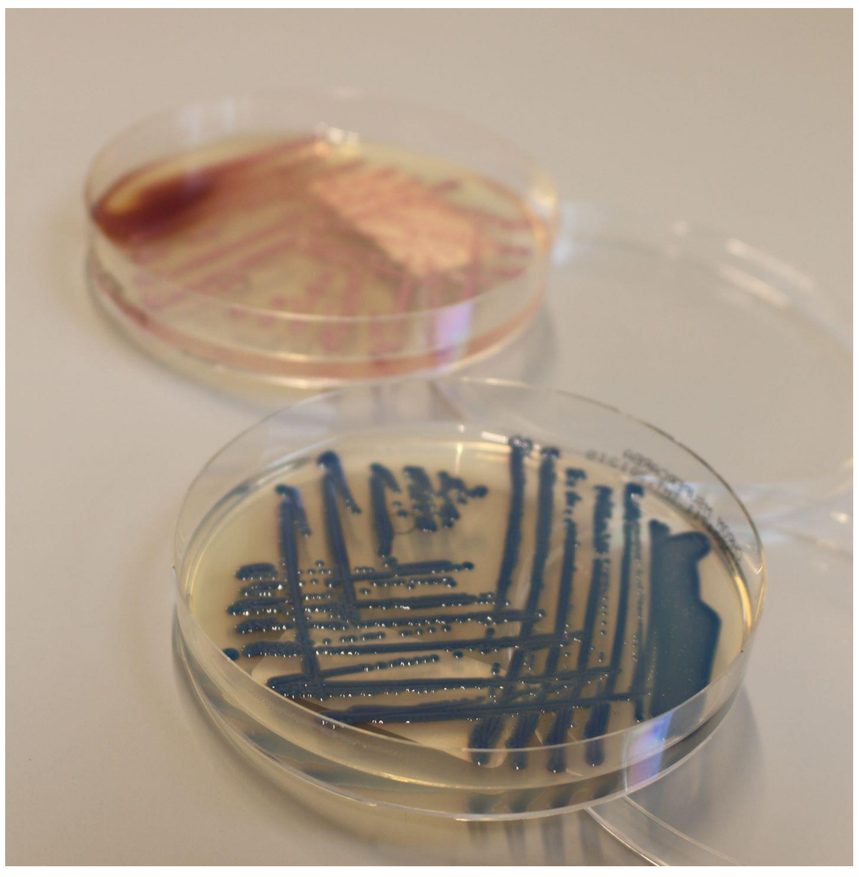
This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

IMPART: Improving Phenotypic Antimicrobial Resistance Testing

Project leader: **Kees Veldman**, Wageningen Bioveterinary Research (WBVR) part of Wageningen University & Research (WUR)
Work package leaders: **Agnès Perrin-Guyomard** and **Sophie Granier**, The French Agency for Food, Environmental and Occupational Health and Safety (ANSES); **Jannice Schau Slettemeås**, Norwegian Veterinary Institute (NVI); **Kees Veldman**, Wageningen Bioveterinary Research (WBVR) ; **Sven Maurischat**, German Federal Institute for Risk Assessment (BfR).

MAIN OBJECTIVES

(1) Harmonising detection methods for specific types of AMR associated with bacteria relevant to public health, such as colistin-resistant and carbapenemase-producing Enterobacterales (CPE).
(2) Establishing new laboratory test criteria (ECOFF) to improve international harmonisation of the monitoring of antimicrobial resistance in bacterial pathogens from animals and humans.

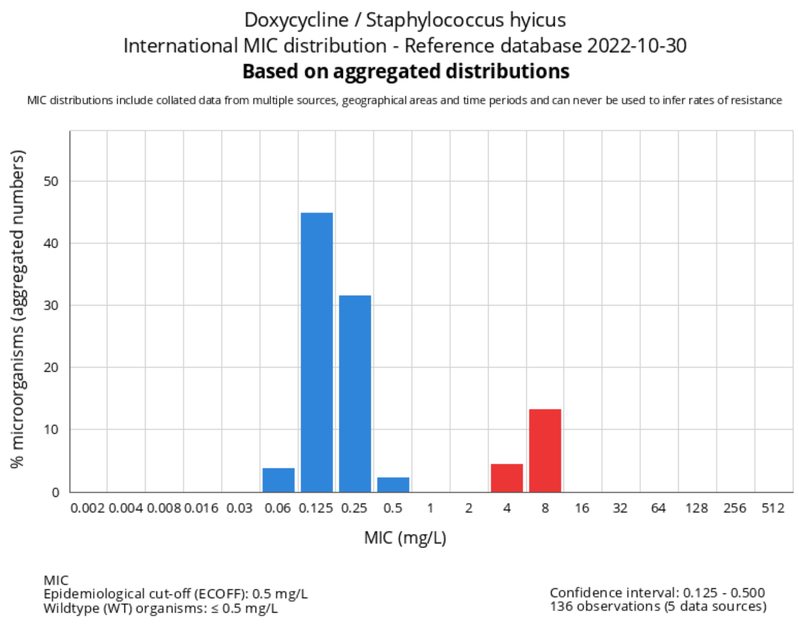


KEY OUTPUTS

WP1: A two-step screening method involving a PCR amplification and culturing on chromogenic agars media was effective to detect most of the *E. coli* or *Salmonella* spp. strains harboring *mcr*-genes.

WP2: Most commercially available agars for the detection of CPE performed adequately. However, the evaluation showed that the investigated agars were not suitable for detection of non-chromogenic CPE strains nor for the detection of CPE with low meropenem MICs.

WP3: Facilitated by the production of >50.000 MIC values new epidemiological cut-off values (ECOFFs) were established for *Staphylococcus hyicus*, *S. pseudintermedius*, *Pasteurella multocida* and *Mannheimia haemolytica*.



SCIENTIFIC IMPACT

WP1: The outcomes of IMPART stimulated different partner institutes to optimize their protocol for detection of colistin resistant bacteria which will result in additional publications on this subject.

WP2: Limitations to detect low-level meropenem resistance using the EURL-AR protocols for detection of ESBL/AmpC and CPE in caecal or meat samples encouraged partner institutes to perform additional CPE screening by using PCR and/or selective enrichment steps.

WP3: ECOFFs of animal pathogenic bacteria are essential for determining clinical breakpoints of veterinary antibiotics. The first clinical breakpoint for florfenicol in cattle was established by VetCAST after setting ECOFFs for *Mannheimia haemolytica* and *Pasteurella multocida*.

POLICY IMPACT

WP1: Implementation of a selective screening protocol will generate valuable data for EFSA regarding the prevalence of *mcr*-carrying bacteria in food-producing animals and meat products to monitor the effect of the recent reduction of polymyxins usage in livestock.

WP2: Based on the outcomes of a questionnaire regarding the non-selective pre-enrichment step within the protocol for detection of ESBL/AmpC and CPE, the EURL-AR decided to stick to the current method because of the multi-purpose application of the broth for other objectives of the AMR monitoring.

WP3: Activities to harmonise susceptibility testing of veterinary pathogenic bacteria are continued within VetCAST and COST action ENOVAT (COST Action CA18217) and will support the EU One Health Action Plan against AMR.

This poster is part of the European Joint Programme One Health EJP.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

Full Plasmid Sequencing for Improved AMR surveillance

Pieter-Jan Ceyssens, Henrik Hasman, Joost Hordijk, Mike Brouwer, Benoit Doublet, Stefan Widgren and the FULL_FORCE consortium*

* The Full Force team includes, apart from the work package leaders listed above: Jean-Yves Madec, Antoine Drapeau, Margo Maex, Vera Manageiro, Jens-Andre Hammerl, Frederik Teudt, Fernando Esperon, Sebastien Leclercq, Henrik Hasman, Aleksandra Giza, Oskar Lindsjo, Antoni Hendrickx, Soren Overballe-Petersen, Emma Ostlund, Amar Telke, Patricia Alba, Aldert Zomer, Manuela Canica, Magdalena Zajac, Arkadiusz Bomba, Magdalena Slomiany-szwarc, Petra Edquist, Egil Fischer, Alma Brolund, Engeline Van Duijkeren, Stefan Borjesson, Jannice Schau-Slettemeas, Amar Telke, Antonio Battisti, Cecilia Jernberg, Eelco Franz, Marissa Haenni and Robert Soderlund.

PROJECT AIM

The technical evolution of surveillance of bacterial infections:

2000s : MLST – based (7 genes)

2010s : cgMLST – based (>500 genes)

2020s: Full genome (incl. plasmids)

Future surveillance of antimicrobial resistance (AMR) will require the capacity for long-read sequencing, but sharp differences in know-how exist across EU countries.

This project aimed to built capacity for plasmid sequencing in 17 EU veterinary and public health institutes, by supplying all partners with a technological toolbox.

The FULL FORCE project setup

Create a common Plasmid Assembler

Python script with best-in-field tools
https://github.com/FullForcePlasmidAssembler

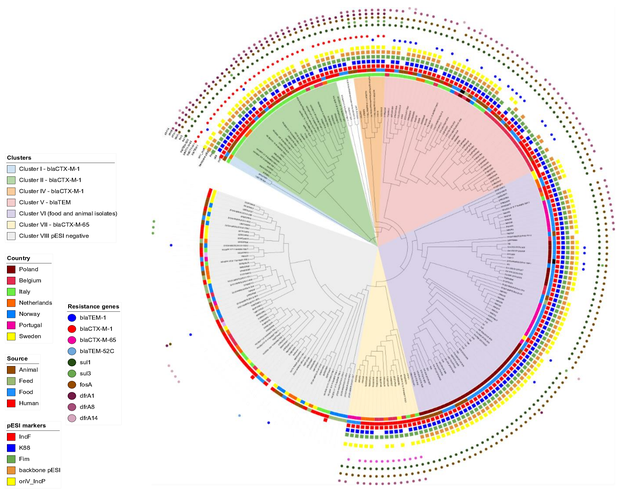
Lead : SSI, DK

Benchmark its performance

Proficiency test of five MDR *E. coli* strains

Case 1: Increasing AMR in *S. Infantis*

pESI megaplasmid in 323 *Salmonella Infantis* Strains from 7 EU countries from animal, food, feed and human infections



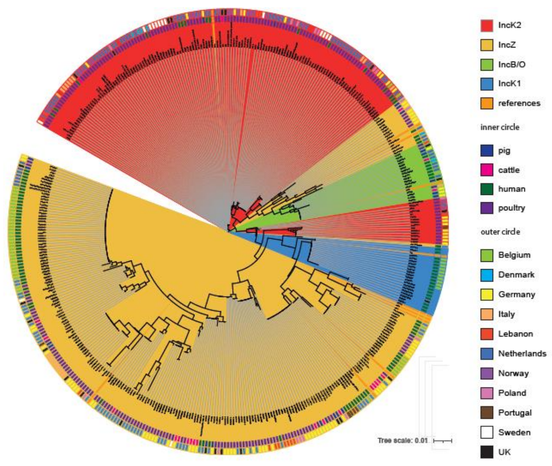
Lead : ISS, IT

KEY FINDINGS:

- Cluster I-V found in all sectors (high risk of infection)
- Cluster VI only found in food and animal isolates (Reduced risk of infection)

Case 2: Deciphering the infamous incK/B/O/Zs plasmids

Full plasmid sequencing of 289 *E. coli* isolates from 11 EU countries, RNAI/II based analysis



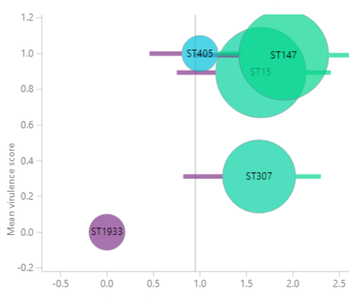
KEY FINDINGS:

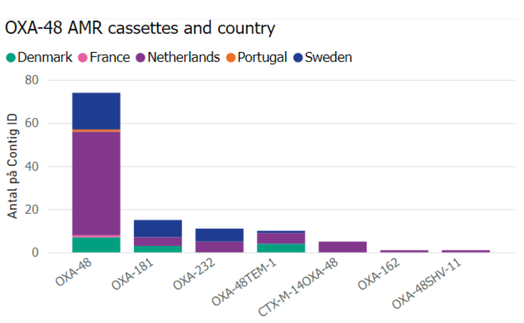
- A high occurrence of MDR IncZ plasmids was found within the IncK/B/O/Z-harboring isolates from human and animal sources in Europe.
- higher occurrence in human clinical samples compared to healthy humans.

Lead : RIVM, NL

Case 3: The *K. pneumoniae* plasmidome

Full sequencing of 850 *K. pneumoniae* strains, with focus on carbapemenase producers





KEY FINDINGS:

- Association between virulence and resistance
- Identification of hybrid formation between resistance and virulence plasmids

Lead : FOHM, SW

Impact on future AMR surveillance

- Build-up of technological capacity for plasmid sequencing across the EU
- Buffer against loss of expertise though pre-encoded assembler
- Detailed characterization of ongoing AMR surveillance, enabling insight in the (complexity of the) drivers of AMR transmission
- Preparing the field for enhanced surveillance of AMR, which includes monitoring of horizontal gene transfer.

This poster is part of the European Joint Programme One Health EJP.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

FED-AMR:
The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain

INTRODUCTION

Free extracellular DNA (exDNA) is assumed to be a major contributor to the environmental resistome. Transformation is an important driver for genetic plasticity of bacterial genes and genomes. Natural transformation does not require physical contact between donor and recipient bacteria and is exclusively regulated by the recipient cell. This may facilitate antibiotic resistance genes (ARG) crossing ecosystem barriers and invading new habitats compared to HGT by conjugation.

Keywords: Bacterial transformation, free extracellular DNA, antimicrobial resistance, horizontal gene transfer, ecosystem boundaries.

QUESTIONS

The design of the experiments are allowing to answer the following research questions:

1. **Question 1:** What are the most prevalent ARGs in the tested environmental compartments?
2. **Question 2:** Which environmental compartments show an especially high contamination with ARGs of anthropogenic origin?
3. **Question 3:** Does ARG-encoding free exDNA overcome ecosystem boundaries and bottlenecks more easily compared to the transfer of non-autochthonous bacteria in newly invaded ecosystems?
4. **Question 4:** What are the main drivers of AMR dissemination via free exDNA in the tested environmental compartments?
5. **Question 5:** Which strategies can be applied to reduce the spread of AMR caused by the transfer of free exDNA between ecosystems highly contaminated with ARGs of anthropogenic origin?

SPECIFIC AIMS

Objective 1 (WP2): Determination of the resistome and microbial biodiversity in the tested environmental compartments – longitudinal study over a crop-growing season (1 year; field studies). Identification of the role of exDNA for HGT in the tested compartments (**Fig. 1**).
Objective 2 (WP3): Elucidating the role of *C. difficile* as an ARG transfer platform over ecosystems boundaries and its linkage between human and non-human (zoonotic) reservoirs (**Fig. 1**).
Objective 3 (WP4): Determination of the selection pressures in the tested compartments of human, animal and environmental ecosystems (**Fig. 1**).
Objective 4 (WP5): Identification of environmental conditions modulating transformation frequencies in soil microcosms and porcine chemostat gut models (laboratory studies)
Objective 5 (WP6): Developing probabilistic and mechanistic models explaining the links between antimicrobial usage in animals, AMR in the environment, and the risks for public health.

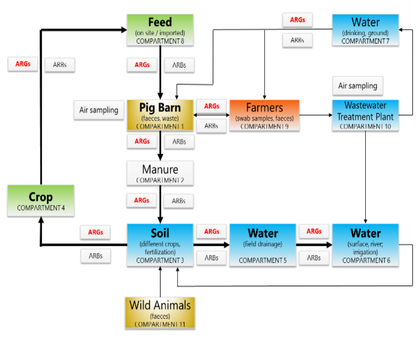


Fig. 1. Potential pathways for ARG dissemination over environmental ecosystem barriers. Bold letters/arrows = compartments and pathways for ARG movement that are monitored on the HOAL testing range; ARGs: antimicrobial resistance genes; ARBs: antimicrobial resistant bacteria; Animal compartments (pigs, stable; wild animals in HOAL catchment area): gold; Human compartments (farmer, workers exposed to animal husbandry in HOAL): red; Compartments associated to plants (crops, animal feed): green; Genuine environmental compartments (soil, water): blue.

Sampling: 6 countries from 4 EU regions (East , North, South, West) collected in 2 HOALs (Hydrological Open Air Laboratory) (AU, PT), 2 conventional agricultural lands (EE, CZ), 1 mixed livestock farm (GB), 1 municipal WWTP & Forest (IE) samples from up to 11 compartments.

KEY OUTPUTS

Objective 1:
The microbial biodiversity and resistome in the tested environmental compartments were evaluated by characterising all 16S V1-V9 regions through 16S rRNA metagenomics and target gene enrichment.
Conclusion: The Phyla in exDNA are dominated by Proteobacteria and Firmicutes. Phyla in **Fig. 2** represent 93.5% of the reads in exDNA, and the samples are ordered by sample data in the x axis which allow us to check if there is an alteration over time. The principal component analysis (**Fig. 3**) suggests separation between three groups: 1) soil; 2) crops; feeds, drinking water, and 3) farmers, pigs, wild animals, manure, waste water. No significant difference was detected between soil in fields with and without manure. In the wild animals compartment we detected ARG conferring resistance to ~30 antibiotic classes, where β-lactams, tetracyclines, aminoglycosides and fluoroquinolones are the most prevalent (**Fig. 4**). In the same compartment, we identified 11 ARG-types conferring resistance to fluoroquinolones (including episomal and chromosomal mutated gene variants) (**Fig. 5**).

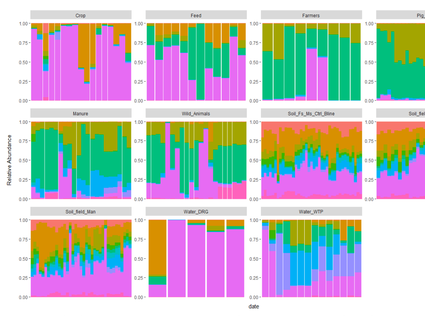


Fig. 2 Phylum percentage in each exDNA samples, by compartment.

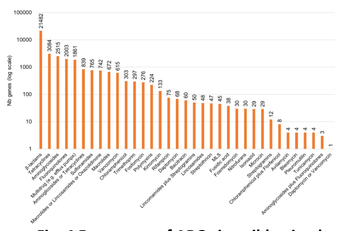


Fig. 4 Frequency of ARGs in wild animals compartment.

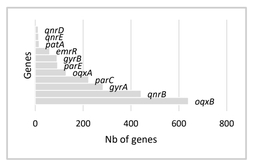


Fig. 5 Frequency of ARG-type conferring resistance to fluoroquinolones, in wild animals compartment.

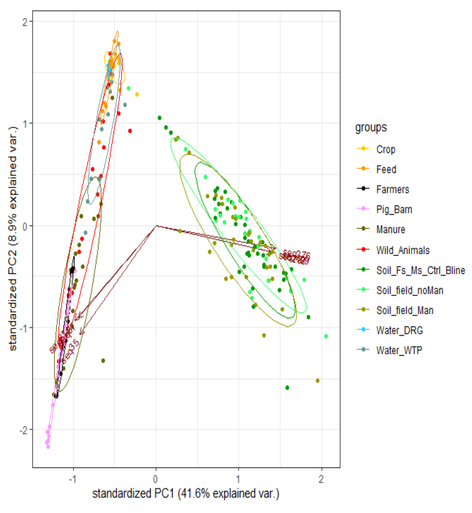


Fig. 3 Biplot of the exDNA samples, among compartments.

Objective 2:
In each HOAL, a different ribotype was dominant.
All compartments connected to the swine production unit (pigs, swine cesspool/manure, agricultural soil and WTP) presented the highest *C. difficile* positivity rate, and RT033 was the predominant type found in those compartments.
Conclusion: The high genetic relatedness found among isolates (0.1 core-SNVs) (**Fig. 6B**) supports a clonal transmission between animal and environmental compartments.

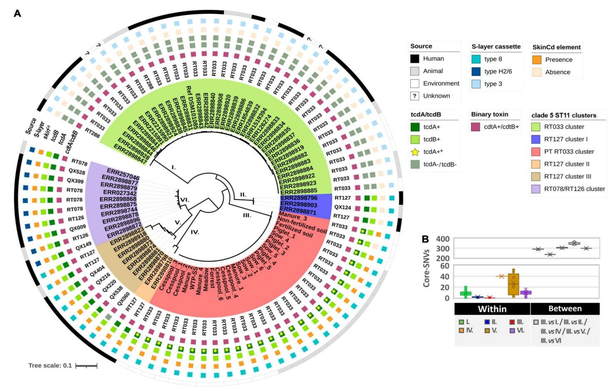


Fig. 6 Phylogenetic positioning of PT RT033 clone within in the *Clostridioides difficile* clade 5 ST11 lineage. (A) Core-genome SNP-based phylogenetic tree. **(B)** Box Plots depicting the number of SNVs observed within and between all clade ST11 clusters.

Objective 5:
We inferred bacterial growth, interaction and antibiotic susceptibility parameters for the Lotka-Volterra model, restricted to the top 10 most abundant genera to avoid overfitting. After simulations we identified a tipping point of the community in response to external perturbation: resulting in a persistent change in its composition and subsequent recovery from colonisation by *C. difficile* (**Fig. 7**). The areas under the curves for both perturbation signals are equivalent.
Conclusion: Ecological modelling of microbial communities affords additional insight into their stability, which cannot easily be determined from experimental studies alone. Our next step is to explore the impact of different perturbation regimes, with the long-term goal to model data obtained from chemostat experiments (WP5).

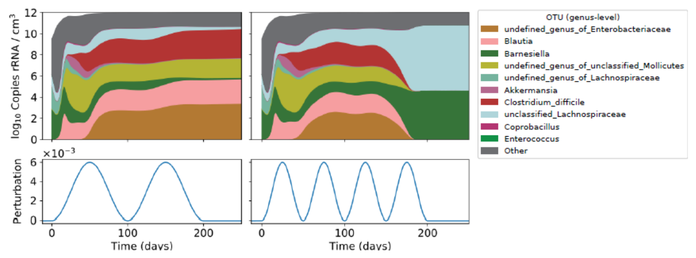


Fig 7. The microbial community composition (top) is influenced by a transient, periodic perturbation signal (e.g. the addition of antibiotics; see bottom). These signals differ in their frequency. (Left) Community displays resilience to this signal. (Right) Community is tipped into a different state, and recovers from colonisation by *C. difficile* (red shaded region).

Publications:

- ✓ Draft Genome Sequence of a Multidrug-Resistant *Escherichia coli* Sequence Type 1193 Pandemic Clone Isolated from Wastewater in Austria. Microbiol. Resour. Announc., 2021, 10:e00762-21. doi: 10.1128/ MRA.00762-21
- ✓ Assessment of the Transmission Dynamics of *Clostridioides difficile* in a Farm Environment Reveals the Presence of a New Toxigenic Strain Connected to Swine Production. Front. Microbiol., 2022, 13:858310. doi: 10.3389/fmicb.2022.858310
- ✓ Characterizing Antimicrobial Resistance in Clinically Relevant Bacteria Isolated at the Human, Animal, Environment Interface Using Whole-Genome Sequencing in Austria Journal. Int. J. Mol. Sci. 2022, 23:11276. doi.org/10.3390/ijms231911276
- ✓ Airborne spores' dissemination of a swine associated *Clostridioides difficile* clone. Anaerobe, 2022, 78, 102651. doi.org/10.1016/j.anaerobe.2022.102651
- ✓ Factors associated with the prevalence of antibiotic resistance in the environment from a One Health perspective: Protocol for a systematic evidence map. Environ. Int. *(in revision, 2022).*
- ✓ Master thesis: Characterization of Antibiotic Resistance in Strains Isolated from Different Environmental Reservoirs *[Caracterização da Resistência aos Antibióticos em estirpes isoladas de diferentes reservatórios ambientais]* http://hdl.handle.net/10451/53801

Other Outcomes:

- ✓ Oral Communications and Posters in national and international congresses and other meetings: **12**
- ✓ Webinars (e.g. "Biotechnology and Safety: Tracking and Analyzing Free-floating Extracellular DNA across Urban Waterways"): **5**
- ✓ Monthly meetings (with all Partners): **27**
- ✓ Supervisory Board meeting (13 Members): **1**
- ✓ Advisory Board meeting (7 Members): **1**
- ✓ Annual meetings (science and management) (with all Partners): 1 face-to-face (2020); 1 online (2021); 1 face-to-face & online (2022)
- ✓ New documents produced, e.g. Protocols (harmonization) (**>30**), Deliverables (**25**), Minutes of meetings (**27**), others: **>82**
- ✓ Data Management Plan: **1**
- ✓ Risk Management Plan: **1 (Fig. 8)**
- ✓ Networking and communication between partners and reference labs from different sectors and countries, leading to new knowledge.
- ✓ Training through research, e.g. a Master, collaborations among different experiences and countries.

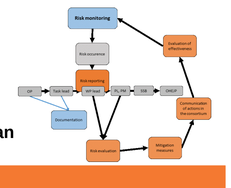


Fig 8. Risk Management Plan

IMPACTS

Scientific impact:

Cross-sector communication of data contributing to the advancement of science, namely e.g.:

- ✓ By a study capitalized on advancements in **high-throughput sequencing methods** and **analytical tools**, as it provides the large scalability necessary to investigate bacterial communities, in a way to explore and mapping AMR in exDNA, either in human, animal, and environmental settings, and in several sample matrices.
- ✓ By sampling campaigns that provided basic information for **establishing ARG monitoring in environmental compartments**, which is recommended by EFSA and has the potential to become compulsory for EU MS.
- ✓ By using the true concept of One Health, namely with an **important environmental component** (farmers, pigs, wild animals, manure, air of pig barns, feeds, crops, soil, water).

Societal, policy and economic impact:

- ✓ Impactful research that **adds value** to an European, national and international level.
- ✓ Decisive **for assessing** the potential of exDNA to serve as **a high-risk source** of resistance determinants in agricultural soils and along the food/feed chain.
- ✓ Impact on **strategies to improve and/or upgrade** wastewater treatment plants, as it is decisive for WWTP engineers to know if they have to design devices that only kill bacteria or if strategies to eliminate bacterial DNA from the waste streams would have to be applied.
- ✓ As reliable and accurate surveillance is fundamental to characterize the risk of AMR in a given region, the results obtained **show how essential it is to track the spread of specific ARGs** geographically and over time, identify new ARGs and support preventive measures and interventions against AMR pathogens.
- ✓ Probabilistic and mechanistic models of the links between antimicrobial usage and AMR in different settings and the risks for public health may be **useful for EU agencies, policy makers, stakeholders, international organizations, others.**

FED-AMR Consortium

Project Leader: W. Ruppitsch. **WP1:** W. Ruppitsch (L); A. C. Rosel (DL). **WP2:** M. Caniça (L); A. C. Rosel (DL). **WP3:** M. Oleastro (L); S. Persson (DL). **WP4:** M. Brandtner (L); A. Gajda (DL). **WP5:** M. Chambers (L); R. La Ragione (DL). **WP6:** G. Lo Iacono (L); M. Chambers (DL)

Acknowledgments: This poster is part of the European Joint Programme One Health EJP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

Other Partners:

TOX-Detect

Development and harmonization of innovative methods for comprehensive analysis of foodborne toxigenic bacteria

Y. NIA[1*] ; J-A HENNEKINNE[1*] ; J. MASQUELIER[2] ; H. FRENTZEL[3] D. CLERMONT[4] ; N RAMA RAO[5] ; K HOGEVEEN[1] ; M. GOHAR[5]; T. SKJERDAL[6]

*** Project leaders**

1. French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France
2. Sciensano, Belgium
3. German Federal Institute for Risk Assessment (BfR), Germany
4. Institut Pasteur, France
5. French National Research Institute for Agriculture, Food and Environment (INRAe), France
6. Norwegian Veterinary Institute, Norway

Introduction

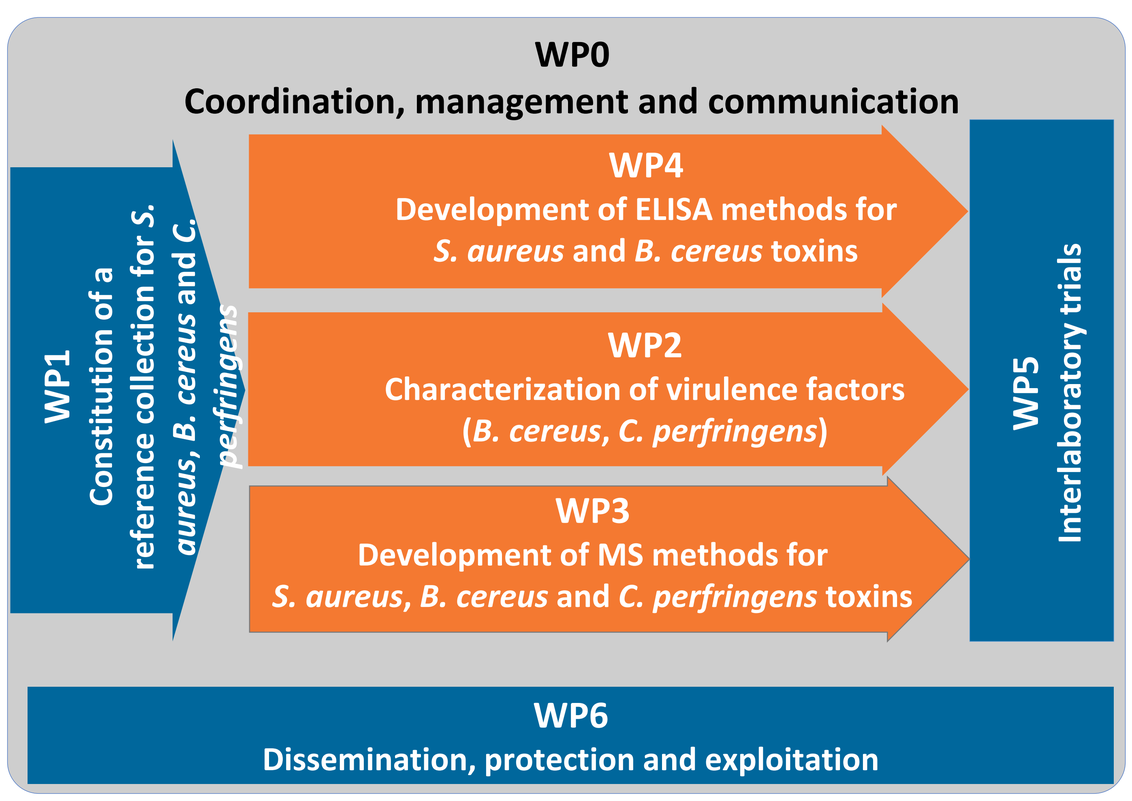
Outbreaks caused by bacterial toxins represent a major proportion of reported FBOs (thousands of cases each year). At EU level, the most frequently reported causes of food-poisoning outbreaks (FPOs) are bacterial toxins produced by *Bacillus spp*. (*Bacillus cereus*), *Clostridium spp*. (*C. perfringens* and *C. botulinum*) and *Staphylococcus spp*. (*S. aureus*). The true incidence of FPOs caused by these toxigenic bacteria is underestimated for many reasons, including misdiagnosis, under-reporting (particularly of minor outbreaks), and improper sample collection or laboratory examination. The complexity and diversity of food matrices involved in FPOs caused by bacterial toxins add to the difficulty. Moreover, FPOs caused by toxigenic bacteria share similar symptoms, making it difficult to clearly identify the causative agent behind the outbreak during investigations. All this may also explain the high proportion of "weak evidence" (90%) FPOs reported by EFSA when bacterial toxins are the causative agent.

The scientific community involved in monitoring foodborne outbreaks caused by bacterial toxins needs harmonized and innovative detection methods for this underestimated One Health issue.

Tox-Detect project design

TOX-Detect project was designed to develop and harmonize methods to detect toxin expression and production by toxigenic bacteria across One Health pillars:

- Collating and fully characterizing a large number of toxin-producing bacterial isolates from environmental, clinical, and food samples provided by all the partner institutes
- Developing dedicated ELISA and mass spectrometry methods, and libraries for the identification of the targeted pathogens
- Harmonizing Standard Operating Procedures (SOPs) for all the targeted virulence factors and methods.



Outputs of TOX-Detect project

76 reference strains were selected and characterized

Strains exchanged between consortium members

New specifc and robust **Maldi-Tof library** was developed

10 oral presentations

6 articles

20 deliverables

14 harmonized Operating Protocol transferred

6 methods were developed and transferred

Production of **4** toxins and dedicated analytical tools

8 transfer and inter-laboratory tests organised

Participants received the 14 operating protocols for method implementation

Participants from the **European Union Reference Laboratory** for Coagulase Positive Staphylococci (DG SANTE) network took part

Impact of TOX-Detect project

European Union Reference Laboratory (DG SANTE)

Other OH EJP projects

Participation of 8 National Reference Laboratories to the Interlab-test: good help in absence of PT organizer

New developed MALDI-ToF library: EURL for CPS network decided to implement inter-laboratory validation study

EJP CARE: strains selected and characterised in WP1 were used in the OH EJP CARE project

This poster is part of the European Joint Programme One Health EJP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

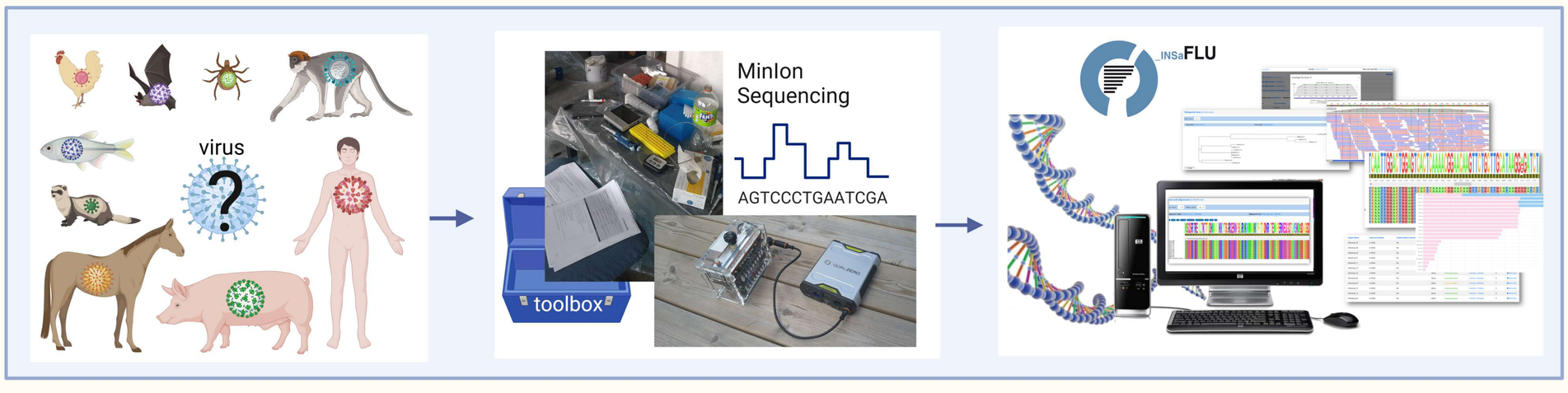
TELEVIR

JRP16-ET2 Point-of incedence toolbox for emerging virus threats

TELEVIR Consortium

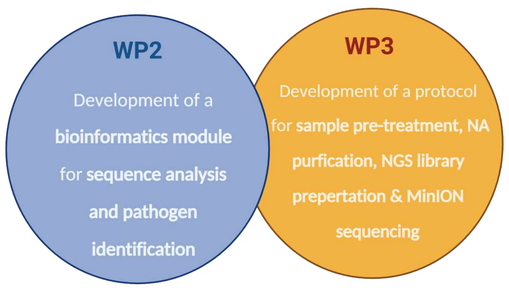
1) ANSES, France - Laurent Bigarré 2) PIWET, Poland - Artur Rzezutka, Eva Kwit 3) INSA, Portugal - Vitor Borges, Joana Isidro, Joao Dourado Santos 4) IZLER, Italy - Alessio Lorusso, Maurila Maracci 5) INIA Spain - Jovita Fernández-Pinero, Pilar Aguilera 6) UoS, UK Daniel Horton, Joaquin Prada, Guido Cordoni 7) SVA, Sweden Tobias Lilja, Mikhayil Hakhverdyan 8) NVI, Norway Øivind Øines, Carlos Goncalo das Neves 9) Sciensano, Belgium Steven Van Borm, Elisabeth Mathijs 10) SSI, Denmark Anders Fomsgaard, Katja Spiess, Morten Rasmussen, Anna Fomsgaard and other TELEVIR consortium members

We developed a Field-employable Protocol for Metagenomic Detection of Viruses



INTRO / BACKGROUND

The world has been regulary facing pandemics caused by pathogens as for example SARS-CoV-2. Viruses infections of animals can lead to zoonotic events, where a virus from a non-human host is transmitted to humans. In our TELEVIR project we developed an unbiased method to detect RNA/DNA viruses. The protocol we established is relatively short and the euipment transportable (**WP3**). As such we can detect viruses in areas of the world with no laboratory infrastructure. We also provided the bioinformatics infrastructure to analyze and indenty virus sequences , by developing the "INSaFLU-TELEVIR" toolkit (**WP2**).



METHODS

WP3

We established a protocol that includes simple to use sample pretreatment, nucleic acid extraction, isothermal random amplification followed by non-targeted sequencing using the Oxford Nanopore Technology system.

WP2

Novel features were implemented into the "INSaFLU-TELEVIR" plaform, with a module for virus detection (TELEVIR).

This includes:

- ✓ Read quality analysis and improvement
- ✓ **State of the art software**
- ✓ Modular Pipeline
- ✓ **Multiple Classification methods**
- ✓ Multiple Viral databases
- ✓ **Summary statistics and intuitive and interactive end-user reports**

RESULTS

TELEVIR partners collected samples and performed field studies in Europe & Africa (**WP4**). They were trained how to use the TELEVIR toolbox developed in WP2 &WP3 at a workshop, which took place at the Statens Serum Institut (Copenhagen/Denmark) in June 2022.

The TELEVIR partners were trained how to use the point of incedence protocol in hands on teaching classes at the TELEVIR workshop. They also evaluated their sequencing results for unbiased virus detection using the INSaFLU-TELEVIR platform.

Field studies performed by the TELEVIR partners NVI and INIA in Norway and Spain. Birds can be vectors for viral diseases. In Norway feces samples were tested for Avian influenza A virus and in Spain chicken were infected with West-Nile Virus in an *in vivo* study, for later on virus detection using fresh sample mateiral.

Field study performed by the TELEVIR partner ANSES on a fish farm in South France. Fish showed sign of unknown illness and were tested for virus infections.

Field studies performed by the TELEVIR partner IZAM on a farm in Tunesia. Diseased cattles where tested for virus infections.

CONCLUSION

In our TELEVIR project we developed a point of incedence toolbox, that we tested as proof of concept in our field studies. We could sucessfully detect viruses in animals, using this metagenomic method combined with the data analysis by the INSaFLU-TELEVIR platform.

REFERENCES Fomsgaard et al., Improvement of field deployable metagenomics virus detection by a simple pretreatment method. Journal of Clinical Virology Plus, 2022.

Acknowledgement This poster is part of the European Joint Programme One Health EJP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830

"Multi-centre study on *Echinococcus multilocularis* and *Echinococcus granulosus s.l.* in Europe: development and harmonization of diagnostic methods in the food chain"

MEME Consortium

Adriano Casulli (**ISS,** coordinator)

WOK PACKAGES:

WP1: SAMPLING STRATEGY

WP2: VALIDATION of PARASITOLOGICAL and MOLECULAR ASSAYS

WP3: DEVELOPMENT/VALIDATION of NEW TOOLS AND PRODUCTION of EPIDEMIOLOGICAL DATA

WP4: TRAINING, DISSEMINATION and PROFICIENCY TESTING SCHEMES

WP5: SCIENTIFIC and ADMINISTRATIVE MANAGEMENT

WP LEADERS:

Jacek Karamon **(PIWET)**

Gerald Umhang, Frank Boue **(ANSES)**

Pavlo Maksimov, **(FLI)**

Adriano Casulli **(ISS)**

Adriano Casulli **(ISS)**

MEME is an international multicentre collaborative project that aims to fill research gaps highlighted by international agencies for the detection and control of zoonotic parasites *Echinococcus multilocularis* (Em) and *Echinococcus granulosus sensu lato* (Eg), causing alveolar echinococcosis (AE) and cystic echinococcosis (CE), respectively. MEME focuses on standardization, harmonization and validation of existing parasitological and molecular methods, and the development and comparative assessment of innovative molecular tools to detect Em and Eg in the food chain. Production of epidemiological data on the presence of Em/Eg eggs in the food chain focuses on vegetables for human consumption and on canine faeces in selected endemic countries.

MEME achievements were:

- The production of Standard Operating Procedures for the sampling of different matrices from naturally or experimentally infected definitive and intermediate animal hosts.
- The validation of the parasitological (Segmental Sedimentation and Counting Technique, SSCT) and molecular diagnostic (multiplex- and MC-RT-PCR) procedures to detect Em and Eg in different matrices along the food chain.
- The development and validation of new molecular tools such as: Comparison of two DNA extraction methods and two PCRs for the detection of Em in stool samples; Bayesian Analysis of three methods for diagnosis of CE in sheep; Microsatellite investigations of Eg cysts; Species detection of Eg by novel probe-based real-time PCRs; Validated method based on PCR-RFLP and multiplex PCR assay for the identification of Eg species; Identification of Eg G1/G3 by SNPs assays.
- Multicentre studies for the production of data relevant for epidemiological assessments: contamination of fresh vegetables for human consumption by eggs of Em/Eg; prevalence of Em/Eg in dog faeces).
- Quantitative assessment on the impact of human CE in Europe by means of systematic review approach.
- Molecular epidemiology studies in selected geographical areas.
- Dissemination of project results at different levels (general public, scientific community, experts, health authorities and media).

MEME impacted on animal health, public health and food safety sectors. Beneficiaries of scientific outputs of MEME are EU reference labs, international organizations and all decision makers.

We provided a set of molecular tools, epidemiological risk assessments and quantitative epidemiological models for the detection, surveillance and control of these parasitic infectious diseases in Europe and beyond.

Funded partners:

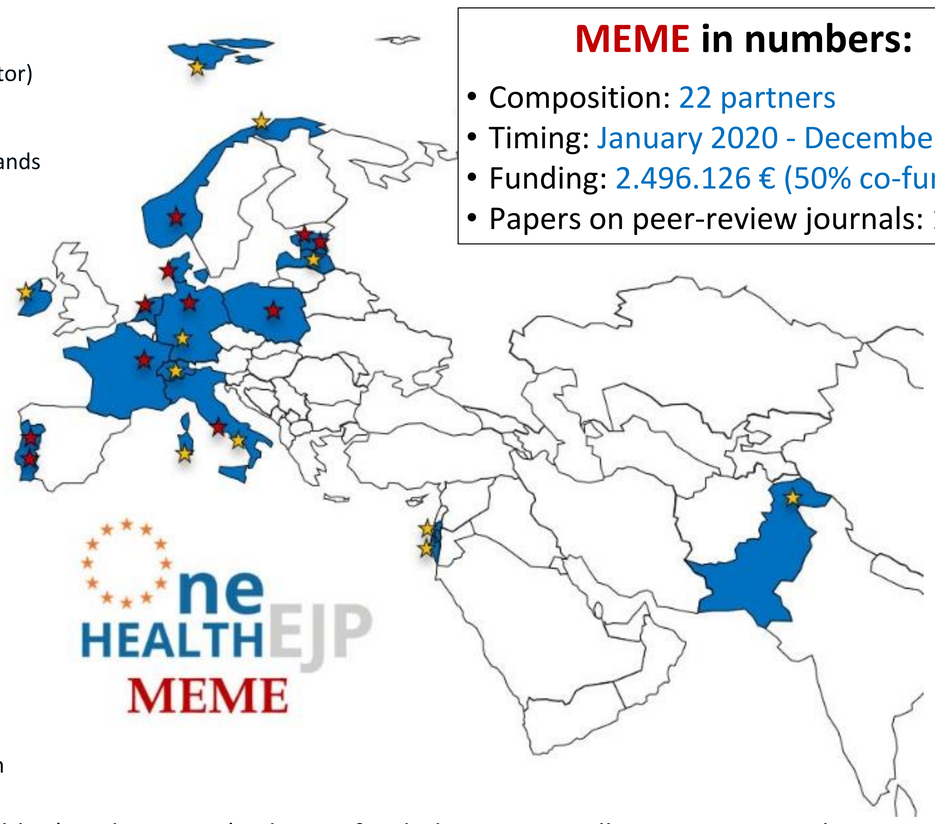
- **ISS,** Italy (Coordinator)
- **FLI,** Germany
- **ANSES,** France
- **RIVM,** The Netherlands
- **PIWET,** Poland
- **NVI,** Norway
- **SSI,** Denmark
- **INIAV,** Portugal
- **INSA,** Portugal
- **UT,** Estonia
- **VFL,** Estonia
- **BIOR,** Latvia

External partners:

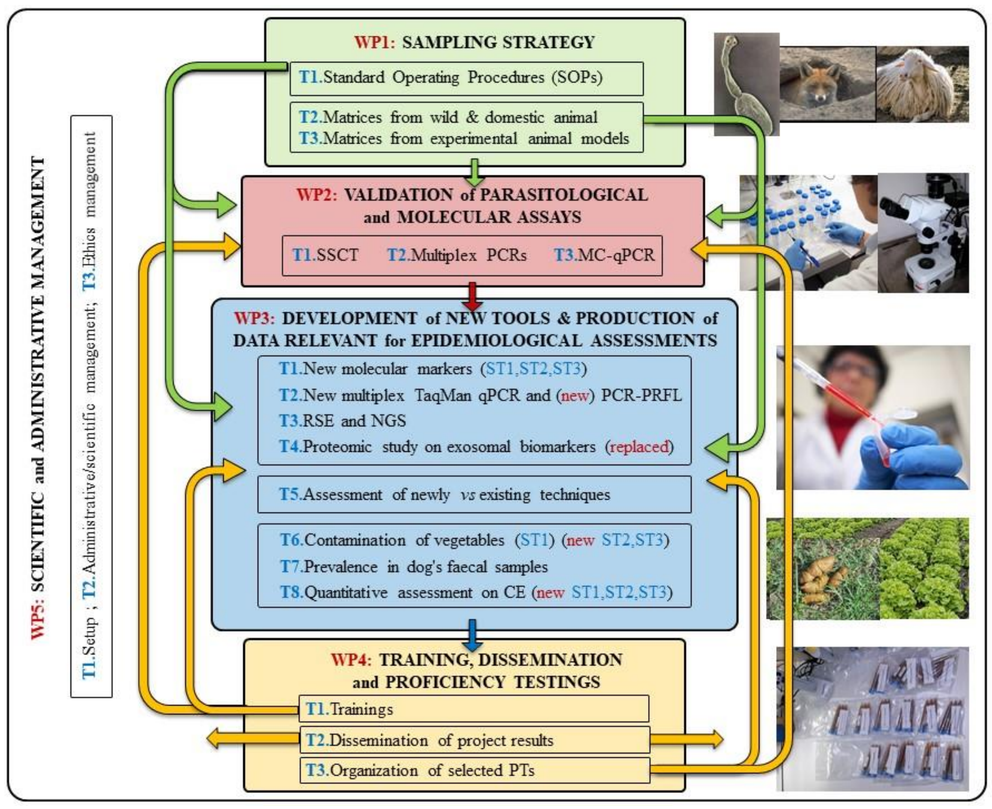
- **CVRL,** Ireland
- **IZSS,** Italy
- **UNF,** Italy
- **UH,** Germany
- **IP,** Switzerland
- **TH,** Israel
- **LRUJ,** Palestine
- **NPI,** Norway
- **SDAS,** Norway
- **COMSATS,** Pakistan

MEME in numbers:

- Composition: 22 partners
- Timing: January 2020 - December 2022
- Funding: 2.496.126 € (50% co-funded)
- Papers on peer-review journals: 17



Countries (in blue) and centres (red star= funded partners; yellow star= external partners) participating in the MEME project.



Work Packages (WPs) and tasks (T) encompassing the MEME project (https://onehealthejp.eu/jrp-meme/).

This poster is part of the European Joint Programme One Health EJP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

The OHEJP PARADISE (PARAsite Detection ISolation and Evaluation) project

Simone M Cacciò (ISS, Italy), Karin Troell (SVA, Sweden), Christian Klotz (RKI, Germany), Yannick Blanchard (ANSES, France), Marco Lalle (ISS, Italy) and the PARADISE Consortium

BACKGROUND

The PARADISE project aimed at delivering informative genotyping schemes and innovative detection strategies applicable to analysing food matrices for both *Cryptosporidium* and *Giardia*, which are important pathogens of humans, livestock, companion animals, and wildlife, with a global distribution.

NEEDS

- Better knowledge of parasite diversity (WP2)
- New high-throughput detection methods (WP2)
- Multi-locus typing schemes with high discriminatory power (WP3)
- New sensitive methods for parasite detection and enrichment (WP4)

ACTIVITIES

WP2

Whole genome study of isolates of *C. parvum* and *G. duodenalis*

Experimental and *in silico* analyses of metagenomes for detection of food-borne parasites

WP3

Development of typing schemes for *C. parvum*

Development of typing schemes for *G. duodenalis*

WP4

Method development for pre-DNA extraction enrichment using nanobodies and aptamers

Method development for post-DNA extraction enrichment using capture probes

OUTCOMES

Generation of hundreds of Whole Genome Sequences (WGS) from isolates of human and animal origin collected across Europe

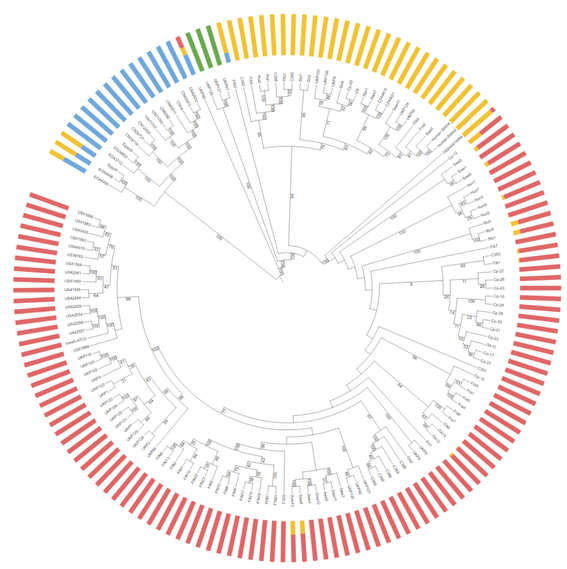
New, robust Multi-Locus Genotyping schemes for *Cryptosporidium parvum* and *Giardia duodenalis* validated and tested (SOP)

New, sensitive hybridization capture probe method for *Cryptosporidium parvum* detection validated and tested (SOP)

New reagents (nanobodies) for detection and capture of *Giardia duodenalis* cysts

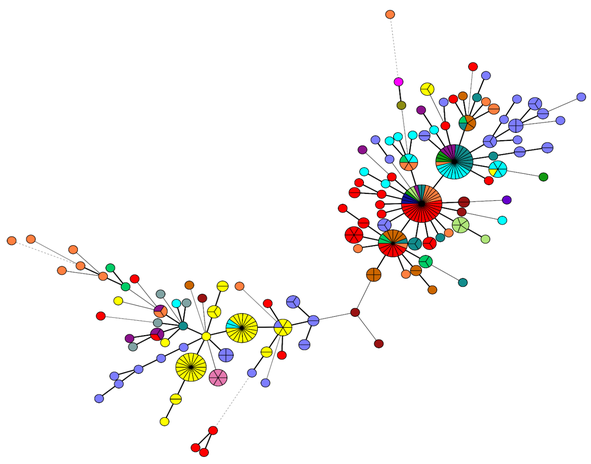
IMPACT

WP2 : Improved knowledge of the population structure, virulence and evolution of *Cryptosporidium* and *Giardia*.
Perspectives opened for functional biology studies



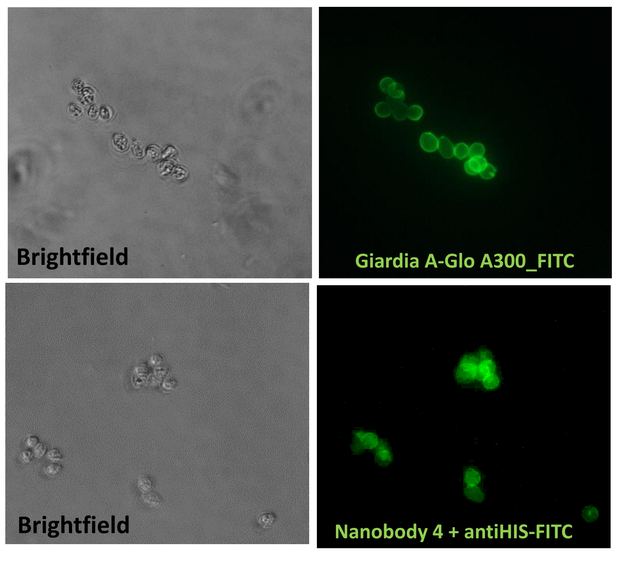
Phylogenetic tree based on genomic SNPs of *Cryptosporidium parvum* showing the presence of four clusters, three of which are comprised of European isolates.

WP3 : Improved characterization of zoonotic transmission and outbreak investigation
Implementation by National Reference Laboratories



Miminum Spanning Tree showing the relationship among the *Cryptosporidium parvum* Multi-Locus Genotypes identified in Europe

WP4 : Improved detectability in environmental and relevant food matrices for better risk assessment, and targeted analysis of parasites in outbreaks of unknown aetiology.
Implementation by National Reference Laboratories



Comparative immunostaining of *Giardia duodenalis* cysts WBC6 (Ass. A), obtained *in vitro*, with either the commercial antibody *Giardia* A-Glo A300_FITC or the nanobody 4

Contact: Simone M. Cacciò simone.caccio@iss.it https://onehealthejp.eu/jrp-paradise/

This poster is part of the European Joint Programme One Health EJP.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.