Thematic report on environmental aspects addressed in One Health EJP activities

Workpackage 5

Science to Policy Translation

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Contributing partners:
PMT members, JRP and JIP project leaders, PhD candidates and supervisors

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THEMATIC REPORT ON ENVIRONMENTAL ASPECTS ADDRESSED IN ONE HEALTH EJP ACTIVITIES

Introduction

Environmental health, in particular ecosystem health, is part of the One Health approach, and environment is part of the classical human-animal-environment triad [1].

The importance of considering the environment when aiming at improving the health and well-being of citizens is highlighted in several EU policies, for example the Green Deal policy frameworks Farm to Fork Strategy [2] and Biodiversity Strategy [3], and the EU Strategic Approach to Pharmaceuticals in the Environment [4]. Recent EU initiatives to prevent and respond to future pandemics acknowledge the role that environmental degradation plays in zoonotic outbreaks (e.g. EU4Health Programme 2021-2027 [5], European Health Union [6]).

The definition of 'environment' in One Health is not fixed. According to the Cambridge Dictionary environment is "the air, water, and land in or on which people, animals, and plants live". Other definition exist, for example the CODEX ALIMENTARIUS AMR Task Force's, defining "Food production environment: The immediate vicinity of the food chain where there is relevant evidence that it could contribute to foodborne AMR". According to the Glossary of the European Environment Agency, environment "includes the built environment, the natural environment and all natural resources, including air, land and water".
For the sake of this report, and considering the remit of the One Health EJP, we adopted the definition as used by the European Environment Agency, and include waterways (both natural and man-made, including wastewater), and wildlife.

The Thematic report on environmental aspects addressed in One Health EJP activities

The main focus of the One Health European Joint Programme (One Health EJP) is to reinforce international collaboration between institutes, enhancing transdisciplinary cooperation and integration of activities in the domains of Foodborne Zoonoses (FBZ), Antimicrobial Resistance (AMR), and Emerging Threats (ET). Public health, animal health, and food safety institutes across Europe collaborate in One Health EJP projects and, in line with the One Health paradigm, produce outcomes that are harmonised across countries and sectors. Some projects deal specifically with integration of approaches (the Joint Integrative Projects, JIPs).

Although the environment is not formally a focus of research conducted in the One Health EJP, as a pillar of the One Health triad, it is often touched upon by research projects to a various degree.

Work Package 5 of the One Health EJP focuses on science to policy translation. In this Thematic Report we provide an overview of the key outcomes produced by the One Health EJP projects on environmental aspects of One Health, by end of April 2021.

Projects presented as examples were selected by scanning the 2020 annual reports or the final reports of all One Health EJP projects for the keywords environment*, wild*, and water*. After excluding non-relevant words, frequency of keywords was calculated, and projects with frequency >0.15% (n keywords/N total words) were included in the report, and ordered based on keyword frequency. Overall, 24 out of the 30 One Health EJP projects mentioned a keyword in their report at least once (3/5 JIPs, 8/8 projects in the domain of AMR, 8/10 of FBZ, 5/6 of ET). 13 out of 17 PhD projects also mentioned a keyword at least once.

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Examples of One Health EJP projects addressing environmental aspects

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

Project leader: Werner Ruppitsch
Start: January 2020, ongoing. Domain: AMR

Over the course of this project, the relevance of horizontal antimicrobial resistance gene (ARG) transfer on free extracellular DNA (exDNA) over ecosystem boundaries relative to bacterial conjugation is evaluated. ExDNA is widely present in the natural environment and sufficiently stable to constitute an important reservoir for ARGs. The dissemination of antimicrobial resistance (AMR) on exDNA will be monitored under controlled but naturally occurring environmental conditions in an open-air agricultural research area. Samples are collected in different regions across the EU covering relevant compartments listed below.

Results on environmental aspects so far:

- Partners have been monitoring quantity and movement of AMR via free exDNA along different compartments of the food/feed chain: "human/animal gut -> manure -> soil -> crop -> drainage -> surface water -> groundwater -> human/animal".
- Ongoing identification of selection pressure in the tested environmental compartments (including soil and water), samples are analysed for traces of antimicrobials, herbicides and heavy metals. Preliminary results are presented in the Annual Scientific Meeting (June 2021).
- Probabilistic and mechanistic models of the links between antimicrobial usage in animals, AMR in the environment, and the risks for public health are in preparation.

More information on FED-AMR here

MedVetKlebs - *Klebsiella pneumoniae*: from ecology to source attribution and transmission control

Project leader: Sylvain Brisse
Start: January 2018, end: December 2020. Domain: FBZ

MedVetKlebs defined the ecology of *Klebsiella pneumoniae* and the sources of infections of humans and animals in order to investigate transmission routes and to find an optimal way to control them.

Results on environmental aspects:

- Protocols were developed for *Klebsiella* isolation from various sources (including water, soil) and for molecular detection through a novel highly sensitive qPCR method, the ZKIR assay, which detects *K. pneumoniae* and its closely related species. Novel biomarkers and laboratory identification tools (based on mass spectrometry, e.g. here and here) were also defined.
- Presence of *K. pneumoniae* and of its virulence and antimicrobial resistance genes were investigated in a broad variety of human, animal and environmental, samples, such as river waters (e.g. here), thus defining major environmental niches that act as reservoirs of *K. pneumoniae*.
- A mathematical model was developed to simulate the diversification of a bacterial lineage that contaminates food, as a function of time and mutation rate of the bacteria. The model is also applicable to environmental bacterial species

More information about MedVetKlebs here

ListAdapt - Adaptive traits of *Listeria monocytogenes* to its diverse ecological niches

Project leader: Sophie Roussel
Start: January 2018, end: June 2021. Domain: FBZ

The ListAdapt project deciphers the molecular mechanisms of the adaptation of *Listeria monocytogenes* to its various ecological niches by comparing both genotypic and phenotypic data from a large and balanced set of strains from environment, animals, foods and clinical cases in several European countries. The project improves scientific knowledge on the ecology of the bacteria by gaining insight into the evolution and genetic make-up of strains that are successful in some environmental niches and not in others.

Results on environmental aspects:

- Strain collections, totalling almost 2000 strains from a number of matrices with high representation from the environment and wild animals (e.g. here), were assembled. After sequencing, clonal complexes of the various reservoirs were defined.
- Strains were phenotypically characterised for a number of features, e.g. antimicrobial resistance profile, biofilm formation and survival capacity in ecological niches (e.g. here).

More information about LISTADAPT here

WORLDCOM - Development of new tools for real-time detection of zoonotic bacteria and antimicrobial resistance in veterinary, human and environmental sources

Project leader: Terry Smith
Start: January 2020, ongoing. Domain: AMR

WORLDCOM aims at the development of real-time diagnostic on-site tools for the detection of zoonotic pathogens focusing on *E. coli*, *Salmonella* and *Campylobacter* together with resistance markers for identification of AMR including CTX-M-15, NDM-5, KPC-2, OXA-48 and MCR-1.

Results on environmental aspects so far:

- The project is optimising DNA extraction protocols from field samples, including water.
- Development of a sensitive singleplex isothermal amplification assays for selected AMR gene targets is under way.

More information about WORLDCOM here

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

Project leader: Jenny Frössling
Start: January 2018, ongoing. Domain: FBZ

The NOVA project strives to develop new surveillance tools and methods and to harmonise and optimise the use of existing surveillance system data. The project consists of five topics concerning the development of targeted surveillance tools, and integrative measures: syndromic surveillance; spatial risk mapping; food purchase data; mathematics and economy; and terminology, data sources and barriers.

Results on environmental aspects so far:

- NOVA is working on methods to integrate signals from different syndromic surveillance systems, including environmental data (e.g. here), to improve outbreak detection in humans.
- An explanatory multivariate syndromic surveillance system that processes multiple time series (including human and animal data, as well as environmental data like rainfall and temperature) is being developed to calculate the value of evidence for outbreak investigation.
- A machine learning algorithm was developed to evaluate the influence of environmental drivers, such as temperature, humidity, precipitation or type of soil and vegetation, on *Salmonella* contaminated farms. The model will also be applied to the identification of environmental risk factors for *Salmonella* infection in wild boar.

More information about NOVA here

IDEMBRU - Identification of emerging *Brucella* species: new threats for human and animals

Project leader: Claire Ponsart
Start: January 2020, ongoing. Domain: ET

The IDEMBRU project aims to develop a toolbox focusing on emerging *Brucella* species and reservoirs in order to ensure rapid detection, identification and characterisation. The project includes i) the detection and investigation of these pathogens from different sources under various epidemiological contexts; ii) the characterisation of the emerging *Brucella* species; iii) the understanding of virulence and zoonotic potentials; iv) the development of a toolkit for the integration of data from emerging *Brucella* and as a resource to guide characterisation of emerging *Brucella*.

Results on environmental aspects so far:

- The project is recording the situation of new *Brucella* species in wildlife and environmental samples as well as new reservoirs for classical *Brucella* species. A database of existing samples from environmental sources and wild mammalian species is being set up and additional sampling is ongoing from a number of different habitats.
- Genomic data are being collected which, adding to DNA and RNA sequencing, and phenotypic characterisation produced in IDEMBRU, will inform novel strategies to characterise *Brucella* spp.

More information about IDEMBRU here

DiSCoVeR: Discovering the sources of *Salmonella*, *Campylobacter*, VTEC and antimicrobial resistance

Project leader: Tine Hald
Start: January 2020, ongoing. Domain: FBZ

DiSCoVeR addresses the challenges of source attribution. It maps the existing knowledge gaps and recommend new studies and/or methods that are needed to fill them. The work includes data from a broad range of reservoirs and sources, including those that are not traditionally part of existing monitoring and surveillance activities, e.g. pets (including reptiles), wildlife, and environmental sources.

Results on environmental aspects so far:

- Partner institutes are assembling *Salmonella*, *Campylobacter,* Shiga-Toxin producing *E. coli* (STEC) and ESBL-*E. coli* strain collections with relative sequencing data from humans, animals, food and environment. Additional sampling is focusing on environmental samples.
- A dataset of *Campylobacter* sequences collected from humans, animals including pets, food and environment is currently being processed through different bioinformatics pipelines. Data will be used in a number of source attribution models to identify host-associated genetic markers.

More information on DiSCoVeR here

TOXOSOURCES: *Toxoplasma gondii* sources quantified

Projects leader: Pikka Jokelainen
Start: January 2020, ongoing. Domain: FBZ

TOXOSOURCES investigates the relative contributions of the different sources of *Toxoplasma gondii* infection. Special focus is on the environmental stage: oocysts. The main outcomes of TOXOSOURCES are quantitative estimates of the contribution of the main sources and transmission routes of *T. gondii* infection based on improved source attribution models (including also the environmental pathway), new data filling the key knowledge gap about the role of ready-to-eat fresh produce, a novel serological method to detect infections caused by oocysts, and a novel typing method to improve preparedness to detect introduction of *T. gondii* strains by import and to trace the infection sources in outbreaks.

Results on environmental aspects so far:

- Summary of available analytical procedures for detection of *T. gondii* oocyst contamination in fresh produce (here) was supported by an extensive literature review (here). SOP on detection of *T. gondii* oocyst contamination in fresh produce is being implemented in laboratories across Europe (described here and here).
- Results of literature reviews on the prevalence of *T. gondii* in animals, including wildlife (report here), and on the prevalence of *T. gondii* oocysts in fresh produce and environment are used for a quantitative microbiological risk assessment that includes also the environmental pathway, and for planning sampling for a multicentre study on fresh produce.
- The project has collected and whole-genome sequenced a high number of *T. gondii* isolates and DNAs from across Europe, including from wildlife and from feline faecal samples.

More information about TOXOSOURCES here

FARMED: Fast Antimicrobial Resistance and Mobile-Element Detection using metagenomics for animal and human on-site tests

Project leader: Manal AbuOun
Start: January 2020, ongoing. Domain: AMR

The FARMED project aims to assess the feasibility of ONT MinION long read sequence technology to rapidly characterise the metagenome and resistome of samples on-site. Initial work will compare long-read sequencing to current gold-standard short-read Illumina metagenome sequencing, to establish the practicability of long read sequencing to detect pathogens and AMR in a range of sample matrices in the laboratory and in the field. Harmonised protocols will facilitate epidemiological investigations by attributing the detected AMR genes to specific species and/or plasmids.

Results on environmental aspects so far:

- A defined microbial community with known complete genome sequences and different AMR profiles was chosen to be tested in water buffalo faeces and pond water as matrices. A panel of lab-based DNA extraction methods were compared using both short- and long-read sequencing has demonstrated application of long-read sequencing.
- Standardisation of sequencing methodologies across the consortium and optimisation data analysis bioinformatics pipelines and outputs is currently in progress.
- A literature review of commonly used DNA extraction kits, a breakdown of the processes to consider for onsite DNA extraction and Long read sequencing and summary of equipment with the potential for on-site DNA extraction and sequencing, is planned to be trialled in FARMED.

More information on FARMED here

MAD-Vir: Metagenomic Array Detection of emerging Virus in EU

Project leader: Anders Fomsgaard
Start: January 2018, end: December 2019. Domain: ET

The project optimised and validated a metagenomics microarray, the Pan-Virus-Array, to improve fast detection of viruses, including all known virus species, and identification of novel virus types or strains belonging to currently known virus families. It was shown to be able to correctly identify a vast majority of PCR-confirmed positive samples with a known viral content. The samples tested during the project (N=1210) included samples from wildlife species (e.g. here and here).

More information about MAD-Vir here

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Examples of One Health EJP PhD projects addressing environmental aspects

SUSTAIN: Scientific UnderStanding of the policy process for Transboundary integration And INstitutionalisation of the One Health across EU member States

PhD candidate: Sarah Humboldt-Dachroeden; supervisors: Olivier Rubin, Ann Lindberg
Start: August 2019, ongoing. Domain: Sustainability

SUSTAIN aims to understand the political drivers and constrains for increased transboundary integration and institutionalisation of the One Health approach across EU member states. A paper analysing the state of One Health research across disciplines and sectors, as well as a paper dealing specifically with environmental factors within the One Health approach were published (available here and here).

More information about SUSTAIN here.

EnvDis: Environment and Diseases: A general method linking Mechanism and Phenomenology

PhD candidate: Laura González Villeta; supervisor: Giovanni Lo Iacono
Start: January 2020, ongoing. Domain: FBZ

EnvDis aims to develop a tool to assess the risk of foodborne zoonosis (FBZ) based on environmental factors. In particular the goals are: to identify the key environmental processes triggering and propagating FBZ, to disentangle the role of animal, human and environmental factors in FBZ, and to identify the delay between variations in the environment and the occurrence of a foodborne outbreak.

More information on EnvDis here

DESIRE: Developing evidence-based surveillance for emerging rat-borne zoonoses in changing environments

PhD candidate: Marieke de Cock, supervisor: Miriam Maas
Start: October 2019, ongoing. Domain: ET

Knowledge of rat-borne pathogens in European cities is scarce. The aim of DESIRE is to design and test an effective surveillance system for rat-borne diseases, using The Netherlands as a testcase. It will provide evidence-based insights in four key elements of this surveillance system: monitoring of populations, monitoring of pathogens, risk-assessment, and intervention.

More information on DESIRE here

WILBR: Contribution of wild birds to AMR in the environment and on farms

PhD candidate: Olivia Turner, supervisor: Muna Anjum
Start: February 2020, ongoing. Domain: AMR

WILBR studies the potential of wild birds to dissemination of resistant microorganisms in the environment in general, and on livestock farms in particular. A literature review was undertaken on the role of wild birds in dissemination and persistence of antimicrobial resistance in the farm environment, and longitudinal studies are ongoing.

More information on WILBR here

Final comments

Environment is a relevant part of One Health. The examples above illustrate that, although not a main focus of the One Health EJP consortium, environmental aspects are indeed considered by One Health EJP research projects, pointing out the alignment of the consortium to recent EU policy initiatives [2-6].

In this report we exemplified the work of projects which, according to our criteria, have a noticeable environmental side, however environmental aspects are touched upon also by a number of projects not included in this report. In fact 24 out of the 29 One Health EJP projects and 13 out of 17 PhD projects mentioned a keyword (environment*, wild*, or water*) at least once in their 2020 annual report or final report. These research projects are: the JIPs CARE, MATRIX, ORION; the projects in the domain of FBZ AIR-SAMPLE, BIOPIGEE, DiSCoVer, LISTADAPT, MedVetKlebs, MOMIRPPC, NOVA, TOXOSOURCES; the projects in the in the domain of AMR ARDIG, FARMED, FED-AMR, FULL-FORCE, IMPART, METASTAVA, RaDAR, WORLDCOM; and the projects in the domain of ET IDEMBRU, MAD-Vir, MEmE, PARADISE, TELE-Vir. The PhD projects which mentioned a keyword at least once are: Codes4strains, DESIRE, ECO-HEN, EnvDis, HME-AMR, KENTUCKY, METAPRO, PEMbo, SUSTAIN, ToxSauQMRA, TRACE, UDoFRIC, WILBR.

Most of the work is ongoing, and updates can be found on the webpages of the projects as well as in the One Health EJP Outcome Inventory. Publications are listed on the publication page.

Upcoming further activities focusing on the environment include e.g. part of the Annual Scientific Meeting 2021, and the One Health EJP Summer School 2021 Environmental Issues in One Health: from risk assessment to surveillance.

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