# D3.11

# Second periodic report on ongoing JRPs

## WP3 Joint Research Projects

Responsible Partner: Sciensano

Contributing partners: ANSES

## GENERAL INFORMATION

| | |
|---|---|
| **European Joint Programme full title** | **Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards** |
| **European Joint Programme acronym** | **One Health EJP** |
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| **Leader** | Sciensano |
| **Other contributors** | ANSES |
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| **Dissemination**<br>*Author's suggestion to inform the following possible interested parties.* | OHEJP WP 1 ☐ OHEJP WP 2 ☐ OHEJP WP 3 ☐<br>OHEJP WP 4 ☐ OHEJP WP 5 ☐ OHEJP WP 6 ☐<br>OHEJP WP 7 ☐ Project Management Team ☒<br>Communication Team ☒ Scientific Steering Board ☒<br>National Stakeholders/Program Owners Committee ☐<br>EFSA ☐ ECDC ☐<br>Other international stakeholder(s): .....<br>Social Media: .....<br>**Other recipient(s):** ..... |

# SECOND PERIODIC REPORT ON ONGOING JRPS

# 1. INTRODUCTION

Together with the joint integrative projects (JIP) and the education and training activities, joint research projects (JRP) are tools that help OneHealth EJP partners to work together in developing new models, new detection and typing methods, better diagnostic tests and intervention techniques…

Consequently, through setting up these scientific collaborations, researchers from public institutions all over Europe identify new possible partners and strengthen links between known colleagues. As such, the JRP help to further create and consolidate the expert network of organizations that have reference tasks in foodborne zoonoses, antimicrobial resistance and emerging threats.

## SUMMARY OF THE JOINT RESEARCH PROJECTS PERFORMANCE

### Project deliverables and milestones

The 11 JRP planned to submit 178 deliverables. At the time of writing this Periodic Report, 56% of the planned deliverables have been finalized and uploaded on the OHEJP Website. There is an improvement since last year when only 32% of the submitted deliverables were uploaded. WP3 will continue to support the project leaders to ensure that all the deliverables are uploaded on time with the right metadata.

44% of the deliverables have been delayed and postponed to 2020, which can be explained since all the projects (except MAD-Vir) have been granted a 6-months extension. MoMIR-PPC has 33 delayed deliverables, but this project was granted a one-year extension in 2020. It will have enough time to produce the expected outcomes and deliverables. The Project Leaders have updated the milestones and delivery times according to the new deadline.

| Deliverables | Total | Finalized | Extended / Delayed to 2020 |
|---|---|---|---|
| Number of Deliverables | 178 | 100 | 78 |
| Percentage | 100% | 56% | 44% |

| Deliverables | Due | Finalized | Delayed to 2020 |
|---|---|---|---|
| *AIRSAMPLE* | 6 | 4 | 2 |
| *ARDIG* | 11 | 6 | 5 |
| *IMPART* | 21 | 14 | 7 |
| *LISTADAPT* | 22 | 10 | 12 |
| *MADVIR* | 10 | 10 | 0 |
| *MEDVETKLEBS* | 15 | 9 | 6 |
| *METASTAVA* | 8 | 7 | 1 |
| *MOMIR PPC* | 43 | 10 | 33 |

| Deliverables | Due | Finalized | Delayed to 2020 |
|---|---|---|---|
| *NOVA* | 22 | 19 | 3 |
| *RADAR* | 11 | 7 | 4 |
| *TOXDETECT* | 9 | 4 | 5 |

Regarding the projects milestones:

55% of the milestones have been achieved on time. The other milestones have been assigned a new delivery date that takes into account the projects extensions.

## Publications

**15 peer-reviewed articles have been published**

Alvarez, Julio, Gema Lopez, Petra Muellner, Cristina Frutos, Christina Ahlstrom, Tania Serrano, Miguel A. Moreno, et al. "Identifying Emerging Trends in Antimicrobial Resistance Using Salmonella Surveillance Data in Poultry in Spain." Transboundary and Emerging Diseases 67, no. 1 (January 2020): 250–62. https://doi.org/10.1111/tbed.13346.

Barbier, Elodie, Carla Rodrigues, Geraldine Depret, Virginie Passet, Laurent Gal, Pascal Piveteau, and Sylvain Brisse. "The ZKIR Assay, a Real-Time PCR Method for the Detection of Klebsiella Pneumoniae and Closely Related Species in Environmental Samples." Edited by Edward G. Dudley. Applied and Environmental Microbiology 86, no. 7 (January 31, 2020): e02711-19, /aem/86/7/AEM.02711-19.atom. https://doi.org/10.1128/AEM.02711-19.

Brouwer, Michael S.M., Stephanie D. Jurburg, Frank Harders, Arie Kant, Dik J. Mevius, Adam P. Roberts, and Alex Bossers. "The Shufflon of IncI1 Plasmids Is Rearranged Constantly during Different Growth Conditions." Plasmid 102 (March 2019): 51–55. https://doi.org/10.1016/j.plasmid.2019.03.003.

Johannessen, Gro S., Giuliano Garofolo, Gabriella Di Serafino, Ivana Koláčková, Renáta Karpíšková, Kinga Wieczorek, Jacek Osek, Julia Christensen, Mona Torp, and Jeffrey Hoorfar. "Campylobacter in Chicken – Critical Parameters for International, Multicentre Evaluation of Air Sampling and Detection Methods." Food Microbiology 90 (September 2020): 103455. https://doi.org/10.1016/j.fm.2020.103455.

Martínez-Avilés, Marta, Macarena Garrido-Estepa, Julio Álvarez, and Ana de la Torre. "Salmonella Surveillance Systems in Swine and Humans in Spain: A Review." Veterinary Sciences 6, no. 1 (February 20, 2019): 20. https://doi.org/10.3390/vetsci6010020

Merla, Cristina, Carla Rodrigues, Virginie Passet, Marta Corbella, Harry A. Thorpe, Teemu V. S. Kallonen, Zhiyong Zong, et al. "Description of Klebsiella Spallanzanii Sp. Nov. and of Klebsiella Pasteurii Sp. Nov." Frontiers in Microbiology 10 (October 25, 2019): 2360. https://doi.org/10.3389/fmicb.2019.02360.

Møller, Frederik T, Kåre Mølbak, and Steen Ethelberg. "Analysis of Consumer Food Purchase Data Used for Outbreak Investigations, a Review." Eurosurveillance 23, no. 24 (June 14, 2018). https://doi.org/10.2807/1560-7917.ES.2018.23.24.1700503.

Mughini-Gras, Lapo, Alejandro Dorado-García, Engeline van Duijkeren, Gerrita van den Bunt, Cindy M Dierikx, Marc J M Bonten, Martin C J Bootsma, et al. "Attributable Sources of Community-Acquired Carriage of Escherichia Coli Containing β-Lactam Antibiotic Resistance Genes: A Population-Based Modelling Study." The Lancet Planetary Health 3, no. 8 (August 2019): e357–69. https://doi.org/10.1016/S2542-5196(19)30130-5.

Patiño-Navarrete, Rafael, Isabelle Rosinski-Chupin, Nicolas Cabanel, Lauraine Gauthier, Julie Takissian, Jean-Yves Madec, Monzer Hamze, Remy A. Bonnin, Thierry Naas, and Philippe Glaser. "Stepwise Evolution and Convergent Recombination Underlie the Global Dissemination of Carbapenemase-Producing Escherichia Coli." Genome Medicine 12, no. 1 (December 2020): 10. https://doi.org/10.1186/s13073-019-0699-6.

Petersen, Andreas, Maiken Worsøe Rosenstierne, Morten Rasmussen, Kurt Fuursted, Henrik Vedel Nielsen, Lee O'Brien Andersen, René Bødker, and Anders Fomsgaard. "Field Samplings of Ixodes Ricinus Ticks from a Tick-Borne Encephalitis Virus Micro-Focus in Northern Zealand, Denmark." Ticks and Tick-Borne Diseases 10, no. 5 (August 2019): 1028–32. https://doi.org/10.1016/j.ttbdis.2019.05.005.

Rebollada-Merino, Agustín, Carmen Bárcena, María Ugarte-Ruiz, Néstor Porras, Francisco J. Mayoral-Alegre, Irene Tomé-Sánchez, Lucas Domínguez, and Antonio Rodríguez-Bertos. "Effects on Intestinal Mucosal Morphology, Productive Parameters and Microbiota Composition after Supplementation with Fermented Defatted Alperujo (FDA) in Laying Hens." Antibiotics 8, no. 4 (November 9, 2019): 215. https://doi.org/10.3390/antibiotics8040215.

Rodrigues, Carla, Virginie Passet, Andriniaina Rakotondrasoa, and Sylvain Brisse. "Identification of Klebsiella Pneumoniae, Klebsiella Quasipneumoniae, Klebsiella Variicola and Related Phylogroups by MALDI-TOF Mass Spectrometry." Frontiers in Microbiology 9 (December 7, 2018): 3000. https://doi.org/10.3389/fmicb.2018.03000.

Rodrigues, Carla, Virginie Passet, Andriniaina Rakotondrasoa, Thierno Abdoulaye Diallo, Alexis Criscuolo, and Sylvain Brisse. "Description of Klebsiella Africanensis Sp. Nov., Klebsiella Variicola Subsp. Tropicalensis Subsp. Nov. and Klebsiella Variicola Subsp. Variicola Subsp. Nov." Research in Microbiology 170, no. 3 (April 2019): 165–70. https://doi.org/10.1016/j.resmic.2019.02.003.

Wieczorek, Kinga, Tomasz Wołkowicz, and Jacek Osek. "MLST-Based Genetic Relatedness of Campylobacter Jejuni Isolated from Chickens and Humans in Poland." Edited by Patrick Jon Biggs. PLOS ONE 15, no. 1 (January 24, 2020): e0226238. https://doi.org/10.1371/journal.pone.0226238.

Wisgrill, Lukas, Sarah Lepuschitz, Marion Blaschitz, Judith Rittenschober-Böhm, Magda Diab-El Schahawi, Sören Schubert, Alexander Indra, and Angelika Berger. "Outbreak of Yersiniabactin-Producing Klebsiella Pneumoniae in a Neonatal Intensive Care Unit:" The Pediatric Infectious Disease Journal 38, no. 6 (June 2019): 638–42. https://doi.org/10.1097/INF.0000000000002258.

**Several manuscripts have been submitted or are in the review** process

The paper entitled "Analysis of COMPASS, a new comprehensive plasmid database revealed prevalence of multireplicon and extensive diversity of IncF plasmids "is under review in the journal Frontiers in Microbiology. The scripts and database of COMPASS, developed in the present study can be found in the following GitHub repository: https://github.com/itsmeludo/COMPASS.

The metagenomics Panvirus microarray as a diagnostic tool for unknown emerging virus in a one-health perspective (working title) Manuscript in preparation.

A Multi-Scale Epidemic Model of Salmonella infection with Heterogeneous Shedding. To appear in ESAIM Proceedings (open access). A first draft was accessible on HAL repository https://hal.archives-ouvertes.fr/hal-02043742

## Impact and relevance of the research projects

Although the principal objective of a research project is the delivery of high quality scientific outcomes, most if not all JRP also cover integrative activities, i.e. capacity building (including expanding expertise through ring trials and developing methodologies that are useful for risk analysis), experimental facilities, detection and typing methods, strains and biobanks collections, reference materials, digital infrastructures and databases, surveillance strategies and legal or policy aspects. Such collaborations help in strengthening the network and building trust among partners, which is of utmost importance for setting up cross-border and cross-sector surveillance and control systems, and in case of outbreak management.

In the 2019 12-month reports, project leaders mentioned many elements to demonstrate the possible impact of their research projects. All project leaders consider that their scientific contributions to the domains of foodborne zoonoses, AMR or emerging threats are valuable, for instance through the development of techniques for MIC performance (IMPART), modelling techniques (NOVA, RaDAR), or genetic (LIST-ADAPT, MAD-Vir, MedVetKlebs, METASTAVA, MoMIR-PPC) and microbiological (AIR-Sample, MedVetKlebs, TOX-DETECT) screening, detection and characterization methods, and by newly developed methods for use in surveillance (METASTAVA, NOVA).

Also the inter-sector, Med-Vet collaboration which develops, validates and tests new methodologies, will probably extend beyond the lifespan of the project. It is a great achievement of the One Health EJP projects, e.g. ARDIG, IMPART, List-Adapt, MAD-Vir, MedVetKlebs, METASTAVA, NOVA.

Similarly, strains collections that are useful for both animal and public health are being created in IMPART, LIST-ADAPT, MedVetKlebs, MoMIR-PPC, and TOX-DETECT.

Some projects describe a direct link with EFSA or ECDC recommendations, EU-Reference Laboratories or even with EU legislation: IMPART, LIST-ADAPT, ListAdapt, MoMIR-PPC and RaDAR.

It is clear that these activities are very relevant for the final objective of the One Health EJP: to create and consolidate a network of public institutes that will become better prepared through improved surveillance and laboratory techniques.

Interactions with other One Health EJP and EU projects, and with national and international networks

Most of the Joint Research Projects reach out to and link with other national and international organisations and networks. Particularly valuable are their interactions with EFSA and ECDC (IMPART, TOX-Detect, ListAdapt and AIR-Sample), other One Health EJP projects (ARDIG with IMPART, FULL-FORCE, WORLDCOM and FARMED; NOVA with ORION, ListAdapt with CARE, METASTAVA with Tele-Vir) and with large EU projects (IMPART with VetCAST and COST ENOVAT, ARDIG with AVANT and JPIAMR, NOVA with COST ASF-STOP, METASTAVA with COMPARE and EFFORT).

The following table gives the details of the connections and cooperations that were described in the 12M-reports.

| Project | Interactions with JRP&JIP and others |
|---|---|
| *JRP01 IMPART* | EU Reference Laboratory for AMR<br>EFSA<br>VetCAST<br>COST ENOVAT<br>AMR surevillance in MS |
| *JRP02 ARDIG* | IMPART, FULL-FORCE, WorldCOM, FARMED, PhD<br>AVANT (UCM)<br>JPIAMR (APHA) |

| Project | Interactions with JRP&JIP and others |
|---|---|
| *JRP05 TOXDETECT* | Link with EREN, EFSA |
| *JRP06 NOVA* | Interaction with various national projects<br>International with COST ASF-STOP<br>OHEJP: ORION |
| *JRP07 ListAdapt* | Results will feed into JIP CARE<br>Use of WGS as proposed by EFSA and ECDC |
| *JRP08 METASTAVA* | COMPARE and EFFORT<br>Led to set up of new JRP: Tele-VIR. |
| *JRP09 AIR-Sample* | Contacts with ISO are established, and also EAAP and FoQQSI<br>Contacts with EFSA, ECDC,WHO and FAO are planned |
| *JRP11 MedVetKlebs* | KlebNET, SpARK, KLEB-GAP and NOR-KLEB-NET |

It is clear that Project Leaders are aware of the role their One Health EJP project can have in the broader spectrum of national and international ongoing and planned projects that deal with foodborne zoonoses, AMR and emerging threats.

## Critical risks

In four joint research projects no risks have been reported (AIR-Sample, ARDIG, MAD-Vir and MedVetKlebs). MAD-Vir is the only project that came to an end in December 2019, after 2 years.

However, a number of project leaders experienced or warned of delays in their work plan execution or reporting associated with loss of key-persons or with potential exit of partners: IMPART, METASTAVA, MoMIR-PPC, RADAR, TOX-Detect and, to a lesser extent NOVA and ListAdapt. The One Health EJP WP3 Team has been consulted and actions have been taken to replace task leaders or to adapt the proposal in such a way that the work plan will be finalised, albeit through extending the original lifespan of the project (without additional budget). In the case of METASTAVA clarification of the role and financing of Erasmus Medical Centre as part of NCOH in The Netherlands caused more time than expected.

## 2. REPORTS OF THE JRP IN THE FIRST ROUND OF PROJECT

### JRP01 - IMPART

#### 1. Summary of the work carried out in year 2 (January to December 2019)

A physical mid-term meeting was held on the 24th of May 2019 in parallel with the One Health EJP annual meeting in Dublin where the progress and plans of the different work packages (WP) were presented and discussed by the different WP leaders. During this meeting, partner institutes of the IMPART consortium were represented by at least one person (physical presence or via Skype).

The design of the final ring trials (including the number of samples, the culture media and the incubation conditions) of WP1 and WP2 were based on the outcomes of the pre-ring trials. Due to a long and careful evaluation of the pre-ring trial results and the need to avoid bank holidays during the final ring trial between the 9 participating countries, the final ring trial had to be rescheduled. For WP1, the samples for the final ring trial were prepared at Anses-Fougères , sent around on 17 June 2019, and received by all partners on 18 June 2019. The samples for WP2 were prepared at Anses-Fougères , sent around on September 9th 2019, and received by all partners on September 10th 2019. The analysis and final evaluation of the WP2 ring trial is postponed to February of 2020.

For WP3, the delivery of the Sensititre plates was seriously delayed. Consequently, the distribution of the Sensititre plates (consisting of three different panels) to the partners took place in March 2019 including the necessary software for partners using a Sensivision reading device. The minimum inhibitory concentration (MIC) testing of bacterial isolates started in May 2019. The list of bacteria to be tested was discussed during the physical kick-off meeting in Dublin and this list was shortened and finalised in June 2019. The majority of the MIC testing was performed by the participants from May to December 2019. The first aggregated data have been sent to the WP3 leader, but some partners have to do some additional testing in January 2020. The data analysis in order to set the ECOFFs is postponed to the first half of 2020.

In WP4, a first draft protocol for disk diffusion was developed based on available descriptions and literature. For optimization and standardization, ten *Clostridium difficile* strains were selected and tested under different conditions. The strain collection was completed and all isolates were confirmed as *C. difficile*, typed and their MICs determined. The ring trial will be organized after the completion of a method recommendation for the participating partners. Inhibition zone diameter distributions and proposed cut-off values for *C. difficile* will be determined in Q1 2020.

Regarding the communication within IMPART a kick-off meeting was held at Schiphol for all consortium members in February 2018 and a mid-term meeting in May 2019. The final meeting will be held in spring 2020. The WP leaders send out emails to all consortium members containing general information on the progress of the different WP's. Furthermore, all WP leaders were in contact via Skype every two weeks discussing the organization of IMPART and the progress of the different WPs. Furthermore, IMPART activities were presented on both Cogwheel meetings organised in 2018 with EFFORT and COMPARE. IMPART will keep on looking for synergies with other research projects in order to avoid duplicate research.

## 2. Wok carried out in the JRP, scientific results

### WP1: Selective isolation, detection and characterization of colistin-resistant Enterobacteriaceae

***JRP1-WP1-T1**: Describe existing methods to be evaluated in a ring trial (M1-M6)*
Task started in July 2018 and was finalized in December 2018, see annual report 2018.

***JRP1-WP1-T2**: Preparation of the samples for the pre-ring trial (WP1 and WP2, M7-M8)*
Task started in July 2018 and was finalized in November 2018, see annual report 2018.

***JRP1-WP1-T3**: Performance of the pre-ring trial and evaluation (M10-M11)*
Performance of the pre-ring trial was evaluated all through the first semester 2019. An alternative protocol has been established to improve the detection of positive strains prior to the final ring-trial. The final report of the WP1 pre-ring trial was posted on the IMPART group private group on 25 November 2019.

***JRP1-WP1-T4**: Preparation of samples for the final ring trial (WP1 and WP2, M12-M17)*
The protocol for the final ring trial, along with a technical questionnaire, were sent out to the 11 participants at draft stage for consultation on 2 May 2019. The final version of the protocol and the result sheet was distributed to the participants by email on 3 June 2019. WP1 ring trial samples were prepared at Anses-Fougères laboratory the week prior to the final ring trial (June 10-14, 2019).

***JRP1-WP1-T5**: Performing the final ring trial (M17)*
The 11 participants received their samples on Tuesday 18 June 2019. Ten participants started immediately the analysis, while one was advised to store the samples for a week at -80°C prior to analysis due to bank holiday.

***JRP1-WP1-T6**: Analysis of the results and reporting (M18-M19)*
The results were sent back from the 11 participants to the WP leader from June to September 2019. Analysis of the WP1 ring trial results started just after organising the WP2 ring trial (September 2019). While we are writing this 12M report, drafting the final report for the IMPART group is ongoing.

***JRP1-WP1-T7**: Publication in peer-reviewed journal (M20-M24)*
Task planned M25-M30.

***JRP1-WP1-T8**: Plan joint implementation (M20-M24)*
Task planned M25-M30.

### WP2: Selective isolation, detection and characterization of carbapenemase-producing Enterobacteriaceae

***JRP1-WP2-T1**: Describe existing methods to be evaluated in a ring trial (M1-M6)*
Task was finalized in December 2018, see annual report 2018.

***JRP1-WP2-T2**: Preparation of the samples for the pre-ring trial (WP2, M7-M8)*
Task was finalized in November 2018, see annual report 2018.

***JRP1-WP2-T3**: Performance of the pre-ring trial and evaluation (M10-M11)*
The samples were prepared at Anses-Fougères laboratory and sent to the three participating laboratories (RIVM, WBVR, NVI) on Tuesday November 20th 2018.

The pre-ring trial focused on testing several conditions to detect carbapenemase-producing (CP) *Enterobacteriaceae* to narrow down different possibilities to test in the final ring trial:

Test all selective agar plates available in all European countries both in-house and ready-to-use.

Test two different temperatures for incubation of the selective agar plates:

Recommended by the different manufacturers: 35±2 °C or 37 °C.

An elevated temperature to try to eliminate unspecific growth: 44 °C.

Preferably, test DNA extracts from the overnight pre-enrichment broth using a direct PCR protocol.

The performance of the pre-ring trial was evaluated through the first months of 2019 and the final report of the WP2 pre-ring trial was posted on the IMPART group private group in July 2019.

***JRP1-WP2-T4**: Preparation of samples for the final ring trial (WP1 and WP2, M12-M17))*

The samples were prepared at Anses-Fougères laboratory on Friday September 6th 2019 and shipped to the eleven participants on Monday September 9th 2019 to arrive in each lab on Tuesday September 10th. Samples were prepared using the same method as for the pre-ring trial (**JRP1-WP2-T2)**.

***JRP1-WP2-T5:** Performing the final ring trial (M17)*

The final ring trial was completed in September 2019 and eleven laboratories in nine countries participated.

The goals for the final ring trial were:

- To test different brands of selective agars for the detection of carbapenemase-producing *Enterobacteriaceae.*
- To isolate the bacterial strains on selective media.
- To confirm both phenotypic and genotypic resistance to carbapenems.
- To screen the samples (overnight broth) direct by PCR (voluntary).

All laboratories have sent their report to the WP leader and the evaluation has started and will be finalized in January 2020.

***JRP1-WP2-T6**: Analysis of the results and reporting (M18-M19)*

This task has been postponed to year 3 (M26) because of the delay in performing the final ring trial.

***JRP1-WP2-T7**: Publication in peer-reviewed journal (M18-M24)*

The task is postponed to M30, June 2020.

***JRP1-WP2-T8** Plan joint implementation (M20-M24)*

Task planned M25-30.

## *WP3. Establishing epidemiological cut-off values (ECOFFs)*

***JRP1-WP3-T1**: Inventory, prioritizing and inclusion criteria (M1-M3)*

This task was finalized in July 2018 but refined in May 2019. Soon after the physical meeting, an Excel sheet was prepared with a list of bacterial species to be tested. After consultation of the partners by email, it was boiled down to a short list consisting of 17 different bacterial species belonging to either staphylococci, streptococci, Enterobacteriaceae, Pasteurellae or Pseudomonas spp.. Based on this short list, a new Excel sheet was sent around by email to all partners on 20 July 2019 in which each partner was asked to fill in exactly which bacterial species they planned to test (or already had tested)

including the number of isolates per panel. In this way, an overview was generated of the total number of isolates per bacterial species tested per panel per partner. For most bacterial species selected, at least 5 partners and > 100 strains were to be tested which should be sufficient for setting ECOFFs for these combinations according to EUCAST guidelines.

***JRP1-WP3-T2:*** *Production of MIC data (M4-M18)*

The first two panels (NLD1GNS and NLD1MAC) were distributed to the partners in February 2019 and the third panel (NLD1GPS) in March 2019, because of an extra delay in the production process. As a consequence, partners performed MIC testing between May and December 2019. One partner mentioned they expected to finish the work in January of 2020 (Q1 of Y3).

***JRP1-WP3-T3****: Collection and quality control of MIC data (M4-M18)*

Partners were asked to send their aggregated MIC results (MIC distributions) to the WP leader as standard formats per panel before 1 December 2019. On December 18th 2019 the WP leader had received MIC distributions from most partners (BfR, UU, Anses, APHA, IZLST), but the total collection of the MIC data was not yet completed. Therefore, the quality control is postponed to Q1 of Y3.

***JRP1-WP3-T4****: Analysis of the data and publication of ECOFFs (M13-M24)*

The analysis of the data has been postponed to Q1 and Q2 of Y3. After finishing the analysis all available ECOFFs will be published on the EUCAST website.

## *WP4: Developing and optimizing a disk diffusion method for antimicrobial susceptibility testing of Clostridium difficile*

***JRP1-WP4-T1****: Establishment of a disk diffusion method for antimicrobial susceptibility testing of C. difficile (M4-M9)*

To establish a robust protocol for disk diffusion testing of *C. difficile*, we reviewed recent literature regarding this topic and identified critical parameters for reliability and repeatability of inhibition zone diameters (IZD) and growth of *C. difficile*. A first draft protocol was developed that is based on the EUCAST disk diffusion method (v 6.0) and includes recommendations from the literature. For optimization and standardization experiments, ten *C. difficile* strains were selected from the strain collection (see WP4-T2) based on different resistance properties. Optimization experiments included the comparison of different media for inoculum preparation (BHI, TPGY, Brucella broth), different turbidity steps (McFarland 0.5 – 4.0) and different solid media (Brucella blood agar, Wilkins-Chalgren-agar, Columbia blood agar) for the disk diffusion itself. Furthermore, different procedures and conditions of anaerobic incubation and pre-treatment were analysed, to be able to propose guidelines later. While the different inoculum and solid media had no significant effect on IZD, the turbidity has to be amended from EUCAST recommendations to reach confluent growth for most of the strains. The biggest variance resulted from different anaerobic conditions and indicates that this factor is the most critical for standardization. The setup which resulted in reliable results and is applicable by most microbiological laboratories, was repeated several times to determine standard deviations and repeatability. Furthermore, the interlaboratory reproducibility will be tested in WP4-T3.

Due to problems in recruiting the applied technician and problems in delivery of a gas mixture for working in an anaerobic workstation as well as the availability of culture media, this task including milestone M-JRP1-4 (M8) has been delayed.

***JRP1-WP4-T2****: Assembly and characterization of C. difficile strain collection (M4-M9)*

Potential partners in IMPART that can contribute *C. difficile* strains from different origins were identified and asked to submit their isolates until end of June 2018. As several partners had to arrange the exchange with third parties, it took until December 2018 to receive all strains at the BfR. So far, we received 497 isolates from SVA, SLU, RIVM, NVWA, INSA, SSI, FLI, FU Berlin and the German NRC for *C.*

*difficile*. Some of the announced strains were not cultivable or showed up not to be *C. difficile*, so that the total strain number remained below 500. Nevertheless, the milestone M-JRP1-5 was fulfilled in M12 (see annual report 2018).

If no corresponding data were available, the isolates were confirmed as *C. difficile* using MALDI-ToF, PCR-ribotype and the toxin genes determined. Furthermore, the MIC was determined for all isolates using the agar dilution method as described by CLSI (M11-A8) for the following antimicrobials: cefotaxime*, clindamycin, imipenem, metronidazole, moxifloxacin, rifampicin, tetracycline and vancomycin. We found that most of the isolates were resistant (according to EUCAST cut-offs) to cefotaxime, the corresponding MICs are in a very narrow range and therefore not suitable for a comparison with IZD from the disk diffusion. Finally, we decided in agreement with the collaboration partners in WP4 to replace cefotaxime by clarithromycin for further testing and especially with regard to the disk diffusion testing.

As several isolates could be incorporated in the strain collection only with delay and because of adaptation in the antimicrobial test panel, we were not able to finish the characterization during the first project year, but have now completed this task by the end of the second year.

***JRP1-WP4-T3**: Performance of a ring trial study (M9-M12)*

The task is postponed to M25-26, January-February 2020.

***JRP1-WP4-T4**: Producing inhibition zone diameter distributions and proposing cut-off values for C. difficile (M11-M22)*

The task is postponed to Q1 2020.

## *WP5: Coordination of the four work packages and knowledge dissemination both internally within and externally beyond the IMPART consortium*

***JRP1-WP5-T1**: Organization of IMPART (M1-M24)*

IMPART consists of five different WP's supervised by WP leaders. The first four WP's have defined scientific goals whereas WP5 is intended for the communication and dissemination of knowledge.

***JRP1-WP5-T2**: Communication within IMPART (M1-M24)*

During the second year, a physical meeting was held for all consortium members at the ASM EJP meeting in Dublin. In addition, the WP leaders sent out emails to all consortium members containing general information on the progress of the different WP's. Furthermore, all WP leaders were in contact via Skype every two weeks discussing the organization of IMPART and the progress of the different WPs. In addition, extra Skype meetings were organized to discuss the planning and results of the ring trials of WP1 and WP2 with all people involved.

***JRP1-WP5-T3**: Communication beyond IMPART (M1-M24)*

Progress of IMPART research has been presented through posters at the ASM EJP meeting in Dublin in May 2019. The preliminary results of IMPART was also presented during the annual workshop of the EURL for AMR, which gathers the European NRL for AMR on a yearly basis. The 3 presentations are available here: https://www.eurl-ar.eu/presentations/workshop-kgs-lyngby-april-2019.aspx Most of the communication through congress and scientific article will be held in 2020 (M25-M30).

## 3. Progress of the research project: milestones and deliverables

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| IMPART | D-JRP1-1.3 | Evaluation of the pre-ring trial | 11 | 13 | - | The evaluation of the pre-ring trial has been postponed to January 2019. discussed with the pre-ring trial participants until may2019, then with all participants during physical meeting in Dublin 2019. Final report posted in November 2019 |
| IMPART | D-JRP1-2.3 | Evaluation of the pre-ring trial | 11 | 19 | - | The evaluation of the pre-ring trial uploaded on the OHEJP website |
| IMPART | D-JRP1-1.4 | Protocol for the final ring trial | 15 | 21 | - | Final protocol will be uploaded asap |
| IMPART | D-JRP1-2.4 | Protocol for the final ring trial | 15 | 21 | - | Final protocol uploaded on the OHEJP website |
| IMPART | D-JRP1-4.1 | Collection of inhibition zone diameter distributions | 15 | - | 21 | Delay due to our problems to hire a technician for the project work as well as to a 3-month delivery problem of a gas mixture, which is required for our anaerobic workstation, and, finally, the late supply of some isolates by the project partners (all problems occurred in year 1). |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| IMPART | D-JRP1-1.5 | Notifications of shipment of the samples for the final ring trial | 16 | 17 | - | Notification uploaded on OHEJP Website |
| IMPART | D-JRP1-2.5 | Notifications of shipment of the samples for the final ring trial | 16 | 17 | - | Notification uploaded on OHEJP Website |
| IMPART | D-JRP1-4.2 | Publication in an open-access peer-reviewed journal | 18 | - | 30 | Delay results from the delayed progress of Tasks 4.1-4.4 |
| IMPART | D-JRP1-5.4 | Protocols and video tutorials online on IMPART EXTRANET | 18 | 17 | - | Protocols uploaded on the OHEJP Website |
| IMPART | D-JRP1-1.6 | Evaluation of the final ring trial | 19 | - | 21 | |
| IMPART | D-JRP1-2.6 | Evaluation of the final ring trial | 19 | - | 26 | The evaluation has been postponed to M26, February 2019. |
| IMPART | D-JRP1-1.7 | Publication in an open-access peer-reviewed journal | 24 | - | 30 | Postponed until Q2 2020 |
| IMPART | D-JRP1-1.8 | Proposal(s) for epidemiological study to monitor resistance to colistin | 24 | - | 30 | Postponed until Q2 2020 |
| IMPART | D-JRP1-2.7 | Publication in an open-access peer-reviewed journal | 30 | - | 30 | Postponed until Q2 2020 |
| IMPART | D-JRP1-2.8 | Proposal(s) for epidemiological study to monitor resistance to carbapenems | 30 | - | 30 | Postponed until Q2 2020 |
| IMPART | D-JRP1-3.3 | Publication of ECOFFs on EUCAST website | 24 | - | 30 | Postponed to Q2 2020 |
| IMPART | D-JRP1-5.3 | IMPART News online on MedVet EJP website | 24 | - | 36 | Postponed to 2020 |
| IMPART | D-JRP1-5.5 | Invitation to the final meeting sent to participants | 21 | - | 27 | Postponed to Q1 2020 |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| IMPART | D-JRP1-5.6 | Final meeting notes sent to participants | 24 | - | 30 | Postponed to Q2 2020 |

## *Milestones*

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| IMPART | M-JRP1-4 | Established disk diffusion method (WP4) | 8 | Yes | 24 | Advertised technician position could not be recruited on time. Delay in gas delivery by manufacturer and availability of culture media. |
| IMPART | M-JRP1-7 | Mid-term video meeting to validate the protocol (WP1, WP2 and WP5) | 13 | Yes | 17 | A physical meeting with the participants in the IMPART consortium was held at the OH-EJP conference in Dublin in May 2019. Participants not present at the conference attended through Skype. Work so far was presented together with draft protocols for the final ring trials for WP1 and WP2. |
| IMPART | M-JRP1-8 | Performing final ring trial (WP1 and WP2) | 17 | WP1: Yes WP2: Yes | WP1: 18 WP2: 21 | Delivery date delayed due to difficulties organizing the final ring trials between all participating labs due to bank holidays. |
| IMPART | M-JRP1-9 | MIC data collection complete (WP3) | 18 | No | 24 | Delivery date postponed due to delay in delivery of the Sensititre plates. (6 months extension requested for the project, submitted 14-06-2019) |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| IMPART | M-JRP1-10 | Proposal of cut-off values based on inhibition zone diameter distributions (WP4) | 18 | No | 26 | Delivery date postponed due to delay in M-JRP1-4. |
| IMPART | M-JRP1-11 | Final meeting (notes of the meeting) | 24 | No | M29 | The project is postponed until M30 and because of this the final meeting. The time and place of the final meeting will be set in January 2020. |

## 4. Publications and patents

No publications in 2019, but two publication planned for 2020.

## 5. Impact & relevance

IMPART stimulates the improvement of methods and exchange of essential practical knowledge between veterinary and medical institutes regarding the detection of bacteria with emerging types of resistances to critically important antimicrobials.

The legislation, 'Commission Implementing Decision on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria' (2013/652/EU)', includes the obligatory monitoring of ESBL- and AmpC-producing *E. coli* and the voluntary monitoring of carbapenemase-producing *E. coli* in meat and caecal samples, according to the most recent version of the protocol of the European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR). Currently, there is no EURL protocols available (yet) for culturing of colistin resistant Enterobacteriaceae. IMPART is in close contact with the EURL-AR and the results of WP1 and WP2 can be used as input for future protocols from the EURL-AR.

Moreover, the outcomes of IMPART are specifically mentioned on page 15-16 in the recently published EFSA report: Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food, EFSA Journal 2019;17(6):5709. This report is currently used as input to update EU legislation 2013/652/EU and will come into force in 2021.

Setting epidemiological cut-off values (ECOFFs) leads to an improved international harmonization of the monitoring of antimicrobial resistance in bacterial pathogens from animals and humans and is an essential first step in the development of missing clinical breakpoints. MIC data will be uploaded by IMPART in the EUCAST database and the subsequent analysis for setting ECOFFs will be performed in close cooperation with EUCAST/VetCAST.

The results generated within WP3 will also be important input for the COST action ENOVAT: European Network for Optimization of Veterinary Antimicrobial Treatment (CA18217). This large European project started at December 1 2019. Within this project, WG2 has two main objectives. The first is to establish a database with information on veterinary pathogens stored across Europe. The second objective is to use selected pathogens from the database for refining their identification by MALDI-TOF MS, and for determining ECOFFs. The mentioned cross-link between the different organisations and research project will stimulate the synergy and thereby increase the impact of IMPART.

By establishing and validating a less laborious method for susceptibility testing for *C. difficile*, the project will also contribute to an improved and harmonized surveillance of AMR in this zoonotic pathogen. Ultimately, ECOFFs will be set for a number of relevant antimicrobials in close cooperation with EUCAST.

## 6. Interactions with other JRPs/JIPs or with external (EU or national) relevant project

Attendance and presentation of the mid-term results of IMPART by three WP leaders at the EURL-AR workshop in Lyngby (DK) held in April 2019. We will probably be invited to present at the next EURL-AR meeting in April 2020.

The project coordinator of IMPART was member of the temporary EFSA AMR working group responsible for writing the technical report (EFSA Journal 2019;17(6):5709) to update the current monitoring and reporting of AMR in livestock and food (Decision 2013/652/EU). As a result the IMPART project is mentioned in this report.

The project coordinator of IMPART is member of the steering committee of VetCAST (the veterinary subcommittee of EUCAST) and member of the national Management Committee of COST action ENOVAT and will be member of the core group of work package 2. As a result, there is direct communication regarding the outcomes of the project with different stakeholders and organisations mentioned.

Three of the four WP leaders (NVI, Anses and WBVR) are involved in the national AMR monitoring activities in their respective countries. Because of this, they are also a part of the NRL-AR network and attend the annual EURL-AR workshop in Lyngby (DK) were they are in contact with people from other NRLs and the EURL-AR to disseminate the IMPART results and thereby increasing the impact. This was especially the case for WP1 and WP2.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements (from ethical reviewers) in 2018 | Measures and actions taken at the end of 2018 | Comments Ethical advisors January 2019 | Comments Project Leader at the end of 2019 |
|---|---|---|---|
| The applicants must clarify the safety mitigation measures in place to protect the environment and staff | The PL of IMPART has asked his supervisor at WBVR if it was possible to arrange this on management level of the partners; because this remarks will account for all JRP's within EJP. | EA did not comment | It is the responsibility of all partners / institutes to make sure that the pathogen-experiments are performed under the correct circumstances (appropriate BSL level and qualified personnel, for instance). The OneHealth EJP management, WP3, the Ethics Advisors and Project Leader can only stress the need for this legal obligation. |

## 8. List of critical risks

| Description of risk | Yes/No |
|---|---|
| Loss of key-persons (staff and / or leaders) | Yes |
| Delay in work plan execution | Yes |
| Conflicts within the consortium | No |
| Lack of commitment of partners | No |
| Delay in duties, tasks or reporting | Yes |
| Poor intra-project (JRP) relationship | No |
| Potential entry/exit of partners | No |
| Other risks (please describe) | No |

## 9. List of dissemination and communication activities

| | | | |
|---|---|---|---|
| Name of the activity: | Two posters at OHEJP Annual Scientific meeting | | |
| Date: | 22-24 May 2019 | | |
| Place: | Teagasc Conference Centre, Ashtown, Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | Yes |
| Organisation of a Workshop | No | Participation to a Workshop | No |
| Press release | No | Participation to an Event other than a Conference or a Workshop | Yes, satellite meeting |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other | Yes |
| Communication Campaign (e.g. Radio, TV) | No | Two poster presentations: (1) overview IMPART project, (2) First results of WP4 (C. difficile). | Yes |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Mid-Term meeting at ASM, physical meeting IMPART | | |
| Date: | 24 May 2019 | | |
| Place: | Teagasc Conference Centre, Ashtown, Dublin (Board Room) | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | No |
| Organisation of a Workshop | No | Participation to a Workshop | No |
| Press release | No | Participation to an Event other than a Conference or a Workshop | Yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other | No |
| Communication Campaign (e.g. Radio, TV) | No | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 15 – 20 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | EURL-AR Workshop 2019 – 40 min presentation | | |
| Date: | April 25-26, 2019 | | |
| Place: | DTU Campus, Kgs. Lyngby | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | No |
| Organisation of a Workshop | No | Participation to a Workshop | Yes |
| Press release | No | Participation to an Event other than a Conference or a Workshop | No |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other | Yes |
| Communication Campaign (e.g. Radio, TV) | No | Joined presentation of mid-term IMPART results by 3 WP leaders. | Yes |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 50 - 70 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 3 - 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | | | |
| Date: | | | |
| Place: | | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

## *10. List of planned tele- or video conferences, face to face meetings in the next year*

In 2020, regular Skype meetings have been planned for WP leaders every two weeks. In May 2020, WP leaders will have a meeting during the One Health EJP annual scientific meeting in Prague (not planned yet). In May or June 2020, a final physical meeting will be organised for all partners (not planned yet).

## 1. Summary of the work carried out in year 2 (January to December 2019)

The ARDIG project has continued to progress and after 24 months there have been substantial achievements made by the project partners, including peer-reviewed publication of papers which are aligned to ARDIG. Details of progress made by each partner for the three scientific work packages (WPs) are described within the report. In the last 12 months, representatives from each participating partner institute met at the Annual Scientific Meeting held in Dublin in May 2019. During this meeting there were detailed discussions on progress as well as partner updates.

Several work package specific meetings took place over the past year. In addition, a two-day AMR genomics workshop was organised at the APHA where partners from WP3 attended. The workshop was enormously successful and included a bioinformatics training component, as well as a discussion between partners on WGS methods used for AMR characterisation of isolates. As a consequence, a priority list of different AMR plasmids and *Escherichia coli* sequence types were selected for genotypic characterisation. There have also been regular email communications within the consortium.

**WP1 (Comparison of AMR and antibiotic sales/usage data collected through existing national surveillance and research programs and assessment of risk factors).** Several of the partners whose data was outstanding have handed overt the information requested by their national surveillance programmes. For a small number of partners this was not possible; therefore their national data will not be included. Based on the collected data, a publication was prepared and submitted. Further analysis of trends within both human and animal data sets has been performed, and included within the deliverables (D-JRP2-1.2 and D-JRP2-1.3).

**WP2** (**Longitudinal studies of AMR persistence).** Both Med and Vet Partners have continued selecting isolates from archived retrospective studies; two manuscripts have been submitted for publication (from the retrospective work by partners). A prospective human study which started in January 2019 has continued throughout the year. The isolates being collected by each partner will be *E. coli* isolated from urinary tract infections from a local hospital and General Practitioner, and will be analysed in Year 3. Veterinary partners have also continued to collect samples prospective longitudinal studies designed within ARDIG. As a result of discussions within the ARDIG workshop in October, partners will examine national collections and prospective studies for WGS from particular MDR plasmid and *E. coli* sequence types which will be compared across different compartments and countries.

**WP3** (**AMR characterization, transmission of plasmids and fitness of MDR isolates**). Partners have been progressing using a combination of molecular techniques, including whole genome sequencing, for AMR characterization of isolates. Most partners have continued characterization of their isolates by WGS (short reads), as well as other molecular techniques. Several partners have also used long read sequencing (MinIon and PacBio) so hybrid data can be used for circularisation of plasmid genomes, which can accurately determine transmission and persistence of different plasmid types. In the ARDIG WGS workshop the different methods currently employed by partner institutes for deriving AMR genotypes was discussed. It is planned that WGS data from ~500 isolates will be run through five different AMR pipelines used by ARDIG partners and the results will be compared, so a harmonized method for AMR genotyping can be considered for future.

## 2. Work carried out in the JRP, scientific results

### WP1 Comparison of antimicrobial resistance (AMR) and antibiotic sales/usage (AMU) data collected through existing surveillance, monitoring and research programs and assessment of risk factors.

#### JRP1-WP1-T1: Exploration and collection of data available on AMR, AMU and potential risk factors (M1-M12)

**NVI**

Data from the Norwegian Veterinary prescription registry and from the Norwegian surveillance program in the veterinary sector for the years 2015 -2018 have been aggregated and sent to BFR for further joint analysis across the consortium. Further on, data from the above mentioned registries are under preparation to be used for a study on possible clustering of resistances and usage of antibiotics at National level where the data could be used less aggregated. The Data transfer/sharing agreement have been signed and sent to BFR. We have contributed to the manuscript review paper "Monitoring antimicrobial resistance and drug usage in the human and livestock sector and foodborne antimicrobial resistance in six European countries" that were submitted to Infection and Drug Resistance 7.11.2019.

**WBVR**

In coordination with BfR, AMU and AMR data for the Netherlands have been aggregated in the supplied format by BfR and submitted for inclusion in the aggregated analysis. The request for inclusion of AMR data of clinical isolates from livestock from the Netherlands from the Dutch Animal Health service has unfortunately been denied.

**UCM**

Data from national surveillance projects carried out during 2014 and 2015 focused on antimicrobial resistance surveillance in different animal production sectors have been arranged to be added to the consortium common data for further analyses. The data sharing agreement has been signed and sent to transfer the processed data with partners responsible for subsequent data analyses. The consumption data of all farms has been forwarded, and resistance data are available from all farms as well.

**UoS**

The UoS has collected historical data on antimicrobial use from a farm located in the South East of England (laying hens, pigs, and dairy cattle). Individual detailed treatments including animal ID, date of treatment, antimicrobial and purpose where submitted to WP1 leaders to be included in the analysis.

**IP**

Monitoring of antibiotic consumption and of antibiotic resistance of bacteria responsible for infections has evolved these two last years in France with new actors. Monitoring is now under the responsibility of Santé Publque France (SPF) by the CPias nouvelle Aquitaine with a new tool called Consores for AMU, and by the CPias Pays de la Loire, with also a new tool called Medqual for AMR. In the past, AMR was monitored by ONERBA (Observatoire National de l'Epidémiologie de la Résistance Bactérienne aux Antibiotiques) which is a private association, collecting information on the voluntary basis. Given this situation, despite much efforts we have not been able to collect additional data on *E. coli* infections beyond what is transmitted by the French authorities to the ECDC (ESAC-net and EARS-net).

**ANSES**

ANSES have collected and sent to BfR, the WP1 leads all relevant data from EU and scanning surveillance of livestock.

**RKI**

The RKI extracted human AMR and AMU from Germany and set up a data sharing agreement between partners. RKI contributes to the joint analysis of the human AMR and AMU of the ARDIG partners (see WP1-T2).

***JRP1-WP1-T2:*** *Investigation of trends, associations and risk factors (M9-M30)*

**BfR**

Phenotypical resistance data on clinical (human and livestock) and non-clinical (livestock) isolates of *E. coli* together with consumption data (human and livestock) has been gathered from ARDIG partners. Data on human isolates were collected on isolates from urine samples.

Data description provided in JRP1-WP1-T2 shows a high degree of data heterogeneity from different countries included in ARDIG regarding: animal categories, consumption units (i.e. kg of substance , defined daiy doses or average daily dose per kg), consumption data sources (i.e. monitoring and projects data), consumption data types (i.e. sales and usage data), antimicrobials and antimicrobial families reported for consumption data, resistance data type reported (i.e. quantitative and qualitative data), standards adopted (i.e. EUCAST, CASFM), interpretation criteria adopted (i.e. ECOFFs, CBPs), laboratory methods used (i.e. disk diffusion vs. broth microdilution), antimicrobial susceptibility testing panels and incomplete data.

Additionally, clinical AMR data from animals are of limited representativeness because of their nature. They come only from cases submitted for diagnostic investigations. As not all clinical cases will be submitted, there is a level of bias and it may not accurately reflect on the whole population.

All available data have been collated in a database and are currently undergoing review and aggregation for reporting in the two deliverables due by the end of month 24. To this end the proportions of isolates of different origins to various antimicrobials has been determined and included in that report. This will allow for analyzing AMR over time and in relation to the development of antimicrobial use in the selected populations. Regional analyses of data will complement the comparisons of data from the different countries and sources. Likewise results from clinical isolates will be compared to non-clinical isolates from animals to analyse potential differences in the resistance patterns that will also help to understand the differences between non-clinical isolates from animals and clinical isolates from humans.

A data sharing contract has been set up by BfR and RKI and it has been sent to the other institutes to be signed. Signing of the contract is necessary for access to certain information.

It is agreed by all institutes involved in task JRP1-WP1-T2 (RKI, APHA and BfR) to focus efforts to describe and analyse clinical data from animals and humans. However, likewise the differences between data on the clinical and non-clinical isolates will be investigated to better understand the peculiarities of the data from different sources.

A manuscript describing monitoring and surveillance systems in the human and livestock sector and foodborne antimicrobial resistance in six European countries has been submitted to a journal for its publication. This manuscript is based on work carried out under task one in the first year and described in the D-JRP2-1.1.

Relevant efforts have also been performed to join forces and cooperate with other projects.

**APHA**

For this task, APHA has performed detailed analysis of clinical AMR data from livestock (cattle, pigs, chicken and turkey) comprising only *E. coli* isolates originating from field cases of clinical disease submitted for diagnostic investigations. Only three countries provided relevant data for this analysis; UK, Germany and France. UK (England and Wales) and German data were available as individual submissions received between 2014 and 2017. French data were available for the respective years only in an aggregated format. Furthermore, the results of susceptibility testing were interpreted by each

country using different standards which had some implications on how these data could be analysed and compared between the countries. To allow these comparisons, first, all the individual country results were transformed based on French CASFM standard. Antimicrobial resistance was then described for each country separately and compared between the three countries for those antimicrobials that overlapped. Resistance to tetracycline and amoxicillin/clavulanic acid was common, especially in cattle across all the three countries. Of the 3rd generation cephalosporins, only resistance to ceftiofur was compared between the three countries. Across the species, the highest proportion of ceftiofur resistant isolates was seen for cattle, especially in Germany. UK chicken isolates were excluded from the comparison due to only small number of isolates tested. A full report describing the results is being prepared.

In addition to the analyses carried out, APHA has contributed to the preparation of manuscript drafted by BfR detailing the results of "Monitoring antimicrobial resistance and drug usage in the human and livestock sector and foodborne antimicrobial resistance in six European countries". The manuscript has now been submitted for publication.

**RKI**

Human AMR and AMU data on human *E. coli* isolates in urine (2014-2017) were collected from the ARDIG partners. Detailed AMR data for previously agreed-upon antibiotics by ARDIG (ampicillin, ceftazidime, cefotaxime, meropenem, ciprofloxacin, gentamicin, cotrimoxazol, trimethoprim, nitrofurantoin, chloramphenicol, azithromycin, nalidixic acid, tigecyclin and tetracyclin, colistin) was extracted from the surveillance systems from Germany and England. The AMR data were interpreted as S, I, R and based on differed standards (EUCAST, CLSI…), while no MIC values were available. Geographical stratification in the German AMR data was made to allow for regional comparisons with the animal AMR data. For the analysis and comparison of human AMU data from ARDIG partners, we used the ESAC-Net-Database (ATC-3 and ATC-4 levels), in addition to AMU data on class level from England. AMU data on substance was only available for Spain (hospital and community) and Germany (community). Large differences were observed in AMU levels between ARDIG partners.

Associations between human AMU and AMR was explored for Germany and England and showed similar patterns for ampicillin trimethoprim, cotrimoxazol and ciprofloxacin from 2014 to 2017.

A data transfer/sharing agreement was drafted together with the BfR and shared with partners for final approval.

The RKI contributed to the manuscript drafted by the BfR "Monitoring antimicrobial resistance and drug usage in the human and livestock sector and foodborne antimicrobial resistance in six European countries", which has been submitted for publication.

***JRP1-WP1-T3:*** *Develop recommendations for improved "One Health" surveillance strategies (M25-M36)*

## *WP2. Longitudinal studies of persistence ESBL/AmpC/carbapenem/mcr-1 and -2/PMQR producing Enterobacteriaceae on farms or hospitals.*

***JRP2-WP2-T1:*** *Assessment and selection of longitudinal data from historical studies (M1-M12)*

NVI

Isolates from a previous study focusing on cephalosporin resistant *Enterobacteriacecae* have been characterized. All broiler flocks raised on ten broiler farms were sampled during the period from May to October in 2016 and a total of 42 positive isolates were obtained (one isolate per flock). These isolates have been sequenced with Illumina technology in order to study a possible on-farm persistence/transmission between bathes of animals on the same farm or broiler house. In total, 11 different *E. coli* STs were identified. blaCMY-2/IncK2 plasmids were the most common gene/plasmid combination (present in nine different STs). A possible clonal persistence of ESC-resistant *E. coli* at

house level was shown for only a minor proportion of the included houses. Isolates from the same house belonging to the same ST could differ by a considerable number of SNPs, shown for ST38 isolates found in three different houses at one farm from several flocks throughout the sampling period. Similar plasmids were detected in different STs, suggesting possible horizontal transfer and/or persistence of plasmids. It is not possible to determine whether different *E. coli* variants and/or ESC resistance genotypes were present simultaneously in a flock, as only a single isolate was characterized per sample. Further analyses of plasmid data will be undertaken and a short communication is planned.

**IP**

IP have analysed two sets of samples from historical studies:

- In order to assess the diversity and the evolution of carbapenemase producing *E. coli*, we have analysed all isolates received by the National Reference Centre laboratory headed by Thierry Naas. 750 isolates were reisolated from glycerol frozen stock and verified. Genomic DNA from the 750 isolates were extracted.
- In order to assess the transmission of *E. coli* among patients in the Berck rehabilitation hospital in collaboration with Didier Guillemot and Lulla Opatowski (Institut Pasteur, Paris). 1422 Gram negative bacteria resistant to third generation cephalosporin were isolated from 329 patients during three months. 420 ESBL expressing *E. coli* were isolated from 67 patients (1 to 25 isolates per patients). Contact between patients and with healthcare workers were monitored by using dedicated personal electronic devices. Among the 420 isolates, we selected 116 isolates for further analyses (at least one isolate from the 67 patients carrier of ESBL-*E. coli*).

***JRP2-WP2-T2:*** *Isolation of resistant Enterobacteriaceae on farms (M1-M30)*

**NVI**

Recent data from monitoring in broilers have demonstrated absence of cephalosporin resistant Enterobacteriaceae in Norwegian broiler production. It was therefore decided that a study in pigs will replace the planned broiler study. A pig study was planned in 2018, but recruiting pig herds for this study has been a great challenge. Recruiting of swineherds has so far not been successful, and last attempts will be made to recruit herds by the end of 2019.

**WBVR**

In the Netherlands, longitudinal sampling was planned on broiler and veal farms. 5 broiler farms were followed for 2 to 5 production rounds resulting in an average prevalence of 20% for ESBL producing *E. coli*. Only one of these farms had several consecutive production rounds in which ESBL producing *E. coli* were isolated from the animals that were sampled. 120 isolates of this farm have been send for next-generation sequencing analysis and will be analysed as part of WP3 to determine the genetic background of the bacteria.

The longitudinal sampling of veal calves at dairy farms and veal fattening farms is carried out in collaboration with Wageningen Livestock Research, partly on national funding. Sampling at the farms is ongoing and is planned to finish in April 2020. So far, the overall prevalence of ESBL producing *E. coli* is 43%. When the sampling has been concluded, the isolates of a subset of farms and animals will be selected for sequencing.

**UCM**

Up to 10 *E. coli* isolates have been collected from 5 pig farms and 5 poultry farms located in different Spanish regions, recovering a total of 94 isolates. All these isolates were further characterized by Minimal Inhibitory Concentration (MIC) to a standard panel of different antimicrobial compounds. Afterwards, the isolates were sequenced by Illumina and Nanopore technologies. We are now analysing the sequencing data to assess the antimicrobial resistance gene dissemination on-farm and the diverse resistance distribution found in different regions according to the antimicrobial

prescription and use. The analyses are described in AMR characterization section. Further, isolates from the same pig farms have been identified, and will be sequenced in order to obtain longitudinal data from the same farms.

**UoS**

The UoS has finalised the collection of *E. coli* isolates from chickens (laying hens) and pigs (sows) over a 12-month period (July 2018 to June 2019) in collaboration with a local farm. *E. coli* isolates from dairy cattle have also been collected over a 3-month period (August 2018 to October 2018) from the same farm. An average of 5 different isolates where collected from non-selective agar culture plates per individual faecal sample (5 faecal samples were collected per animal per month). At two time points during the 12-month period (in December 2018 and in June 2019), additional isolates where collected from selective plates containing cefotaxime, ciprofloxacin, colistin or meropenem. To date, a total of 661 commensal *E. coli* has been isolated during the study and their AMR profiles have been determined by broth microdilution or disk diffusion. In addition, a subset of resistant isolates has been genotypically characterised by WGS. Additional isolates will be sequenced to identify the most prevalent AMR genes and mobile genetic elements present during the study. Data on antibiotic use and farm characteristics has also been collected to determine any risk factors affecting AMR.

**APHA**

APHA has finalised the collection of *E. coli* and Salmonella isolates from a large pig enterprise. This farm was visited 5 times over the duration of the project, and the last 2 visits were carried out in the last 12 months. Faecal samples from all pig classes (weaner, grower, finisher pigs, farrowing and dry sows and gilts) were collected and cultured for Salmonella and on non-antibiotic selective and antibiotic (ciprofloxacin and cefotaxime) selective media for E .coli. Numbers of colony forming units per gram of faeces of resistant *E. coli* were estimated through serial dilutions and colony counts. The use of antibiotics in this pig enterprise is low and the proportion of *E. coli* resistant to ciprofloxacin and cefotaxime is very low. *E. coli* isolates were purified and the Minimum Inhibitory concentrations of approximately 140 strains per visits were determined through broth microdilutions against a panel of antibiotics of relevance to human health (as specified in the EU Harmonised monitoring programme). Resistance in this isolates was low and for the majority of the antimicrobials the proportion of resistant isolates declined over time. The same panel of isolates was investigated genotypically in WP3.

***JRP2-WP2-T3:*** *Isolation of resistant Enterobacteriaceae in hospitals and care facilities (M1-M30)*

**NVI**

*E. coli* isolates from humans with UVI in a large centrally located hospital in Norway, and from GP in the same area are collected (the 20 first isolates of each category, from each month in 2019). The isolates are sent us monthly and they are stored at NVI, together with relevant/requested data. Planning of further investigations of the isolates will start in 2020. Funding for WGS can be covered by the project.

**UCM**

All *E. coli* isolates recovered from urinary tract infections during years 2018 and 2019 in the ICU of a reference hospital located in Madrid have been collected for further analysis. For this purpose, we collaborated with Dr Rafael Cantón, President of EUCAST. We have characterized the phenotypic antimicrobial resistance profile of all isolates according to standard surveillance antimicrobial panels. We are sequencing all the isolates by Illumina and Nanopore technologies to carry out the genetic characterization.

We are collecting the last samples from December, in order to sequence all the human isolates from ARDIG in a batch.

**UoS**

The UoS is undertaking the collection of *E. coli* isolates from human urinary tract infections over a 12-month period in collaboration with two local hospital trusts (January 2019 to December 2019 and June

2019 to May 2020, respectively). Each hospital is gathering a total of 240 pathogenic *E. coli* from hospital and community-acquired infections (10 isolates per month of each type). These isolates will shortly be sent to the University of Surrey for further analysis and WGS. Additional metadata from the isolates such as AMR profile, as well as patient data (e.g. age, gender, antibiotic use and other potential risks factors) will be provided as part of the study. Ethical approval by HRA for anonymised patient data sharing is in place, and participating sites have confirmed capability and capacity.

In addition, the UoS has collected and analysed by WGS a set of 120 isolates from human blood bacteraemia cases from July 2017 to June 2018 through a local hospital. AMR profile of the isolates has been provided, and patient data will be received too.

The UoS has started to analyse data of the *E. coli* isolates collected during the longitudinal studies over a 12-month period in farms and hospitals, specifically isolates from healthy animals (pigs, laying hens and dairy cattle) and human blood bacteraemia. Phenotypic AMR data, molecular data from WP3, and the corresponding metadata (e.g. source, disease, date of isolation, or antibiotic use, among other), are currently being integrated to determine associations and risk factors affecting AMR spread and prevalence. Additional data from isolates being collected will be incorporated to the analysis once ready.

**IP**

We have collectively defined rules for a longitudinal collection of *E. coli* isolates from urinary tract infection. Since January 2019, we are collecting each month 20 isolates: 10 from the community and 10 from hospital wards. Isolates should be from adults and not isolated from a urinary catheter. Hospital isolates were collected at the Bicêtre Hospital. Due to difficulties to collect isolates from a community medical laboratory from Le Kremlin Bicêtre, we have collected isolates from the emergency of the Bicêtre Hospital. Antibiotic susceptibility testing for 16 antibiotics was done on 120 isolates.

**PHE**

PHE have attended teleconferences and agreed for sequencing and analysis of urinary isolates collected from one of a pair of hospitals in England by consortium partners (UoS).

***JRP2-WP2-T4:*** *Data analysis of collected resistant Enterobacteriaceae on national levels (M22-M34)*

**Bfr**

3,425 *E. coli* isolates from the German National Reference Laboratory for Antimicrobial Resistance were investigated. Antimicrobial resistance was determined and six different *qnr*-PCRs were conducted. Further, XbaI-PFGE macrorestriction profiling, S1-PFGE and whole genome sequencing (WGS) were accomplished. One particular aim was the examination of the presence of different *qnr*-genes within *E. coli* isolates recovered from livestock and food in Germany in 2017.

More than 1,000 colistin-resistant *E. coli* isolates from the annual monitoring programs were investigated for the presence of *mcr*- genes 1 to 5. All *mcr-4* and *mcr-5* positive isolate were further subjected to XbaI and S1-PFGE analysis and whole-genome sequencing.

**ALL WP3 PARTNERS**

All WP3 partners were part of an ARDIG Bioinformatics workshop organised by APHA in October 2019 (see details in WP4). It was agreed in the workshop that both medical and veterinary partners will look within their national data sets, as well as those collected specifically through WP2 T2 and T3, for presence of Enterobacteriaceae harbouring particular resistance genes, plasmid and multilocus sequence types for further analysis in WP2-T5.

***JRP2-WP2-T5****: Comparative analysis of collected isolates on a Europe-wide level (M30-M36)*

Europe wide comparison for particular resistance genes, plasmid and multilocus sequence types have been agreed by partners during an ARDIG Bioinformatic workshop. These include the following comparisons:

1) Plasmid comparison:
    a. IncI1 & CTX-M-1
    b. IncK/I & CMY-2
    c. Any Inc & MCR 1&4
    d. IncL/M & IncK NDM1 plasmids
    e. CTX-M-14/15 in non-typed plasmids
2) Isolate comparison :
    *a.* *E. coli* ST744
    *b.* *E. coli* ST38
    *c.* *E. coli* ST1196

## *WP3. AMR characterization, transmission of plasmids and fitness of MDR isolates*

### ***JRP2-WP3-T1**: Detailed molecular characterisation of AMR genes present in human, animal, food and environment isolates from WP1 and WP2 (M6-M18)*

**ALL WP3 PARTNERS: WGS analysis harmonisation**

All WP3 partners were part of an ARDIG Bioinformatics workshop organised by APHA in October 2019 (see details in WP4). As part of this workshop methods used for WGS analysis performed on isolates to determine their AMR genotypes was discussed. It was found that a myriad of methods are used by partners, which makes comparison of data across partners difficult. In an attempt to harmonise analysis of AMR data it was agreed that all WP3 partners will submit WGS data from 50 isolates per partner (450 isolates in total) for further analysis by the five different AMR pipeline/WGS analysis methods used within this group. This will be performed by five different partners (APHA, PHE, WBVR, NVI and UCM). Once the data has been analysed for all 450 isolates, the genotype results from each pipeline will be compared so a single harmonised approach may be considered in future for WGS data analysis within ARDIG.

**NVI**

A collection of more than 260 cephalosporin resistant Enterobacteriaceae has been sequenced using short-read NGS (archived isolates). The strains were isolated from broilers between 2012 and 2016 and can be available for the project. WGS data is also available from a selection of quinolone resistant *E. coli* from Norwegian animals (pigs, broilers, red fox, wild bird). Isolates from WP2 (task 2.1) have been characterized and main findings are described above. For a more complete analysis of circulating ESBL plasmids/strains in broilers in Norway a selection of isolates have been sequenced using both short and long-read sequencing. The isolates were sampled in 2016 and originate from a study were all broiler flocks raised in Norway were screened for cephalosporin resistant Enterobacteriacecae during the period May to October. Approximately 10% of the flocks were positive, and blaCMY-2 was the dominating gene responsible for cephalosporin resistance. However, a minor proportion were classical ESBLs all containing the blaCTX-M-1 gene, located on IncI1-Iγ plasmids. All isolates have been sequenced using Illumina technology, in addition a subset of isolates (one isolates per sequence type) have been sequenced with PacBio. The isolates belonged to nine different sequence types (STs), with the largest groups being ST57 and ST297. The blaCTX-M-1/ IncI1-Iγ plasmids grouped into two main plasmid lineages, namely clonal complex (CC)-3 and CC-7. Our data showed that dissemination of blaCTX-M-1 in Norwegian broiler production is due to both clonal expansion and horizontal transfer of plasmids carrying blaCTX-M-1. The genetic diversity at both strain and plasmid level indicates multiple introductions to Norwegian broiler production.

**WBVR**

In the Netherlands, molecular characterisation of plasmids through WGS using short-read and long-read platforms has been carried out on > 130 MCR positive isolates dating from 2010-2018. Analysis

of these plasmids will be compared to a set of WGS data of MCR-encoding plasmids from Spain from the same time period to see how much overlap exists in plasmids that circulated in these countries. This complete dataset will also be compared to MCR encoding plasmids globally through sequences that were deposited in public databases such as Genbank.

**UCM**

All collected *E. coli* isolates from *WP1* and *WP2* either have been sequenced or are planned to be sequenced using short-read WGS (Illumina technology). With Illumina data we are extracting the resistance gene content to all antibiotic families applying different programs and parameters, which allows us the identification of both known and predicted resistance genes. Furthermore, we are assembling the reads obtained by Illumina sequencing to perform the detection of antimicrobial resistance genes following different approaches and to identify the different *E. coli* sequence types involved in the dissemination of specific resistance genes at farm/hospital, regional and national levels. We are developing phylogenetic analysis to put in context the epidemiological links of all multi-drug resistant isolates found. In addition, we have selected predominant isolates in different isolate sets to be sequenced by Nanopore technology (long-read WGS) to resolve the genomic structure of these isolates.

**UoS**

The Uos has carried out detailed molecular characterization of 275 *E. coli* isolates by Illumina short-read WGS technology. The set includes 94 isolates from human urinary tract infections, 111 from the longitudinal human blood bacteraemia isolates, 20 from healthy human faeces, 13 from healthy pig faeces, 10 from healthy laying hens and 27 from avian colibacillosis. The reminder of the isolates from the animal and human longitudinal studies will be sequenced in the coming months. A subset of the isolates carrying high-risk AMR genes will be selected to be re-sequenced using Nanopore long-read WGS technology to reconstruct the mobile genetic elements involved in AMR transmission.

Bioinformatic analysis of the sequenced isolates has been performed, including phylogenetic analysis, pangenome analysis and database searches using reads and/or assemblies have been used to create a profile of plasmids, AMR genes, serotype, sequence type, phylogroup and virulence genes for each isolate. The integration of this information together with the available metadata and phenotypic data is currently being analysed to look for patterns useful to understand AMR prevalence in humans and animals.

In addition, the UoS has prepared questionnaires to harmonise methods and results for WP3 between partners. The completed questionnaires have been summarised and discussed in WP3-specific meetings. As a result, a workshop was organised in October by APHA to harmonise WGS bioinformatic pipelines. Five representative pipelines were decided to be compared using 50 sequenced isolates from each partner. In addition, sequences from plasmid-specific, AMR-specific, or sequence type-specific isolates will be gathered to compare their prevalence in different sectors from the participant countries. The UoS has agreed to lead the study on CMY-2 cephalosporinase gene.

**APHA**

The APHA has performed detailed molecular characterisation of 386 isolates from both pig and wild birds from 3 time-points over 12 months of the large, low AMU pig enterprise outlined in WP2. This has been performed using short-read Illumina sequencing of all isolates, alongside long-read Nanopore sequencing of a subset of 15 isolates based on the AMR gene content followed by bioinformatic analysis. A further 441 isolates, including 142 isolates from an associated clinical pig farm have recently completed Illumina sequencing, increasing the farm sampling timeline to 5 time-points over 24 months to align sampling periods with partner agencies, to enable future data comparisons.

Phylogenetic, sequence type, core and accessory genome analysis has been performed in silico on the primary 386 isolates, in addition to profiling of AMR genes and mobile genetic elements. Further analysis of the remaining 441 isolates, including long-read sequencing, is underway. Analysis of the available data has allowed the characterisation of mobile genetic elements on-farm and elucidation of

the role of vertical transmission and strain persistence in a reduced AMU environment both as a standalone and in comparison to a clinical setting.

The APHA has agreed to lead the comparative analysis of the five representative WGS bioinformatics pipelines as arranged during the October workshop and to perform a study on the potential clonal spread of ST 744 *E. coli* isolates that will be provided by ARDIG partners.

**BfR**

We chose *E. coli* isolates exhibiting qnrS1 as well as heterogeneous genetic basis and different amounts of extrachromosomal elements of diverse size ranges. For comparative analyses, we sequenced the isolates with Illumina® NextSeq, Illumina® MiSeq and with the Pacific BioScience® Sequel System. The reads were assembled with different assembly strategies and evaluated for their outcome in bacterial typing. We used Unicycler for assembling short reads and conducting hybrid assembly. Further, we used the long-read assemblers HGAP4 and Flye. Long read assemblies were additionally polished with Pilon. All assembled reads were used for in silico typing and were also compared with the results obtained from experimental data. While PacBio Sequencing resulted in larger and more circularized contigs, it missed information on smaller extrachromosomal DNA elements. Here, short-read assembly led to proper detection for determination of the sequence type or the abundance of resistance genes as well as the prediction of the phenotype, but did not resulted in closed genomes. The use of preassembled long reads for other assemblers than HGAP4 did not prove to be fruitful. Contig polishing with Pilon could reduce erroneous gene detection for Flye assemblies. In conclusion, we found hybrid assembly with Unicycler well suited to combine the advantages of short- and long-read sequencing and recommend the use of it for closing genomes, as well as for detecting extrachromosomal DNA elements of different size ranges.

**IP**

Isolates from the study were phenotypically analysed for antibiotic susceptibility by disk diffusion, Etest and broth microdilution for colstin. Molecular characterization was performed by whole genome sequencing and by in depth analysis by using freely available bioinformatics tools and in-house developed pipelines. We have sequenced 986 isolates (750 carbapenemase producers, 116 from the Berck Hospital and 120 from the longitudinal sampling of UTI *E. coli* from Bicêtre Hospital. We have analysed these genome sequences together with 12000 *E. coli* genome sequences publically available (NCBI and Enterobase). A major result was the identification of three evolutionary trajectories for the acquisition of carbapenemase genes (Patino Naverrete et al. Genome Medicine, in press). The analysis of the 750 genomes of CP-Ec from *E. coli* shows a broad diversity of ST, with 3 lineages representing 45% of the isolates (CC10, CC23 and ST38). ST131 isolates were extremely rare with only 16 isolates belong to this clade. On the other hand, analysis of isolates from the Berck Hospital showed that almost two third of the isolates belong to this ST (in agreement with previous studies). Genome analysis allowed to trace in this hospital, strain transferred between patients as well as three cases of plasmid transfers from *K. pneumoniae* to *E. coli*. Preliminary analysis of the UTI *E. coli* isolates showed that 47% are resistant to ampicillin and express a broad range of β-lactamase. Only one isolate devoid of β-lactamase gene was resistant to ampicillin due to overexpression of an efflux pump: an ST131 isolate belonging to the C1 lineage (FQR).

**PHE**

As part of the two day ARDIG workshop PHE representatives identified human isolate comparators with ESBL and AmpC AMR genes, and also encoding carbapenemases present in MLST types of E.coli found in animal compartments.

PHE analysis of widely distributed plasmid types encoding AMR identified incL/M plasmid types associated with transferable AMR, particularly NDM carbapenemases. These plasmid / resistance combinations were associated with outbreaks in different regions of England in different species of Enterobacterales. ARDIG partners are examining sequence databases to identify IncL/M plasmids in isolates elsewhere in Europe and in other one-health compartments.

***JRP2-WP3-T2**: Characterisation of prevalent circulating plasmids and their transfer in vitro (M6-M18)*

**NVI**

Comparison studies showed that blaCTX-M-1 plasmids circulating in Norwegian broiler production are highly similar to plasmids previously described from broiler production in other countries. Reconstruction of blaCTX-M-1/ IncI1-Iγ plasmids from broilers in Norway showed that a plasmid from an ST57 isolate harboured both IncI1-Iγ and IncFIB replicons. Further characterization implied that this was an IncI1-Iγ/IncFIB co-integrated plasmid that consisted of a complete IncI1-Iγ plasmid and a fraction of an IncFIB plasmid. Several virulence determinants, including sit, iroN and hlyF, were encoded on the IncFIB fraction of the plasmid. The IncFIB specific part was inserted into the accessory module on the IncI1-Iγ plasmid. Co-integrated IncI1-Iγ/IncFIB plasmids were found to be present exclusively in ST57 and were detected from a total of five different farms during the six months sampling period in 2016; this could indicate a successful plasmid-host combination. The IncI1-Iγ/IncFIB co-integrated plasmid and additional IncI1-Iγ plasmids containing genes encoding cephalosporin resistance have been subjected to transfer experiments. Recipient strains used are laboratory strains as well as wild type strains from broilers. Further experiments including stability and fitness-cost will be performed. A manuscript entitled "blaCTX-M-1/IncI1-Iγ plasmids circulating in Escherichia coli from Norwegian broiler production are related, but distinguishable" is in revision in Frontiers in Microbiology.

**UCM**

As for the antimicrobial resistance content determination, we have determined the plasmid content of all collected *E. coli* isolates from *WP1* and *WP2*, identifying all different plasmid incompatibility groups and plasmidic replication structures using short-read data. Furthermore, we have selected and sequenced by Nanopore technology (long-read WGS) the most prevalent *E. coli* sequence types found in different settings. The genomic structures from hybrid assemblies have allowed us to assign the diverse resistance genes to the plasmids and the mobile genetic elements involved in the dissemination of these resistance genes among these bacteria at farm/hospital, regional and national levels.

**UoS**

A close collaboration with the project partner PHE has been established to characterise the transfer of the carbapenemase gene NDM-1 via IncL/M broad host range plasmids. These plasmids carry several AMR genes and have been detected by UoS and PHE in local hospitals, in different species of Enterobacteriaceae. *In vitro* transfer experiments using different bacteria as donors and recipients are being performed by the UoS to determine the transfer risks of each host in disseminating these plasmids. Additional plasmids carrying high-risk AMR genes, including other broadly disseminated IncL/M plasmids carrying the carbapenemase OXA-48 will be studied in the same analysis for comparison purposes.

**BfR**

Within this sub-project all available mcr-4 and mcr-5 carrying *E. coli* isolates from livestock and food in Germany (210-2018) were subjected to whole-genome sequencing and plasmid determination. We found a highly conserved genetic basis of the mcr-4 carrying plasmids. Differences among the determined plasmids seemed to be represented by insertions and deletion of non-essential plasmid DNA regions that did not affect the maintenance of the respective plasmid. In contrast to mcr-4 carrying plasmids, the genetic basis of mcr-5 plasmids is more diverse. Among the analysed isolates, we found three different plasmid prototypes that carried mcr-5. These plasmid-prototypes belong to different incompatibility groups and exhibit different replication functions. Interestingly, up to now only one of the mcr-5 plasmids could be determined to be transmissible (self-transmissible).

**PHE**

Detailed genetic plasmid investigations of IncL/M plasmids with NDM carbapenemases by PHE have indicated priority plasmids for further functional characterisation for their frequency of transfer by ARDIG partners (UoS; T4).

***JRP2-WP3-T3**: Fitness cost of AMR and stability of plasmids in different host strain backgrounds (M18-30)*

**UCM**

Plasmids conferring resistance to colistin through *mcr-4* have been analysed. Interestingly, these plasmids belong to a new family of replicons that has not been characterised. We identified the plasmid-copy-number, as well as transduction and stability assays. Further, fitness experiments showed the reason for the prevalence of these plasmids in enteroibacteria. A manuscript with this information is being produced. Further, transduction experiments have been performed with small plasmids from other isolates belonging to our ARDIGF samples. The manuscript is currently under review.

**UoS**

The UoS is carrying out preliminary fitness, mutation and stability studies of the NDM-1 positive IncL/M plasmids in different bacterial hosts.

**BfR**

Detailed investigations were performed to determine the impact of the *mcr-4*/*-5* carrying plasmids on *E. coli* isolates. Therefore, we tested isogenic *E. coli* either with or without the respective plasmid. Within the analysed we found no significance changes in the growth properties of the used *E. coli* isolates. Furthermore, we also detect no significant differences in the growth performance of *E. coli* isolates with and without the plasmid.

**IP**

We are systematically analysing plasmid carrying carbapenemase genes from the 750 sequenced isolates. Plasmid were predicted for 447 isolates. Their in-depth verification and comparison are on-going.

***JRP2-WP3-T4:** Measuring AMR plasmid dissemination in mouse and Galleria, and chicken and pig in-vitro models (M24-M36)*

**UoS**

The UoS has set up chicken and porcine *in vitro* gut models for plasmid transmission analysis in complex microbiome conditions. UoS has also access to *Galleria* and mouse infection models to study fitness and transfer of AMR *in vivo*.

## *WP4: Project coordination and management.*

***JRP2-WP4-T1:** Steering committee quarterly meeting (M1-M36)*

Regular teleconference meetings and updates by email have been made to all members in the steering committee within ARDIG.

***JRP2-WP4-T2**: Consortium members annual meeting (M1-M36)*

We took advantage of partners attending the Annual Scientific Meeting in Dublin to arrange the annual ARDIG meeting where at least one member from each partner organization attended. Several work package associated subgroup meetings were also held to provide time for more in-depth face-to-face discussion between partners.

Several WP3 partners attended the ARDIG WGS workshop which was held at the APHA on 21st to 22nd October, 2019. It included partners from: NVI; ANSES; IP; UCM; UoS; WBVR; PHE; APHA. Sadly, Jens-Andre Hammerl from BfR was ill so could not attend the workshop.

During the workshop use of the APHA AMR SeqFinder pipeline, which is a Linux based tool, was trialled by partners; it included providing tutorials to partners that were less proficient with Linux. This was followed by discussion on the AMR WGS data analysis that partners currently perform, and possible harmonisation in future (see WP3 T1 for details).

There was also a session on AMR plasmids and *E. coli* STs that are of interest for analysis by ARDIG for comparison of their AMR profiles and other characteristics in the different partner institutes across Europe (see WP2 T5 for details).

It was agreed that isolates will be shared between partners for AMR analysis for the pipeline work, as well as AMR plasmid and *E. coli* ST analysis; currently partners are signing an MTA for agreement of transfer of data.

***JRP2-WP4-T3**: Reporting and communication (M1-M36)*

For Year 1 ARDIG 9M and 12M reports were submitted in full and in a timely manner. The Year 2 ARDIG 9M report was also submitted in a timely manner. In addition ARDIG submitted their Data Management Plan in full and in a timely manner for Year 1.

There have been a number of publications from ARDIG partners which has included work performed within ARDIG.

## 3. Progress of the research project: milestones and deliverables

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| ARDIG | D-JRP2-2.1 | Assessment of criteria for inclusion of retrospective and prospective longitudinal studies. | 2 | M25 | M36 | A questionnaire has been completed by all partners to assess the criteria for inclusion. For both the animal and human isolates to be used from retrospective studies the criteria is still under discussion and will be finalized when data from all the strain collections become available.<br><br>For the prospective studies for the human isolates the criteria is that the first 20 E. coli isolated from urine from each hospital at each month over a year will be included.<br><br>There are no criteria for the animal isolates and we will attempt to harmonise methods as much as possible across partners. |
| ARDIG | D-JRP2-3.1 | Prevalent AMR genes and platforms in enterobacteria from humans, animals, food and environment. | 20 | M20 | M36 | All partners have started to assess and report on the AMR gene content of their isolates, especially those already collected in prospective studies and from historical collections. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | However, as isolates are still being collected in several of the prospective longitudinal studies for up to another 12 M, their analysis will not be complete by September 2019. Also, a workshop to harmonise AMR gene analysis within ARDIG took place in October 2019. |
| ARDIG | D-JRP2-1.2 | Description of the specified AMR prevalence/frequency and AMU at population/country/regional level. | 24 | M24 | - | A report has been delivered by BfR, who are WP1 lead, which includes this deliverable. |
| ARDIG | D-JRP2-1.3 | A list of the regions identified for in-depth analysis, and a report including the assessments of parallel trends and estimates of potential associations between AMR and AMU. | 24 | M24 | - | A report has been delivered by BfR, who are WP1 lead, which includes this deliverable. (See D1.2) |
| ARDIG | D-JRP2-2.3 | A project isolates database accessible to all member of the consortium | 24 | - | M36 | As part of WP2 and WP3, a hub has been created at the ENA for depositing WGS data for analysis across partners, which will also be a database of isolates. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| ARDIG | D-JRP2-3.1 | Prevalent AMR genes and platforms in enterobacteria from humans, animals, food and environment. | M24 | - | M36 | All partners have started to assess and report on the AMR gene content of their isolates, especially those already collected in prospective studies and from historical collections. However, as isolates are still being collected in several of the prospective longitudinal studies for up to another 12 M, their analysis will not be complete until December 2020. Also, a workshop to harmonise AMR gene analysis within ARDIG in October has helped plan work for the next 12 months.<br><br>Within the ARDIG workshop partners have agreed a plan of work which will examine AMR genes present in 450 isolates tested through different pipelines, which will help determine prevalence of AMR genes in the different compartments. |
| ARDIG | D-JRP2-3.2. | Circulating plasmids in humans, animals, food and environment | 24 | - | M36 | A plan has been made to examine more closely circulating plasmids harbouring AMR genes which have been found to be present in isolates from different partners. |
| ARDIG | D-JRP2-4.2 | Annual communication to stakeholders | 24 | - | M25 | The stakeholder is aware of the work being performed and will receive a copy |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | of the 12M report from Y2 once it is finalised at the end of January. |

## *Milestones*

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| ARDIG | M-JRP2-6 | Assessment of AMR genes and platforms in enterobacteria collected from humans, animals, food and environment. | M30 | No | 36 | All partners have started molecular characterisation of isolates. WP3 provides details of the work. An ARDIG workshop was undertaken and differences between AMR gene analysis identified. The five pipelines used by partners will be compared to each other using WGS data from the same set of 500 isolates. |
| ARDIG | M-JRP2-4 | Assessment of AMR prevalence/frequency and AMU at population/country/regional level. | M34 | Yes | - | With WP1 AMR phenotypes have been correlated with AMU at regional or country level. Details are provided in D-JRP2-1.2 and 1.3. |
| ARDIG | M-JRP2-5 | Identification of MDR isolates circulating in humans, animals, food and environment | M36 | No | - | An assessment has been made by partners of common MDR isolates circulating within the different regions/countries. Based on this assessment partners have agreed to look in more details at the WGS of three different E. coli STs collected from partner institutes. |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| *ARDIG* | M-JRP2-7 | Identification of circulating plasmids in humans, animals, food and environment | 24 | No | M36 | An assessment has been made by partners of some of the most common circulating plasmids within the different regions/countries. Based on this assessment partners have agreed to look in more details at the genomes of a number of different E. coli STplasmids collected from partner institutes. |

## 4. Publications and patents

Brouwer, Michael S.M., Stephanie D. Jurburg, Frank Harders, Arie Kant, Dik J. Mevius, Adam P. Roberts, and Alex Bossers. "The Shufflon of IncI1 Plasmids Is Rearranged Constantly during Different Growth Conditions." *Plasmid* 102 (March 2019): 51–55. https://doi.org/10.1016/j.plasmid.2019.03.003.

Patiño-Navarrete, Rafael, Isabelle Rosinski-Chupin, Nicolas Cabanel, Lauraine Gauthier, Julie Takissian, Jean-Yves Madec, Monzer Hamze, Remy A. Bonnin, Thierry Naas, and Philippe Glaser. "Stepwise Evolution and Convergent Recombination Underlie the Global Dissemination of Carbapenemase-Producing Escherichia Coli." *Genome Medicine* 12, no. 1 (December 2020): 10. https://doi.org/10.1186/s13073-019-0699-6.

Another couple of manuscripts are in preparation.

Further collaborative work on mcr-genes together with Wageningen (M. Brouwer) is being organised.

## 5. Impact & relevance

One of the central goals of ARDIG has been to consider current approaches recommended by the EU for its policy to tackle the problem of antimicrobial resistance. This has included examining a variety of surveillance activities and laboratory methods. Within WP1 the ARDIG project has been reviewing the antimicrobial resistance (AMR) and antimicrobial usage (AMU) data collected through EU harmonised surveillance for both humans and animals from partners participating within ARDIG; the human data is collected from patients, whilst the animal data is collected from healthy livestock. In addition to the EU surveillance data several veterinary partners also perform AMR and AMU surveillance on diseased animals, and so have clinical data available. A paper has recently been published by ARDIG partners, in addition to reports/deliverables that have been submitted, which details the different surveillance activities undertaken by partners in different sectors and countries. Further work is also being performed in analysing the clinical data. We believe these activities will provide valuable information to help support EU policy in this area.

In addition both WP2 and WP3 will also help towards EU policy. WP2 includes retrospective and prospective collection of E. coli isolates from hospitals and farms, which are analysed further by molecular methods in WP3. A Whole Genome Sequencing (WGS) workshop that was held at APHA in October for ARDIG partners is a good example as it has helped partners to look at similarities/differences in the methodologies used for analysis of AMR by genotyping. Such comparisons are of extreme importance to EFSA and ECDC as they are moving to reporting of AMR data by genotyping. By performing this comparison within ARDIG, which will be reported on in due course through a publication, we expect to make a valuable contribution to EU policy and also demonstrate the closer working between animal and human sectors in this context of One Health.

## 6. Interactions with other JRPs/JIPs or with external (EU or national) relevant project

ARDIG partners are involved in a number of other projects within OHEJP including IMPART, FULL-FORCE, WorldCOM and FARMED. Further, there are a number of OHEJP PhD grants: WILBR, with co-supervision between APHA, SVA and Univ. of Exeter; METAPRO, with co-supervision between UCM, UoS and IP.

UCM are involved in the new H2020 project AVANT, alternatives to antibiotics, starting Jan 2020.

APHA are involved in two JPI-AMR projects on AMR in the environment.

There has been interaction between ARDIG colleagues and ECDC and EFSA at the COGWHEEL workshop for WGS, which was organised in September. APHA colleagues Muna Anjum and Manal

Abuoun presented both on the AMR pipeline for WGS that is being used within APHA and ARDIG, and also on the WGS AMR workshop that APHA was leading and hosting with ARDIG colleagues, in the UK.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements (from ethical reviewers) in 2018 | Measures and actions taken at the end of 2018 | Comments Ethical advisors January 2019 | Comments Project Leader at the end of 2019 |
|---|---|---|---|
| The applicants must confirm that ethics approvals for the use of biological samples have been sought. | Ethics approval is not required as the animal samples will be collected from the farm environment rather than animals themselves or national surveillance activities. Human samples will be collected from hospital reference laboratories. | The appropriate ethics approval is in place (in the process of submission to IRAS, the Integrated Research Approval System, for ethics and approvals for health and social care / community care research in the UK) | / |
| The applicants must confirm the application of 3Rs and the ethical approvals (approval letters, etc) for animal work at a national / institutional level level. The applicants must confirm the process for the application of the 3Rs across the whole programme of work to ensure 3Rs coordination across the programme (e.g. in-vivo mouse work, etc). Please elaborate. | The applicants can confirm the application of 3Rs. Partners who will undertake any animal work e.g. in vivo mouse work, will do so in a justifiable way with full ethical approval, applying the principles of 3Rs. | No details are given on how the 3Rs are applied. The team was asked to provide the licence approval number / ethical approval code. This is not provided. This information should also include the name of the approving body (e.g. site and name of the AWERB). This can be done through the provision of the approval letter.<br><br>Limited response, more information needed. | The University of Surrey will be collecting faecal material and tissues at post-mortem for in vitro assays. This work is in line with the 3Rs as it is a significant refinement (IVOC and gut models). The work was approved through NASPA – NERA-2018-011 |
| The applicants must confirm the compliance with GDPR. | The applicants can confirm GDPR compliance. | No detail has been provided to ensure the correct implementation of the GDPR. The beneficiaries must provide the contact address of the Data Protector Officer of the institution in charge of processing the data obtained. | All ARDIG partners comply with GDPR and have specific teams. For example, at the APHA the Privacy and Data Sharing team evaluates all GDPR requests and advices on the appropriate action. |

| Requirements (from ethical reviewers) in 2018 | Measures and actions taken at the end of 2018 | Comments Ethical advisors January 2019 | Comments Project Leader at the end of 2019 |
|---|---|---|---|
| The applicants must document the safety mitigation measures in place to protect the staff. | All partners work in laboratories that perform risk assessments of reagents and experimental procedures, following the correct health and safety procedures. | No material has been provided to ensure the correct implementation of the safety measures | It is not clear what type of material is required. Please provide clarification. |
| The applicants must specify whether the samples used for genetic analysis permit to identify the sample donors. If so, then an incidental / adverse finding policy must be prepared and available. | No personal or farm identifiers will be used so any sample owner will not be identified. | Satisfactory reply | / |
| The applicants must specify why the misuse issue has been identified in the ethical self-assessment, and how they will address this issue. | The applicants do not foresee any misuse of ethical or other data and results and believe this was a misunderstanding. All applicants will be vigilant and ensure that there is no misuse of data of any type. | Satisfactory reply, although it's still unclear why the applicants have raised this misuse issue in their initial ethical self-assessment | The applicants did not intend to raise any concerns in this area, and have already stated that this was a misunderstanding. |

## 8. List of critical risks

| Description of risk | Yes/No |
|---|---|
| Loss of key-persons (staff and / or leaders) | No |
| Delay in work plan execution | No |
| Conflicts within the consortium | No |
| Lack of commitment of partners | No |
| Delay in duties, tasks or reporting | No |
| Poor intra-project (JRP) relationship | No |
| Potential entry/exit of partners | No |
| Other risks (please describe) | No |

## 9. List of dissemination and communication activities

| | | | |
|---|---|---|---|
| Name of the activity: | Annual ARDIG meeting | | |
| Date: | 21/05/2019 | | |
| Place: | Teagasc Conference Centre, Ashtown, Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | Yes | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | >10 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | >10 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | WGS analysis workshop | | |
| Date: | 21-22 October 2019 | | |
| Place: | APHA | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | yes | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | >10 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | ARAE Conference | | |
| Date: | 1-3rd July 2019 | | |
| Place: | France | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | Yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | >10 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | >10 | | |

## *10.List of planned tele- or video conferences, face to face meetings in the next year*

We plan to have at least one face to face meeting and three teleconferences for the ARDIG project.

## JRP03 - RADAR

### 1. Summary of the work carried out in year 2 (January to December 2019)

The RADAR project was intended to run for 2 years primarily because no (large-scale) new data will be produced and the focus is on the development of modelling methodology. Significant progress has been made (bioinformatics of plasmid typing, pharmaco-kinetic and risk assessment models, source attribution, and disease burden models). However, additional time is expected to be needed to finish these models to the point that they can be shared with the community (sharing of model frameworks, publications etc.). Hence, a request for 6 months extension has been submitted.

WP1: Complete plasmids and associated metadata available in public databases have been retrieved and curated to create a comprehensive plasmid database. The analysis of this database gave a global overview of plasmid diversity and classification and has provided information on plasmid host range and transmission routes. The project will continue to integrate new datasets into the database.

WP2: Model frameworks and datasets have been developed and completed to model the relation between antimicrobial use and development of resistance, and on-farm transmission of resistance. We are performing a systematic review on the difference in estimated importance of transmission routes of antimicrobial resistant bacteria according to different methodologies.

WP3: Regarding the inventory of available exposure assessment models we realized the structure of the model inventory, as described in the annual report 2018, on a BfR test server. This server is currently hosting an alpha version of the web application in order to gather feedback on functionality, design and user experience of already implemented (https://nolar.bfr.berlin). Further constructive work has been realized on the QMRA models for poultry, pork and mussels.

WP4: We were able to retrieve two relevant AMR related data sets, which are already analysed with standard methods and were published, and necessary for our evaluation of machine learning approaches. We reviewed and screened for all ML algorithms accessible via the R-package caret, and we were able to find the class of parametric models, regularized via the elastic net, to be most promising.

WP5: Following the work completed last year on the development of a disease burden approach we have worked on creating a mathematical framework to determine the extent at which infections with AMR bacteria add to the burden of infections and to what extent there is replacement of infections with AMS-bacteria by AMR-resistant bacteria. In addition, we are performing a systematic review to estimate the parameters for a burden model regarding urinary tract infections. Regarding source attribution of AMR, a paper was submitted to **Lancet Planetary Health** on the source attribution of ESBL-carriership among humans in the general population (60% of carriership can be attributed to human-human transmission, and roughly 20% due to food sources; presented at One Health EHP Annual Scientific meeting, Dublin). In addition, methodology was explored for attribution based on metagenomics. The results indicate an inconsistent and most often non-significant correlation between antibiotic treatment incidence and the proportion of resistant isolates of the microorganism currently used as indicator in surveillance.

WP6: We performed a literature meta-analysis on dose-response studies for *E. coli* carriership (presented at One health EHP Annual Scientific meeting, Dublin) which was added (together with an updated source attribution calculation) to the evidence synthesis framework. QMRA and EPI estimations of carriership are now more in agreement, constructively contributing to an overall estimation of human ESBL *E. coli* carriership. Slaughterhouse contamination model needs yet to be included.

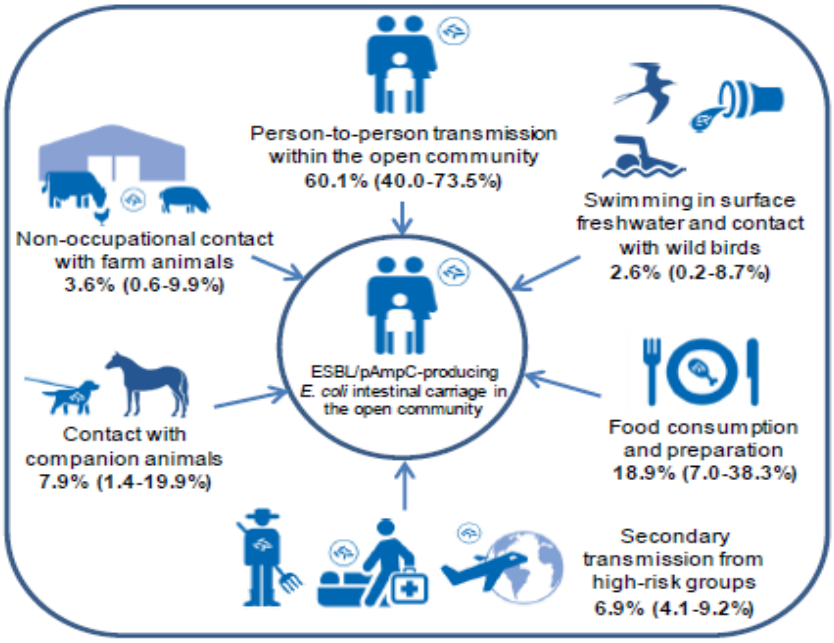


*Fig.1. Summary results of ESBL source-attribution (submitted to Lancet Planetary Health)*

## 2. Work carried out in the JRP, scientific results

### WP0: Coordination and communication

#### JRP3-WP0-T1: Coordination and project management (M1-M30)

Extension was requested and granted for 6 months. The RADAR project was intended to run for 2 years primarily because no (large-scale) new data will be produced and the focus is on the development of modelling methodology. Significant progress has been made (bioinformatics of plasmid typing, pharmaco-kinetic and risk assessment models, source attribution, and disease burden models). However, additional time is expected to be needed to finish these models to the point that they can be shared with the community (sharing of model frameworks, publications etc.). Main reason is delay in acquiring personnel and/or the loss of key personnel on several WPs/tasks and change in scientific approach of some WPs/tasks. Therefore, a six month extension will help compensate for the time taken for recruitment and new staff getting up to speed with what has already been done.

#### JRP3-WP0-T2: Consortium meetings (M1-M30)

***JRP3-WP0-T2-ST1**: Kick-off meeting*

January 2018 Schiphol Airport, The Netherlands. Important first alignments and directions were consolidated.

***JRP3-WP0-T2-ST2**: Mid-term meeting (M10-M12)*

Held January 18th Schiphol Airport, The Netherlands. Scientific progress was presented and discussed. Good agreements for WP alignments were made.

***JRP3-WP0-T2-ST3** Final meeting (M30-M30)*

Due in summer 2020.

#### JRP3-WP0-T3: Annual reports (M1-M30)

***JRP3-WP0-T3-ST1**: First annual report (M10-M12)*

Submitted in January 2019

***JRP3-WP0-T3-ST2** Second annual report (M22-M24)*

See this document.

***JRP3-WP0-T3-ST3*** *Third annual report (M28-M30)*

## *WP1. New genomic information to feed AMR transmission models*

### ***JRP3-WP1-T1:*** *Build collections of high throughput sequencing (HTS) data needed for project-specific milestones and deliverables (M1-M15)*

Conjugative plasmids are predominantly responsible for the global dissemination of antimicrobial resistance, representing an important threat to global health. As the number of plasmid sequences grows exponentially, it becomes critical to depict the global diversity and decipher the distribution of circulating plasmids in the bacterial community. To this end, we created **COMPASS**, a **novel and comprehensive database** compiling 12 084 complete plasmids with associated metadata from 1 571 distinct species isolated in 126 different countries over more than 100 years.

The curation of the database allowed us to identify identical plasmids shared between different species revealing their disseminating potential and stability. We outlined and analysed all relevant features, plasmid properties, and host range. This curated plasmid database was integrated, as a reference, into our new developed pipelines (JRP3-WP1-T2) aiming at identifying plasmids from sequencing data. This novel resource will help researchers and clinicians understand the genetic plasticity and transmission routes of plasmids, which are crucial in the fight against the spread of antimicrobial resistant pathogens.

The paper entitled “Analysis of COMPASS, a new comprehensive plasmid database revealed prevalence of multireplicon and extensive diversity of IncF plasmids “is under review in the journal Frontiers in Microbiology (Fig 2).

New environmental genomic datasets relevant to various AMR challenges have also been collected and organized in three subprojects on pilot species (Salmonella enterica and E. coli), antimicrobials resistance (Cephalosporin and Fluoroquinolone resistance) and origins (human, animal-food sectors, environment) (JRP3-D-1.1.2). These include:

- *Salmonella* surveillance network (Anses, 2000-2016) contained 2839 isolates from all food, animal and environmental sectors. This dataset have a particular focus on fluoroquinolone resistance and plasmid-associated *qnr* genes.
- 59 QREC (quinolone resistant *E. coli*) from animals (poultry, pigs, wild birds, foxes) in Norway.
- Cephalosporin resistant *E. coli* (containing blaCMY-2) from poultry in Norway

Plasmid *de novo* assembly and characterization (plasmidome and resistome) of these genomic datasets were performed using our novel plasmid pipeline **(JRP3-WP1-T2**). These databases will also be used in **JRP3-WP1-T3** and **JRP3-WP1-T4** for testing and validating the methods.

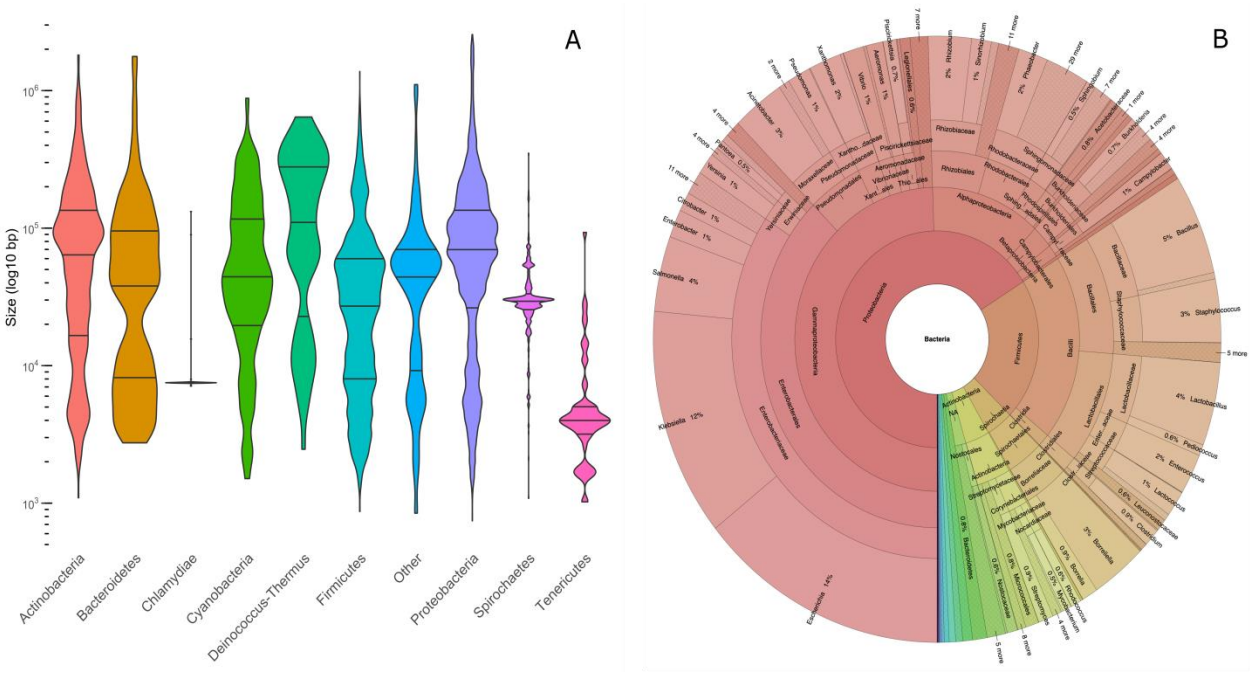


***Fig. 2.*** *Description of the COMPASS database. (A) Violin plot displaying plasmid size distribution (log10) among the main phyla (n=12 084). The phylum entitled "Other" is composed of 57 plasmids from 12 minority phyla (n<25) and 129 plasmids missing taxonomy data. (B) Krona plot showing the compositions of taxa and taxonomic ranks (n=12 084).*

***JRP3-WP1-T2:*** *Develop an innovative automated bioinformatic pipeline integrating de novo plasmid reconstruction and identification (M1-M18)*

Identifying and tracking circulating plasmids is heavily relying on the ability to accurately assemble their whole genome sequences and differentiate chromosomal from plasmid contigs. Even though several bioinformatics tools can be applied to reconstruct plasmid sequences from short reads, a contiguous assembly is still difficult to obtain. Plasmid *de novo* assembly based from the coverage and the assembly graph was first perform by SPAdes. In order to assess the performance of different plasmid detection tools, we built a "test dataset" composed of 56 known genomes of *Salmonella enterica* (Chromosome and plasmids) and tested against MOBrecon, PlaScope, Plasflow and HyAsP programs (**M-JRP3-14 & D-JRP3-1.3**).

Our COMPASS database was integrated in the detection tools as a reference database to help discerning the plasmids contigs from the chromosomal sequences and to identify the closest plasmid neighbour present in COMPASS. Core plasmid genes necessary for replication (replicon) and mobilization (Relaxase, MPF and *oriT*) were annotated by the MOBtyper program from the MOBsuite package and the plasmid resistome was characterized in silico by detecting resistance genes of the Resfinder database.

Finally, we developed an automated plasmid identification pipeline by integrating plasmid assembly (SPADES) and detection programs (PlaScope), annotation tools (MOBtyper and Resfinder) and reference-based identification (Mash program against the COMPASS database).

The outputs of the pipeline (plasmid contigs / genes and resistance genes) were designed for handling by microbiologists and modellers to facilitate biological interpretation (**JRP3-WP1-T3)** and integration into transmission models (**JRP3-WP6)**. Following the validation of our pipeline on the "test dataset", our novel plasmid tool was used to detect and identify plasmids from the new environmental datasets collected in **JRP3-WP1-T1 (M-JRP3-24 & D-JRP3-1.4).**

***JRP3-WP1-T3**: Plasmidome: biological annotation and risk assessment (M12-M24)*

With the objective of getting a global overview of the diversity of circulating plasmids, we characterized the COMPASS database by *in silico* typing (MOBtyper) based on replication and mobilization systems and predicted the mobility from the analysis of dissemination markers. The distribution in the bacterial community was carefully examined to highlight particular features and specific associations between core genes and the plasmid host range.

In order to characterize the plasmid resistome, resistance genes were searched against the Resfinder database. Particular associations between resistance genes and plasmid types / mobility / host range were carefully examined. In total, 13 812 resistance genes were detected among the 12 084 plasmids and they included 503 different resistance variants. From our dataset, we found that 3 438 plasmids (28%) carry at least one resistant gene and resistance to Betalactams, aminoglycosides and sulphonamides are the most frequent. Among these resistant plasmids, 41% are multiresistant of which 80% were isolated from *Enterobacteria*. A particular attention was made on Betalactams-resistant plasmids, a major health concerns. Overall, 179 different Betalactamase variants were detected in 2 155 plasmids (18%). The Betalactamases included the four molecular classes (A-D) and belonged to 33 different enzyme families. The 2 155 plasmids were isolated from 127 distinct species. The majority (n=1 673), were associated with *Enterobacteriaceae*. The in silico typing analysis demonstrated that Betalactams-resistant plasmids are very diverse (100 different REP-types and all MOB-types identified) and highly mobile (68% conjugative and 14% mobilizable) **(*paper in preparation*)**.

Sequencing data from *Salmonella enterica* strains gathered from **JRP3-WP1-T1** were run through our plasmid assembly and identification pipeline **(JRP3-WP1-T2)** and characterized the same way as the COMPASS database (replicon / mobilization / resistome) **(D-JRP3-1.5)**. Outputs will be examined and curated by microbiological experts and phenotypic characteristics such as MIC values will be used for verifying the genomic findings. The knowledge will be used to predict risk factors associated to different plasmids/plasmid genes /AMR profiles and to inform the exposure assessment modelling of (**JRP3-WP3-T3)**, as well as the evidence-based synthesis attribution in **JRP3-WP5.**

***JRP3-WP1-T4:** Methods to identify genetic traits associated to AMR (M12-M24)*

Two different approaches will be used for identifying the genetic traits associated to AMR.

Whole-genome association studies (WGAS) will be performed on sets of sequencing data issued of epidemiologically comparable population to identify SNP's and Indels statistically associated to AMR traits whether plasmid-borne or not. (***D-JRP3-1.6)***

A second approach will be through regression model that will focus on the presence/absence of gene belonging to the accessory genomes (chromosomal and plasmid-borne). (***D-JRP3-1.7)***

The results of the analysis performed in **JRP3-WP1-*T3 and* JRP3-WP1-*T4*** will be used to predict risk factors associated to different genetic markers linked to plasmids and/or AMR profile and to inform the exposure assessment modelling of **JRP3-WP3**, as well as the evidence-based synthesis attribution in **JRP3-WP5**.

## *WP2. Pharmacodynamics and transmission models*

***JRP3-WP2-T1:** On-farm transmission models (M1-M24)*

***JRP3-WP2-T1-ST1**: PK/PD model to assess relationship between animal exposure and change in antimicrobial resistance (M1-M20)*

The PKPD models initially developed for colistin (with a "simple" intestinal PharmacoKinetic) allowed us to identify key points for the development of a PKPD model for amoxicillin and ESBL *E. coli* (***Fig. 3)***. However data gaps are a major limit to get a complete realistic predictive model. The work is still ongoing in association with partners of the sub task JRP3-WP2-T1-ST3, in order to get the optimal connection between PKPD model and transmission model.

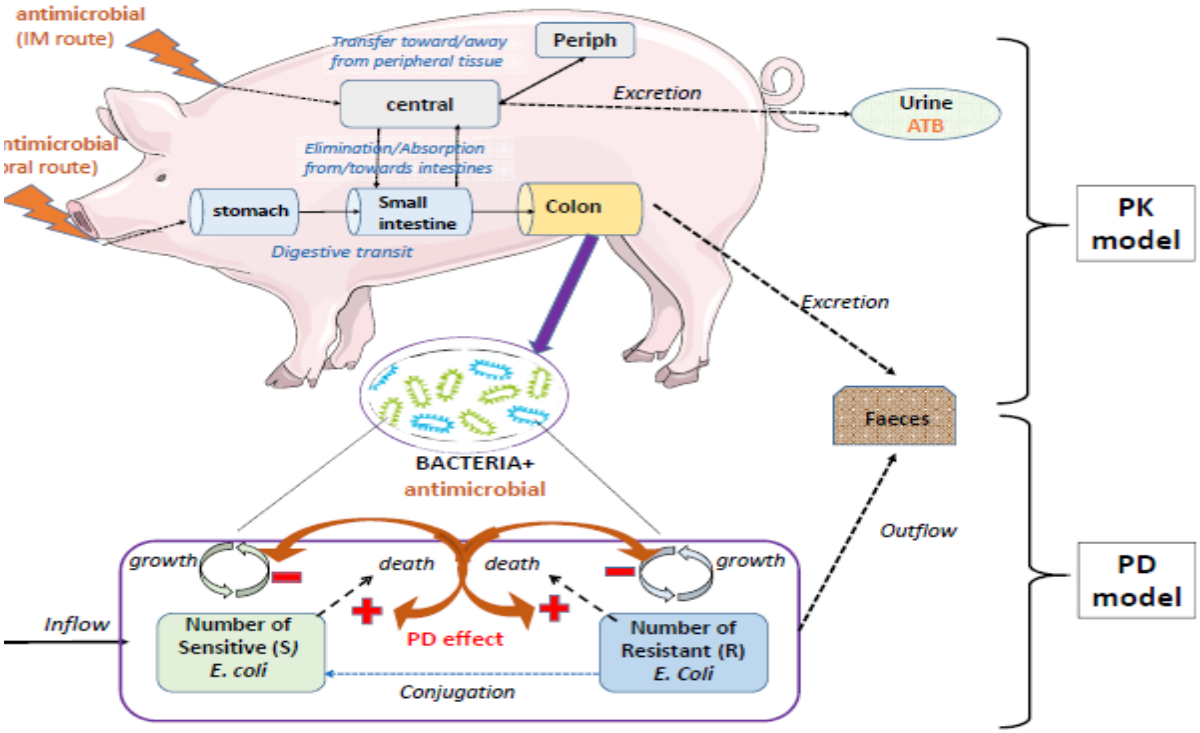


***Fig. 3.*** *Schematic diagram for the proposed Pharmacodynamic model for pigs*

***JRP3-WP2-T1-ST2:*** *Assess relative importance of AMU and clonal dissemination for resistance occurrence (M1-M20).*

Recent publications in human medicine have investigated the relative importance between AMU and plasmid/ clonal dissemination and we are currently reviewing these papers to identify the key parameters to be the most influential on this phenomenon. The impacts of their associated variabilities are currently under investigation.

***JRP3-WP2-T1-ST3:*** *Development of on-farm transmission model (M1-M20)*

Due to resource issues there has been limited progress this year. These issues have now been resolved. With the six month extension we are on track to meet the revised milestone and deliverable dates.

The framework of the on-farm model has been built and draft results have been produced to confirm it is functional. The model now needs to be fully parameterised and validated for the case study of ESBL *E. coli* in the UK.

We have discussed with the consortium members working on the PK/PD model (JRP3-WP2-T1-ST1) to ensure that the models can link up. We have also been discussing with the members working on the slaughterhouse model (JRP3-WP3-T2-ST2), to ensure that we can provide relevant outputs of the farm model that can be used as inputs to this model. This will also require additional parameter estimates so that the model is relevant for the case study of The Netherlands.

***JRP3-WP2-T1-ST4:*** *Scenario analysis to assess hypothetical on-farm intervention measures (M6 M20)*

The majority of this work is yet to be done. We have been in discussion with our colleagues in the Veterinary Medicines Directorate and the APHA Pig Health and Welfare Council as to relevant intervention measures that would be good for the model to assess. Suggestions included, sick picks always being moved to a sick pen for antimicrobial treatment and ban on on-farm use of

antimicrobials. It was suggested that the problem is likely multifactorial, so a combination of measures may be appropriate

***JRP3-WP2-T1-ST5**: Communication of results (M18-M24)*

A poster detailing the proposed connections between the models from **JRP3-WP2-T1-ST1**, **JRP3-WP2-T1-ST3** and **JRP3-WP3-T2-ST2** was presented at the OHEJP conference in Dublin in May 2019.

***JRP3-WP2-T2:** Models for transmission between livestock and human populations*

***JRP3-WP2-T2-ST1**: Development of mathematical models for source-attribution (M1-M22)*

We conduct a review which compares the different methods to determine source attribution (registered in Prospero, CRD42019136298). All articles are read and summarized, and we are currently performing the analyses. We hope to finalize the manuscript in the next 3 months. We are especially interested whether some methods give consist higher/lower estimates of the source attribution than others.

We also contributed to a publication on source attribution (see JRP3-WP2-T2-ST3).

***JRP3-WP2-T2-ST2**: Assessment of intervention measures (M13-M22)*

The majority of the work is to be done when the review (see JRP3-WP2-T2-ST1) is finished.

***JRP3-WP2-T2-ST3**: Communication of results (M18-M24)*

A poster was presented at the Epidemics Conference in December 2019 (P1.069)

## *WP3. Transmission through the food chain.*

This WP focuses on risk assessment models that calculate risks of exposure/infection with ESBL *E. coli* from different sources and through different food chains ***(Fig. 4)***.

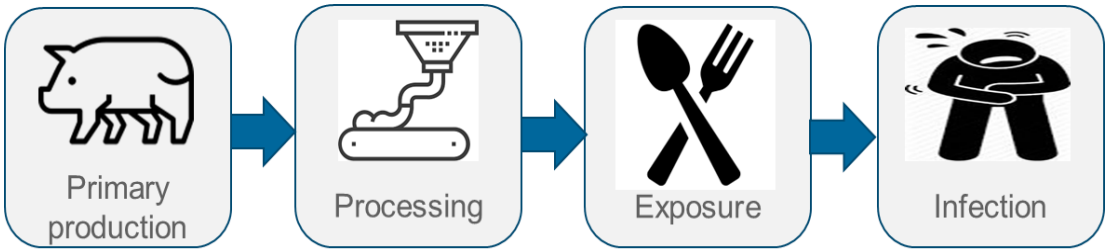


***Fig. 4.** Schematic simplified overview a risk assessment models to assess risks of exposure/infection with ESBL E. coli from different sources and through different food chains.*

***JRP3-WP3-T1:** Inventory of available exposure assessment models and related data and transfer to FSK Standard (M1-M24)*

***JRP3-WP3-T1-ST1**: Inventory of available exposure assessment models (M1-M12)*

The task of taking stock of models for exposure assessment available among partners has been completed, see annual report 2018. We have also achieved the milestone of developing the structure for the RaDAR model inventory, see annual report 2018.

Additional work is ongoing to technically facilitate usage of the inventory. The structure of the model inventory, as described in the annual report 2018, is planned to be realized on a stand-alone BfR server. However, the setting up of a dedicated server is delayed. Therefore the migration of web application towards a BfR server is scheduled for January 2020. A Google server (https://test-cb8b2.web.app) currently hosts a beta version of the web application in order to gather feedback on functionality, design and user experience of already implemented features.

During the implementation of the application the requirement list from the annual report 2018 has been extended due to new ideas that completed the concept and feature requests from users. As of December 31st 2019 following features has been implemented:

- Display model results and upload them
- Change of model parameter and execution models
- Upload new models from script files
- Upload new models from FSK files
- Guide to create FSK files
- Sort and display models filtered by metadata
- Access to an individualized user space
- Simple and intuitive user interface
- Assessments of models by users
- Comment section for each model
- Display, Execute and change model code within the inventory
- Version control
- Information on new Uploads and Comments
- Invitation system
- Group management system
- Save feature for unfinished models

Besides the migration of the inventory towards a BfR server and an implementation of secure login and upload routines, the main focus during the extension period will be to improve the performance and durability of the inventory. Therefore computing-intensive methods should be replaced and potential weak spots in the design fixed. Summarized, it is planned to finish the following tasks by April 30th 2020:

- Migrating the inventory to an dedicated BfR server
- Audited login and upload routines
- Dynamic plots/charts feature
- Improvement performance and durability
- Implementation additional feature requests

***JRP3-WP3-T1-ST2**: Transfer of available exposure assessment models developed in R (or Matlab) to FSK Standard for at least one type of AMR bacteria and at least one animal (chicken, pig or mussels) (M10-M24)*

A comparative Exposure Assessment model of ESBL-producing E. coli through meat consumption (https://doi.org/10.1371/journal.pone.0169589) has been transferred to FSK standard. During the transfer of the model on the spread of ESBL/AmpC *E.coli* in the broiler production chain (https://doi.org/10.1111/risa.13145) to the FSK standard we identified a limitation of our current IT infrastructure that we are currently working to resolve. The limitation consists in the current server infrastructure which cannot handle models that contain larger amounts of data (in our current case "larger amounts" refers to sizes of about 40 megabytes and more). This limitation should be overcome as soon as the migration of the application to a stand-alone server is completed. Then also the transfer of the model (https://doi.org/10.1111/risa.13145) to the FSK standard will be completed.

***JRP3-WP3-T2**: Exposure assessment models for different production chains (M1-M24)*

***JRP3-WP3-T2-ST1:** Exposure assessment model for the chicken production chain (M1-M24)*

So far the model for the chicken production chain has two main parts. First, the primary production model which looks at the spread of ESBL/AmpC *E.coli* from the hatcheries over fattening up to arrival at the processing plant and was published under https://doi.org/10.1111/risa.13145. Second, the processing model, which follows ESBL/AmpC *E.coli* colonization of broiler carcasses through a generic slaughtering process consisting of the steps scalding, plucking, evisceration, washing and chilling and which was an reimplementation of a model for Campylobacter formerly published under https://doi.org/10.1111/j.0272-4332.2005.00569.x. This model has been adapted from Campylobacter to *E. coli*.

In the last months we connected the two independently developed models for primary production and processing, so that the end result from the primary production model has a corresponding impact on the initial conditions of the processing model.

***JRP3-WP3-T2-ST2:** Exposure assessment model for the pork production chain (M13-M24)*

Development is underway of two sub-models for the pork chain. Firstly, an existing consumer-phase model describing inactivation, growth, and cross-contamination of ESBL *E. coli* during preparation and consumption of pork has been modified for inclusion in the current project. Modifications consist of 1) translation of the model into R and JAGS, 2) identification of key uncertainties 3) identification of generic parameters that may be used Europe-wide. These modifications are implemented for compatibility with WP6 - the pork production chain model will be the case study for the evidence synthesis work. The work on the consumer phase model is completed. Furthermore, first steps have been made for transferring the consumer phase model to the FSK repository (JRP3-WP3-T1-ST2).

The second sub-model is the pork slaughterhouse model. Here we are in the process of amending the existing EFSA Salmonella in Pork model. Since also this model will be integrated in WP6, we greatly simplify the model structure, and replace Salmonella specific parameter values with a parametrisation more appropriate for ESBL *E. coli*. The model structure has been implemented in R, in such a way as to be easily used in the model of WP6. We are currently in the process of gathering appropriate data and parameter distributions. Input for the slaughter model will come from the farm model developed in JRP3-WP2-T1-ST3, a common format for interfacing of the models has been established, and a prototype coupling of the models was established using preliminary farm data.

***JRP3-WP3-T2-ST3**: Exposure assessment model for the mussel production chain (M13-M24)*

Laboratory experiments regarding the association between the cooking time and temperature of the eatable parts of the blue mussels are finished. Further, blue mussels have been contaminated with E. coli and the concentration were analysed on blue mussels before and after the contamination as well as after different cooking times. The temperature was measured simultaneously. The experiments were repeated three times. Experiments where *E.coli* have been taken up by blue mussels (*Mytilus edulis*) have been performed in an aquarium setting, and the mussels subsequently heat-treated in intervals before survival of *E.coli* has been assessed. A model of exposure risk as a function of contamination level and heat treatment regime has been developed. Experiments addressing potential effects of ESBL elements on heat treatment survival and persistence of genetic elements for horizontal gene transfer are ongoing.

***JRP3-WP3-T3**: Generic comparative exposure assessment model (M13-M24)*

This task builds on the work performed in WP2 and WP3-T2. During the second half of the year we discussed the potential for deriving or creating generic methods for exposure assessment for different production types. Discussion addressed how different bacterial species, plasmids and genes might be covered in this generic approach.

We gathered a dataset of sequenced isolates collected from live animals, food and humans with foodborne infection and we are currently preparing the pan-genome dataset for those isolates. This dataset will be fit to machine learning classification algorithms in order to identify genetic markers

associated with pathogen persistence throughout the food chain and their eventual association with different animal reservoirs.

## *WP4: Machine learning methods for quantification of risk and health effects*

### ***JRP3-WP4-T1:** Add state of the art ML models for risk profiling to an inventory of exposure risk assessment models (M1-M2)*

***JRP3-WP4-T1-ST1**: Definition of the aims and requirements for literature research (M1-M2)*

This task has been completed, see annual report 2018

***JRP3-WP4-T1-ST2:** Decision on the model inclusion criteria (M2-M5)*

This task has been completed, see annual report 2018

***JRP3-WP4-T1-ST3**: Decision on how the models are to be represented (described for the end user) and development of a template (M5-M6)*

This task has been completed, see annual report 2018

***JRP3-WP4-T1-ST4:** Repository setup including setup of a Github repository(M7-M12).*

This task has been completed, see annual report 2018.

Remark: When applying for the project, a system was sought with which the results of the investigations could be made publicly available. The system to be used should consider the FAIR principle ("Findable, Accessible, Interoperable, and Re-usable"). This can be realized with a GitHub repository. Since the results of the investigations are now to be made available according to a data management plan (DMP) to be created, the establishment of a Github repository was postponed to a later point in time after clarification of the exact design of the DMP. Nevertheless, the repository can be set up at short notice, as all preparations has been completed.

### ***JRP3-WP4-T2:** Methods for testing model -validity, -sensitivity and –robustness (M13-M21)*

***JRP3-WP4-T2-ST1**: Selection of test data set(s) to be used (M13-M14)*

Data sets were selected according to our needs: one well behaved data set, one misbehaved data set that exceeds the possibilities of current fitting routines. Both data sets are already analysed and published.

***JRP3-WP4-T2-ST2**: Defining a work bench for assessing model (M15-M17)*

Task completed.

The workbench for the evaluation of the ML algorithms uses publications on microbiological resistance in journals with peer review system. It is guaranteed that the data used for the publication can be accessed (in the concrete case the data were made available by the authors under restrictions). The data are evaluated with different ML algorithms and the results are compared with the results of the original work regarding the determined influencing variables and their impact. On the basis of these criteria a similarity measure can be developed.

***JRP3-WP4-T2-ST3**: Model Analysis (M18-M21)*

We concluded that regularized parametric algorithms would satisfy our needs. For the regularization we realized that the elastic net is the most appropriate approach. Three algorithms were selected: logistic regression with elastic net, linear discriminant analysis using the elastic net, and sparse distance weighted discrimination. The last two models need additionally a final probability calibration.

### ***JRP3-WP4-T3:** Literature review of methodologies and compilation of the selected methods (M22-M24)*

***JRP3-WP4-T3-ST1:** Literature review of methodologies and compilation of the selected methods (M22-M24)*

Our literature review showed that the elastic net is indeed a modern approach to the problem of model selection. It could overcome the limitations of the LASSO, stepwise regression, and univariate filtering.

***JRP3-WP4-T4**: ML and causality. Does it fit together? (M28-M36). Ongoing.*

## *WP5: The burden of disease caused by AMR exposure*

***JRP3-WP5-T1:** Methodological framework for AMR burden (previously "Identify data gaps and define target questions for SEJ (Structured Expert Judgment)"(M1-M12)*

Completed in Y1 (see 12M report ).

***JRP3-WP5-T2:** Comparison of AMR burden methods (previously "Defining the seed questions") (M1-M12).*

Completed in Y1 (see 12M report )

***JRP3-WP5-T3**: Application of AMR disease burden framework to urinary tract infections (previously "Identifying, enrolling and interviewing the experts") (M4-M30)*

We have further developed the burden of disease methodology (***fig. 5***) to compute antibiotic resistance-attributable burden as excess burden, and have determined how to best carry out the computations using existing software. To supply the necessary input data (national-level incident cases of resistant ESBL *E coli* UTI, we made a data request to the Netherlands national AMR surveillance system (ISIS-AR), and this dataset has now been delivered. The systematic literature review conducted for model parameter values (transition probabilities, durations, case fatality) that are specific to the Netherlands healthcare setting has been completed. This review identified knowledge gaps for certain parameters (applicable to either susceptible or resistant infection, or both), for which decisions and/or further action must be taken. Parameter value decisions will be made by end Jan 2020.The final step, which will be completed by end of Feb 2020, will be to set the UTI disease progression model parameters to match the Netherlands situation, and so estimate the national excess burden of UTI attributable to AMR. The report on this work will be completed by end of March 2020.

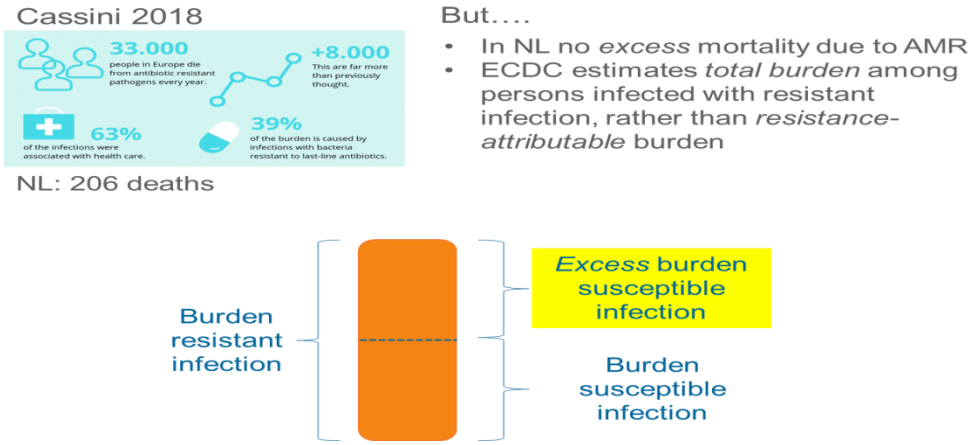


***Fig. 5.*** *The rationale and basis of the approach to propose a new paradigm for estimating disease burden of AMR.*

***JRP3-WP5-T4:** Source attribution of AMR for attribution of disease burden to sources (previously Analysing the data to obtain aggregated responses to the target questions") (M0-M30)*

**ESBL *E. coli* attribution**

Completed, see D5.3 Publication: Attributable sources of community-acquired carriage of *Escherichia coli* containing β-lactam antibiotic resistance genes: a population-based modelling study. 2019. Mughini-Gras L, Dorado-García A, van Duijkeren E, van den Bunt G, Dierikx CM, Bonten MJM, Bootsma MCJ, Schmitt H, Hald T, Evers EG, de Koeijer A, van Pelt W, Franz E, Mevius DJ, Heederik DJJ;

ESBL Consortium. Lancet Planetary Health. 2019 Aug;3(8):e357-e369. doi: 10.1016/S2542-5196(19)30130-5 (*fig. 6. And fig.7).*

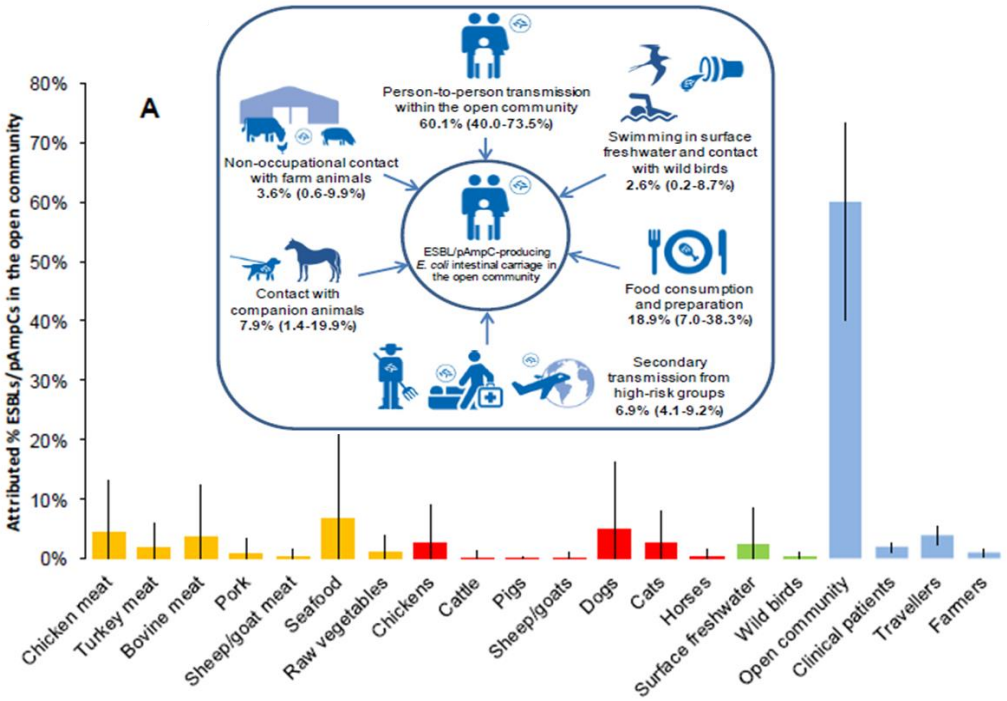


***Fig. 6.*** *Attributable fraction of sources contributing to human carriership of ESBL E. coli.*

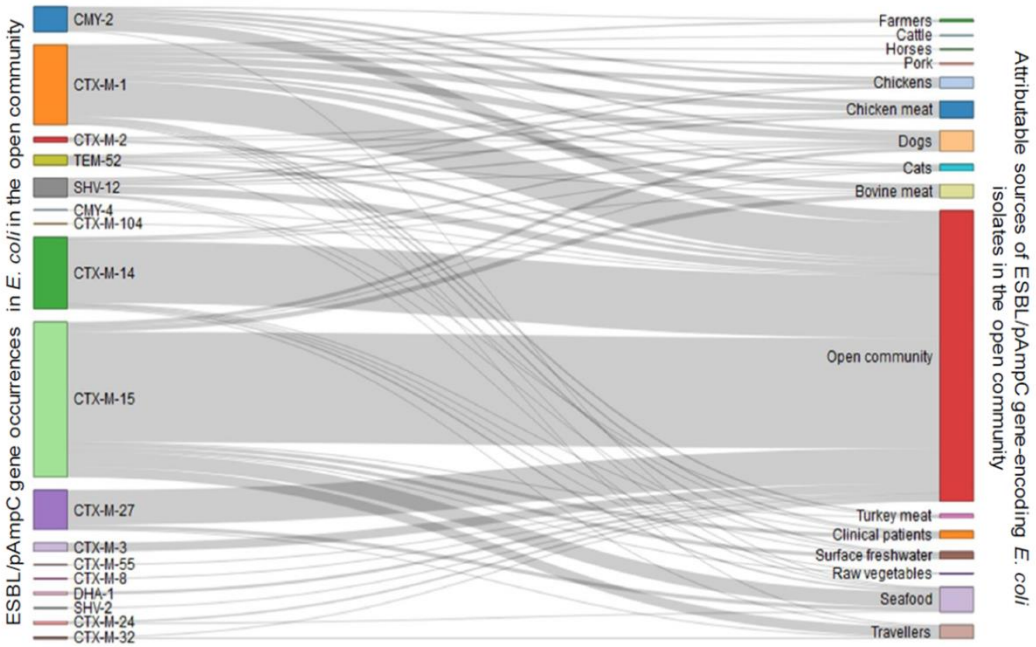


**Fig. 7**. Attribution of different ESBL genotypes to sources (gene-flow)

**Metagenomic source attribution**

The metagenomics-based model for source attribution of AMR was able to predict the source of the antimicrobial resistance genes observed in the resistomes of three human groups – pig farmers, broiler farmers and pig slaughterhouse workers. The results show that the majority of the exposure cannot be attributed to the animal reservoirs considered in the model (broilers, pigs, veal calves and turkey), but that there is a higher proportion of attribution to pigs among humans working in direct contact with pigs, especially among pig farmers, suggesting the occurrence of occupational exposure to AMR determinants. A similarity percentage analysis allowed identifying the individual AMR determinants

which mainly contributed to dissimilarity between every two reservoirs, and determinants specifically associated to individual reservoirs (fig 8).

We are presently gathering additional human resistome data representative of human groups without occupational exposure to the reservoirs considered in the model.

Future work will consist on applying the model for source prediction with the gathered data and comparing the predictions to those previously obtained.

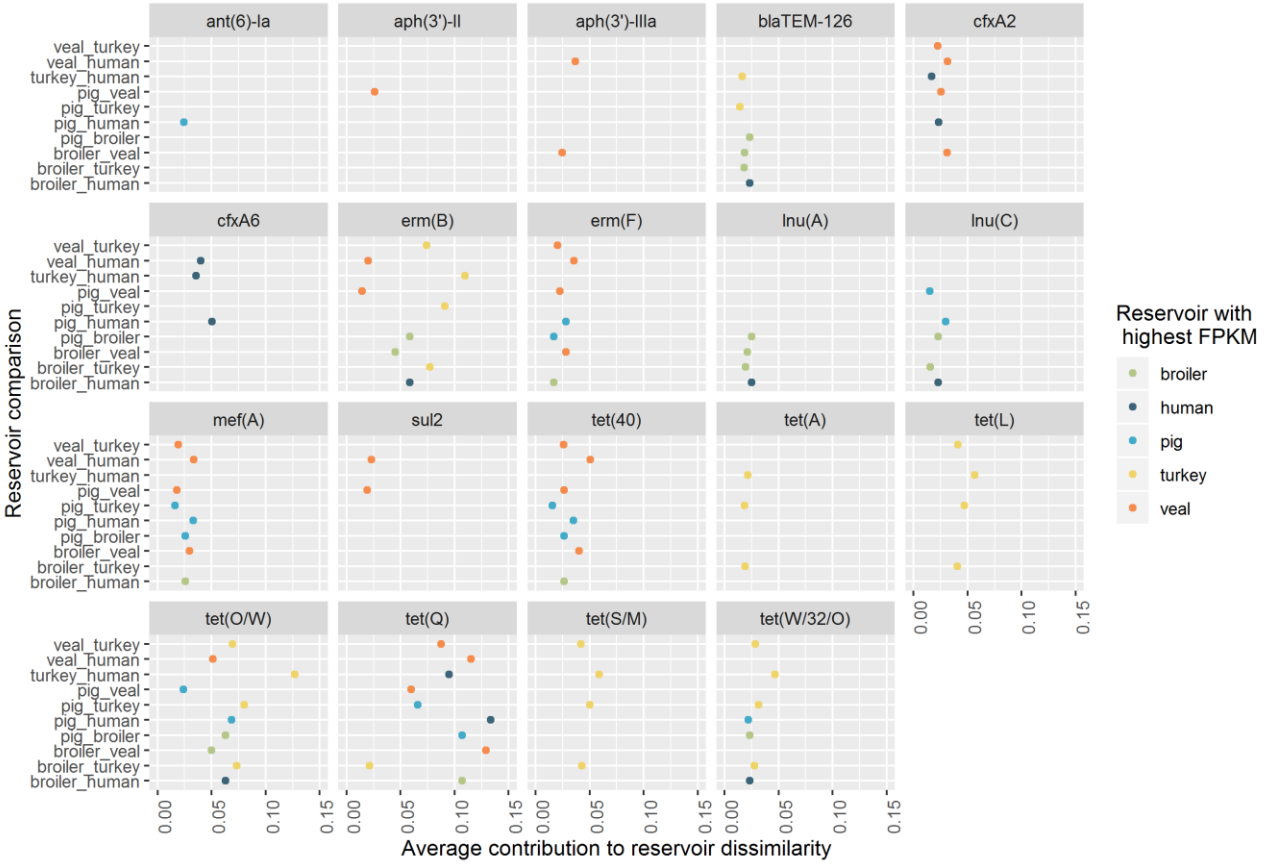


***Fig. 8.*** *The genes indicated represent the AMR determinants identified as major contributors to dissimilarity between reservoirs. The points represent the reservoir for which the AMR determinant had a higher relative abundance (FPKM) in those pairwise comparisons where the determinant was among the top ten contributors for reservoir dissimilarity. The x-axis represents the average proportional contribution of individual AMR determinants to the overall average dissimilarity between two reservoirs.*

***JRP3-WP5-T5:*** *Propose and assess a new paradigm for AMR surveillance in pigs (M18-M30)*

This is a newly designed tasks (in reaction to changed co-financing demands). Faecal samples collected under Denmark's AMR surveillance program (DANMAP), representing two past periods of 5 years (2000-2004) and 4 years (2015-2018) were metagenomically sequenced. Additionally, historical data of antimicrobial usage (AMU) in pigs and results of minimum inhibitory concentration assays performed under DANMAP were gathered.

We performed a multicorrelation analysis between the proportion of resistant isolates (*Campylobacter coli*, *Enterococcus faecium*, *Enterococcus faecalis*, *Escherichia coli* and different serotypes of *Salmonella enterica* subsp. *enterica*) and the treatment incidence for different antibiotic classes. The results indicate an inconsistent and most often non-significant correlation between antibiotic treatment incidence and the proportion of resistant isolates of the microorganism currently used as

indicator in surveillance – *E. coli* (see example for tetracycline resistance in **fig. 9**). Further work is needed to assess the impact in correlation of different units of treatment incidence.

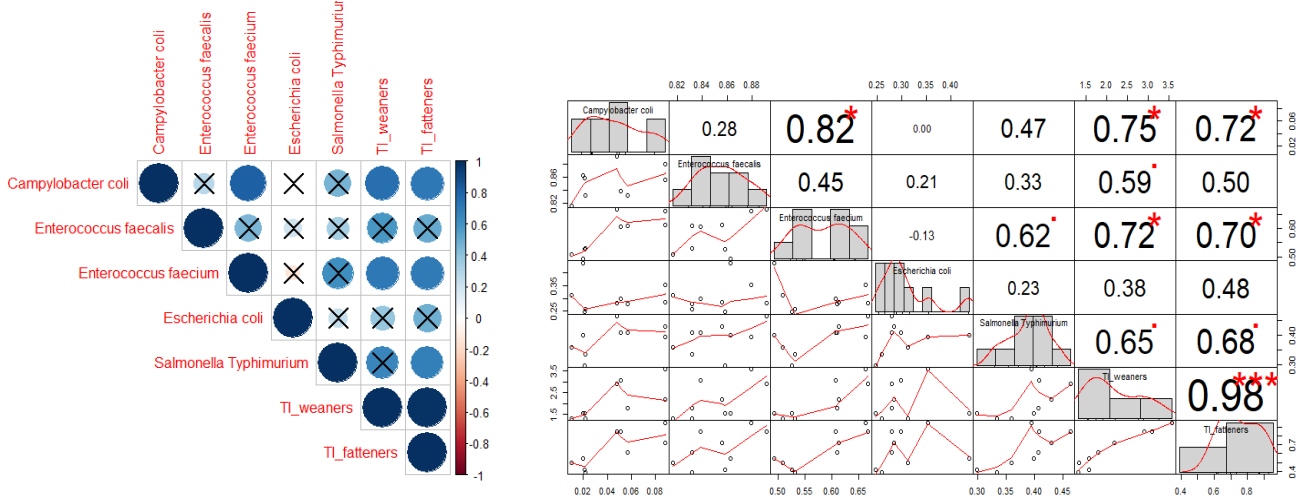


***Fig. 9.*** *The correlogram (left) and the correlation chart (right) indicate the results for the multicorrelation analysis (Spearman correlation coefficients) between proportions of resistant isolates and the treatment incidence with tetracycline in weaners and fatteners between the years 2001 and 2009 in Denmark. The crosses on the correlogram indicate correlation coefficients non-significant at the significance level of 5%. The correlation chart indicate correlation coefficients values between every two variables (top-right corner), with significant results indicated with red symbols (different levels of significance).The correlation chart also shows the distribution of each variable (diagonal) and the relationship between every two variables (bottom-left corner).*

The metagenomic sequences were mapped to the ResFinder database and results were selected for analysis based on a criterion of a minimum of 20% gene coverage. The reads are annotated by antibiotic class. See fig. 10 for the distribution of (non-normalized) read counts over time for each class and of different genes within a particular class (example with glycopeptide resistance). Future work will consist on normalization of the read counts, characterization of the resistomes, extending the multicorrelation analysis to include resistome observations (total abundance of resistance for an antibiotic class). We will also perform time-series analysis of the trends in antibiotic use, proportion of resistant isolates and total abundance of resistant genes, for each antibiotic class.

## WP6: Integration of information by Bayesian evidence synthesis

### JRP3-WP6-T1: Collect current status data (M1-M6)

This task has been completed, see annual report 2018

### JRP3-WP6-T2: Build evidence synthesis network for current status database (M1-M12)

This task has been completed, see annual report 2018

### JRP3-WP6-T3: Update evidence network for information developed in the other work packages (M12-M18)

We performed a literature meta-analysis on dose-response studies for *E. coli* carriership (presented at One health EHP Annual Scientific meeting, Dublin) (fig. 11)).

Updated source attribution calculation and updated data on dose-response relation for ESBL *E. coli* carriership have been included under the evidence synthesis framework. QMRA and EPI estimations of carriership are now more in agreement, constructively contributing to an overall estimation of human ESBL *E. coli* carriership with lower uncertainty than the separate models (fig.12). Models for different phases of the chain are now being extended. The slaughterhouse model is ready, but data collection for parameter estimates is still under way. The consumer phase model is finished, also in terms of data. The farm phase model results, being used as input, will be delivered by WP2 at short

notice. Different options for evidence synthesis are being discussed and evaluated, both from a theoretical and practical viewpoint.

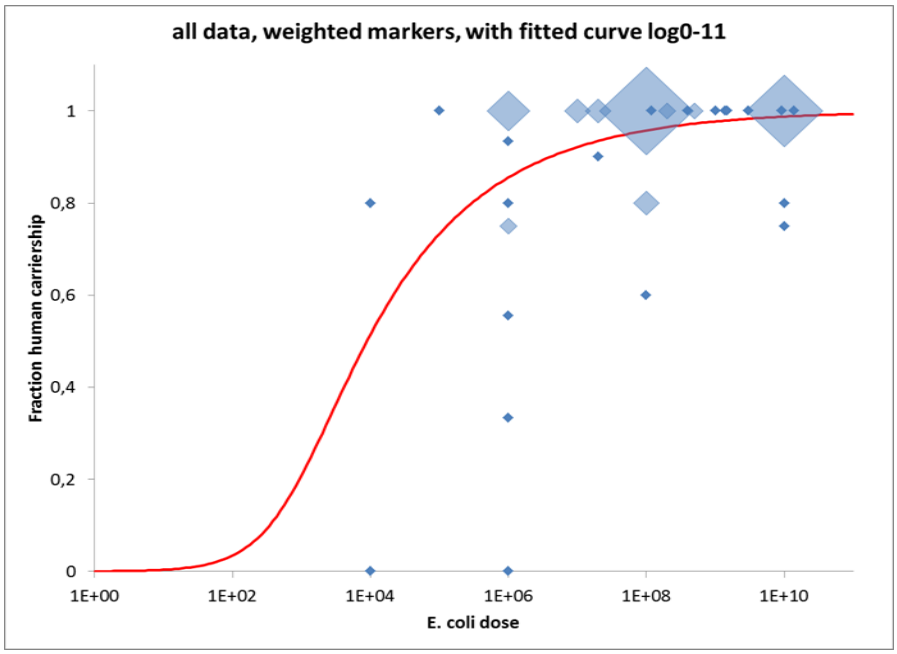


***Fig. 11**. Data and fitted dose-response curve for human ESBL colonisation. The probability of colonization for one colony-forming-unit ESBL E. coli was estimated at 3.74x10-4. The dose at which there is 50% probability of colonization after ingestion was estimated at 8846 CFU.*

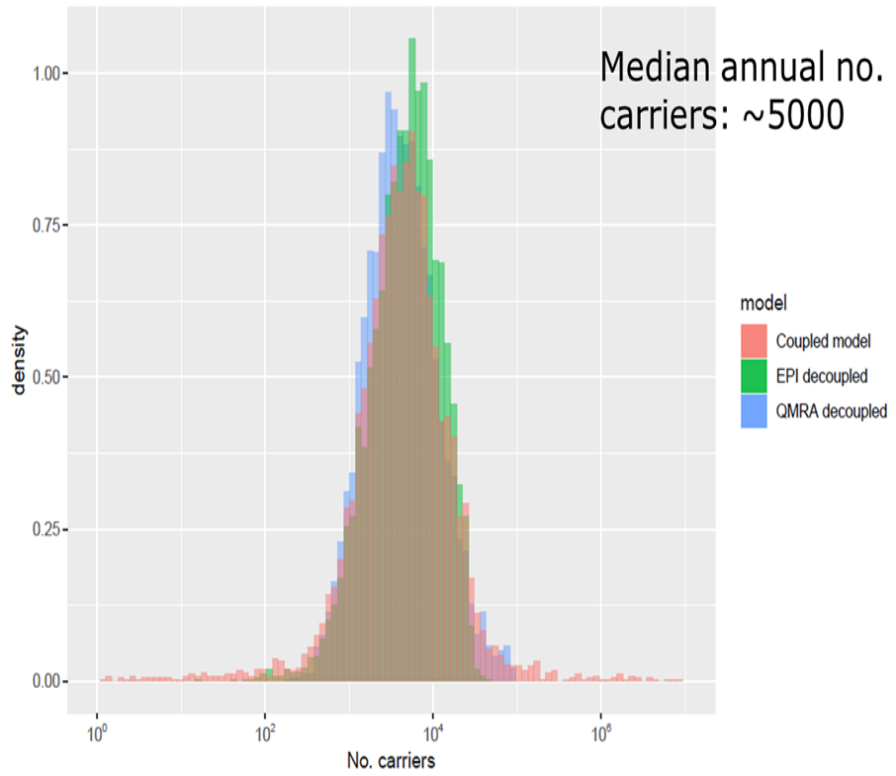


***Fig 12**. Distributions of the estimated number of human ESBL E. coli carriers in the general population as estimated by separate epidemiological model, separated QMRA model, and a combined model.*

***JRP3-WP6-T4**: Define endpoint for the current project and report results for the evidence synthesis model in its endpoint state (M18-24)*

Different options for evidence synthesis are being discussed and evaluated, both from a theoretical and practical viewpoint. These different options are and will be applied to pilot versions of the model and in the end to the full model when this is finished. Then implications in terms of policy and interventions will be evaluated in the end report.

## 3. Progress of the research project: milestones and deliverables

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| RaDAR | D-JRP3-0.2 | Second annual report | 24 | 24 | - | This report |
| RaDAR | D-JRP3-1.2 | Establishment of a database of field (meta)genomic data | 18 | 18 | - | COMPASS plasmid database & WGS dataset from environmental samples completed (see Deliverable document D-JRP-1.2) |
| RaDAR | D-JRP3-1.3 | Automated assembly pipeline integrating de novo plasmid reconstruction | 12 | 24 | - | |
| RaDAR | D-JRP3-1.4 | Test and parameterization of the assembly pipeline for metagenomics data | 24 | 24 | - | |
| RaDAR | D-JRP3-1.5 | Biological annotations of plasmid identified in the pipeline | 24 | 24 | - | |
| RaDAR | D-JRP3-1.6 | WGAS-based method for genomic data analysis | 30 | | - | The WGAS will be performed from M24 to M30 |
| RaDAR | D-JRP3-1.7 | Development of regression model for genomic data analysis | M30 | - | - | The regression model will be performed from M24 to M30, according project extension |
| RaDAR | D-JRP3-2.1 | Report/draft paper on generic PK/PD model | M30 | - | - | |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| RaDAR | D-JRP3-2.2 | Report/draft paper on on-farm model | M30 | - | - | |
| RaDAR | D-JRP3-2.3 | Report/draft paper on transmission model | M30 | - | - | |
| RaDAR | D-JRP3-2.4 | Report/draft paper on intervention strategies | M30 | - | - | |
| RaDAR | D-JRP3-3.1 | Inventory with models and related data in FSK standard | M30 | - | - | |
| RaDAR | D-JRP3-3.2 | Scientific report on a generic model for the chicken production chain | M30 | - | - | |
| RaDAR | D-JRP3-3.3 | Scientific report on an adapted model for the pork production chain developed | M30 | - | - | |
| RaDAR | D-JRP3-3.4 | Scientific report on an model for the exposure assessment of AMR through mussels | M30 | - | - | |
| RaDAR | D-JRP3-3.5 | Scientific report on a generic comparative exposure assessment model | M30 | - | - | |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| RaDAR | D-JRP3-4.1 | Model repository of state of the art ML methods for risk profiling available | 12 | 12 | - | |
| RaDAR | D-JRP3-4.2 | Recommended methods for risk profiling in investigations on antibiotic resistance available | 24 | - | 30 | |
| RaDAR | D-JRP3-5.1 | The'excess burden' approach for computing the burden of disease attributable to AMR: Application to urinary tract infection | 24 | - | 30 | Delayed; extension requested according the overall project extension |
| RaDAR | D-JRP3-5.2 | A proposal for a new paradigm for AMR surveillance (NEW) | 30 | - | 30 | |
| RaDAR | D-JRP03-5.3 | Publication (submitted) on source attribution ESBL E. coli general population (NEW) | 18 | - | 18 | |
| RaDAR | D-JRP3-6.1 | Publication on final evidence network | 24 | - | 30 | Delayed. Model and results are finished, publication is being written |
| RaDAR | D-JRP3-6.2 | Policy-targeted report | 24 | - | 30 | On schedule according project extension |

***Milestones***

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes/No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| RaDAR | M-JRP3-12 | Formulate CM/NGM (CVI, NCOH) and SD (RIVM) transmission models | 13 | Yes | - | |
| RaDAR | M-JRP3-13 | Produce case study (collected datasets) baseline results for PK/PD (ANSES) | 15 | Yes | - | Datasets have been collected to estimate unknown phamarcokinetic and pharmacodynamic parameters. Simulations of case studies has begun. |
| RaDAR | M-JRP3-14 | Test and validation of assembly pipeline on synthetic and reference genomic data | 15 | yes | - | Described in WP1-T2 Validation of our pipeline on a “test dataset” composed of 56 Salmonella enterica known genomes (Chromosome and Plasmid) |
| RaDAR | M-JRP3-15 | Establish connection between the PK/PD model and the on-farm model (ANSES, APHA) | 16 | Yes | - | Connections between the PK/PD and farm model formed part of a poster presented at the OHEJP ASM. |
| RaDAR | M-JRP3-16 | Establish connection between the PK/PD model and the on-farmmodel (ANSES, APHA) | 16 | Yes | - | |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes/No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| *RaDAR* | M-JRP3-17 | Calibrate models using data library (CVI) | 17 | Yes | - | Metagenomic sequencing of 9 years of DANMAP data completed (DTU) |
| RaDAR | M-JRP3-18 | Dataset with geno- and phenotypic AMR data and AMU data completed and Metagenomic sequencing of 9 years of DANMAP data | 17 | yes | - | |
| RaDAR | M-JRP3-19 | Develop model frameworks for PK/PD and on-farm model (ANSES, APHA) | 18 | Yes | - | The model framework has been completed, and formed part of a poster presented at the OHEJP ASM. |
| RaDAR | M-JRP3-20 | Simulations of the PK/PD model for green AMDs (ANSES) | 18 | No | 27 | This will be done. Extension to milestone requested |
| RaDAR | M-JRP3-21 | Concept for a improved model for the chicken production chain developed | 18 | yes | - | The concept was presented during the annual meeting |
| RaDAR | M-JRP3-22 | Concept for an adapted model for the pork production chain developed | 18 | Yes | - | |
| RaDAR | M-JRP3-23 | Concept for an model for the exposure assessment of AMR through mussels | 18 | Yes | - | |
| RaDAR | M-JRP3-24 | Analysis of HTS field data with assembly pipeline | 18 | Yes | 24 | Described in WP1-T2 Detection, identification and characterization of plasmids |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes/No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | from *Salmonella* enterica genomes |
| RaDAR | M-JRP3-25 | Analysis of field genomic data with WGAS-based method | 18 | No | 30 | GWAS will be performed from M24 to M30 |
| RaDAR | M-JRP3-26 | Analysis of field genomic data with regression model | 18 | No | 30 | The regression model will be performed from M24 to M30 |
| RaDAR | M-JRP3-28 | Formulate/investigate interventions (RIVM, NCOH, CVI, BfR43) | 21 | No | 28 | Delayed according project extension |
| RaDAR | M-JRP3-29 | Structural adaptations of the PK/PD model according to AMR mechanisms (ANSES) | 21 | No | 28 | Delayed according project extension |
| RaDAR | M-JRP3-30 | Concept on a generic comparative exposure assessment model | 21 | No | 28 | Delayed according project extension |
| RaDAR | M-JRP3-31 | Model development and model assessment completed | 21 | Yes | | Delayed according project extension |
| RaDAR | M-JRP3-32 | Assimilate project-generated data (NCOH) | 22 | No | 28 | Delayed according project extension |
| RaDAR | M-JRP3-33 | Assess hypothetical intervention measures using on-farm model (APHA) | 22 | No | 28 | Delayed according project extension |
| RaDAR | M-JRP3-34 | Final meeting and report | 24 | No | 30 | Delayed according project |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes/No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| RaDAR | M-JRP3-35 | Make recommendations to fill data gaps (RIVM, NCOH, CVI) | 24 | No | 30 | Delayed according project |

## 4. Publications and patents

Mughini-Gras, Lapo, Alejandro Dorado-García, Engeline van Duijkeren, Gerrita van den Bunt, Cindy M Dierikx, Marc J M Bonten, Martin C J Bootsma, et al. "Attributable Sources of Community-Acquired Carriage of Escherichia Coli Containing β-Lactam Antibiotic Resistance Genes: A Population-Based Modelling Study." *The Lancet Planetary Health* 3, no. 8 (August 2019): e357–69. https://doi.org/10.1016/S2542-5196(19)30130-5.

Simons, R., Viel, A., Swart A., Gavin, C., Dewar, R., Snary, E., Evers, E.G., Sanders, P. (2019). Building a Combined Model for Transmission of Antimicrobial Resistance Along the Pork Production Chain. Poster at *One Health EJP Annual Scientific Meeting*, Dublin, Ireland.

The paper entitled "Analysis of COMPASS, a new comprehensive plasmid database revealed prevalence of multireplicon and extensive diversity of IncF plasmids "is **under review in the journal Frontiers in Microbiology**. *The scripts and database of COMPASS, developed in the present study can be found in the following GitHub repository:* https://github.com/itsmeludo/COMPASS.

## 5. Impact & relevance

Antimicrobial resistance threatens the effective prevention and treatment of an ever increasing range of infections. It is an increasingly serious threat to global public health that requires action across all government sectors and society. Furthermore, resistance mechanisms emerge and spread globally. Containment of AMR spread is part of the EC action plan against the rising threats from AMR, and improvement of attribution and risk assessment activities should be encouraged according to EFSA. The most important aim of the RaDAR project is to adapt existing and produce new modelling methodologies to improve our understanding of the complex AMR problem. These include

- A curated repository of plasmid genomic data that can subsequently be used by other projects for various research questions,
- The development of new transmission and risk assessment models specifically for AMR transmission among different food chains,
- Comparison and evaluation of statistical methods used in the field of AMR research and suggest best practices,
- The (further) development of methods to estimate the "excess" burden of AMR
- To develop source attribution models for AMR for data and evidence-based intervention strategies
- To develop methodology to integrate risk assessment and epidemiological models in order to identify datagaps, to produce consensus estimates of risk, and to reduce uncertainty in the risk estimates.

All these activities have a strong focus on developing these methodologies rather than actually providing true answers, which very much depend on the data available and likely vary between countries. This provides the EJP network (and beyond) with state-of-the-art innovative model frameworks that can subsequently be applied in various contexts with the benefit that harmonized frameworks are used.

## 6. Interactions with other JRPs/JIPs or with external (EU or national) relevant project

- The RaDAR model inventory uses the FSK standard that has been developed by the **RAKIP initiative**. In order to create a stable and sustainable result, it was needed to anticipate future developments in the FSK standard. For this reason there's been a close collaboration with the RAKIP initiative.

- The RADAR poultry risk model was demonstrated in a workshop with **German stakeholders** (poultry production, slaughterhouses, retailers) in the context with the national research project EsRAM (funded by Ministry of Agriculture). Around 20 experts participated.
- We are currently working on testing ML methods on a data set from Norwegian farms to test the ability of methods to detect risk factors for AMR presence and, if possible, quantifiable correlations between resistotypes measured as MIC values for different antimicrobials, aiming for a synergy between the RaDAR and **ARDIG** EJPs.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

No ethical recommendations were given for the RaDAR project.

## 8. List of critical risks

| Description of risk | Yes/No |
|---|---|
| Loss of key-persons (staff and / or leaders) | yes |
| Delay in work plan execution | yes |
| Conflicts within the consortium | No |
| Lack of commitment of partners | No |
| Delay in duties, tasks or reporting | No |
| Poor intra-project (JRP) relationship | No |
| Potential entry/exit of partners | No |
| Other risks (please describe) | No |

### Additional information

ISS left the RADAR consortium. However this will not lead to critical problems since all tasks where SSI should work on are covered by other partner.

## 9. List of dissemination and communication activities

| | |
|---|---|
| Name of the activity: | The EJP RaDAR project: data integration and modelling frameworks for increased quantitative understanding of AMR risk, source attribution and burden.(oral presentation) |
| Date: | 22/05/2019 |
| Place: | Teagasc Conference Centre, Ashtown, Dublin |

| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |

| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
|---|---|---|---|
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Building a Combined Model for Transmission of Antimicrobial Resistance Along the Pork Production Chain (Poster) | | |
| Date: | 21 – 24 May 2019 | | |
| Place: | Teagasc Conference Centre, Ashtown, Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Press release on humans as most important reservoir of ESBL-producing E. coli | | |
| Date: | Sept 2019 | | |
| Place: | Bilthoven, NL | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | yes | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | Whole of NL | Other | |
| Policy Makers | | | |

| | |
|---|---|
| Name of the activity: | Poultry risk model demonstration German stakeholder |
| Date: | Sept 2019 |
| Place: | Bilthoven, NL |

| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |

| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
|---|---|---|---|
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | 20 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Integration QMRA en Epidemiology | | |
| Date: | 9-1-2020 | | |
| Place: | - | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | Yes (policy report) |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 10 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Antimicrobial resistance in the environment: new methods, ongoing work and further ideas | | |
| Date: | June 12 2019 | | |
| Place: | Namibia | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | y |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | Yes (policy report) |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 15 | Media | |
| Industry | 7 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | *RaDAR – Risk and disease burden of antimicrobial resistance* | | |
| Date: | October 9th, 2019 | | |
| Place: | Meeting of the SCAR CWG on Animal Health & Welfare Research, DTU, Copenhagen, 8-9 October 2019 | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | +/- 10 | Media | |
| Industry | +/- 10 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | +/- 40 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | One Health EHP ASM 2019 - Comprehensive Database of Complete Bacterial Plasmids (Poster) | | |
| Date: | 21 – 24 May 2019 | | |
| Place: | Teagasc Conference Centre, Ashtown, Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |
| | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Foodborne pathogens & whole genome sequencing: impact on public health protection - Curated Database of Complete Bacterial Plasmids (Poster) | | |
| Date: | 26-28 March 2019 | | |
| Place: | Maison de la RATP - Paris | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |
| | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Société Française de Microbiologie 2019 - Global analysis of betalactams resistant plasmids (Poster) | | |
| Date: | 30/09 – 2/10 | | |
| Place: | Cité des sciences et de l'industrie, Paris | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 710 | Media | |
| Industry | 12 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |
| | | | |

## *10. List of planned tele- or video conferences, face to face meetings in the next year*

A final scientific symposium is planned for summer/autumn 2020 I which the results of RADAR will be disseminated. We are exploring the combination with the final meeting of the EJP JRP ARDIG.

## JRP04 - MADVIR

In contrast to the other projects reported here, MAD-VIR terminated at the time as foreseen in the proposal (after 24 months). The following report is therefore the final report.

### 1. Consortium composition

**1. SSI, Denmark (participant no. 13). Project Coordinator**

1a) Anders Fomsgaard, MD DMSc Professor, Chief of Virus Research & Development, Statens Serum Institut (SSI), 5 Artillerivej, DK-2300 Copenhagen, Denmark, T: +45-32683460, M: +45-40634638, Email: afo@ssi.dk.

1b) Maiken Worsøe Rosenstierne, Statens Serum Institut (SSI), 5 Artillerivej, DK-2300 Copenhagen, Denmark, T: +45-32683971, MWR@ssi.dk

**2. ANSES, France (participant no. 1)**

2a) Sylvie Lecollinet PhD, Chef de Projet Recherche, Laboratoire de Santé Animale de Maisons Alfort, JRU 1161 VIROLOGY - Neurovirology of Zoonoses Team, 14 Rue Pierre et Marie Curie, 94701 Maisons-Alfort Cedex, Tel : 01 43 96 71 11, email : sylvie.lecollinet@anses.fr.

2b) unité pathologies virales des poissons, France: Dr Laurent Bigarré, Fish virologist, laboratoire de Ploufragan-Plouzané, Technopôle Brest Iroise, site IFREMER, 29 280 Plouzané, France, T (33 0) 2 98 22 49 82, laurent.bigarre@anses.fr.

2c) Sara Moutailler PhD, Laboratoire de Santé Animale de Maisons Alfort, UMR BIPAR - Equipe Vectotiq, 14 Rue Pierre et Marie Curie, 94701 Maisons-Alfort Cedex, Tel : 01 49 77 46 50, email : sara.moutailler@anses.fr

**3. PIWET, Poland (participant no. 35)**

3a) DVM, PhD Jowita Samanta Niczyporuk, National Veterinary Research Institute, Department of Poultry Viral Disease, Al. Partyzantow 57, 24-100 Pulawy, Poland, phone: 608 442 522, +48 81 889 30 76, e-mail: Jowita.niczyporuk@piwet.pulawy.pl,

3b) Artur Rzeżutka DVM, PhD, ScD, National Veterinary Research Institute, Department of Food and Environmental Virolog, Al. Partyzantow 57, 24-100 Pulawy, tel. +48 81 8893036. arzez@piwet.pulawy.pl

**4. APHA, Animal and Plant Health Agency (participant no. 20):**

4a) Prof Falko Steinbach, MRCVS, Head of Mammalian Virology Group, Virology Department, Tel: +44 1932 357-566, falko.steinbach@apha.gsi.gov.uk Website: www.gov.uk/apha | Twitter: @APHAgovuk | Facebook: aphagov , Address: Woodham Lane, New Haw, Addlestone, KT15 3NB, UK

4b) DVM, PhD, Akbar Dastjerdi, Department of Virology, Animal and Plant Health Agency (APHA), Akbar.Dastjerdi@apha.gov.uk

**5. NCE, National Center for Epidemiology, Budapest, Hungary (participant no. 24):**

5a) Mária Takács, PhD, Deputy Director-General , Honorary Professor, National Center for Epidemiology, Division for Virology, Albert Flórián út 2-6, Budapest, Hungary, H-1097, T: +36 1 476 1383, takmar@gmail.com

5b) Anna Nagy, National Public Health Centre; Budapest, National Reference Laboratory for Viral Zoonoses, +36/1/476 1100/2111, Albert Flórián út 2-6.H-1097 Budapest; Hungary, nagy.anna@nnk.gov.hu

**6. VRI, Veterinary Research Institute, Czech Republic (participant no. 8):**

Daniel Ruzek, Ph.D. Associate Professor of Medical Microbiology, Head of the Department of Virology, Veterinary Research Institute, Hudcova 70, CZ-62100 Brno, Czech Republic, and Head of the Laboratory

of Arbovirology, Academy of Sciences of the Czech Republic, Institute of Parasitology, Branisovska 31, CZ-37005 Ceske Budejovice, Czech Republic, ruzekd@paru.cas.cz, ruzek@vri.cz, T: +420-38-777-5458;

**7. Surrey Univ. UK (participant no. 22)**

7a) Daniel Horton MA VetMB MSc PhD MRCVS DipECZM, European Veterinary Specialist in Zoological Medicine (Wildlife Population Health), Lecturer, Veterinary Virology, School of Veterinary Medicine, Faculty of Health & Medical Sciences, Vet School Main Building, Daphne Jackson Road, University of Surrey, Guildford, GU2 7AL, Tel: 01483 689888, Fax: 01483 686711, http://www.surrey.ac.uk/vet/index.htm, d.horton@surrey.ac.uk,

7b) Roberto Marcello La Regione, Department of Pathology and Infectious Disease, University of Surrey. Director of the Veterinary Pathology Centre, University of Surrey. r.laragione@surrey.ac.uk.

7c) Elizabeth Royall PhD, Visiting Researcher, School of Veterinary Medicine, Faculty of Health and Medical Sciences, Vet School Main Building, Daphne Jackson Road, University of Surrey, Guildford GU2 7AL UK, Tel: +44 (0)1483 684315, e.royall@surrey.ac.uk

**8. IZSLER (Brescia headquarter), Italy (participant no. 29):**

Antonio Lavazza, head of the Virology Unit , Direttore Sanitario, Istituto Zooprofilattico Sperimentale, Della Lombardia e dell'Emilia Romagna, Via Bianchi 9, 25124 Brescia (Italy), Tel +390302290234, cell +393351436343, www.izsler.it, Antonio.lavazza@izsler.it

**9. IZSAM eramo, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale"(participant no. 28):**

Giovanni Savini, Scientific Director, Head of Virology Department, OIE and National Reference Laboratory for BT, OIE and National Reference Laboratory for WND, National Reference Centre for Foreign Animal Diseases, Via Campo Boario, 64100 Teramo, Italy, g.savini@izs.it

**10. INIA, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (participant no. 14)**

10a) Miguel Ángel Jiménez-Clavero, Head of Service for P3 Coordination and Head of the Group of Emerging and Infectious Diseases, Animal Health Research Center (CISA), Ctra Algete-El Casar, s/n, 28130, Valdeeolmos, Madrid, Spain, majimenez@inia.es.

10b) Jovita Fernandez-Pinero, Centro de Investigación en Sanidad Animal (CISA-INIA), Madrid, Spain. fpinero@inia.es.

10c) Dr. Francisco Llorente, Centro de Investigación en Sanidad Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CISA), Ctra Algete-El Casar s/n, Valdeolmos, Spain. dgracia@inia.es

## 2. *Summary of the work carried out in the JRP*

The aim of the MAD-Vir Project was to further develop and validate a metagenomics PanVirus microarray for fast detection of viral FBZ agents and emerging threats. The PanVirus microarray provides a rapid, simultaneous and unbiased detection of all known virus species and identification of novel virus types or strains belonging to currently known virus families (viral genomes present in Genbank). This metagenomics PanVirus microarray is very fast, easy and cheap (compared to NGS), does not require any bioinformatics skills or super computers and can eliminate the need for a specific clinical hypothesis regarding the disease causing viral pathogen.

The PanVirus microarray technology has been implemented in four EU reference laboratories and a standardized PanVirus microarray S.O.P has been harmonized between three of the four laboratories. Ring-test trials and molecular EQAs have ensured the specificity and sensitivity of the method in all four laboratories. Several other European reference laboratories have expressed an interest in implementing the PanVirus microarray technology and it is expected that at least five additional Institutes will implement the technology in 2020, beyond the end of the MAD-Vir project.

In the MAD-Vir project, 1210 samples of human and animal origin have been analysed with the PanVirus microarray. Of these samples, 432 samples contained a known virus content previously identified by either qPCR/RT-qPCR, sequencing, negative EM staining, serology (ELISA) or other microarrays. The remaining samples contained an unknown virus content that either had not previously been analysed or was tested negative using standard diagnostic methods.

The PanVirus microarray detected 94% of all PCR confirmed positive samples with a known viral content. A 100% detection was observed for cell culture isolates, gastrointestinal samples, respiratory samples, serum samples, plasma samples and external materials such as skin swaps, biopsies etc. For urine samples and sample belonging to the central nervous system and internal organs the detection was 91%, 95% and 98%, respectively. The lowest detection was observed in samples from water-living animals such as fish (40%) and whole blood-samples (53%). A key element in the PanVirus microarray S.O.P is whole genome amplification (WGA) of DNA virus and whole transcriptome amplification (WTA) of RNA virus. Analysis of the WTA/WGA showed that the amplification was inhibited/lowered in fish and whole blood samples, for unidentified reasons. These results show that almost all sample materials from all species can be analysed by the PanVirus microarray.

The metagenomics PanVirus microarray also detected additional unknown co-infections in 48% of the samples with a known virus content. Co-infections were predominantly identified in domestic farm animals (70%) and in samples from the gastrointestinal system (76%) with up to 10 different viruses belonging to different virus genus and species. In the samples with unknown etiology, the PanVirus microarray identified virus in 38% of these samples with a viral content of up to 10 different viruses. In samples from insects and domestic farm animals, 64% and 58% respectively, were virus-positive. These finding are currently being investigated further by the interested MAD-Vir partners and will continue beyond the end of the MAD-Vir project.

In summary, the MAD-Vir project showed that the dispersed, implemented and harmonized PanVirus microarray method/technology has a clinical sensitivity comparable to diagnostic RT-qPCR/qPCR assays in all sample materials tested except fish and whole blood. These results show that the metagenomics PanVirus microarray is a fast, easy, and inexpensive alternative to NGS for detection of all unknown viral FBZ agents, multiple viruses and novel emerging threats, might aid in an early identification of zoonotic pathogens, and will aid in sustained European outbreak preparedness and response.

## *3. Work carried out, the main results*

This part is confidential. To be disclosed after publication.

## 4. Achievements of the JRPs

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| MAD-Vir | D-JRP4-1.1a | Kick-off meeting | 6 | 6 | - | Deliverable available on MAD-Vir group of the OHEJP website. |
| MAD-VIR | D-JRP4-1.1b | First annual meeting for the participating Institutes | 24 | 17 | - | The first annual meeting (2nd meeting in total) was held at the 1st ASM OHEJP in Dublin in May. Deliverable available on MAD-Vir group of the OHEJP website. |
| MAD-Vir | D-JRP4-2.1 | Collection of samples (maximum 1500 samples in total from year 2017 to 2019) | 18 | 18 | - | Deliverable available on MAD-Vir group of the OHEJP website. |
| MAD-Vir | D-JRP4-3.1 | Implementation of MAD-VIR to INIA and APHA | 6 | 6 | - | Deliverable available on MAD-Vir group of the OHEJP website. |
| MAD-Vir | D-JRP4-3.2 | QA-validation of MAD-VIR to INIA and APHA | 12 | 10 | - | Deliverable available on MAD-Vir group of the OHEJP website. |
| MAD-Vir | D-JRP4-3.3 | MAD-VIR microarray analysis of minimum 500 samples | 12 | 12 | - | Deliverable available on MAD-Vir group of the OHEJP website. |
| MAD-VIR | D-JRP4-3.4 | MAD-VIR microarray analysis of all collected samples (max. 1500) | 24 | 24 | - | A total of 1210 samples have been analyzed in the MAD-VIR project. Deliverable available on MAD-Vir group of the OHEJP website. |
| MAD-Vir | D-JRP4-4.1 | Functional Website | 12 | 12 | | The common EJP website (https://onehealthejp.eu) is used for the MAD-Vir project. All |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | | | | - | documents, reports, newsletters, metadata and results are available on MAD-Vir group of the OHEJP website (https://onehealthejp.eu/groups/mad-vir/). All partners joined the website |
| *MAD*-VIR | D-JRP4-4.2 | Fully updated Website | 24 | 24 | - | All documents, reports, newsletters, metadata and results are available on MAD-Vir group of the OHEJP website (https://onehealthejp.eu/groups/mad-vir/). |

***Milestones***

| *JRP name* | *Milestone number* | *Milestone name* | *Delivery date from AWP* | *Achieved (Yes / No)* | *If not achieved: Forecast achievement date* | *Comments* |
|---|---|---|---|---|---|---|
| *MAD*-Vir | M-JRP4-1 | Collection of samples (minimum 750 samples) | 12 | yes | - | The final sample collection is described in the final metadata file, the D-JRP4-3.4 and the final report available on MAD-Vir group of the OHEJP website (https://onehealthejp.eu/groups/mad-vir/). |

## 5. Publications and patents

Petersen, Andreas, Maiken Worsøe Rosenstierne, Morten Rasmussen, Kurt Fuursted, Henrik Vedel Nielsen, Lee O'Brien Andersen, René Bødker, and Anders Fomsgaard. "Field Samplings of Ixodes Ricinus Ticks from a Tick-Borne Encephalitis Virus Micro-Focus in Northern Zealand, Denmark." *Ticks and Tick-Borne Diseases* 10, no. 5 (August 2019): 1028–32. https://doi.org/10.1016/j.ttbdis.2019.05.005.

**The metagenomics Panvirus microarray as a diagnostic tool for unknown emerging virus in a one-health perspective (working title).** Rosenstierne MW, Lecollinet S, Gonzalez G, Bigarré L, Moutailler S, Niczyporuk JS, Rzeżutka A, Kozyra I, Steinbach F, Dastjerdi A, Takács M, Nagy A, Ruzek D, Horton DL, Royall E, La Ragione R, Lavazza A, Savini G, Jiménez-Clavero MÁ, Fernandez-Pinero J, Llorente F, Fomsgaard A. Manuscript in preparation.

## Additional output

The most relevant dissemination are highlighted here, although also reported under point 6.

Rosenstierne MW, MAD-Vir consortium collaborates, Fomsgaard A. **Metagenomic Array Detection of emerging Virus in EU (MAD-Vir)**. Meeting: 16th Smögen Summer Symposium on Virology, Smögen, Sweden, 21-24/08/2019

Rosenstierne MW, MAD-Vir consortium collaborates, Fomsgaard A. **Metagenomic Array Detection of emerging Virus in EU (MAD-Vir)**. Meeting: 4th EVD LabNet annual meeting, Thessaloniki, Greece, 4-6/11/2019

Rosenstierne MW, MAD-Vir consortium collaborates, Fomsgaard A. **Metagenomic Array Detection of emerging Virus in EU (MAD-Vir)**. Meeting: SVA research day, Uppsala, Sweden, 12/11/2019

Rosenstierne MW, Lecollinet S, Bigarré L, Moutailler S, Gonzalez G, Niczyporuk JS, Rzeżutka A, Kozyra I, Steinbach F, Dastjerdi A, Takács M, Nagy A, Ruzek D, Horton DL, La Ragione R, Lavazza A, Savini G, Jiménez-Clavero, MA, Fernandez-Pinero J, Llorente F, Fomsgaard A. **Metagenomic Array Detection of emerging Virus in EU (MAD-Vir).** Poster at 1st OHEJP ASM, Dublin, Ireland, 2019

Jiménez Clavero, M.A.; Llorente, F.; Fernández Pinero, J.; Fernández-Pacheco, P.; Cano-Gano-, C; Gallardo-Frontaura, C.; Pérez Ramírez, E.; Fomsgaard, A. and Rosenstierne, M.W. **Testing of Pan-virus Metagenomic Array Detection assay (MAD-Vir) using panels of samples from animals experimentally infected with known viruses.** Meeting: 13th EPIZONE Annual Meeting, Berlin, Germany, 26-28/08/2019.

Dastjerdi A. Detection of porcine circovirus-3 (pcv-3) in stillborn piglets with multi-systemic inflammation. Poster at Epizone, Berlin, Germany, 26-28/08/2019.

## 6. One Health Impact

The metagenomics PanVirus microarray was developed at SSI and is a very fast and simple non-NGS-based metagenomic method for detection of unknown virus in human and animal samples. The major advantages of the method are;

Analysis time (1½ day from sample to result)

A simple excel based data analysis method (does not require any bioinformatics skills or super computers)

Simple basic laboratory equipment (Hybridization oven and Array scanner for glass slides containing a 532 nm laser (Cy3) and a 633/647 nm laser (Cy5))

Simultaneous and unbiased detection of different virus species and identification of novel virus types or strains belonging to currently known virus families (viral genomes present in Genbank)

The simplicity of the method is a huge benefit when used as a screenings tool for detection of unknown viral FBZ agents, emerging threats and identification of new (zoonotic) human and animal pathogens.

The MAD-Vir project has validated the metagenomics PanVirus microarray, using a whole range of samples materials from different animal and human species. This has not only brought MED-VET partners closer together but also resulted in the identification of new virus types and viral pathogens in many different animal species. The many new results are currently being investigated in collaborations between the MED-VET partners, which will continue beyond the end of the MAD-Vir project.

The MAD-Vir project has also resulted in capacity building, training and implementation of a harmonized metagenomics technology in 3/4 MED-VET Institutes. The simplicity and robustness of the PanVirus microarray method have resulted in an easy implementation in the different laboratories, and the analysis of ring trials and molecular EQAs have, and will in the future ensure a specific and sensitive method. One disadvantage of the method is that viruses mutate which may influence the detection of new virus strains; however, the microarray can easily be updated with new probes for specific virus strains. All new future updated PanVirus microarray versions will be available for laboratories using the technology (free of charge).

The dissemination of the MAD-Vir project at International meetings have resulted in an enormous interest in the technology and several other European MED-VET Institutes and laboratories have expressed an interest in implementing the method. It is expected that at least five additional Institutes will implement the technology in 2020 (IZSAM, Italy; CSMM, Cyprus; HOH, Finland; DNPHI, Netherlands; IRBA, France). Hence, the implementation of the metagenomics PanVirus microarray as a virus detection method will continue beyond the end of the MAD-Vir project, which will lead to sustainability of the PanVirus microarray as a diagnostic assay.

The MAD-Vir project has so far resulted in an enormous interest within the scientific MED-VET community. It is expected that the publication of the manuscript describing the MAD-Vir project (and all the following manuscripts) will result in even more interest in the PanVirus microarray as a screening tool for detection of viral FBZ agents, emerging threats, multiple infections and identification of new (zoonotic) pathogens.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements Ethical Reviewers in January 2018 | Measures and actions taken by Project Leaders at the end of 2018 | Comments Ethics Advisors in January-February 2019 | Measures and actions taken (2019) |
|---|---|---|---|
| The applicants must confirm the national ethical approval for patient access and any adverse effect protocol as appropriate | The MAD-Vir participants confirm that National ethical approval has been obtained.<br>In Czech Republic (VRI) human patients has signed informed consent for the analysis.<br>In accordance with the Hungarian Scientific and Research Ethics Committee; The Chief Medical Officer of Hungary approved the use of human samples and sending them to the SSI.<br>All information regarding the diagnosis of HIV has been removed from analysis. | EA did not comment | |
| The applicants must confirm that ethics approvals for the use of biological samples have been sought | The MAD-Vir participants confirm that ethics approvals for the use of biological samples has been obtained.<br>In accordance with the Hungarian Scientific and Research Ethics Committee; The Chief Medical Officer of Hungary approved the use of biological samples and sending them to the SSI.<br>ANSES – Protocol evaluated by ANSES/ENVA/UPE ethic committee in February 2018, with a positive evaluation | Satisfactory reply | |
| The applicants must document the safety mitigation measures in place to protect the staff. | All samples are inactivated or purified before shipment. Inactivation is performed according to (Rosenstierne et al. 2016; Vinner et al., 2009)<br>Samples provided by ANSES Animal Health Laboratory are handled in a BSL-3 laboratory, Icube ZO, conceived for the handling of class 3 zoonotic viruses. SOPs pertaining to the functioning of this BLS-3 are available upon request.<br>The INIA-CISA group works in a high-biosecurity (BSL-3) laboratory compliant with the biosafety | Satisfactory reply | |

| Requirements Ethical Reviewers in January 2018 | Measures and actions taken by Project Leaders at the end of 2018 | Comments Ethics Advisors in January-February 2019 | Measures and actions taken (2019) |
|---|---|---|---|
| | and biosecurity rules of the INIA-CISA (Biosafety Reference Laboratory for FAO). Biorisk assessment of the different pathogens used in the project has been evaluated by INIA-CISA Biosafety & Biocontainment service. | | |
| The applicants must confirm the compliance with GDPR. | The MAD-Vir participants confirm the compliance with GDPR.<br>Data sharing between MAD-Vir participants is anonyms and coded with the name of the Institute and a consecutively number.<br>All sample data in results sheet in the private group "MAD-Vir" at the OHEJP website are anonyms coded with the name of the Institute and a consecutively number. | Satisfactory reply, however the beneficiaries must provide the contact address of the Data Protector Officer of the institution in charge of processing the data obtained. | The contact of the DPO at SSI is Helle Ginnerup-Nielsen (hgn@sum.dk). |
| The applicants must confirm the application of 3Rs and the ethical approvals (approval letters, etc) for animal work at a national / institutional level.<br>The applicants must confirm how they will apply the 3Rs across the programme of work (e.g. clarify the sampling contribution in terms of numbers or endpoints, etc - Reduction management protocols for the programme, as well as the standard project level). Please elaborate. As appropriate this should include 3R protocols for feral / wild animals as well as laboratory animals (e.g. IZSLER). | The MAD-Vir applicants confirm that the application of 3Rs and the ethical approvals for animal work at a national / institutional level has been obtained.<br>The samples from IZSLER included in the MAD-VIR project all come from diagnostic routine work that has been performed at Veterinary Public Institute to support the institutional stakeholders. None of the tested samples were obtained from experimental animals or experimental infections. Anyhow, even in such cases, all the experiments with animals should be previously authorized by Ministry of Health in agreement with the National Law "DECRETO LEGISLATIVO 4 marzo 2014 , n. 26", which is the application of the European Law 2010/63/UE.<br>Animal samples from INIA-CISA used for this project have been obtained from experimental procedures performed at the group's lab (INIA- | Satisfactory reply<br><br>No specific details are given on how the 3Rs are applied.<br><br>The team can provide the licence approval number / ethical approval code, this is not provided. They have included information on the approving body. An approval letter with date and code could be provided. | None of the animals investigated come from experimental trials performed in IZSLER, since all the samples (either organ tissue from necropsied carcasses or excretion and secretions taken from ill, live animals) have been taken for diagnostic purposes and examined within the routine diagnostic service offered by IZSLER to different stakeholders (farmers, food industries, private citizens, public veterinary officers, zoos, rehabilitation centres, universities etc etc).<br>Since the whole sampling was not specifically programmed as experimental study and none of the animal samples included in the project were obtained from animals maintained in experimental condition at IZSLER but originating from diagnostic activity, we believed that it does not fall in the provisions of the National Law (e.g DLSG 4/3 2014 , n. 26. Application at national level of the EU Directive 2010/63/UE). As consequence, we cannot |

| Requirements Ethical Reviewers in January 2018 | Measures and actions taken by Project Leaders at the end of 2018 | Comments Ethics Advisors in January-February 2019 | Measures and actions taken (2019) |
|---|---|---|---|
| | CISA). These procedures were approved and supervised by the Ethics and Animal Welfare Committee of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) in strict accordance with the guidelines of the European Community 86/609/CEE. | | include any approval by ethical committee or provide any specific details on how the 3Rs are applied.<br>For samples obtained from experimental procedures performed at the group's lab (INIA-CISA). These procedures were approved and supervised by the Ethics and Animal Welfare Committee of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) in strict accordance with the guidelines of the European Community 86/609/CEE. 3Rs rules were always applied in the experiments reducing the number of animals to the minimal required. Experiments have been performed only if an alternative method was not possible and avoiding the repetition of experiments previously performed by other laboratories. Ethical approval codes for the experiments used for samples in this project are: PROEX 212/17; PROEX 045/16; PROEX 019/16; PROEX 068/15; PROEX 191/14; 10/384153.9/12; 10/389203.9/12; 10/389200.9/12 and MAJ/ce2 15-10-10 |

## 8. List of dissemination and communication activities

| Name of the activity: | Press release | | |
|---|---|---|---|
| Date: | April 20th 2018 | | |
| Place: | www.SSI.dk | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | yes | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | yes | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation and implementation of PanVirus microarray at INIA | | |
| Date: | April 16th-25th 2018 | | |
| Place: | INIA, Valdeolmos, Spain | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | yes | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 5 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Implementation of PanVirus microarray at PIWET | | |
| Date: | June 11th-20th 2018 | | |
| Place: | PIWET, Pulawy, Poland | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | yes | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 5 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at PIWET (a scientific meeting gathering the institute employees and members of the Polish Society of Veterinary Sciences) | | |
| Date: | June 14th 2018 | | |
| Place: | PIWET, Pulawy, Poland | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 30 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Implementation of PanVirus microarray at APHA | | |
| Date: | June 25th 2018 | | |
| Place: | APHA, , Addlestone, UK | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | yes | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 1 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Poster at the Danish Virology society | | |
| Date: | November 22nd 2018 | | |
| Place: | SSI, Copenhagen, Denmark | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 100 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at a Network meeting at Lindholm | | |
| Date: | February 2nd 2019 | | |
| Place: | The island of Lindholm, Denmark | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 20 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at the One Health course at the Veterinary Institute | | |
| Date: | April 1st 2019 | | |
| Place: | The Veterinary Institute, Copenhagen, Denmark | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |

| | | | |
|---|---|---|---|
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 75 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at the MAD-Vir 1st annual meeting | | |
| Date: | May 21st 2019 | | |
| Place: | At the OHEJP 1st ASM, Dublin, Ireland | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 12 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Poster the 1st ASM of OHEJP | | |
| Date: | May 21st-24th 2019 | | |
| Place: | Dublin, Ireland | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |

| | | | |
|---|---|---|---|
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at the Swedish virology society | | |
| Date: | August 21st 2019 | | |
| Place: | Smögen, Sweden | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 150 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at the EVD-LabNet meeting | | |
| Date: | November 4th 2019 | | |
| Place: | Thessaloniki, Greece | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |

| | | | |
|---|---|---|---|
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 80 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at SVA research day | | |
| Date: | November 12th 2019 | | |
| Place: | Uppsala, Sweden | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 60 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at EPIZONE 2019 meeting | | |
| Date: | 28/8/2019 | | |
| Place: | Berlin (Germany) | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |

| | | | |
|---|---|---|---|
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 200 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Poster presentation at EPIZONE 2019 meeting | | |
| Date: | 28/8/2019 | | |
| Place: | Berlin (Germany) | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 200 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

## JRP05 - TOXDETECT

### 1. Summary of the work carried out in year 2 (January to December 2019)

Important deliverables of the Tox-Detect project have been released in 2019. Among these ones, the consortium decided to update (version 2) the reference strain collection of *S. aureus*, *B. cereus* and *C. perfringens* including new strains of *S. aureus* and *B. cereus* to focus on the new targets designated during the second general meeting of the project which took place on March 2019. It has also been decided to launch MALDI-ToF analyses to generate a library of reference spectra. For this purpose, all partners were invited to prepare biomasses according to the commonly approved protocol and to send them to ANSES platform MALDI ToF. The MALDI ToF platform received strains and associated MTA (if relevant). Results were generated during autumn 2019 and a library of reference spectra is now available.

### 2. Work carried out in the JRP, scientific results

#### *WP0. Coordination, management and communication*

***JRP5-WP0-T1:*** *General coordination and management of the project (administrative and financial) (M0-M36)*

The overall purpose of the management structure is to ensure the timely implementation of the tasks and the smooth running of the project as a whole. Its primary goal is to identify arising opportunities and detect the occurrence of obstacles as early as possible, hence maximise the outcome of the project while preventing delays in its implementation. This will ensure that all tasks and research objectives are performed in due time.

Face to face meeting on Task 3.1 at Pasteur Institute (France) (17 January 2019)

Face to face meeting at Sciensano (Belgium) WP 3 organisation (08 February 2019)

**First general meeting** at Anses (France) on 20 and 21 March 2019
TC WP3 on 15 February 2019

TC WP4 on 12 March 2019

TC WP3 on 14 March 2019

TC WP1 on 15 March 2019

Face to Face meeting on Task 3.1 at Sciensano (Belgium) (29 April 2019)

TC WP4 on 06 June 2019

TC WP5 16 July 2019

TC WP4 24 October 2019

TC WP3 06 November 2019

TC WP2 08 November 2019

TC WP0 06 November 2019: with WP1 (Coordination) and WP3 (JRP) of OH-EJP

TC WP1, Task 1.4 (Maldi-TOF) on 13 December 2019

***JRP5-WP0-T2 to JRP5-WP0-T5:*** *Organisation of four face-to-face meetings with all partners (M0-M36)*

During the 2nd year of Tox-Detect project, a general face to face meeting was organized on 20 and 21 March 2019 at Anses (Maisons-Alfort, France).

15 participants representing all Tox-Detect partners were present during this general meeting. All participants presented their institutions, activities and involvement in the Tox-Detect project.

The aims of this meeting were:

- to introduce new project members;
- to provide information on administrative and financial issues by representatives of EJP coordination team;
- to give an overview of the results obtained
- to discuss the next steps for each WP.

Report of this meeting (deliverable D0.3) is available on https://onehealthejp.eu/groups/tox-detect/documents/.

***JRP5-WP0-T6**: Mandatory reports on network activities: interim activity report, final report (M0-M36)*

M9 and M18 reports have been uploaded on the dedicated website on September and December 2019, respectively.

## *WP1. Constitution of a reference strain collection for S. aureus, B. cereus and C. perfringens*

***JRP5-WP1-T1**: Constitution of S. aureus strains collection (M1-M3)*

As it was decided to focus on toxins SEM, SEN and SEO, strains of *Staphylococcus aureus* have been proposed by BfR partner to be included in the list previously established.

***JRP5-WP1-T2**: Constitution of B. cereus strains collection (M1-M3)*

Some minor changes have been done in the list previously established by partners. Thus, Bacillus pseudomycoides CIP 105701 was added as control in WP3-T2 (development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from B. cereus), because it is not supposed to express any toxin.

***JRP5-WP1-T3**: Constitution of C. perfringens strains collection (M1-M3)*

Some of the strains selected for the project by partners have been replaced or not studied by MALDI-ToF mass spectrometry due to problem of cultivation. At this moment, forty biomasses of strains of *C. perfringens* and the MTA corresponding have been sent to Anses (Nancy).

**For these three tasks (WP1-T1 to T3), the Deliverable D.01 was updated (version 2) and uploaded on OH-EJP website.**

***JRP5-WP1-T4:** Transfer of libraries of MALDI-ToF reference spectra (M3-M3)*

According to conclusion of face-to-face meeting, MTA were drafted by partners and sent to Anses Nancy (Partner 1).

Partners prepared Biomasses according to the developed protocol and sent these samples to Partner 1 for Maldi-TOF analysis.

Libraries of MALDI-ToF reference spectra have been generated and cleaned during 2019. All data were assessed during December. This library is currently available in Partner 1.

During the next general face-to-face meeting (15 and 16 January 2020), technical and data processing transfer will be discussed with partners for an export planned on March 2020 before organization of a dedicated PT trial (cf WP5).

## *WP2 Characterization of toxins/virulence factors*

***JRP5-WP2-T1**: Characterization of candidate toxin and/or virulence genes using toxicity tests (M4-M24)*

***JRP5-WP2-T2**: Assessment of virulence and toxin gene expression using RT-PCR and transcriptomic assays (M4-M24)*

***JRP5-WP2-T3**: Correlation of specific toxicity profiles with expression patterns of bacterial toxins/virulence factors (M25-M30)*

Data obtained during WP2 tasks are not publicly available due to protection and valorisation purposes.

## *WP3: Development of Mass Spectrometry-based proteomics procedures for detection of bacterial toxins and virulence factors*

An important change has occurred for WP3 in the first 18 months of the project with the change of leadership to take into account an internal reorganization at Sciensano and the fact that Mirjana Andjelkovic was no more in charge of the MS team. She has been replaced by Julien Masquelier, who is now taking care of all the proteomics experiments planned in WP3 but who could not take the lead on WP3.

For this reason, Julia Chamot-Rooke (IP) became WP3 leader after 12 months. A meeting has been organized at IP the 17/01/2019 to discuss this reorganization.

A part of the budget allocated to Sciensano for leading WP3 is planned to be reattributed to IP (J. Chamot-Rooke).

***JRP5-WP3-T1**: development of Mass Spectrometry-based methods for the detection of new enterotoxins (eg SEG, SEH, SEI) from S. aureus (M4-M27)*

After being busy with the implementation of a new method for SEA & SEB enterotoxins by bottom up proteomics, based on an “on filter” digestion, the method will be extended to SEM, SEN & SEO enterotoxins. Indeed it was clearly decided to switch from SEG, SEH & SEI to SEM, SEN & SEO during general project meetings.

Moreover, besides the “on filter” digestion/purification, a first step of purification with antibodies/magnetic beads is also considered, in case of limitations with the filter method. The development of the method for enterotoxins SEM, SEN & SEO is planned, with standards and antibodies that will be provided by BfR.

This work is also carried out in close collaboration with ANSES (P1 – EURL for coagulase positive staphylococci) & BfR (P9 – responsible in particular for the production of enterotoxin standards) with pooling of sequences for enterotoxins & discussions on reference sequences, and in particular for the determination of peptides of interest, needed for a future quantification.

LC-MS protocol was developed and optimized on SEA and SEB. In the absence of SEM, SEN and SEO standards it was decided to work on native toxins produced on CPS culture supernatants.

***JRP5-WP3-T2**: development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from B. cereus (M4-M27)*

First of all a selection of various strains of Bacillus expressing toxins of interest (or not) has been made by the IP in order to perform the MS experiments on the appropriate samples. Among the 10 strains available we decided to choose 3 of them.

*Bacillus cereus* CRBIP 3.5606 and Bacillus cereus CRBIP 3.262; these strains are supposed to express various toxins such as non-hemolytic enterotoxins (Nhe), haemolysin BL (HBL), haemolysin, sphingomyelinase C and cytotoxin K2 (cytK2). They are not supposed to express cereulide or CytK1.

*Bacillus pseudomycoides* CIP 105701: this strain is chosen as a control and is not supposed to express any toxin.

All strains were cultivated in BHI and lysates were prepared in PBS, filtered and the supernatants were recovered in all cases (4 replicates of each). Both lysates and supernatants were analysed. Samples were then trypsin digested (using a standard protocol used at the IP) and the digests were analysed in nanoLC-MS/MS on a Q-Exactive Plus. This bottom-up analysis is required before any top-down analysis to make sure that the targeted proteins are present in the sample. It can happen than the genes are present but the proteins are not expressed.

The analysis of all data indicated that the following toxins could be retrieved in the *B. cereus* samples: NHE, sphingomyelinase C, haemolysin, haemolysin BL and enterotoxin. Only CytK2 could not be found. Many more peptides were obtained in the supernatants compared to the lysates confirming that these toxins are secreted. Excellent sequence coverage could be achieved in some cases. However, for some toxins (in particular haemolysins and enterotoxins) the database used for the search comprises several closely-related sequences that are not reviewed, which complicates the data analysis. Since these sequences share the same peptides a precise assignment cannot be done.

Moreover, thanks to these results, we now know that CytK2 is not a relevant target for the project but the other toxins could be appropriate ones. This information has been communicated to the partner in charge of the immune-enzymatic assays (INRA, M. Gohar) in WP4.

Top-down experiments are currently performed by Mathieu Dupré and Magalie Duchateau (IP) to evaluate if characterization of intact proteoforms for the various toxins could be performed. The instrument on which the experiments are performed has experienced issues that delayed the results.

***JRP5-WP3-T3**: Development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from C. perfringens (M4-M27)*

The Q-Exactive HF-X (ThermoScientific) mass spectrometer was implemented during summer 2019 on the Proteomic and Metabolomic Exploration Facility (PFEM) and is now operational. A one-year post-doctoral fellowship has been recruited in October 2019 to carry out methodological developments and analyses of the CPE toxin and proteins expressed and secreted in *C. perfringens* culture supernatants, in close coordination with the other two laboratories involved in the project.

Two supernatants were provided by ANSES (P1, Maisons-Alfort) in November 2019. They were obtained from cultures of two *C. perfringens* isolates grown in TPG broth containing bile salt : *C. perfringens* 16SBCL940 and *C. perfringens* 17SBCL79.

Prior to nanoLC-MS/MS analysis, supernatants were concentrated and proteins were in-gel digested by trypsin. The digests were first analyzed on a LTQ-Orbitrap Velos mass spectrometer. CPE was identified with a very good sequence coverage in 16SBCL940 sample. However CPE was not found in 17SBCL79 sample despite its detection by RPLA kit assay (performed by ANSES). As CPE production is dependent on sporulation, presence of proteins involved in sporulation in both samples was checked. A lower amount of sporulation-related proteins was identified in 17SBCL79 sample than in 16SBCL940 sample. So, a lower amount of CPE in 17SBCL79 was then expected and confirmed by ANSES.

In a second time, by performing nanoLC-MS/MS analysis on Q-exactive HF-X mass spectrometer and by improving our conditions of measurements, we were also able to identify CPE with a good coverage in 17SBCL79 sample. The results of the final method developments/optimization and supernatant analyses are in progress and should be expected to be released at the first quarter of 2020.

***JRP5-WP3-T4:** Transfer of LC-MS/MS methods (M24-M30)*

Not relevant

## *WP4: Development of new immuno-enzymatic assays for detection of S. aureus and B. cereus toxins and virulence determinants*

### ***JRP5-WP4-T1:*** *Development of quantitative immunoassays for five known S. aureus and B. cereus toxins and virulence factors (M4-M32)*

### ***JRP5-WP4-T2:*** *Development of a quantitative immunoassay on a new B cereus toxin or virulence factor (M18-M32)*

Data obtained during WP4 tasks are not publicly available due to protection and valorisation purposes.

## *WP5. Inter-laboratory ring trial scheme (M24-M36)*

First TC on WP5 was organized on 16 July 2019. The aim of this TC was to select methods for the interlaboratory tests (mass spec, Maldi-Tof, ELISA), and to establish a schedule according to the progress of each WP. During this TC, objectives of WP5 was presented and discussed. It was decided to draft a SOP dealing with interlaboratory test organization. This one will be presented and discussed during the next general face-to-face meeting. Moreover, coordination asked to WPL to draft their developed method under the same SOP template that will be prepared by the WP0.

Finally, the next steps of WP5 over the 3rd year will be discussed during the general face to face meeting (15 and 16 January 2020).

## *WP6. Dissemination, protection and exploitation of results*

All dissemination activities on the EJP ToxDetect have been uploaded on the dedicated portal https://onehealthejp.eu/internal-events-survey/

### ***JRP4-WP6-T1****: dissemination of information within the partners (M0-M36)*

/

### ***JRP4-WP6-T2****: dissemination of information to the outside (M0-M36)*

Various presentations were given to promote Tox-Detect project. During this second year, five oral presentations were accepted in different meetings including both scientific and decision makers audiences:

EURL for CPS annual Workshop (26 to 28 June 2019, Maisons-Alfort): Enlargement of scope of the methods for the detection of new toxin types (Y Nia). 40 scientific attendees.

Australie Group Meeting (4 June 2019, Paris): Questioning trade controls for Staphylococcus aureus (Y NIA and O Chesneau). 40 scientific and policy makers' attendees.

Workshop Emerging Risks Exchange Network (EREN), and European Food Safety Authority (EFSA) onNatural toxin" (20 November 2019, Maisons-Alfort): Development and harmonization of innovative methods for comprehensive analysis of foodborne toxigenic bacteria, ie. *Staphylococcus* spp., *Bacillus cereus* and *Clostridium perfringens* (M Michaut). 30 scientific and policy makers' attendees.

Italian NRL annual Workshop (5 November 2019, Turin): Non solo CPS: lo stato dell'arte per quel che riguarda gli altri batteri tossigenici (J-A Hennekinne). 50 scientific attendees.

Italian NRL annual Workshop (5 November 2019, Turin): Nuovi metodi per la detection SE (Y Nia). 50 scientific attendees.

SFM conference (1 October 2019, Paris): Development and harmonization of innovative methods for comprehensive analysis of foodborne toxigenic bacteria, ie. *Staphylococcus* spp., *Bacillus cereus* and *Clostridium perfringens* (M Michaut). 40 scientific attendees.


Fig 3: EJP Tox-Detect at SFM conference (1st Oct 2019)

## 3. Progress of the research project: milestones and deliverables

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| Tox-Detect | D-JRP5-1.2 | Libraries of MALDI-ToF reference spectra | 3 | 24 | 24 | It was proposed to postpone the analysis of strains due to a need of preparation and signature of MTA between all partners. MTA were signed and Biomasses were sent to MALDI-ToF platform (Partner 01). Analysis have been performed during summer 2019. All data have been assessed to build the reference spectra library. This one is available at partner 01 location. The transfer has been discussed during the 2nd general meeting (15-16 January 2020). It hase been decided to share this library among partners who will participate to the Inter lab study Maldi-Tof (WP 5, task 5.1) |
| Tox-Detect | D-JRP5-2.1 | Report on results from toxicity assays (classical toxicity tests and High Content Analysis) | 24 | No | 32 | Due to technical issues, and according to WP2 TC (nov 2019). It was decided to postpone this deliverable to M 32.<br>Also, OH EJP was advised that 6 months extension asked by the beginning of 2020 (TC nov 2019) |
| Tox-Detect | D-JRP5-2.2 | Report on TR-PCR and RNAseq data analysis | 24 | No | 32 | Due to technical issues, and according to WP2 TC (nov 2019). It was decided to postpone this deliverable to M 32.<br>Also, OH EJP was advised that 6 months extension asked by the beginning of 2020 (TC nov 2019) |

## *Milestones*

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| Tox-Detect | M-JRP5-04 | Exchange of libraries of MALDI-ToF reference spectra | 3 | No | 27 | All data were assessed during December 2019. This library is currently available in Partner 1 laboratory. During the next general face-to-face meeting (15 and 16 January 2020), technical and data processing transfer will be discussed with partners for an export planned on March 2020 before organization of a dedicated PT trial (cf WP5). |
| Tox-Detect | M-JRP5-05 | Reference materials available | 5 | 18 | 18 | Reference strains are available for WPs depending on the availability of MTA and results of M-JRP5-04. Reference materials available after MTA signature |
| Tox-Detect | M-JRP5-06 | High content analysis methods developed | 18 | No | 32 | Due to technical issues, and according to WP2 TC (nov 2019). It was decided to postpone this deliverable to M 32.<br>Also, OH EJP was advised that 6 months extension asked by the beginning of 2020 (TC nov 2019) |
| Tox-Detect | MS2.2 | RT-PCR assays developed | 14 | - | 32 | Protocols tested and will be shared among partners soon. |
| Tox-Detect | MS2.4 | RNAseq data analysed | 24 | - | 32 | Protocol of RNA extraction and experiments are in progress.<br>Due to technical issues, and according to WP2 TC (nov 2019). It was decided to postpone this deliverable to M 32.<br>Also, OH EJP was advised that 6 months extension asked by the beginning of 2020 (TC nov 2019) |

## 4. Publications and patents

No publication was submitted during year 2.

## 5. Impact & relevance

In 2019, EJP Tox-Detect consortium finalized the characterization of a reference strains library (D1.1) including Maldi ToF reference Spectra (D1.2). This is the first time that such spectra library and reference collection of strains were developed and characterized across the EU. These relevant tools will enable partners to develop methods to improve food outbreak characterization due to bacteria producing toxins.

According to the annual EFSA Zoonosis reports, bacterial toxin outbreaks remain poorly characterized. This could be due to the lack of relevant tools to characterize bacteria as well as toxins. Tox-Detect will upgrade the characterization scheme by developping reliable methods focusing on toxins produced by S. aureus, B. cereus sensu lato and C. perfringens. The first delivrable D1.2 (Libraries of MALDI-ToF reference spectra) enables to characterize bacteria from various origins (One Health concept). The other tools (coming from WP2, 3 and 4) will provide relevant methods to detect / quantify toxins in order to improve food poisoning outbreaks characterization.

Finally, D1.1 and D1.2 deliverables would be useful to feed the EJP CARE project which began on January 2020.

## 6. Interactions with other JRPs/JIPs or with external (EU or national) relevant project

Not relevant. However, the Tox-Detect project has been presented during the 2019 EREN meeting, which took place in Partner 1 location on November 2019. In fact, EJP-Tox-Detect project focuses on staphylococcal enterotoxins and some Bacillus cereus toxins possibly involved in food borne outbreaks that cannot be currently detectable due to a lack of relevant tools.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements (from ethical reviewers) in 2018 | Measures and actions taken at the end of 2018 | Comments Ethical advisors January 2019 | Comments Project Leader at the end of 2019 |
|---|---|---|---|
| The applicants must confirm the source of tissues. | Cell culture needs administrative authorization | Satisfactory reply | / |
| The applicants must document the safety mitigation measures in place to protect the staff. | BSL2 working conditions | Satisfactory reply | / |

## 8. List of critical risks

Risks have been discussed with OHEJP Coordination team.

## 9. *List of dissemination and communication activities*

| | | | |
|---|---|---|---|
| Name of the activity: | TOX-Detect Annual meeting | | |
| Date: | 20-21 March 2019 | | |
| Place: | ANSES, Paris | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | Yes |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 15 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | EURL for CPS annual Workshop | | |
| Date: | 26-28 May 2019 | | |
| Place: | Maisons-Alfort | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 40 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Australie Group Meeting (bio threat weapons) | | |
| Date: | 4 june 2019 | | |
| Place: | Paris | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 5 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 35 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Workshop EREN “Natural toxin” | | |
| Date: | 20 november 2019 | | |
| Place: | Anses, Maisons-Alfort | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 15 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 15 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Italian NRL annual Workshop | | |
| Date: | 5 november 2019 | | |
| Place: | Turin, Italy | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 50 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | SFM conference | | |
| Date: | 1 October 2019 | | |
| Place: | Paris | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 40 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

## 10. List of planned tele- or video conferences, face to face meetings in the next year

The next general meeting will be organized on January 15-16, 2020 in Maisons-Alfort, France.

TC dedicated to each WP will be organized between March and May 2020.

## JRP06 - NOVA

### 1. Summary of the work carried out in year 2 (January to December 2019)

On project management level, the main accomplishments during spring 2019 have been to finish a more detailed data management plan and to organise a second annual meeting. By the end of the year, we have also organised a scientific webinar to share research results within the project.

WP1 has focused on aligning our one health terminology work with ongoing work in the ORION project. The conclusion from dialogue with ORION and COHESIVE is that a joint one health glossary with contributions from all projects would be preferable, and this is now the aim. Work to investigate surveillance opportunities and barriers across the food chain is also ongoing. In this work we aim for synergy effects by collaboration across work packages (WP1 combined with WP2, WP3 and WP4). Plans to interview OHEJP participants, to collect information and identify perceived opportunities and barriers in surveillance is ongoing.

In WP2, the availability and barriers for using consumers' food purchase data (CPD) or food data from hospitals/healthcare for outbreak investigations are being explored, e.g. in questionnaire studies. A framework for reporting outbreak investigations using CPD, as well as a network of stakeholders from across Europe that have experience from or interest in such data/investigations, have been initiated. Preparations have also been made to enable access to data, simulation studies of outbreaks and case-control studies. In addition, a systematic review of outbreaks within health institutions have been conducted.

In WP3, different strategies have been put in place depending on the degree of progress of syndromic surveillance (SyS) monitoring tools within each partner institute. Monitoring of *Salmonella* and *Campylobacter* from animal and food production to human population is used as case studies by all partners. The univariate analysis of time series has included retrospective analysis with a full assessment of temporal effects. Preparations for multivariate SyS, i.e. analysing different data sources jointly, is ongoing.

In WP4, the spatial aspects of surveillance in high and low prevalence regions are being investigated based on spatial analysis and modelling of data on *Salmonella* in production animals in Spain and Sweden. In different tasks, the risk of introduction to pig farms from feed suppliers and the role of the environment (wild boar) are also being explored.

In WP5, spread models based on different modelling approaches are being adapted to assess the effect of surveillance in animal production on consumer exposure to foodborne pathogens. Assessment of the effect of using metagenomics in surveillance has also been started by gathering existing metagenomic data from different parts of the animal production. In addition, a retail-to-DALY model for pig meat products has been simplified to be able to accommodate data from Denmark, France, the Netherlands, Sweden and the United Kingdom, and to model the effect of surveillance programs in the food production on human health. Data collection in these countries is in progress.

### 2. Work carried out in the JRP, scientific results

#### WP0: Coordination and project management

***JRP6-WP0-T1:*** *Project management (M1-M36)*

Monthly meetings with WP leaders have been held. The coordinator (and other project participants) has also attended the annual scientific meeting of the OHEJP and has taken part of collaboration

meetings with the representatives from the ORION and COHESIVE projects. The deputy coordinator has presented research from NOVA at the OHEJP Programme Managers' Committee meeting in May, and the WP leader of WP3 has presented the NOVA project at the 2019 stakeholder meeting organised in Brussels by Sciensano, also in May. In November, the project leader presented the project at SVA's Research Day 2019 in Uppsala.

During spring, project management has included work to deliver a data management plan for the project. This required attendance of educational sessions organised by the OHEJP management, and additional meetings to plan and complete this work process. The DMP was finalised in March and updated in December 2019.

***JRP6-WP0-T2:*** *Organise annual assemblies (M1-M34)*

A second annual assembly was organised in collaboration with Sciensano and held in Brussels, March 7-8, 2019. A complete documentation of this meeting is included in D-JRP6-0.2 Documentation of consortium assembly and steering committee meeting (M20). The purpose of the meeting was to meet, plan and discuss ongoing and coming tasks. To focus this work, three specific topics were addressed in the discussions: 1. International aspects and opportunities for international collaboration, 2. Scientific publications, 3. Novelty of methods and approaches used.

To share research results across WPs, a scientific webinar was also organised for all project participants in November.

***JRP6-WP0-T3:*** *Economic reporting and financial management (M1-M36)*

No major tasks to report.

## *WP1: Food chain surveillance mapping*

***JRP6-WP1-T1:*** *Definition of a joint food borne zoonosis surveillance terminology (M1-M24)*

Given the work on a Med-Vet glossary of the participants of ORION project WP2 Integration in which members of NOVA project WP1 also participated in the first year, to complete the deliverable of this WP, a collaboration has been established with the respective ORION team, as well as COHESIVE.

The NOVA Glossary has been delivered to ORION project leader, BfR, in order to incorporate this into the common OH Glossary. There has been several exchanges with BfR in order to adjust the NOVA Glossary into the common version. The final result can be found here: https://ckan-aginfra.d4science.org/organization/about/orionknowledgehub

As an outcome of this cooperation (of all three projects) is a common article that is currently being drafted. This publication work is coordinated by BfR.

***JRP6-WP1-T2:*** *Mapping of surveillance: data, regulatory framework, key stakeholders, opportunities and barriers (M1-M36)*

An overall mapping exercise on zoonotic foodborne pathogens (with a focus on Salmonella) has been performed. A discussion with NOVA WP3 has been initiated to explore a possible collaboration regarding this exercise and its use.

Based on the overall mapping exercise, it was agreed initially (first half of 2019) to draft a questionnaire addressing the OH EJP participants, in order to collect information and identify perceived opportunities and barriers in food-borne disease surveillance through an online survey. Several drafts of this online questionnaire were prepared and discussed among the participants of WP1 and the project leader.

This extensive work helped the WP1 participants to conclude (during summer 2019) that a qualitative study based on interviews of selected people would be more appropriated for our aim. This aim is to understand the barriers and opportunities in food-borne disease surveillance from a One Health perspective. Since September 2019, efforts were made to identify collaborators who are experts in qualitative studies and further work has been focused on methodological aspects.

## *WP2: Analysis of food purchase data*

***JRP6-WP2-T1:*** *Data availability and barriers (M1-M14)*

Within this task, the availability of purchase data for outbreak investigations has been explored through two questionnaires and has been described in detail in D.2.1.1. The next step has been to focus on barriers on use of food purchase data. This has been done in collaboration with Task 2, below.

***JRP6-WP2-T2:*** *Food purchase data for outbreak investigations (M2-M34)*

This task aims to describe the use of consumer purchase data (CPD) as an outbreak investigation methodology.

***JRP6-WP2-T2-ST1:*** *Identify existing use of CPD for outbreak investigations, including a survey of EU public health institutes, conducted in cooperation with JRP6-WP2-T1.*

In the second year, we have worked to form a network consisting of stakeholders from across Europe that have experience or interest in working with CPD in relation to outbreak investigations. This was done via several channels, including a general appeal via the EPIET Alumni Network. A survey among the stakeholders was followed by focus group meetings conducted via Skype. The results are being written up in a manuscript for publication. Overall, we see much possibility for further utilisation of the method and few real barriers.

***JRP6-WP2-T2-ST2:*** *Develop Best Practice descriptions for CPD for outbreak investigations.*

The design of a framework for reporting outbreak investigations using CPD and utilising the outputs from WP2-T2 has been initiated. A Short Term Mission from FHI hosted by SSI was held in the last week of June and attempts to exchange data and code between Denmark and Norway have followed. The plan for now is to include the Best Practice descriptions in the publication mentioned above.

***JRP6-WP2-T3:*** *Big data analysis of risk factors for sporadic disease (M1-M34)*

This task aims to explore if consumer purchase data can be used for analytical studies beyond the outbreak setting; something has not previously been done. The electronic web module in which Danish users can be securely invited to sign up and give consent for their purchase data to be used (described in D.2.3.1) has now been finalised. The system is undergoing testing for errors and deployment is pending various permissions and acceptance from the Danish body of health IT before it can be launched.

***JRP6-WP2-T3-ST1:*** *Achieve supermarket CPD to study the ways to structure data on foods and run simulation studies for the risk of outbreak/sporadic foodborne infections.*

Model description for running simulations on CPD is currently underway, with the aim to have running model by year 3. We are hoping to obtain a supermarket dataset from Norway.

***JRP6-WP2-T3-ST2:*** *Case control study, food posing and campylobacter*

Build a research infrastructure to achieve consent from sporadic cases of Salmonella and healthy controls in order to use CPD to conduct analytical studies of foods posing a risk for sporadic Salmonella infections. A protocol for a planned case-control study with this aim have been finalised and we aim to publish this protocol.

***JRP6-WP2-T4:*** *Food distribution data for hospital outbreaks (M1-36)*

This task aims to use electronic food purchase data at the institutional level for investigation of nosocomial foodborne outbreaks. A systematic review of foodborne outbreaks within health institutions have been conducted and an MS is close to the submission stage. A questionnaire was developed and distributed among health institutions in Germany and Italy with the aim to describe the availability, usability, and traceability of food/catering data in healthcare settings in order to support foodborne outbreak investigations.

***JRP6-WP2-T5:*** *Trace back and food risk mapping (M1-36)*

This task aims to develop improved tools for food risk mapping and integrate them into the state-of-the-art tracing tool software FoodChain-Lab. In agreement with the work plan, this work has only begun in the second half of 2019.

***JRP6-WP2-T5-ST1:*** *Investigate the availability and usefulness of data from WP2*

The development of the likelihood method so that it handles data on time together with GTIN, has begun. BfR and NVI are working together on how to achieve improvement of the likelihood method.

***JRP6-WP2-T5-ST2:*** *Develop the likelihood method and guidance for selection*

The implementation of the likelihood method as cloud service, and integration of the results of the likelihood method into the analysis of FoodChain-Lab (FCL) is being prepared. A handover of the likelihood method R script for integration in FCL is planned for March 2020 at the latest.

## *WP3. Syndromic surveillance*

WP3 continues based upon what was achieved during the first annual period of the EJP. The WP has three tasks, where T-3.2 and T-3.3 are dependent from T-3.1.

***JRP6-WP3-T1:*** *Identify the opportunities for SyS of FBD (M1-M10)*

***JRP6-WP3-T1-ST1:*** *Food chain mapping (M1-M8)*

***JRP6-WP3-T1-ST2:*** *Data source screening: availability, quality and suitability for SyS (M1-M10)*

These tasks have been completed, see annual report 2018

***JRP6-WP3-T2:*** *Univariate syndromic surveillance development for FBD (and AMR) (M11-M30)*

In Sweden, *Campylobacter* data from broiler monitoring program was selected for the univariate analysis. Three different aberration detection algorithms commonly used in public health and animal health surveillance (Farrington by {surveillance}, Holt-Winters and Exponentially weighted moving average (EWMA) algorithm by {vetsyn} package in R) were used on the weekly positive number of *Campylobacter* slaughter batches. All three algorithms were successful in generating alarms for the same period when there was a long period of human outbreaks reported in Sweden, showing the potential of serving as a syndromic surveillance indicator for *Campylobacter* outbreaks in humans. A rigorous comparison of the performance between the algorithms along with adjustment of parameter settings will be done against human *Campylobacter* outbreaks data in Task 3.3 where we will be working with the Swedish Public Health Agency (FOHM). We will also assess weather data, e.g. temperature, as another component in the development of multivariate syndromic surveillance for human *Campylobacter* outbreaks in Sweden in Task 3.3.

In Norway, univariate surveillance is used in the NorSySS to detect signals in the number of consultations with gastro-intestinal symptoms reported from GPs and out-of-hours GPs. The main method used in this surveillance system is a quasi-poison (QP) regression model. We compared the alarms generated by this method with the set of surveillance algorithms used for *Campylobacter* in Sweden. We obtained clear similarities in which weeks alarms are raised for the different algorithms, but also clear differences. Implementing the Farrington methods may help to better detect alarms at certain period of the year.

In France, data from four data sources were described and analysed for livestock production. Access to data on human infections is being finalised. The three algorithms for alarm detections are currently tested for the four data streams, taking into account geographical levels and Salmonella serovars.

***JRP6-WP3-T3:*** *Evaluation of multivariate syndromic surveillance for FBD (M11-M36)*

WP3-T3 has started for the Norwegian case study. Three main approaches to multivariate surveillance were identified. The three strategies will be tested during Year 3, first on the Norwegian case study

and then on the Swedish and the French case studies. A post-doc proposal has been open since December 2019 to contribute on task 3.3 in France.

## *WP4: Spatial risk mapping*

### ***JRP6-WP4-T1:*** *Identification of spatial relationships and patterns in Salmonella prevalence*

#### ***JRP6-WP4-T1-ST1:*** *Surveillance in high prevalence regions to detect introduction and changes in prevalence (M1-M24)*

The analysis in this subtask has been finalised as planned. Data included in the model consisted of the information on pigs sampled for isolation and antimicrobial susceptibility typing of Salmonella in slaughterhouses, and the results of such tests. Several spatial analyses to identify a spatial pattern in the probability of isolation of Salmonella were applied and suggested a West-East increasing risk of Salmonella infection in swine at the province level in Spain. Association of risk areas with human cases could not be explored because human data does not contain epidemiological information on the possible source of salmonellosis infection. Moreover, from the exploration of the surveillance sources we concluded that even if food alerts were mainly in meat (particularly pork) and seafood (particularly mollusks), Salmonella-related food-borne outbreaks were mainly related to eggs.

Value of antimicrobial susceptibility testing to identify specific Salmonella strains that could be used as targets of the surveillance systems was assessed using information from poultry (where a larger number of isolates are available) as proof of principle with a positive result (Alvarez et al., 2019). A postdoctoral researcher at the VISAVET surveillance centre in Madrid, has visited the Centre for Statistics at the University of Hasselt. During the short term mission, data related to the aforementioned analyses on the antimicrobial susceptibility patterns of the swine isolates from 2008 to 2013 have been also analysed, revealing different patterns and spatial clusters depending on the antimicrobial (detailed results will be reported in deliverable report).

#### ***JRP6-WP4-T1-ST2:*** *Surveillance in low prevalence regions to reduce prevalence (M1-M24)*

The aim of the work was to compare surveillance strategies to detect herds infected with S. Dublin in cattle in a low prevalence region such as Sweden. A stochastic disease spread model was developed to simulate S. Dublin infections within and between Swedish dairy herds. Alternative surveillance options (both traditional and risk-based) to detect infected herds have been evaluated deterministically using simulated disease data. Traditional surveillance was capable to detect 25% of the infected herds after one year, while risk-based surveillance detected around 15% of the cases. However, risk-based surveillance was economically more convenient, as it utilized one third of the samples used by traditional surveillance.

### ***JRP6-WP4-T2:*** *Risk of introduction of Salmonella in pig farms through animal feed (M1-M36)*

Information on feed movement patterns has been gathered for one of the biggest systems in the country, located in central Spain. Movement data from a province in the same region (Segovia) from 2015 to 2017 have been also obtained from the Ministry of Agriculture and analysed. In total, there were 80,868 movements across the three years. It is likely that the network of pig movement in Segovia and potentially in the whole Spain will help to elucidate the transmission of Salmonella between pigs located in different premises.

### ***JRP6-WP4-T3:*** *Role of the environment in the occurrence and maintenance of Salmonella infection in extensive farming (M1-M36)*

Salmonella data on wild boar gathered from official sources (“Wildlife Epidemiological Surveillance Program” from the Autonomous Community of Andalusia) have been explored. A cartographic map of hotspot areas for Salmonella transmission between wild boar and extensive systems has also been produced based on the extensive pig farm produced in 2018 and the wild boar density map from Bosch et al (2015). Association between hot spots and salmonella in wild boar was not found.

Finally, biosecurity measures guideline has been produced for extensive farming in cooperation with ASFSTOP eCOST action.

## *WP5: Evaluation of surveillance programs & cost efficiency*

***JRP6-WP5-T1**: Adapt infectious disease models for assessing the effect of surveillance programs in primary animal production on consumer exposure to foodborne pathogens (M1-M36)*

The backbone of this work is the development and application of three disease models – ParaTB in cattle; Salmonella in broiler production and Salmonella in pig production. The aim of each of these models is to support the relevant authorities in decision-making about how to optimize surveillance of the diseases modelled, both for monitoring and control. The models describing the transmission are dynamic compartment models, taking into account transmission both within farms and between farms. Some of the outputs of this task have been submitted as conference abstracts to an international conference on animal health surveillance taking place in 2020.

***JRP6-WP5-T2**: Assessing the effect of using metagenomics in surveillance of foodborne zoonoses (M1-M24)*

The assessment is focusing on using metagenomics on fecal samples in surveillance of emerging occurrence of AMR in the primary production. The metagenomics approach is compared with surveillance of emerging AMR based on phenotypic characterisation of bacteria isolated from the primary production.

The following factors has been identified as factors where decisions can be taken in the design of the surveillance: sampling schedule, frequency of sampling, pooling of samples, sequencing depth

Next step is to build the simulation code for stochastic simulation. Building a stochastic model is complex, but it is necessary to integrate the likelihood of chance in any surveillance program. The code will be generic in respect to population size and structure as well as occurrence of AMR, so the code can be utilised as a tool in different populations with different occurrence of AMR. As an initial case, the code is adjusted to the Danish pig industry.

Output of interest from the simulation is observed presence or absence in the population given varying true occurrence in the population (sensitivity and predictive value of results).

Finally, we will also simulate the time until detection, given a new AMR clone/gene is introduced into the population.

***JRP6-WP5-T3**: Modelling the effect of surveillance programs in the food production on human health (M1-M36)*

In this task, a model developed in the Netherlands has been applied in a more general context, assessing the effect of risk-based sampling in other EU countries.

The results show that in principle the risk-based sampling method can be applied to countries other than The Netherlands, but also that severe data availability problems are apparent. As was the original intention, the results should be seen as a proof of principle of a new method (being a goal of the EJP-NOVA project). To solve the data problems, a significant time investment and (more) involvement of experts from the food processing and food consumption working fields would be necessary.

## 3. Progress of the research project: milestones and deliverables

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| NOVA | D-JRP6-0.2 | Documentation of consortium assembly and steering committee meeting | 20 | 2019-08-30 | - | |
| NOVA | D-JRP6-1.1 | Glossary of terms based on a common food borne zoonosis surveillance terminology | 24 | 2019-10-31<br>2019-12-20 | - | Second half of 06/2019: NOVA Glossary delivered to ORION project leader, BfR, 07-10/2019: exchanges with BfR to adjust the NOVA Glossary into the common version |
| NOVA | D-JRP6-2.2 | Description by member state of barriers for use: legal, political, economic, practical and technical obstacles. | 14 | 2019-12-20 | 24 | Initially M14 (updated in AWP2), first forecast M22 which was later changed to M24. |
| NOVA | D-JRP6-2.9 | Studies using the German results extended to the partner institutes. | 24 | 2019-12-20 | - | |
| NOVA | D-JRP6-3.3 | Description of the SS components implemented and guidelines for their use | 24 | 2019-12-20 | - | |
| NOVA | D-JRP6-4.2. | Identification of periods with higher probability of detection of infection identified in high prevalence regions and temporal evidences for an association with human cases | 24 | 2019-12-20 | - | |
| NOVA | D-JRP6-4.4. | Evaluation of optimal surveillance strategies. | 24 | 2019-12-20 | | |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| NOVA | D-JRP6-4.5. | Characterization of the spatial network structure of the pig industry in a Mediterranean scenario and Salmonella data mapped and analysed. | 24 | 2019-12-20 | - | |
| NOVA | D-JRP6-4.8. | Cartographic map of hot spot areas for Salmonella transmission between wild boars and low biosecurity systems. | 24 | 2019-12-20 | - | |
| NOVA | D-JRP6-5.3 | An assessment of the public health effects of very different surveillance strategies to detect emerging foodborne infections in a MS or at European level. | 24 | - | 32 | The assessment will focus on using metagenomics in surveillance of emerging occurrence of AMR in the primary production. |

***Milestones***

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| NOVA | D-JRP6-0.2 | Documentation of consortium assembly and steering committee meeting | 20 | 2019-08-30 | - | |
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| NOVA | D-JRP6-2.9 | Studies using the German results extended to the partner institutes. | 24 | 2019-12-20 | - | |
| NOVA | D-JRP6-3.3 | Description of the SS components implemented and guidelines for their use | 24 | 2019-12-20 | - | |
| NOVA | D-JRP6-4.2. | Identification of periods with higher probability of detection of infection identified in high prevalence regions and temporal evidences for an association with human cases | 24 | 2019-12-20 | - | |
| NOVA | D-JRP6-4.4. | Evaluation of optimal surveillance strategies. | 24 | 2019-12-20 | - | |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| *NOVA* | D-JRP6-4.5. | Characterization of the spatial network structure of the pig industry in a Mediterranean scenario and Salmonella data mapped and analysed. | 24 | 2019-12-20 | - | |
| *NOVA* | D-JRP6-4.8. | Cartographic map of hot spot areas for Salmonella transmission between wild boars and low biosecurity systems. | 24 | 2019-12-20 | - | |
| *NOVA* | D-JRP6-5.3 | An assessment of the public health effects of very different surveillance strategies to detect emerging foodborne infections in a MS or at European level. | 24 | - | 32 | The assessment will focus on using metagenomics in surveillance of emerging occurrence of AMR in the primary production. |

## 4. Publications and patents

Martínez-Avilés, Marta, Macarena Garrido-Estepa, Julio Álvarez, and Ana de la Torre. "Salmonella Surveillance Systems in Swine and Humans in Spain: A Review." *Veterinary Sciences* 6, no. 1 (February 20, 2019): 20. https://doi.org/10.3390/vetsci6010020.

Møller, Frederik T, Kåre Mølbak, and Steen Ethelberg. "Analysis of Consumer Food Purchase Data Used for Outbreak Investigations, a Review." *Eurosurveillance* 23, no. 24 (June 14, 2018). https://doi.org/10.2807/1560-7917.ES.2018.23.24.1700503.

## 5. Impact & relevance

The project contains cultivation of new methods as well as more efficient utilisation of existing data and methods. The tools and methods are primarily developed with a focus on the currently most important/frequent zoonotic diseases but may also be adapted to control other hazards or emerging agents.

The methods/tools for surveillance of food exposures and trace-back developed, includes tools that presently do not exist anywhere in the world (WP2). This project's development and combination of syndromic surveillance systems and use of new data sources, is a first bridge across the Med-Vet gap in the syndromic surveillance context (WP3). Understanding the geographical transmission of diseases is a cornerstone in epidemiology, yet surprisingly rarely used in practise – the focus on spatial mapping and analysis will provide better possibilities for actual utilisation in the zoonotic disease community (WP4). Mathematical modelling is another cornerstone of modern disease transmission understanding that we are now using to find ways to actually measure surveillance performance, and thereby be able to compare the cost-efficiency of different surveillance strategies (WP5). Finally, our mapping of available data sources and identification of surveillance key actors across the food chain, including the underlying reasons for sub-optimal surveillance, should help clarify surveillance structures and challenges across the EU (WP1).

This work relates to ECDC's Long-term surveillance strategy that highlights the need to increase the use of analytical epidemiology, including spatial analysis, and to consider alternatives to traditional surveillance, including data not normally available for public health investigation. In addition, NOVA includes both machine-learning methods and address social aspects of surveillance, which is in line with research needs identified by EFSA (Food Safety Regulatory Research Needs 2030). During 2019, we have also had contact with EFSA's Advisory Forum Task Force on Data Collection and Data Modelling, and we foresee that our results will be of relevance to them.

## 6. Interactions with other JRPs/JIPs or with external (EU or national) relevant project

### National projects:

**Syndromic surveillance**

WP3 is linked to "Real-time data analyses and information systems to support animal disease prevention and control". The project is funded by FORMAS, a Swedish research council for sustainable development (project ID: 2017-00779).

EG17-141. Assignment from Ministry of Agriculture and Fisheries, Food and Environment to INIA in R&D animal health activities and technical support to the national reference laboratories (2017-2019).

It favours and support the exchange of information with the Ministry of Agriculture and Fisheries, Food and Environment on 1) data on salmonella surveillance in animal health, and b) technical and scientific meetings.

Vet+i Foundation, Spanish Technology Platform for Animal Health. http://www.vetmasi.es/plataforma-tecnologica-espanola-de-sanidad-animal/menu-superior/inicio_37_1_ap.html.

Vet+I is an interdisciplinary forum that integrates all relevant stakeholders from academia, research, farmers, veterinarians, industry, regulators, etc. interested in animal health. This is an efficient instrument to facilitate the networking and discussion in order to achieve its main goal: to enable the efficient transfer of research developed in Spain and accelerate the development and delivery of the most effective tools for controlling the animal diseases of priority for Spain, thereby improving human and animal health, food safety and quality, animal welfare and market access.

XI Annual Conference. Zoonosis and emerging diseases. 30th May, 2019. http://www.vetmasi.es/plataforma-tecnologica-espanola-de-sanidad-animal/videos/video-xi-conferencia-anual-de-la-fundacion-veti_4198_27_4456_0_1_in.html

**Spatial modelling of VTEC/EHEC.**

WP4 has had minor/some support from a Swedish research project financed by the Swedish Research Council Formas. This project uses the same modelling framework as in task 4.1.2 to model the spatial distribution and spread of VTEC/EHEC. EHEC human case data collected in the Swedish research project will be used for spatial mapping and spatial analysis.

**AMR in Danish pig production**

The activities in WP5 is interacting with research activities related to surveillance in the participating institutes/countries. In particular the work can be linked to a national project on predicting the occurrence of AMR in Danish pig production when altering the AMU. 2018-2022 (founded by the Ministry of Environment and Food of Denmark).

**Surveillance of salmonella in poultry meat**

The title of another project that the WP5 is connected to is: Evaluate and Establish Surveillance programs of Salmonella in imported and domestic Poultry Meat in Jordan. 2016-2020 (founded by the Islamic Development Bank)

## *EU projects*

COST ASF-STOP CA15116. Understanding and combating African Swine Fever in Europe. https://www.asf-stop.com

Some of the NOVA WP4 members are participating in this e-cost action. Within this project an assessment of biosecurity measures to prevent the spread of infection diseases has been conducted for intensive, non-commercial and extensive pig farms.

ASF-STOP WG3 workshop: Biosecurity guidance in outdoor pig-production systems. 31 January 2019. Tallin, Estonia.

## *One Health EJP*

Within the EJP, we have had collaboration with the integrative project **ORION**, in particular as regards One Health surveillance terminology. To avoid the risk that the same or similar work is being done in parallel, we have also had discussions to clarify how our work connects and how we make use of potential overlap.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements (from ethical reviewers) in 2018 | Measures and actions taken at the end of 2018 | Comments Ethical advisors January 2019 | Comments Project Leader at the end of 2019 |
|---|---|---|---|
| The applicants must confirm that ethics approvals for the use of isolates from human origin have been sought. | Isolates from human origin will not be used in NOVA. Analysis in WP4 only includes anonymous public data of serotype results from clinical human cases. Ethic approvals for the use these data will not be needed. | Satisfactory reply | / |
| The applicants must confirm the compliance with GDPR. | Work to confirm compliance with GDPR is on-going in all partner institutes. As experts working in NOVA, we continuously check that we follow the national regulations and institute routines that are adjusted or updated due to GDPR. As part of the One Health EJP, the project is participating in our common web-learning programme, to ensure that the details of our data management plan comply with GDPR. | Satisfactory reply | Each institution is in charge of processing the data obtained by its project participants. The Data Protector Officer at the coordinating institute (SVA) is Jerker Plobeck. |

## 8. List of critical risks

| Description of risk | Yes/No |
|---|---|
| Loss of key-persons (staff and / or leaders) | Yes |
| Delay in work plan execution | Yes |
| Conflicts within the consortium | No |
| Lack of commitment of partners | Yes |
| Delay in duties, tasks or reporting | No |
| Poor intra-project (JRP) relationship | No |
| Potential entry/exit of partners | No |
| Other risks (please describe) | No |

***Additional information:***

The risk to lose key persons exists but is weak. The problem has rather been that recruitment of staff that will work in the project has been delayed. This connects to the second point, i.e. the delay in work plan execution, which we consider a more immediate risk. Our assessment is that now that staff has been recruited, we will be able to catch up in most tasks that have had a slower start than expected. The risk of lack of commitment of partners is also weak, but we want to acknowledge that risk, as we are a relatively wide project with several institutes being involved, but on a varied budget.

## 9. List of dissemination and communication activities

| | | | |
|---|---|---|---|
| Name of the activity: | Second Annual Assembly | | |
| Date: | 7-8 March 2019 | | |
| Place: | Eurostation Brussels (Sciensano) | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | yes | Participation to a Conference | No |
| Organisation of a Workshop | No | Participation to a Workshop | No |
| Press release | No | Participation to an Event other than a Conference or a Workshop | No |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other (presentations book) | Yes |
| Communication Campaign (e.g. Radio, TV) | No | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 39 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Project presentation at OHEJP PMC meeting | | |
| Date: | 9 May 2019 | | |
| Place: | ANSES, Paris | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | No |
| Organisation of a Workshop | No | Participation to a Workshop | No |
| Press release | No | Participation to an Event other than a Conference or a Workshop | yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other (abstract book) | Nos |
| Communication Campaign (e.g. Radio, TV) | No | Other (QDR code) | No |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | ? | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | OHEJP: How is Sciensano involved? | | |
| Date: | 5 June 2019 | | |
| Place: | Sciensano Uccle, Brussels | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | Yes |
| Organisation of a Workshop | No | Participation to a Workshop | No |
| Press release | No | Participation to an Event other than a Conference or a Workshop | No |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other (abstract book) | Nos |
| Communication Campaign (e.g. Radio, TV) | No | Other (QDR code) | No |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 35 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 11 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | 1st annual scientific meeting of the one health European joint programme on food-borne zoonoses, antimicrobial resistance and emerging threats | | |
| Date: | 22-24 May 2019 | | |
| Place: | Dublin, Ireland. | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other (abstract book) | |
| Communication Campaign (e.g. Radio, TV) | | Other (QDR code) | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | |
|---|---|
| Name of the activity: | XXXVII Annual scientific meeting from SEE<br>XVIII Congress SESPAS<br>XIV Congress APE |
| Date: | September 2019 |
| Place: | Oviedo, Spain |

| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | Yes |
| Organisation of a Workshop | No | Participation to a Workshop | No |
| Press release | No | Participation to an Event other than a Conference or a Workshop | No |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other (abstract book) | Yes* |
| Communication Campaign (e.g. Radio, TV) | No | Other (QDR code) | No |

| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
|---|---|---|---|
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 500 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

* Iglesias, I; Martínez, M; de la Torre, A. 2019. Soluciones a la falta de datos en fauna silvestre dentro del concepto one health aplicando el análisis espacial. Gaceta Sanitaria, 33:210. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=2ahUKEwiW66jj8JvmAhUSkRQKHcHNDHwQFjAAegQIBRAC&url=http%3A%2F%2Fwww.gacetasanitaria.org%2Findex.php%3Fp%3Drevista%26tipo%3Dpdf-simple%26pii%3DX0213911119000670&usg=AOvVaw1Hup8osFJVc40rDWJAQ9oQ

* Martínez, M; Álvarez, J; Garrido, M; de la Torre, A. 2019. "One health" concept applied to surveillance systems of Salmonella in swine and humans. Gaceta Sanitaria, 33:79. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=2ahUKEwiW66jj8JvmAhUSkRQKHcHNDHwQFjAAegQIBRAC&url=http%3A%2F%2Fwww.gacetasanitaria.org%2Findex.php%3Fp%3Drevista%26tipo%3Dpdf-simple%26pii%3DX0213911119000670&usg=AOvVaw1Hup8osFJVc40rDWJAQ9oQ

| | | | |
|---|---|---|---|
| Name of the activity: | GeoVet 2019 | | |
| Date: | October 8-10, 2019 | | |
| Place: | Davis, California, USA. | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | Yes |
| Organisation of a Workshop | No | Participation to a Workshop | No |
| Press release | No | Participation to an Event other than a Conference or a Workshop | No |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other (abstract book) | Yes* |
| Communication Campaign (e.g. Radio, TV) | No | Other (QDR code) | No |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 1000 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

* Teng K, Martinez Aviles M, Ugarte M, Barcena C, De La Torre A, Lopez G and Alvarez J (2019). O, Salmonella, Where Art Thou? Modelling Salmonella infection in swine farms in Spain using Hamiltonian Monte Carlo methods. Front. Vet. Sci. Conference Abstract: GeoVet 2019. Novel spatio-temporal approaches in the era of Big Data. doi: 10.3389/conf.fvets.2019.05.00007

| | | | |
|---|---|---|---|
| Name of the activity: | SETAC Europe 29thAnnual Meeting | | |
| Date: | 26-30 May 2019. | | |
| Place: | Helsinki, Finland | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | Yes |
| Organisation of a Workshop | No | Participation to a Workshop | No |
| Press release | No | Participation to an Event other than a Conference or a Workshop | No |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other (abstract book) | No |
| Communication Campaign (e.g. Radio, TV) | No | Other (QDR code) | No |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

* Carballo, M; Esperón, E; de la Torre, A. 2019. Use of mapping tools for defyning scenarios for the environmental risk assessment of antibiotic residues, antimicrobial resistances and resistance genes. SETAC Europe 29thAnnual Meeting. 26-30 May 2019. Helsinki, Finland

| | | | |
|---|---|---|---|
| Name of the activity: | Project presentation at SVA Research Day | | |
| Date: | Nov 12, 2019 | | |
| Place: | Uppsala, Sweden | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | Yes |
| Organisation of a Workshop | No | Participation to a Workshop | Yes |
| Press release | No | Participation to an Event other than a Conference or a Workshop | No |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | No | Pitch Event | No |
| Training | No | Trade Fair | No |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other (abstract book) | No |
| Communication Campaign (e.g. Radio, TV) | No | Other (QDR code) | No |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 60 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Norwegian Public Health Conference | | |
| Date: | 2019, Oct 15 | | |
| Place: | Oslo, Norway | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | No | Participation to a Conference | Yes |
| Organisation of a Workshop | No | Participation to a Workshop | No |
| Press release | No | Participation to an Event other than a Conference or a Workshop | No |
| Non-scientific and non-peer-reviewed publication (popularised publication) | No | Video/Film | No |
| Exhibition | No | Brokerage Event | No |
| Flyer | Yes | Pitch Event | No No |
| Training | No | Trade Fair | |
| Social Media | No | Participation in activities organized jointly with other H2020 projects | No |
| Website | No | Other (abstract book) | No |
| Communication Campaign (e.g. Radio, TV) | No | Other (QDR code) | No |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 100 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

## *10. List of planned tele- or video conferences, face to face meetings in the next year*

The coordinator and WP leaders will have monthly one-hour (or longer, if needed) video conferences. The preliminary dates (all at 13:00 CET) for these are:

12 February, 10 March, 14 April, 13 May, 10 June, 19 August, 16 September, 14 October, 11 November, 9 December

An annual assembly with an opportunity for face-to-face meetings across and within WPs will be held 23-24 April 2020, in Madrid, Spain.

In addition, we plan to have at least two scientific web conferences for project participants; one in Spring, before the annual meeting, and one in Autumn. The dates for these have not yet been decided.

## JRP07 - LISTADAPT

### 1. Summary of the work carried out in year 2 (January to December 2019)

In December 2019, we have closed the WP1 dedicated to the collection of 1738 *Listeria monocytogenes* (*Lm*) strains. All the strains collected by the different partners (*Task 1.2*) are now centralized, long-term-maintained and stored within the bacterial collection of ANSES (Maisons-Alfort). The LISTADAPT collection, unique in Europe, is composed of three compartments, one of which (First compartment C1) very original, as it regroups 1051 strains isolated in the environment (soil, river, farm environment) along with strains from wild and farm animals (both healthy and animal presenting clinical symptoms). It was very challenging to collect such strains. That is why we needed to (i) collaborate with more partners and (ii) perform additional sampling campaigns. This has led to a substantial delay in the project. The second compartment (C2) is composed of 577 strains from five ready-to-eat (RTE) food categories. C2 includes also genomes from LISTADAPT partners from previous studies or from sequencing activities carried out in the context of NRL's activities (*Task 2.2.3*). The last compartment (C3) is composed of 110 strains involved in human sporadic cases.

Regarding the WP2, the second batch of sequencing was done at the beginning of 2019, the third in July, the fourth in September and the last will be done in January 2020. The total genome collection comprises 2557 genomes with 1738 new genomes generated for this project. Of these genomes, 1383 are now *de novo* assembled and annotated through a harmonized in-house workflow called ARTwork (Radomski et al., 2019; https://github.com/afelten-Anses/ARtWORK) adopted in the ANSES laboratory of food safety. Here, we also received and centralized the 782 genomes (raw reads) of partners which performed in-house sequencing (*Task 2.1.3 and T 2.2.2.2.3*). We will complete assembling and annotations of all these genomes by February 2020.

The Data Management Plan of LISTADAPT was successfully deployed with the notable creation of a Zenodo repository (10.5281/zenodo.2617258). We plan to make available to the scientific community all the produced genomes in an umbrella *Bioproject* submitted to the European Nucleotide Archive (ENA). We aim to write a paper describing the whole LISTADAPT collection in the review "Scientific data" of the Nature Journal research (draft paper expected by March 2020).

The three LISTADAPT partners involved in the phenotypic characterization (WP3) received the second set of 100 strains (those isolated from environment) in April 2019 (*Task 3.1*). Several studies were therefore carried out on the susceptibility of strains to antibiotics and biocides (Task 3.2.1) as well as on the survival of strains in the soil microcosm (*Task 3.4.2*). These phenotypic data were compared with the genomic data (*Task 4.4*) and preliminary results obtained by Yann SEVELLEC, the ANSES post-doctoral student, were presented at a seminar organized by the Italian NRL (ISZAM) (4 July 2019). This work will be finished by February 2020. We plan to end the study on the effects of biocides on strains' adaptation by January 2020 (*Task 3.2*).The remaining phenotypic tests will be completed in May 2020.

Regarding the WP4, we analysed in depth the *Lm* clonal complexes (CCs) genetic diversity between the compartments (*Task 4.1*) and we showed the obtained results during a 2-days meeting held at ANSES in April 2019 including all the partners and some external partners. Linking the WP3 and WP4, we also started a comparative analysis on ~200 genomes to unveil the causal genomic variants for antibiotics and biocides resistance phenotypes using integrated pangenome-wide-association study (panGWAS) approaches. This study will also investigate the genomic environment of resistance genes on mobile genetic elements (e.g. plasmid, transposons, etc.). We plan to display all the results during two face-to-face meetings: (i) the first one day -17$^{th}$ January 2020- meeting ANSES-INRA specifically dedicated to WP3-WP4 (ii) the second of half a day -24$^{th}$ January 2020- with all the partners, just before the annual workshop EURL / NRL meeting.

The EJP project management team and the EJP Scientific Steering Board have both validated the extension of LISTADAPT for six months (January-June 2020). At ANSES (Maisons-Alfort), we decided (i) to extend the post-doctoral student, Yann Sevellec, contract by additional 6 months (until December 2020) and (ii) to recruit another post-doctoral student, Federica Palma, for six months (until June

2020). This will help to carry out the latest task of WP4 and to gain more time for publishing the obtained results in peer-reviewed scientific journals. The first results obtained in 2019 on LISTADAPT were summarized in a total of four oral communication and eight posters presented during national and international conferences. Moreover, IZSAM and ANSES are writing a paper on the occurrence of a ST121 strain isolated from a dolphin brain, for publication in Frontiers in Microbiology.

## 2. Work carried out in the JRP, scientific results

### WP0: Coordination

The LISTADAPT leader, Laurent Guillier, left the project during the summer of year 2 (Y2) for another position in ANSES. The project lead has been transferred to Sophie Roussel, the successor of Laurent Guillier. Sophie Roussel is a senior scientist that has maintained close relationships with all the LISTADAPT partners. Indeed, she was a scientist at ANSES from 2007 to 2017 working as the head of the molecular typing team of different food-borne bacteria, including *Listeria monocytogenes*. She hold grants from EU-DG Santé, EFSA and national grants. She was closely involved in European projects funded by EFSA and by the European Union (Horizon 2020 PHC7-"COMPARE"). In 2016, with her team, she wrote and set up LISTADAPT. Sophie Roussel worked then during two years (2017-2019) as Research Director at INRA and was coordinator of the International Centre of Microbial Resources (CIRM) set up by INRA. In this frame, she was involved in different European programs (H2020 - "CIRCLES" project and H2020 EJP One Health "CARE").

As LISTADAPT leader, Sophie Roussel coordinated and organised two face-to-face meetings: (i) the first – one day -17th January 2020- meeting Anses-INRA specifically dedicated to WP3-WP4 (ii) the second - half a day -24th January 2020- with all the partners, just before the annual workshop EURL / NRL *Listeria* meeting.

### WP1: Constitution of a strains collection representative of the different reservoirs of Listeria monocytogenes

#### ***JRP7-WP1-T1****: Strains collection (M1-M12)*

This task has been completed in December 2019. All the 1738 strains collected by the different partners (*Task 1.2*) are now centralized, long-term maintained and stored within the ANSES bacterial collection. At ANSES, typing data and associated epidemiological information of all the strains are centralized in a molecular database under the software BioNumerics version 7.6.3. Typing data included MLST data and cgMLST data. In addition, a standardized nomenclature has been established with well-defined categories for all epidata (country, origin, matrix and animal condition) in order to improve the homogeneity of strains IDs.

#### ***JRP7-WP1-T2****: Campaigns to collect additional animal and environmental strains (M1-M10)*

It was very challenging to find strains from soil and animals, consequently more partners have been involved, and additional sampling campaigns performed. This explains the delay in the project.

#### ***JRP7-WP1-T2-ST1****: External collaborations (M1-M2)*

This task has been completed in November 2019. As we had not enough strains from soil and animals, we extended this task and we looked for more partners. In total, we have successfully collaborated with fifteen external European partners. They provided either *Lm* strains (Table 1) (that have been sequenced in LISTADAPT) or sequences (Table 2).

**Table 1. List of 13 European partners who have provided the strains that have been sequenced in LISTADAPT**

| Partners | Country | Strain origin | Number of strains provided | Strain Isolation period | References |
|---|---|---|---|---|---|
| Faculty of Veterinary Medicine/ department of Food Hygiene and Environmental Health, Helsinki | Finland | Animal (birds, healthy animals) | 180 | 1978-2015 | Castro et al., 2018 doi: 10.1128/AEM.02000-17 |
| BIOR, Institute of Food Safety, Animal Health and Environment | Latvia | Animal sector (cattle and farm strains) | 33 | 2010-2018 | |
| West Pomeranian University of Technology Szcecin (ZUT) | Poland | Soil | 60 | 2010 and 2016 | Szymezak B et al., 2014. doi: 10.1007/s12223-013-0260-8 |
| University of Munich | Germany | Wild animal (deer and wild boar) | 55 | 2011-2012 | Weindl et al., 2016 doi: 10.1089/fpd.2015.2061 |
| Institute of Hygiene and Meat Technology (IHMT) | Germany | Healthy animals at slaughterhouse | 19 | 2009-2018 | |
| Neiker Tecnalia<br>Instituto Vasco de Inves | Spain | Farm animal (cattle, sheep) | 68 | | -Esteban JI et al., 2009 doi:10.1186/1746-6148-5-2<br>-Hurtado A et al., 2017 doi.org/10.1016/j.vetmic.2017.09.003 |
| INIAV Animal Pathology Laboratory | Portugal | Farm animal (cattle, sheep, poultry) | 14 | | |
| Veterinary faculty of Ljubjana (NRL for Lm) | Slovenia | Farm animal (cattle, sheep) | 163 | 2010-2019 | |
| VWA –laboratory Feed and Food and Product Safety (NRL for Lm) | The Netherlands | Farm animal | 37 | 2016-2018 | |
| Veterinary and Food Laboratory | Estonia | Farm animal healthy animals | 29 | 2015-2018 | |
| Institute for Agricultural, Fisheries and Food research, ILVO | Belgium | Animals | 120 | | |
| Veterinary Faculty of Zaghreb | Croatia | Animals | 30 | 2016-2017 | |
| State Veterinary and food Institute Dolny Kubin | Slovakia | Animals | 30 | 2014-2018 | |

**Table 2. List of the three European partners who have provided with sequences not made publically available at the time of the project**

| Partners | Country | Strain origin | Number of genomes provided | Strain Isolation period | References |
|---|---|---|---|---|---|
| Public Health England (PHE) | United Kingdom | Animal (Hedgehogs) | 5 | | -Hydeskov et al., 2019 https://doi.org/10.1638/2018-0093 |
| Vetsuisse Faculty University of Bern | Switzerland | Farm environment Animal | 228 | | -Aguilar-Bultet et al. doi: 10.3389/fcimb.2018.00020 -Dreyer et al., 2018 10.1038/srep36419 |
| Veterinary faculty of Ljubjana (NRL for Lm) | Slovenia | Farm animal (cattle, sheep) | 78 | 2010-2019 | -Papic et al. 2019 BMC Microbiology accepted for publication the 4th December 2019 |

***JRP7-WP1-T2-ST2**: Sampling campaigns (M1-M10)*

This task has been completed. The last sampling campaign was carried out in June 2019.

***JRP7-WP1-T3**: Strategy for sequencing (M1-M12)*

This task has been completed, see annual report 2018. The last batch of strains to sequence was sent in December (Deliverable D-JRP7-1.3 uploaded on web site)

## *WP2: Whole genome sequencing of Listeria monocytogenes strains*

***JRP7-WP2-T1**: Purification of Lm DNA from 2000 Lm strains (M2-M14)*

***JRP7-WP2-T1-ST1: First batch Purification of DNA from Lm strains available (M2-M4)***

This task has been completed, see annual report 2018.

***JRP7-WP2-T1-ST2**: Second batch Purification of DNA from additional Lm strains (M13-M14)*

This task has been completed.

***JRP7-WP2-T1-ST3**: Purification of DNA from routine surveillance systems at IZSAM, DTU, AGES (M1-M12)*

This task has been completed, see annual report 2018.

***JRP7-WP2-T2**: Whole Genome Sequencing (WGS) (M3-M14)*

***JRP7-WP2-T2-ST1**: First batch WGS for available Lm strains (M3-M6)*

This task has been completed, see annual report 2018.

***JRP7-WP2-T2-ST2**: Second batch WGS for additional Lm stains (M13-M14)*

This task will be completed in January 2020.

***JRP7-WP2-T2-ST3**: Ad hoc WGS (M3-M14)*

This task has been completed. The genomes were centralized at ANSES.

***JRP7-WP2-T3**: Genome Assembling and Annotation (M5-M2)*

We will have finished the assembling and annotation of all the genomes by January 2020.

## *WP3 Phenotypic characterisation of Listeria monocytogenes strains*

***JRP7-WP3-T1**: Strategy for selection of strains for phenotyping (M1-M12)*

The three LISTADAPT partners (INRA, NVI and ANSES) involved in phenotypic characterization (WP3) received the second set of 100 strains (those isolated from environment) in April 2019. The selection was done according to the MLST-CC data. The most prevalent CCs were selected. A total of 200 strains were then analysed.

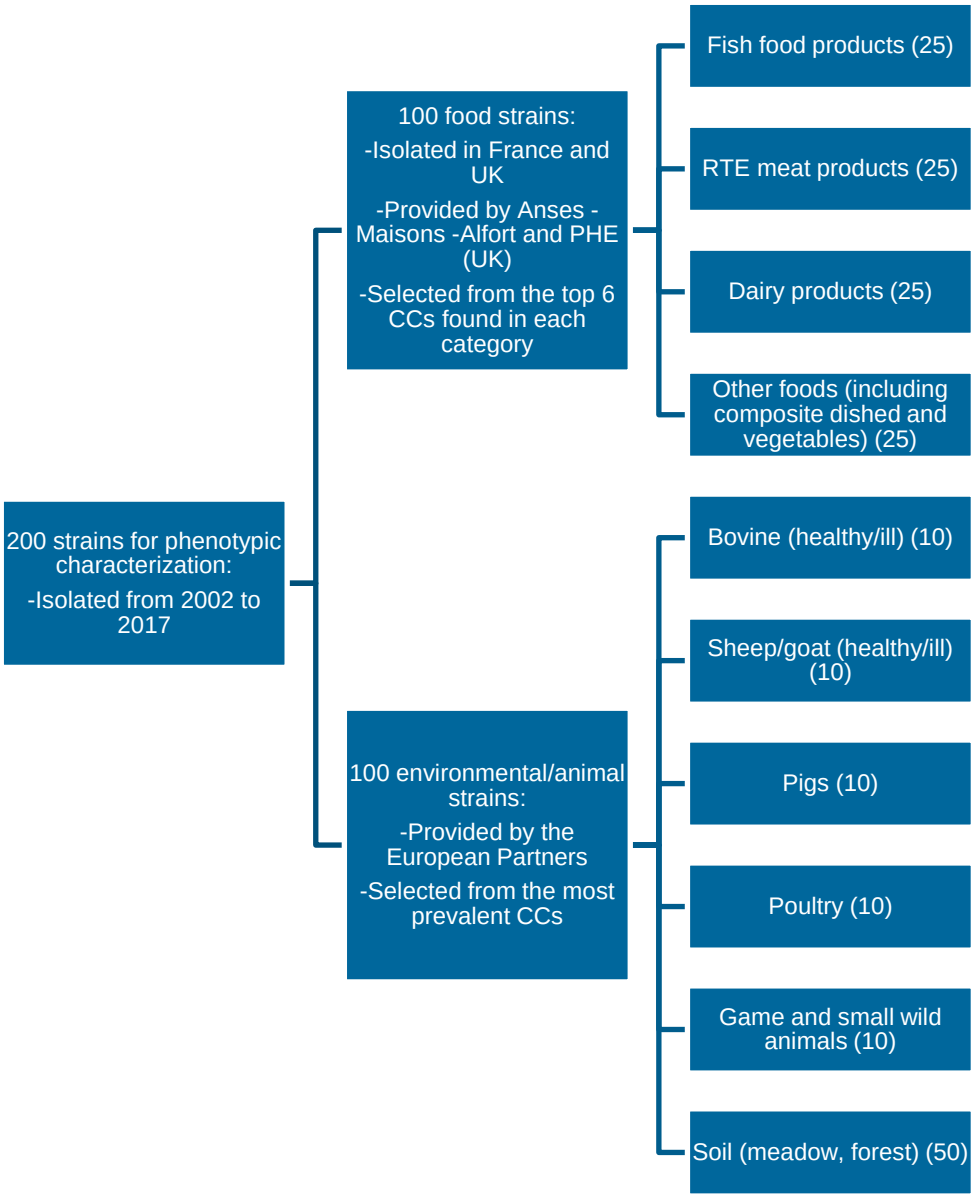


Figure 1: Description of the 200 strains selected for phenotypical tests.

***JRP7-WP3-T2**: The effects of biocides on Lm strains adaptation (M3-M29)*

***JRP7-WP3-T2-ST1**: Antibiotics and biocides resistance profiles of Lm strains (M3-M22)*

The characterization of MICs values for 14 antibiotics and 8 biocides was achieved in August 2019 (report available). ). The Deliverable D-JRP7-3.1 was uploaded on web site on 17 December 2019.

***JRP7-WP3-T2-ST2**: Adaptation to biocides and cross-resistance development to antibiotics of relevant Lm strains (M12-M22)*

Thirty-four isolates have been already tested on three disinfectants (seven different concentrations). The disinfectants used were Didectyl Dimethyl Ammonium Chloride (DDAC), Sodium hypochlorite (HS) and Hydrogen peroxide (Hper). This task will be completed in January 2020.

***JRP7-WP3-T2-ST3**: The effect of biocides on Lm strains in biofilm (M12-M29)*

This task will be completed in May 2020 (M29).

***JRP7-WP3-T3**: Bacterial adhesion and biofilm formation of Lm strains (M3-M29)*

This task will be completed in May 2020 (M29).

***JRP7-WP3-T4**: Survival and persistence of Lm strains in different ecological niches (M3-M29)*

***JRP7-WP3-T4-ST1**: Survival of Lm in food products and gastro-intestinal environment (M3-M29)*

The growth in broth in response to lactate and acetate, both frequently used preservatives, have been carried out for 130 isolates (including all isolates in the culture collection from food). The study was performed with two concentrations of each additive and three temperatures in the range from 4 to 12 °C. The characterization of survival in gastro-intestinal conditions will be ended in May 2020.

***JRP7-WP3-T4-ST2**: Survival of Lm in soil microcosm (M3-M16)*

This sub-task has been completed in November 2019. The Deliverable D-JRP7-3.6 was uploaded on web site on 17 December 2019. As shown Figure 1, the ability of *Lm* to survive in soil was strain dependent. Survival ranged from zero to 22% (Figure 2).

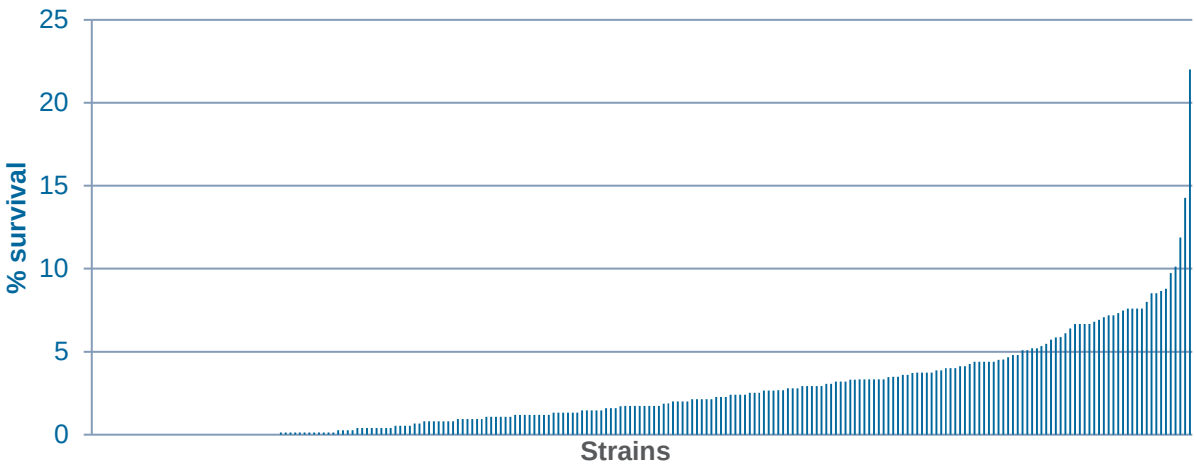


Figure 2. Soil survival phenotype of 230 isolates of *Listeria monocytogenes.*

Ascending Hierarchical Clustering clearly identified 3 groups of phenotypes (Figure 3), possibly indicating that some isolates (Phenotype 3) may be better competitors in complex habitats such as soil.

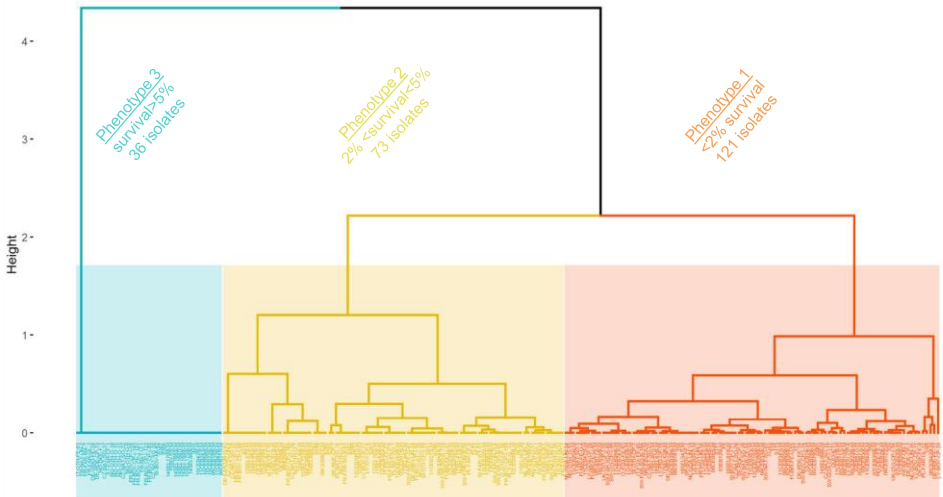

Figure 2. Ascending Hierarchical Clustering of soil survival data.

## *WP4: Identification of genetic traits in Listeria monocytogenes underlying adaptation to the ecological niches*

### ***JRP7-WP4-T1:*** *Analyze the distribution / prevalence of clonal complexes among the reservoirs (M1-M14)*

This task has been completed in April 2019. We analysed in depth the strains genetic diversity between the three compartments and we showed the obtained results during (i) a 2-days-meeting at ANSES in April (2019) including all the partners and some external partners and (ii) the EJP general meeting in Dublin (2019).

This characterization helped to determine the two sets of strains for phenotypic studies. The final distribution would marginally evolve with the last batch of sequenced strains.

### ***JRP7-WP4-T2:*** *Literature search of genes or genetic mechanisms responsible for virulence, adaptation and survival (M9-M12)*

This task has been completed. The list of genes involved in adaptation and survival was produced from research data obtained during the H2020 COMPARE project (cf point 6 "Interactions with other JRPs / JIPs or with external relevant project"). The Deliverable D-JRP7-4.1 was uploaded on web site on 16 December 2019.

### ***JRP7-WP4-T3:*** *Biostatistics analysis of annotated genomes (M6-M29)*

#### ***JRP7-WP4-T3-ST1:*** *Identification of statistically relevant methods and development of analysis (M6-M16)*

During the workshop of Y1, a list of relevant tools for identifying markers of adaptation to niches (environment, food industry) was established. The LISTADAPT partners has identified two alternative methods (DBGWAS and machine learning method from DTU) (Jaillard et al., 2018). Within the full lists, at least three methods are tested (Machine learning, GWAS based on presence/absence matrix and TreeWAS for SNP) (Brynildsdrud et al., 2016; Collins et al., 2018). For the research of genes identified in JRP7-WP4-T2, the LISTADAPT partners have chosen to use ABRICATE method (https://github.com/tseemann/abricate). The Deliverable D-JRP7-4.3 was uploaded on web site on 16 December 2019.

References :

DBGWAS:https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1007758

Scoary: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-1108-8

TreeWAS: https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1005958

#### ***JRP7-WP4-T3-ST2:*** *Processing of all isolates (M22-M27)*

The final analysis is postponed to year 3 considering the delay for the other tasks.

### ***JRP7-WP4-T4****: Comparative analysis of phenotypic data / genotypic data (M24-M29)*

We are investigating the correlation between the phenotypes obtained for survival in soil and the characteristics of the genomes from these isolates. When GWAS was performed on closely related strains (notably, belonging to the same CC), significant correlations were evidenced and putative mechanisms important for soil survival were highlighted. The final analysis is postponed to year 3 considering the delay for the other tasks.

## *WP5 : Trainings and dissemination*

***JRP7-WP5-T1:*** *Implementation of a workshop (M1-M2)*

Statistical and bio-informatic tools useful for the project were discussed during the Kick off meeting (March 2018).

***JRP7-WP5-T2:*** *Trainings (M3-M6)*

This task has been completed, see annual report 2018. An additional training has been organized by the LISTADAPT coordinator in April 2019, in parallel with the meeting. It aimed to train 20 participants to R package methods.

***JRP7-WP5-T3****: Proficiency Testing Trials (M19-M22)*

Given the delay in the project, we decided that there will be no Proficiency testing trial WGS as planned in WP5 (Task 5.3). However, as part of the activities carried out by ANSES as EURL / NRL Listeria, a PT trial WGS was organized in 2018, then in 2019, during which four LISTADAPT partners (AGES, IZSAM, NVI, ANSES) participated.

***JRP7-WP5-T4:*** *Dissemination (M1-M36)*

The first results obtained in LISTADAPT were summarized in two oral communications and in five posters presented at the International Symposium on Listeriosis Problems (ISOPOL XX/ 24-27 September) in Toronto.

Moreover, IZSAM and ANSES are writing a paper on the occurrence of a ST121 strain isolated from a dolphin brain, for publication in Frontiers in Microbiology. As soon as we have finished annotating and assembling all the sequences, we will write a paper describing the LISTADAPT collection in the review "Scientific data" of the Nature Journal research (draft paper expected by March 2020). All the genomes will be thus made available to the scientific community with associated epidata in public repositories (ENA). In parallel, we will start writing other research articles, in collaboration with LISTADAPT partners, focusing on the results from the different WPs, for publication in international peer-review scientific journals. We plan also to present our results during (i) the **One Health EJP ASM 2020** meeting (May 2020-Pragues), (ii) the **6$^{th}$ World One Health Congress (June 2020- Edinburg) and (iii)** the "Food Micro" congress (September 2020,-Athens).

## 3. Progress of the research project: milestones and deliverables

***Deliverables***

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| LISTADAPT | D-JRP7-0.3 | Plan for dissemination and exploitation of results. | 14 | 16 | - | The dissemination plan was validated during the face-to-face meeting of April 2019. |
| LISTADAPT | D-JRP7-1.3 | Description of the second panel of strains to sequence. | 12 | 24 | - | The last panel of strains to sequence was sent in December (11 December 2019) --Deliverable uploaded on web site on 18 December 2019. |
| LISTADAPT | D-JRP7-1.4 | Report on strain collection and strategy for selection of strains for sequencing. | 14 | 14 | - | The strategy was presented during the OHEJP conference in May 2019 (Dublin). -Deliverable uploaded on website on 16 December 2019 |
| LISTADAPT | D-JRP7-2.1 | Annotation of Lm genomes already sequenced (genomes available before the start of the project). | 6 | - | 26 | The assemblies and annotations of a part of genomes (genomes available before the start of the project) were carried out using Prokka V.1.12 (Seemann 2014). |
| LISTADAPT | D-JRP7-2.2 | Annotation of the Lm assembled genomes from 1st batch sequencing. | 10 | - | 26 | Annotation of a part of genomes is finished. It has been carried out with Prokka (V.1.12) software. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| LISTADAPT | D-JRP7-2.3 | Annotation of the Lm assembled genomes from 2nd batch sequencing. | 15 | - | 26 | |
| LISTADAPT | D-JRP7-2.4 | Annotation of the Lm assembled genomes from ad hoc WGS | 17 | - | 26 | December 2019: The partners sent us all the sequences. We started the assembly and annotation of these sequences. |
| LISTADAPT | D-JRP7-3.1 | Resistance profiles to biocide and antibiotics for the 200 Lm strains. | 12 | 20 | - | --Deliverable uploaded on web site on 17 December 2019.<br>-Poster presented during the OHEJP conference in May 2019 (Dublin) and during the IAFP's European Symposium on Food safety in April 2019 (Nantes). |
| LISTADAPT | D-JRP7-3.2 | Assessment of the ability to adapt to biocides and develop cross-resistance to antibiotics for some illustrative Lm strains. | 22 | - | 25 | |
| LISTADAPT | D-JRP7-3.3 | Data on the effect of biocides on Lm strains in biofilm. | 22 | - | 29 | |
| LISTADAPT | D-JRP7-3.4 | Biofilms phenotypes for the 200 Lm strains. | 22 | - | 29 | |
| LISTADAPT | D-JRP7-3.5 | Collection of data on survival of Lm as planktonic cells in various ecological niches. | 22 | - | 29 | |
| LISTADAPT | D-JRP7-3.6 | Collection of data on survival of Lm in soil microcosms. | 22 | 20 | - | -Deliverable uploaded on web site on 17 December 2019. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | -Poster presented at ISOPOL XX in September 2019 (Toronto). |
| LISTADAPT | D-JRP7-4.2 | Report on prevalence and distribution of clonal complexes among the reservoirs. | 13 | 17 | 25 | -Oral communication at OHEJP conference in May 2019 (Dublin).<br>Not yet uploaded on website |
| LISTADAPT | D-JRP7-4.3 | Software chosen for bioinformatics analysis. | 16 | 16 | - | -Validated during the LISTADAPT meeting (April 2019).<br>-Deliverable uploaded on website on 16 December 2019 |
| LISTADAPT | D-JRP7-5.1 | "LISTADAPT" workshop program. | 2 | - | - | No workshop was organised. Exchanges and discussions were held during the kick off meeting on methodologies and bioinformatics and statistical tools used in LISTADAPT; |
| LISTADAPT | D-JRP7-5.2 | Minutes of the training sessions. | 10 | - | - | There has been no minutes on the training sessions. |
| LISTADAPT | D-JRP7-5.3 | Publications and communications. | 24 | - | from M29 | |

***Milestones***

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| LISTADAPT | M-JRP7-2 | Selection of the 200 Lm strains based on genomic analyses in WP2. | 3 | Yes | - | 100 strains have been selected based on their genomic characteristics and context of isolation. These strains correspond to samples collected along the food production chain.<br>For the remaining 100 strains (from environment), the selection was based on the prevalence of CCs in C1 compartment and chosen to best represent the diversity at the European level. The strains were sent to partners in May 2019. |
| LISTADAPT | M-JRP7-7 | Face-to-face meeting -2018 | 8 | Yes | - | Kick off meeting (March 2018) |
| LISTADAPT | M-JRP7-14 | Selection of some representative Lm strains for the study of adaptation to biocides. | 12 | Yes | 12 | 15 strains were selected. |
| LISTADAPT | M-JRP7-15 | WGS raw data produced. | 13 | No | 25 | The last batch of sequencing will be done by January 2020. |
| LISTADAPT | M-JRP7-16 | Second batch Lm genomes assembly completed. | 14 | No | 26 | This work will be completed by February 2020. |
| LISTADAPT | M-JRP7-17 | The database includes MLST data (CC and ST) of all the strains. | 14 | Yes | - | This work led the first communication of results in May 2019 (OHEJP / Dublin). |
| LISTADAPT | M-JRP7-18 | Second batch of Lm genomes annotation completed. | 15 | Yes | 26 | |
| LISTADAPT | M-JRP7-19 | Ad hoc batch of Lm genomes assembly completed | 16 | No | 25 | In December 2019, we received and centralized all the genomes of the partners. |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | Assembling will be completed by January 2020. |
| LISTADAPT | M-JRP7-20 | Bioinformatics analysis done for all the strains. | 16 | No | 28 | |
| LISTADAPT | M-JRP7-21 | Bioinformatics analysis done for all the strains. | 17 | No | 28 | |
| LISTADAPT | M-JRP7-22 | Ad hoc batch Lm genomes annotation completed | 17 | No | 22 | |
| LISTADAPT | M-JRP7-23 | Face-to-face meeting -2019 | 17 | Yes | 16 | A 2-days face-to-face meeting has been organized in April 2019 (9-10). This meeting was held at ANSES and included all the LISTADAPT partners along with 7 of the external partners. |
| LISTADAPT | M-JRP7-24 | WGS Proficiency Testing trial (PT trial) done | 22 | No | - | Given the delay in the project, Rene Hendriksen (DTU), which was in charge of the WGS PT trial, left LISTADAPT at the beginning of the year 2. The WGS PT trial has been therefore cancelled. |

## 4. Publications and patents

### Oral communications (4)

Felix B, Feurer C, Maillet A, Guillier L, Boscher E, Kerouanton A, Denis M and Roussel S (2019). Population genetic structure of *Listeria monocytogenes* strains isolated from the pig and pork production chain in France. 26-27 August 2019, Safepork 2019, Berlin.

Félix B, Feurer C, Maillet A, Desmonts M H, Hickey B, Jankuloski D, Karpíšková R, Skjerdal T, Denis M, Gareis M, Zdovc I, Pietzka A and Guillier L (2019). Typing and persistence of *Listeria monocytogenes* strains in food processing environments, prophages identified as major persistence markers. ISOPOL XX 2019, 24 – 27 September 2019, Toronto.

Guillier L (2019). Assessment of the Genomic Diversity of a Large Collection of *Listeria monocytogenes* Strains Isolated in EU Natural Environments. OHEJP Annual scientific meeting, Dublin, 22-24 May 2019

Guillier L (2019). Proposal of an Original Method for Selecting Strains to Include in Source Tracking or Source Attribution Based on their Metadata. OHEJP Annual scientific meeting, Dublin, 22-24 May 2019

### Posters (8)

Oevermann A, Hurtado A, Papić B, Karpíšková R, Piveteau P, Wullings B, Bulawova H, Castro H, Lindström M, Korkeala H, Šteingolde Ž, Bērziņš A, Avsejenko J, Kramarenko T, Cabanova L, Szymczak B, Torresi M, Leroux A, Sevellec Y, Guillier L and Félix B (2019). European-wide study reveals high prevalence of hypervirulent *Listeria monocytogenes* clones in farmed ruminants and their environment. ISOPOL XX (24 – 27 September 2019 Toronto)

Skjerdal T, Sevellec Y, Guillier L, , Zdovc I, Pate M, Torresi M, Riacao M, Boysen M, Lindstrøm M, Castro H, Gareis M, Bulawova H, Amar C, Grant K, Leroux A, Pomillo F, Camma C, Di Pasquale A, Lagesen K, Osland Mohr A, Rinaldi A, Karpiskova R, Pietzka A, Ruppitsch W, Szymczak B, Ascencio-Schultz E, Piveteau P and Felix B (2019). Occurrence and diversity of *Listeria monocytogenes* strains in environment and wild life in Europe. ISOPOL XX (24 – 27 September 2019 Toronto)

Ascencio-Schultz E, Gal L, Garmyn D, Szymczak B, Karpiskova R, Pietzka A, Ruppitsch W, Boysen M, Pomilio F, Torresi M, Camma C, Di Pasquale A, Pate M, Skjerdal T, Sevellec, Y., Felix B, Guillier L and Piveteau P (2019). Investigation of genome characteristics underlying fitness of *Listeria monocytogenes* in soil. ISOPOL XX (24 – 27 September 2019 Toronto)

Sévellec Y, Torresi M, Orsini M, Centorotola G, Bilei S, Senese M, Terracciano G, Felix B, Guillier L and Pomilio F (2019). Investigation of a dolphin infection by L*isteria monocytogenes* CC121. ISOPOL XX (24 – 27 September 2019 Toronto)

Vranckx K, Sevellec Y, Deneweth J and Felix B. Phages in *Listeria*: Who are they, what do they do? ISOPOL XX (24 – 27 September 2019 Toronto)

Guérin A, Le Grandois P, Bridier A, Soumet C (2019). Evolution of antibiotics and biocides resistance of *Listeria monocytogenes* from diverse ecological niches following *in vitro* exposure to biocides disinfectants. OHEJP Annual Scientific meeting, XX (24 – 27 September 2019 Toronto)

Guérin A, Le Grandois P, Bridier A, Soumet C (2019). Evolution of antibiotics and biocides resistance of *Listeria monocytogenes* from diverse ecological niches following *in vitro* exposure to biocides disinfectants. IAFP's European Symposium on Food Safety, Nantes, 24-26 April 2019

Guérin A, Le Grandois P, Bridier A, Soumet C (2019). Evolution of antibiotics and biocides resistance of *Listeria monocytogenes* from diverse ecological niches following *in vitro* exposure to biocides disinfectants. OHEJP Annual Scientific meeting, Dublin, 22-24 May 2019

## 5. Impact & relevance

LISTADAPT allows continuing stimulation or implementation of WGS as the method of surveillance of *Lm* in the European countries, as recommended by ECDC and also by the EFSA Biohaz panel in 2019 (https://doi.org/10.2903/j.efsa.2019.5898).

The genomic approaches implemented in LISTADAPT project aim to identify molecular markers of interest such as mobile genetic elements harbouring antimicrobial resistance factors as well as provide insight into the population structure and evolutionary history of *Lm* for epidemiologic investigation. This information could be used for the development of **new diagnostic tests** to screen food, processing environment and animal reservoirs for contamination by *Lm* strains. These new tests represent **key tools** to improve the *Lm* surveillance system and to assist the food industry decision-making around food processing for improving food safety.

The LISTADAPT project makes it possible bridging the gap between "Med" and "Vet": this is the first time a project has focused on such a large and diverse collection of *Lm* strains isolated from farm animals in different European countries. The detailed characterization of these strains at phenotypic and genotypic level will help to assess the true importance of these strains as sources of foodborne infections for public health.

LISTADAPT's partners now share a well-characterized collection of 1139 strains representative of the different ecological niches. This collection is unique in Europe. As soon as it is completed, the database centralizing metadata, genes and genomic markers will be available to LISTADAPT partners. In addition, the protocols adopted in LISTADAPT to select strains and evaluate their adaptation to different ecological niches were shared with all partners.

## 6. Interactions with other JRPs/JIPs or with external (EU or national) relevant project

The project has resulted in:

- The compilation of a very well-characterized collection of Lm strains representative of the different ecological niches. This collection is unique in Europe.
- The production of ~3000 new Lm genomes available to the scientific community.

These strains and genomes will be useful for the EJP One Health Project "CARE" (2020-2023) (Cross-sectoral framework for quality Assurance Resources for countries in the European Union). One of the objective of CARE is to set up reference panel (strains and genomes) for food-borne bacteria.

In the H2020 COMPARE project, the genes involved in the adaptation of ST121 and ST14 strains were investigated (Pasquali et al., 2018-Frontiers in Microbiology). Mobile genetic elements were also studied in depth. It has been shown that some clones harbour highly conserved mobile genetic elements, probably due to selective pressures and the availability of compatible genetic elements. Interestingly, horizontal transfer of plasmids contributing to the survival of associated stressors has been identified between Lm clonal complexes (Palma et al., submitted to BMC microbiology in 2019). These results obtained in COMPARE will help guide the search for markers associated with adaptation (WP4 of LISTADAPT).

In addition, LISTADAPT stimulated Reference laboratories to use WGS for the surveillance of *Lm* in their countries, as recommended by EFSA and ECDC.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements (from ethical reviewers) in 2018 | Measures and actions taken at the end of 2018 | Comments Ethical advisors January 2019 | Comments Project Leader at the end of 2019 |
|---|---|---|---|
| The applicants must confirm that ethics approvals for the use of isolates from human origin have been sought. | No isolates of human origin has been included in LISTADAPT right now. The phenotypic tests will be carried out from strains from environment or food. Sequences from strains isolated in listeriosis context might be used in year 2, but the sequences will be gathered in open Bioproject with ethics approvals. | EA did not comment | / |
| The applicants must document the safety mitigation measures in place to protect the staff. | Every partners has the biosafety level associated with the handling of Listeria. | Satisfactory reply | / |
| The applicants must document there are no environmental safety issue from the sampling etc. and protocols are in place. | A video has been proposed to partners that samples environment (soil, water). | Satisfactory reply | / |
| 'Animal samples' are collected, please re-confirm no animal experimentation approvals are required for the animal sampling protocols (e.g. do to interventions, restrictions, etc.). | No animal experimentation have been done. Samples corresponds to faecal samples. | EA did not comment | / |

## 8. List of critical risks

| Description of risk | Yes/No |
|---|---|
| Loss of key-persons (staff and / or leaders) | Yes/No (1) |
| Delay in work plan execution | Yes (2) |
| Conflicts within the consortium | No |
| Lack of commitment of partners | No |
| Delay in duties, tasks or reporting | Yes |
| Poor intra-project (JRP) relationship | No |
| Potential entry/exit of partners | Yes/No (3) |
| Other risks (please describe) | No |

### Additional information

1. The LISTADAPT leader, Laurent Guillier, left the project in June of Y2 for another position in ANSES. The project lead has been transferred to Sophie Roussel, from 1 October 2019 when she was recruited.
2. It was very challenging to find enough strains isolated from natural environment and animals. We needed to do additional sampling campaigns and seek more external partners than expected. This led to delays in the project.
3. Given the delay in the process, René Hendriksen (DTU), leader of the entire WP4 and the WP5 *task 5.3*, left this project at the beginning of Y2.

## 9. List of dissemination and communication activities

| | | | |
|---|---|---|---|
| Name of the activity: | Poster / IAFP's European Symposium on Food Safety | | |
| Date: | 24–26 April 2019 | | |
| Place: | La Cité des Congrès de Nantes, France | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | X |
| Industry | 15 | Investors | X |
| Civil Society | X | Customers | X |
| General Public | X | Other | X |
| Policy Makers | 10 | | X |

| | | | |
|---|---|---|---|
| Name of the activity: | 1 Poster and 1 oral communication / OHEJP Annual Scientific meeting | | |
| Date: | 22-24 May 2019 | | |
| Place: | Teagasc Conference Centre, Ashtown, Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at OHEJP Annual Scientific meeting: Proposal of an Original Method for Selecting Strains to Include in Source Tracking or Source Attribution Based on their Metadata | | |
| Date: | 22-24 May 2019 | | |
| Place: | Teagasc Conference Centre, Ashtown, Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation OHEJP Annual Scientific meeting: First Assessment of the Genomic Diversity of a Large Collection of *Listeria monocytogenes* Strains Isolated in EU Natural Environments | | |
| Date: | 22-24 May 2019 | | |
| Place: | Teagasc Conference Centre, Ashtown, Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | |
|---|---|
| Name of the activity: | 1 Oral presentation and 6 posters<br>International Symposium on Problems of *Listeria* and Listeriosis (ISOPOL) |
| Date: | September 24 - 27, 2019 |
| Place: | The Peter Gilgan Centre for Research and Learning, Toronto |

| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |

| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
|---|---|---|---|
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 500 | Media | 3 |
| Industry | 20 | Investors | 0 |
| Civil Society | 0 | Customers | 0 |
| General Public | 0 | Other | 0 |
| Policy Makers | 20 | | X |

## 10. List of planned tele- or video conferences, face to face meetings in the next year

- 17th January 2020: A face-to-face meeting on one day, IRSTEA, Rennes (France): between the French partners (INRA and ANSES)
- 29th January 2020: An half-day meeting, ANSES, Maisons-Alfort (France): between all the partners
- 29th-31th January 2020: Workshop EURL / NRL Listeria, ANSES Maisons-Alfort. The seven NRLs partners of LISTDAPAT will attend the workshop. Sophie Roussel will make a presentation on LISTADAPT.
- Teleconferences will be planned during 2020 to advance on the valorization of LISTDAPAT outputs.

## JRP08 - METASTAVA

### 1. Summary of the work carried out in year 2 (January to December 2019)

Metastava obtained a 6 months cost free extension to allow more time for data analysis and valorisation. As a result, several deliverables can be delayed until M30 and will be reported in the final report.

WP1: Tasks 1.1 and 1.2 have been previously delivered. Task 1.3 (documentation of publicly available datasets) shifted its focus to datasets we are producing ourselves in Metastava due to the limited metadata available for most public datasets. Task 1.4 is scheduled for the last 6 months of the project (prolongation) , and is heavily linked to task 2.1; a joined effort to document existing guidelines and norms was started at the Metastava progress meeting in Brussels in February 2019.

WP2:Task 2.1 is linked to 1.4 where we have started an effort to document currently available norms and guidelines for diagnostic metagenomics and NGS QC and interpretation in general. Task 2.2: external controls for metagenomics QC: datasets have been produced for representative sample matrices (tissue, swab, fecal, serum) metagenomics analysis (read classification using several algorithms) is finished, and the resulting output is being analysed and compiled . Task 2.3 data on batch effects collected, analysed, and conclusions made. Deliverable was submitted. Task 2.4 (applying QC metrics to parallel projects) awaits conclusions from T2.1. Task 2.5. several partners participated in a COMPARE PT. In addition a Metastava PT was prepared and send out in july 2019. Data collection is being finalized and analysis is ongoing.

WP3: task 3.1 all samples listed and documented (D3.1 finalised). task 3.6. procedure for analyzing the data has been agreed (D3.2 finalised). Tasks 3.2-3.5 ongoing (most sequencing data finalized and analysis ongoing).

WP4: in addition to initiatives reported in annual report: participation in discussion OHEJP-IRIDA-INNUENDO about WGS data, and presentation at the International Association for Biological Standardisation (IABS) meeting on NGS use for adventitious agent detection in biologicals. Stakeholder dissemination activities included presentations @ OHEJP Programme managers committee (9.5.2019), a national (Belgium) stakeholders info meeting about OHEJP (5.6.2019), OHEJP programme owners committee (19.6.2019), national (Sweden, SVA Research Day 12.11.2019) scientific dissemination meeting. General dissemination in addition to 12 mth report: 2 posters at ASM OHEJP, talk at IABS NGS meeting.

WP5: heavy management workload with Data management plan v.1; application for 6 mth extension; 21 month progress report and formalization of the roles of Dutch partners WUR and third partner ErasmusMC.

### 2. Work carried out in the JRP, scientific results

#### *WP1. Collect reference data from other metagenomic projects, select the metagenomic methods to be used for the project, and provide guidance data for informed metagenomic workflow design.*

***JRP8-WP1-T1**: broad survey to collect information about sample selection and data generation methods for metagenomics (M1-M12)*

This task has been completed, see annual report 2018

***JRP8-WP1-T2:** broad survey to collect information about data analysis methods for metagenomics (M1-M12)*

This task has been completed, see annual report 2018

***JRP8-WP1-T3:*** *identifying available sequence datasets (M1-M12)*

As reported in the 2018 annual report, a list of publicly available metagenomic sequence read deatasets from sample types relevant to Metastava was previously produced (D-JRP8-1.3), but indicated an issue with poor metadata (both in terms of sampling metadata and sequencing methodology metadata). We will focus on using metagenomic datasets produced in WP2 and WP3 of Metastava. The datasets produced for the PT (task 2.5) are good candidates to serve as reference datasets with excellent metadata.

***JRP8-WP1-T4:*** *Propose a standardised framework for the description of the application scope and analytical properties of a metagenomics assay (M18-M24)*

During the annual meeting (february 2019, Brussels), we decided to join efforts of WP1-T4 and WP2-T2 to provide diagnostic laboratories envisaging a diagnostic use of metagenomic methods with a schematical overview providing guidelines for:

- the proper selection of a sample preparation, sequencing and analysis method in function of the diagnostic question
- registration of critical parameters, and relevant quality control metrics to register and evaluate during the workflow
- interpretation of critical workflow and sequencing results metrics in order to evaluate the significance of the outcome of a metagenomic experiment
- potential follow-up strategies.

Participants in this effort started to share links to relevant existing review papers and guidelines. The resulting guidance document will be completed by the end of the prolongation period (M30)

## *WP2. Quality assurance tools for the validation and interpretation of metagenomics.*

***JRP8-WP2-T1:*** *The development of quality metrics to evaluate the significance of the outcome of a metagenomics experiment (M10-M24)*

See paragraph above: **JRP8-WP1-T4:** Propose a standardised framework for the description of the application scope and analytical properties of a metagenomics assay (M18-M24)

***JRP8-WP2-T2****: development and evaluation of external controls for metagenomics (M1-M18)*

Mengovirus extraction control kit (bioMérieux), originally marketed for the QC of targeted molecular diagnostics of foodborne viruses, was evaluated as a potential spike-in control for individual sample result validation in virus metagenomics (always using WP1 modules for data generation and analysis). A first experiment (sequencing of replicates of the mengovirus control reagents diluted in molecular biology grade water) proved that the reagent was free of contaminating viruses that may interfer with diagnostic metagenomics. A second experiment investigated the reproducibility of the use of the control (in terms of normalised mengovirus control reads) for clinical sample panels representing relevant sample matrices for Metastava. These included pig fecal material as a model for a matrix with complex and variable microbiome and host genomic content (also model for human feces), pig serum as a model for a matrix with a limited microbiome and variable host genomic content (according to the degree of hemolysis in the serum samples, highly variable host genomic contents were observed in the datasets), pig lung tissue as a matrix with predomination of host genomic information, and finally wild bird cloacal swabs as a model for a matrix containing highly variable microbiome and host genome content due to highly variable sampling. Two QC measurements were investigated: (1) a mengovirus qRT-PCR measurement after nucleic acid extraction (to validate the sample and viral nucleic acid quality) and (2) normalised mengovirus readcounts in the resulting NGS datasets. While the qRT-PCR metric validated the correct extraction of viral nucleic acids, the normalised readcounts provided an additional level of QC, allowing the detection of issues where viral reads were outcompeted by host or bacterial reads and helping to identify false negative results in these cases. We have also shown that the addition of mengovirus did not have an effect on the detection of other viral taxa compared to a

control (without mengovirus) condition. As expected, the quantitative reproducibility of mengovirus readcounts varied with the complexity of the samples, necessitating a sample matrix specific interpretation of their use and usefulness.

***JRP8-WP2-T3**: reproducibility and batch effect evaluation (M1-M12)*

Data on batch effects were collected, analysed, and conclusions were made. Deliverable was submitted. Three experiments using porcine feces as sample matrix and several porcine astrovirus taxa as analyte investigated: (1) the effect of sample preparation method on the sensitivity of metagegenomic virus detection, (2) repeatability of metagenomic virus detection in function of the viral load , and (3) batch effect of the viral nucleic acid extraction kit.

As previously documented, the sample preparation methodology has an effect on the amount of viral reads recovered. The detection of astrovirus species was reproducible irrespective of the viral load, however metagenomics was less sensitive than RT-QPCR and the normalized (reads per million reads of data) read counts varied between replicates. No extraction kit batch effect could be demonstrated.

***JRP8-WP2-T4**: evaluation of QC metrics on additional parallel datasets (M13-M24)*

We will critically evaluate metagenomic results from our parallel projects in the view of the guidelines that will result from the joined efforts of WP1-T4 and WP2-T1. Planned for the prolongation (M30)

***JRP8-WP2-T5**: Metagenomic proficiency test (M13-M24)*

Three out of 5 partners participated in the COMPARE 2018 food metagenomics proficiency test. In addition, WP3 tracked in its sample questionnaire the availability of material to be included in a PT. We (annual Metastava progress meeting, february 2019 in Brussels) decided to use a panel of porcine fecal samples available at SVA for the metastava PT. Sufficient sample aliquots (blinded samples) were prepared and QC'd , as well as an RNA extract (to isolate the effect of the extraction from other factors) and was provided to all partners in july 2019. The use of Metastava-WP1 modules for data generation and analysis was obligatory and participants could add additional data using in house methods if so wished. In collaboration with WP3-T6, an exact procedure for analysis (both metagenomically and read mapping to references) was distributed, as well as a fixed bioinformatic database version (refseq microorganisms) to be used for all analyses. Participants were asked to provide raw sequence data, read classification data, and a report including their interpretation to the PT organiser. The collection of data is being finalised and the overall analysis is planned for M25-26.

## *WP3. evaluation of the analytical properties of metagenomics workflows*

As highlighted in previous reports, D-JRP8-3.1 highlights all sample panels that will be used for the evaluation of the analytical properties of them metagenomic workflows for model pathogens in selected sample matrices. The collection of data within the different pathogen subtasks is being finished , and the analysis and interpretation will be finalised during the 6 months extension (where not already done so).

***JRP8-WP3-T1:** analytical sensitivity, HEV (M1-M24)*

Ongoing > M30. See above

***JRP8-WP3-T2:** analytical sensitivity, norovirus (M1-M24)*

Ongoing > M30. See above

***JRP8-WP3-T3:** analytical sensitivity, large DNA viruses (M1-M24)*

Ongoing > M30. See above

***JRP8-WP3-T4**: analytical sensitivity, STEC (M1-M24)*

Ongoing > M30. See above

***JRP8-WP3-T5:*** *analytical sensitivity, detection of ABR genes (M1-M24)*

Ongoing > M30. See above

***JRP8-WP3-T6:*** *bioinformatics and statistical analysis of analytical performance experiments (M1-M24)*

A procedure (D-JRP8-3.2, live deliverable allowing updates) for standardised read mapping and metagenomic analysis was shared, as well as a set database version to remove the effect of unsynchronised databases on sensitivity (where needed).

## *WP4: Concertation with ongoing efforts and dissemination.*

***JRP8-WP4-T1:*** *concertation with ongoing initiatives (M1-M24)*

In addition to the previously reported efforts for concertation with COMPARE and EFFORT: participation in discussion OHEJP-IRIDA-INNUENDO about WGS data, and participation in the International Association for Biological Standardisation (IABS) meeting on NGS use for adventitious agent detection in biologicals (providing interesting insights in the huge efforts the pharmaceutical industry is doing to develop, standardize and validate the analytical use of NGS for virus detection in biological products).

***JRP8-WP4-T2:*** *formal dissemination (M1-M24)*

Two posters at OHEJP ASM in Dublin. One oral presentation at a national scientists meeting (SVA Research Day, Sweden).

***JRP8-WP4-T3****: dissemination of recommendations to stakeholders (M1-M24)*

One presentation at national stakeholders information workshop (Belgium). One presentation at the 2nd IABS (International Association for Biological Standardisation) conference on next generation sequencing for adventitious virus detection in biologics for humans and animals (stakeholders from industry and international organisations).

***JRP8-WP4-T4:*** *Organization of a scientific meeting (M20-M24)*

Participation in OHEJP annual scientific meetings. Output will maximally be presented during the 2020 ASM.

## *WP5: Project management*

***JRP8-WP5-T1:*** *Consortium agreement (M1-M6)*

This task has been completed, see annual report 2018

***JRP8-WP5-T2:*** *Internal communication (M1-M24)*

Annual progress meeting 18.02.2019, Brussels, Belgium. WP specific mailings. Teleconferences for planning and progress evaluation.

***JRP8-WP5-T3:*** *reporting and liaising with the EU (M20-M24)*

Annual report 12M. midyear (18M) progress report. Annual report 24M.

Financial project management with evaluation of unused budget in Y1 and re-attribution. heavy management workload with Data management plan v.1; application for 6 mth extension; 21 month progress report and formalization of the roles of Dutch partners WUR and third partner ErasmusMC.

## 3. Progress of the research project: milestones and deliverables

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| METASTAVA | D-JRP8-1.3 | List of sequence datasets | 10 | - | 27 | Partially delivered (public datasets). Partly to realize (partner's datasets from parallel projects). Focus will be on own generated data in Metastava due to better metadata documentation. Reporting and sharing of valuable reference datasets (from PT) towards end of project. |
| METASTAVA | D-JRP8-1.4 | SOP: guidelines for the description of scope and analytical properties of a metagenomic method in a diagnostic context | 24 | - | 30 | Use 6 month prolongation. & will be integrated with DJRP8-2.1 and DJRP8-1.5 resulting in a general comprehensive guidance document |
| METASTAVA | D-JRP8-1.5 | Review paper | 24 | - | 30 | Use 6 month prolongation. & will be integrated with DJRP8-2.1 and DJRP8-1.4 resulting in a general comprehensive guidance document |
| METASTAVA | D-JRP8-2.1 | SOP: use of quality metrics for metagenomics dataset evaluation | 24 | - | 30 | Use 6 month prolongation. & will be integrated with DJRP8-1.4 and DJRP8-1.5 resulting in a general comprehensive guidance document |
| METASTAVA | D-JRP8-2.2 | Report and guidelines for the use of exogenous | 18 | - | 26 | All data collected and 1st line analysis ok. Conclusions to be written down in report. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | process controls in metagenomics | | | | |
| METASTAVA | D-JRP8-2.3 | Report on batch and contamination effects in metagenomics | 12 | 19 | | Data generated. Analysis done. Deliverable submitted. |
| METASTAVA | D-JRP8-2.4 | Report on proficiency test | 24 | - | 27 | Report to be written |
| METASTAVA | D-JRP8-3.2 | Procedure for analyzing analytical sensitivity and robustness datasets | 12 | 17 | | D-JRP8-3.1 Deliverable report uploaded |
| METASTAVA | D-JRP8-3.3 | Report: analytical sensitivity and robustness , hepE | 24 | - | 30 | Use prolongation |
| METASTAVA | D-JRP8-3.4 | Report: analytical sensitivity and robustness , NoV | 24 | - | 30 | Use prolongation |
| METASTAVA | D-JRP8-3.5 | Report: analytical sensitivity and robustness , pox | 24 | - | 30 | Use prolongation |
| METASTAVA | D-JRP8-3.6 | Report: analytical sensitivity and robustness , STEC | 24 | - | 30 | Use prolongation |
| METASTAVA | D-JRP8-3.7 | Report: analytical sensitivity and robustness , ABR genes detection | 24 | - | 30 | Use prolongation |
| METASTAVA | D-JRP8-4.2 | SOP's, guidelines, scientific papers, presentations | 24 | - | 30 | Use prolongation. All output will be summarised in Djrp8-4.2 at the end of the project |
| METASTAVA | D-JRP8-4.3 | Minutes of scientific meeting | 24 | - | 30 | Attention shifted to maximum presentation at OHEJP ASM 2020. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | Djpr8-4.3 will summarise at end of project all our dissemination efforts. |
| METASTAVA | D-JRP8-5.2 | Progress and final meeting minutes | 24 | - | 30 | Deliverable will list all metastava annual meeting minutes. Minutes from AM1 (2018) and AM2 (2019) already available. Prolongation results in one additional AM. |
| METASTAVA | D-JRP8-5.3 | Half term report | 13 | 13 | | Submitted 12 month report |
| METASTAVA | D-JRP8-5.4 | Final report | 24 | - | 30 | Prolongation: Moved to M32. The present intermediate report document reports all progress until M24 |

## *Milestones*

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| METASTAVA | M-JRP8-M2 | Public and own dataset identified | 8 | - | 30 | Partially delivered (public datasets). Partly to realize (partner's datasets from parallel projects). Focus will be on own generated data due to better metadata documentation. Reporting and sharing of valuable reference datasets towards end of project. |
| METASTAVA | M-JRP8-M4 | Procedure for analysing analytical sensitivity and robustness datasets agreed | 12 | 17 | - | |
| METASTAVA | M-JRP8-M5 | Progress meeting | 12 | 13 | - | Feb.2019. Brussels live meeting. |
| METASTAVA | M-JRP8-M6 | Proficiency test panel ready for shipping | 18 | 18 | - | Samples ready to start PT in summer 2019 |
| METASTAVA | M-JRP8-M7 | Scientific meeting | 24 | - | 30 | ASM OHEJP 2019 done. Focus on OHEJP ASM 2020 for maximal scientific dissemination. |
| METASTAVA | M-JRP8-M8 | Dissemination to various stakeholders including joint communication with ongoing initiatives | 24 | - | 30 | Intermediate progress: see this report |
| METASTAVA | M-JRP8-M9 | Final meeting | 24 | - | 30 | prolongation |

## 4. Publications and patents

Liu L, Hakhverdyan M, Leijon M. The influence of sample preparations on high-throughput sequencing detection of viruses in clinical samples. The 11th International Congress for Veterinary Virology. Vienna, Austria, 27-30 August 2018.

Sander van Boheemen. Sample Pretreatment: Challenges in Virology. Workshop: ESCV Next Generations Sequencing in Clinical Virology. 20-21 November, 2018

Van Borm S. OHEJP-METASTAVA: Joint efforts in standardization and analytical validation of diagnostic metagenomics approaches in public (animal) health laboratories. 2nd IABS (International Association for Biological Standardisation) conference on next generation sequencing for adventitious virus detection in biologics for humans and animals, Ghent, 13-14 Nov. 2019

## 5. Impact & relevance

Efforts to standardize and validate metagenomics methods and their implementation in Vet and Med reference laboratories will allow better understanding of the added value of these methods and their complementarity to other diagnostic methods, as well as increased preparedness for emerging threats. Metastava also provides a platform for diagnostic labs from vet and med health institutes to jointly discuss the critical parameters to consider when implementing metagenomic NGS methods for diagnostic purposes, hereby encouraging the one health approach. The project's specific outcomes include, besides scientific publications, practical information allowing laboratories to bring metagenomic workflows for pathogen identification closer to diagnostic interpretation, including guidelines for quality assurance, reference datasets, reference material, protocols, standard operating procedures, etc.

## 6. Interactions with other JRPs/JIPs or with external (EU or national) relevant project

See report of WP4 that focuses on integration with other efforts. Constructive discussions with other JRPs also resulted in a new JRP starting in 2020: TeleVIR with several Metastava participants.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements | Measures and actions taken by Project Leaders at the end of 2018 | Comments Ethics Advisors in January-February 2019 | Measures and actions taken by Project Leaders at the end of 2019 |
|---|---|---|---|
| The applicants must confirm that ethics approvals for the use of biological samples, human and non-human animal (as appropriate) have been sought. | Each partner provided a detailed statement describing their measures to safeguard data protection, ethics, safety and biosafety and the way this is integrated with national and EU legislation. These statements are available on request form the JRP leader. | Satisfactory reply | / |
| The applicants must confirm the compliance with GDPR | Each partner provided a detailed statement describing their measures to safeguard data protection, ethics, safety and biosafety and the way this is integrated with national and EU legislation. These statements are available on request form the JRP leader. | Satisfactory reply. As a reminder of one important part of GRPD, the beneficiaries must provide the contact address of the Data Protector Officer of the institution in charge of processing the data obtained | Data protection officer contacts:<br>Sciensano InformationSecurity@sciensano.be<br>FLI datenschutz@fli.de<br>ANSES data protection officer : Director of Legal Affairs (saisine-daj@anses.fr)<br>SVA registrator@sva.se<br>WBVR functionarisgegevensbescherming@wur.nl<br>EMC unctionaris.gegevensbescherming@erasmusmc.nl |
| The applicants must document the safety mitigation measures in place to protect the staff | Each partner provided a detailed statement describing their measures to safeguard data protection, ethics, safety and biosafety and the way this is integrated with national and EU legislation. These statements are available on request form the JRP leader. | Satisfactory reply | / |

## 8. List of critical risks

| Description of risk | Yes/No |
|---|---|
| Loss of key-persons (staff and / or leaders) | NO |
| Delay in work plan execution | YES |
| Conflicts within the consortium | NO |
| Lack of commitment of partners | NO |
| Delay in duties, tasks or reporting | YES |
| Poor intra-project (JRP) relationship | NO |
| Potential entry/exit of partners | YES |
| Other risks (please describe) | NO |

## 9. List of dissemination and communication activities

| | | | |
|---|---|---|---|
| Name of the activity: | Metastava annual Progress Meeting | | |
| Date: | 18/02/2019 | | |
| Place: | Eurostation Brussels (Sciensano) | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | Yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 10 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | OHEJP – IRIDA – INNUENDO teleconference on organisation of joint WGS workshop | | |
| Date: | 14/02/2019 | | |
| Place: | Teleconference (SVA) | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | y |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | y |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 10 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | 2 Posters at OHEJP ASM 2019 | | |
| Date: | 22-24 May 2019 | | |
| Place: | Teagasc Conference centre in Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Project presentation at Programme Management Committee | | |
| Date: | 9 May 2019 | | |
| Place: | ANSES, Paris | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | Yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 25 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Project presentation at “OHEJP: How is Sciensano Involved?” | | |
| Date: | 5 June 2019 | | |
| Place: | Sciensano Uccle, Brussels | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 35 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 11 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Project presentation at Programme Owner Committee | | |
| Date: | 19 June 2019 | | |
| Place: | ANSES Paris | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 25 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Project presentation at SVA Science day | | |
| Date: | 12 november 2019 | | |
| Place: | Uppsala | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | y |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 55 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Project and scientific presentation at IABS 2nd Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animals . | | |
| Date: | 13-14 november 2019 | | |
| Place: | Ghent | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | y |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 55 | Media | |
| Industry | 70 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

## *10.List of planned tele- or video conferences, face to face meetings in the next year*

Metastava progress meeting (teleconference) – jan/feb 2020

Metastava final meeting (Brussels or parallel to OHEJP-ASM 2020) – may/june 2020

Presentation of output at OHEJP-ASM 2020

## JRP09 - AIR SAMPLE

### 1. Summary of the work carried out in year 2 (January to December 2019)

**General:** The project is proceeding according to the second year annual work plan (AWP). There is a good sense of collaboration and active dialogue by e-mail, exchange of protocols, Skype meetings and phone calls. Six newsletters have been circulated in 2019, one successful hands-on workshop on metagenomics bioinformatics has been organized by in Oslo, and the Second Annual Meeting was held on Oct 15$^{th}$ -16$^{th}$ , 2019, in Brno, Czech Republic. The Oslo workshop is expected to propose a metagenomics test that consists of DNA extraction from air filters, and a subsequent bioinformatics solution to DNA sequence analysis. The project is extended 6 months into 2020 in order to finalize manuscripts and SOPs.

**Company agreement:** The project has re-negotiated the agreement with Sartorius (Germany) to continue with supporting the partners with gelatine filters and air sampling device worth of ca. € 20,000 into the second year of the project.

**Lab work:** Overall, the project has successfully moved into the evaluation phase (below):

Harmonization ->Implementation > **Evaluation >** Validation.

We have completed the method harmonization and implementation phases. During the summer 2019, we have focussed on farm sampling, sample analysis and data generation. All the protocols, including a consensus real-time PCR have been fully implemented by the partners. The results of farm sampling have been discussed at the second annual meeting. Further statistical analysis will be performed before preparing the second manuscript for publication. No further lab work is planned for 2020, but using bioinformatics for diagnostic metagenomics, writing up SOPs and manuscripts.

**Deliverables:**

- SOPs for harmonized protocols : prepared and implemented.
- Demonstration of the air sampling protocol - online free-access video : https://youtu.be/S9mapXSM8tw
- Manuscript in review by Food Microbiology : describing the outcome of 2018-farm sampling.

**Dissemination activities:** Oral presentation of the project results by Gro Johannessen (Norway) at the OneHealthEJP first annual scientific meeting in Dublin (slides enclosed). Oral presentation of the project by Jeffrey Hoorfar at the 70$^{th}$ annual meeting of the European Association of Animal Production (EAAP2019), Ghent, Belgium.

### 2. Work carried out in the JRP, scientific results

#### WP1. Method Development.

***JRP9-WP1-T1**: Sampling activities and creation of a sample bank (air and boot-swab samples) from different regions (M1-M6)*

This task has been completed, see annual report 2018

***JRP9-WP1-T2**: Development of a protocol for non-complex DNA extraction for diagnostic qPCR and metagenomics analysis from gelatine-filter samples (M3-M13)*

Based on the intensive in-house studies at several participating labs, the consensus protocol enclosed was agreed. It consists of two steps: Pre-treatment of gelatine filters taken by AirPort8 device, and DNA extraction using QIAgen DNeasy Blood&Tissue Kit, according to manufacturer's instructions with several modifications. The main step in the filter preparation is the addition of the Protex protease to completely dissolving the gelatine.

Some important modifications were applied to the commercially available DNA extraction kit:

- Addition of RNAse (due to our intended later use of the DNA product for downstream metagenomics analysis);
- Replacement of kit elution buffer with TE-EDTA buffer;
- Addition of AL buffer. A metagenomics pilot project was performed that included a standardized MOCK community. Based on the pilot project sequencing technology, sequencing depth and bioinformatics analysis for a larger metagenomics project has been agreed upon, including samples from all partners. The samples from all partners were collected and sequenced. The bioinformatics analysis and interpretation of data including writing manuscripts will be performed in 2020.

## *WP2 Validation and Standardization.*

***JRP9-WP2-T1**: Validation of air sampling and DNA extraction methods (M13-M21)*

Three harmonized protocols were prepared for :

- field air sampling,
- DNA extraction,
- PCR testing.

The protocols were validated on the field air samples from chicken farms around Europe (collected during the summer 2019). A sample bank was established. It will be used later for metagenomics diagnostics. The protocols will be used to submit draft standards to CEN and EFSA as part of the continued standardization work for Campylobacter detection in chicken farms. This part of the work is planned for Spring 2020. A second manuscript is planned based on the 2019 summer sampling outcome.

***JRP9-WP2-T2**: Statistical analysis, Standardization and dissemination (M17-M24)*

This Task is moved to the first half of 2020 (see the third-year plan submitted and approved earlier).

*Country reports:*

**Denmark**

June and July 2019 : two rounds of farm sampling have been conducted . The sock swabs were negative, while some of the air filter were PCR positive.

A third round of sampling has been planned for August in 4 chicken flocks that are just one week from slaughtering. This should increase the chance of finding Campylobacter-positive samples on agar plates. A large metagenomic bioinformatics follow-up study is planned for autumn 2019, which should hopefully result in a new joint manuscript by all partners.

**Norway**

In 2019, according to the project plans and protocols, 10 flocks were sampled twice by air filters and once by sock samples. PCR was not performed on the 2018 Norwegian samples; it was performed the samples collected in 2019. All the Norwegian samples of 2019 were negative for Campylobacter, both on culture and PCR. An in-house verification of the “Josefson” 16S PCR with Agilent Brilliant III UF master-mix has been performed to assess amplification efficiency, annealing temperature and primer probe/concentrations to be able to optimize DNA extraction for air filters.

On the 11th / 12th of March, NVI hosted a metagenomics workshop for the project partners. It resulted in the development of a protocol for non-complex DNA extraction for qPCR and metagenomics and a discussion regarding the metagenomics pilot and final (The protocol has been circulated by Giuliano).

The metagenomics pilot project will result in a planned manuscript (2020).

The final metagenomics project with samples from all partners was led by NVI. Samples sequencing was outsourced to the Norwegian Sequencing Center. Gro and Mona participated at the OH EJP ASM 2019 in Dublin, with an oral presentation of the pilot study results (by Gro). A manuscript on the air sampling pilot study in cooperation with all the partners in the project has been prepared, submitted to Food Microbiology and resubmitted in a revised version.

**The Czech Republic**

According to the second year AWP, we cooperated with other partners to optimize the protocols for the second round of air sampling (sample preparation, cultivation and PCR detection).

In May and June 2019 : farms samplings were performed. Altogether 12 broiler flocks were visited in 3 different biosecured farms (E, F, G) in 3 separated sampling days.

Farm E consisted of 5 houses with 26.000 up to 55.000 birds at the age of 20 or 24 days in each house. Inner temperature in the houses during sampling was 24°C and humidity ranged from 63% to 70%. Outside temperature was 11°C. Farm F consisted of 4 houses with 55.000 animals at the age of 21, 22 and 25 days in each house. Inner temperature in the houses during sampling was 24°C to 26°C and humidity ranged from 54% to 65%. Outside temperature was 9°C. Farm G consisted of 9 houses, when only 3 were sampled. The selected houses differed in their size/number of animals – 11.500, 16.500, 34.000. All broilers were at the same age (21 days). Inner temperature during sampling in the houses was 28°C to 29°C and the humidity was monitored only in one house (63%). Outside temperature was 25°C. In all farms straw was used as the bedding and ROSS as the breed.

A pair of sock samples and two air filters were taken in each flock. All samples were processed according to the agreed protocol using ISO 10272 cultivation method with three different approaches – direct plating, enrichment in Preston medium and enrichment in Bolton medium.

The analyses were completed for 9 flocks. Only two boot swabs (E_14, E_15) were Campylobacter positive from direct plating and enrichment in Preston medium. For the flock E_15 the corresponding air filter was positive from enrichment in Preston. DNA for qPCR was extracted from filters but results of PCR detection are in process. National results (CZ) of the project (2018 and 2019) were presented in the 28$^{th}$ Congress of Czechoslovak society for microbiology (September 26-27, 2019, Slovakia).

**Poland**

Sampling was started on the 16$^{th}$ of May 2019. The samples were taken from four chicken houses located at two conventional farms. The flocks sizes ranged from 23,600 to 39,500 chickens. Birds ages were 14 days (two houses) and 20 days (two houses).

Each flock was sampled with a pair of boot socks (boot swab sample) and two corresponding air filters. The second sampling round is planned at ca.25 of June (two chicken houses), and the third one by the end of July or the first week of August (four chicken houses).

For the sample tested the consensus protocol for DNA extraction from gelatine filters and protocol for the second round of sampling – AIR Sample was used. For confirmation of the suspected colonies, PCR was used according to Denis M. et al.: Development of a PCR assay for simultaneous identification of Campylobacter jejuni and C. coli. Lett. Appl. Microbiol., 1999, 29, 406-410. Manuscript entitled: MLST-based genetic relatedness of Campylobacter jejuni isolated from chickens and humans in Poland was sent to PLOS ONE. It contains information that the study was in part supported by the Air Sampling project. The manuscript is being modified after the first review.

**Italy**

The IZSAM activities ran on time respecting the schedule. In the referred period, we were involved in the following activities: (i) development of methods. (ii) sampling sections, (iii) bacteriological experiments, (iv) real time PCR tests, (v) metagenomics.

First, we implemented microbiological methods referred to ISO standards and to the common protocols for analyzing the air sample filters, which represent a new and innovative system for

Campylobacter monitoring. We also modified the Real-time PCR protocol to load the air filter samples. Briefly, we tested the DNA extraction methods for the isolation of nucleic acid from air filters and boot swabs. Raw data from the first real time PCR experiments have been used for finalizing the consensus protocol of the DNA extractions. Then, we carried out the sampling from September 2018 to May 2019 visiting 4 different poultry farms with a total of 16 chicken flocks.

The sampling was carried out using one pair of boot socks and one air filter for each flock. Samples from the chicken houses were analyzed by means of bacteriological and molecular methods following the abovementioned protocols. We also developed a diagnostic procedure for the detection of Campylobacter DNA using metagenomics approach based on ONT MinION technology.

Spiking experiments were conducted on air filters to check the sensitivity and sensibility of the metagenomics approach using MinION.

All the strains isolated by cultivation method during the project contributed to implement the samples bank of Campylobacter in our laboratory, either in term of strains bank or DNAs. Results obtained with the metagenomics analyses have been presented at 29$^{th}$ European Congress of Clinical Microbiology and Infectious Diseases (ECCMID).

On the 27$^{th}$ and 28$^{th}$ of September 2018, IZSAM hosted the intermediate meeting to discuss Air-sample project preliminary results. The meeting was an opportunity of a great sense of collaboration, communication and active dialogue through the partners involved in the project.

## 3. Progress of the research project: milestones and deliverables

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| AIR Sample | D-JRP9-2.1. | Online video - demonstration of air sampling for farmers. | M24 | M18 | - | Youtube demonstration video https://youtu.be/S9mapXSM8tw |
| AIR Sample | | Standard Operating Procedure (SOP) for air sampling. | M24 | M24 | - | Protocol uploaded at the project website |
| AIR Sample | | Draft manuscript for publication in EFSA Journal. | M30 | - | M30 | The work has been shifted to 2020 |
| AIR Sample | D-JRP9-2.4. | Hands-on, wet-lab workshop for relevant EJP partners. | M25 | - | M29 | Will be done during the ASM meeting, Prague. |

### Milestones

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| AIR Sample | M-JRP9-3 | Local field studies completed. | M18 | Yes | - | All partners have conducted local field sampling in chicken farms, as described the project plan. (Initially M15, adapted in AWP-Y2) |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| AIR Sample | M-JRP9-4 | Statistical analysis completed. | M21 | No | M29 | Initially M17, adapted in AWP-Y2 May 2020 |

## 4. Publications and patents

Johannessen, Gro S., Giuliano Garofolo, Gabriella Di Serafino, Ivana Koláčková, Renáta Karpíšková, Kinga Wieczorek, Jacek Osek, Julia Christensen, Mona Torp, and Jeffrey Hoorfar. "Campylobacter in Chicken – Critical Parameters for International, Multicentre Evaluation of Air Sampling and Detection Methods." *Food Microbiology* 90 (September 2020): 103455. https://doi.org/10.1016/j.fm.2020.103455.

Wieczorek, Kinga, Tomasz Wołkowicz, and Jacek Osek. "MLST-Based Genetic Relatedness of Campylobacter Jejuni Isolated from Chickens and Humans in Poland." Edited by Patrick Jon Biggs. *PLOS ONE* 15, no. 1 (January 24, 2020): e0226238. https://doi.org/10.1371/journal.pone.0226238.

Metagenomic MinION sequencing for Campylobacter surveillance in air samples from Italian poultry farms. A. Peserico, M. Di Domenico, V. Curini, F. Marotta, G. Di Serafino, E. Di Giannatale, C. Cammà, G. Garofolo, L. Di Marcantonio. 29th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 13-16 April 2019.

## 5. Impact & relevance

The air filtering in combination with PCR has the potential to become part of future action plans to monitoring and surveillance of Campylobacter in chicken farms (EC, 2003; EFSA, 2011). It could replace the current sock-sampling, due to its much higher detection and diagnostic sensitivities found by the muticenter study that was carried out by the present project.

Since the basic instruments and material are already commercially available at a low cost, such implementation would be economically viable and sustainable. The method has also potential for exploration of High-Throughput Sequencing (HTS) and Metagenomics in the future.

EC (2003). 2003/99/EC Directive of the European Parliament and of the Council of 17th November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. Off. J. Eur. Union, L 325 of 12.12.2003, 31-40.

EFSA (2011). Scientific Opinion on Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA Journal 9:2105 (141 pp.).

## 6. Interactions with other JRPs/JIPs or with external (EU or national) relevant project

The project has been presented at the recent ISO committee meeting by Gro Johannesen (Norway) who is also member of the ISO Microbiology subcommittee. We are in close contact with ISO, national food safety authorities, European Association of Animal Science (EAAP (Belgium) and Feed and Food Quality Safety and Innovation Centre FFoQSI (Austria). Further contact to EFSA, ECDC, WHO and FAO is planned for early 2020, when the first manuscript is published.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements | Measures and actions taken by Project Leaders at the end of 2018 | Comments Ethics Advisors in January-February 2019 | Measures and actions taken by Project Leaders at the end of 2019 |
|---|---|---|---|
| Due to the sampling protocol, the programme team may be collecting personal and business data. Please confirm compliance with GDPR. | This project does not make use of animals and will not impact any societal or ethical aspects.<br>The identity of farms participating in the sampling plans will not be revealed.<br>All personal and business data collected will be treated confidentially and in accordance with strict national and EU data law. | More information needed | Each participating lab has strictly followed the GDPR rules and no personal or business info has been shared within or among the partners. |
| 'Samples' are collected in an animal unit, please confirm no animal experimentation approvals are required for the animal sampling protocols (e.g. do to interventions, restrictions, etc) | This project does not have animal experimentations. | More information need from the team<br>The answer is not adequate as the ethics question was not asking if there are animal experiments but was asking since:<br>"'Samples' are collected in an animal unit, please confirm no animal experimentation approvals are required for the animal sampling protocols (e.g. interventions, restrictions, etc). Some of these procedures can require approval (for example from an AWERB under Schedule 1 requirements). | No manipulation or contact to animals are done in this project. We just sample the air in chicken houses. |

## 8. List of critical risks

| Description of risk | Yes/No |
|---|---|
| Loss of key-persons (staff and / or leaders) | No |
| Delay in work plan execution | No |
| Conflicts within the consortium | No |
| Lack of commitment of partners | No |
| Delay in duties, tasks or reporting | No |
| Poor intra-project (JRP) relationship | No |
| Potential entry/exit of partners | No |
| Other risks (please describe) | No |

### *Additional information*

We have no issues in the project. Just the standardization and publication tasks have been shifted to 2020.

## 9. List of dissemination and communication activities

| | | | |
|---|---|---|---|
| Name of the activity: | Oral presentation at OHEJP ASM: A Multi-center Pilot Study of an Air Sampling Method for *Campylobacter* in Broiler Houses | | |
| Date: | 20-24 May 2019 | | |
| Place: | Teagasc Conference Centre, Ashtown, Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop: | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training: | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | |
|---|---|
| Name of the activity: | 70th annual meeting of the European Association of Animal Science (EAAP) |
| Date: | 26 - 30 August 2019 |
| Place: | Ghent, Belgium |

| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | NO | Participation to a Conference | yes |
| Organisation of a Workshop: | | Participation to a Workshop: | |
| Press release | | Participation to an Event other than a | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |

| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
|---|---|---|---|
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 3000 | Media | |
| Industry | 100 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 100 | | |

| | |
|---|---|
| Name of the activity: | AIR-Sample annual meeting |
| Date: | October 15-16, 2019. |
| Place: | Brno, CZ |

| Specify the Dissemination and Communication activity linked to the One Health EJP project | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | Yes | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop: | |
| Press release | | Participation to an Event other than a Conference or a Workshop: | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 10 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |
| | | | |

| | | | |
|---|---|---|---|
| Name of the activity: | FFoQSI annual meeting | | |
| Date: | Oct 10-11, 2019 | | |
| Place: | Vienna, Austria | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 30 | Media | |
| Industry | 15 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 2 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | 29th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) | | |
| Date: | 13-16 April 2019 | | |
| Place: | Amsterdam, the Netherlands | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | NO | Participation to a Conference | yes |
| Organisation of a Workshop: | | Participation to a Workshop: | |
| Press release | | Participation to an Event other than a | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 13000 | Media | |
| Industry | 100 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 100 | | |

## *10. List of planned tele- or video conferences, face to face meetings in the next year*

One face-to-face meeting during the OneHealth EJP ASM meeting in Prague and one demonstration workshop for the ASM participants.

## 1. Summary of the work carried out in year 2 (January to December 2019)

The **MoMIR project** aims to develop new approaches to predict, identify and prevent the appearance of animal and human Super-shedders based on immune response and gut microbiota composition. In order to achieve this aim the project will focus on four objectives.

1. Defining **Predictive markers** that will signal the risk of both animals and Salmonella isolates becoming a super-shedder of *Salmonella*.
2. Immune and microbiota **biomarkers of excretion** to detect animal super- shedders and/ or human prolonged carriers.
3. **Preventive measures and /or control measures** of this zoonotic problem by the characterisation of prebiotics, probiotics and nutraceutical products, for use in both animals and humans
4. Development of **mathematical models to provide new risk management tools**. These tools will lead to creation of a **pool of biosecurity measures** at the farm levels, each with a **cost effectiveness** consideration.

In order to meet these objectives a consortia of partners from across Europe are working together. However, initiation of the project was delayed due to unavoidable issues with obtaining ethical approval of animal studies and the replacement of two project partners.

To date the project team has undertaken *in vivo Salmonella* infection studies in both chickens and pigs. Serological analysis of these studies has now been completed, with Immunological and microbiome analyses currently ongoing. These studies have enabled the consortia to identify predictive biomarkers based on gut microbiota composition in the chicken. The *Salmonella* isolates recovered from high and low shedding pigs are currently undergoing characterisation, with various *Salmonella* associated virulence studies. Recruitment of participants in the human aspect of the project is also now ongoing, with analysis being carried out on a rolling basis.

Concerning the development of preventive measures, the comparison of the gut microbiota compositions of the *probiotic*-inoculated and control groups revealed an overall impact of the inoculated strains. Besides, one of the assays performed noticed that when *Bacteroides* are absent from piglet microbiota during first week of life, *Clostridium perfringens* is abundant. However, when *Bacteroides* spp. reach around 10% abundance in piglet gut microbiota, *C. perfringens* decreases below 1 % of total microbiota. Nutraceuticals (alperujo) are currently tested in broiler chicks experimentally infected. Similarly, pro and prebiotics (lactobacilli and GlucoOligoSaccharides (GOS)) are currently being tested in field conditions. A first version of the generic mathematical model of the dynamic interplay between the gut microbiota, the pathogen and the host's immune response at the within and between-host scale was developed. New experiments will also enable us to validate (and possibly refine) the mathematical model describing indirect transmission by testing its predictions in an experimental setting. Finally, a draft inventory of relevant intervention measures against *Salmonella* in laying hens has been developed within the framework of a HACCP analysis, and the cost effectiveness (utility) of intervention strategies using probiotics to reduce *Campylobacter* prevalence in broilers has been calculated. The model has been parameterized for several countries.

The partners met for their midterm meeting in February 2019, hosted by VISAVET, Madrid. The majority of partners represented and able to give updates on the progress of the aspect of the MOMIR project. During the meeting it was agreed that the project should be extended by 12 months, due to the unexpected delays encountered at the start of the project. The extension has been accepted by the EJP Project Management Team and the Scientific Steering Board. It was also agreed that at the end of the project Partner 23 (University of Surrey) will host a final project meeting, with the date of this meeting to be agreed at a later date.

## 2. Work carried out in the JRP, scientific results

This part is confidential. To be disclosed after publication.

## 3. Progress of the research project: milestones and deliverables

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| MoMIR-PPC | D-JRP10-0.4 | Minutes of project meetings (mid term meeting) | 13 | 18 | 18 | Completed |
| MoMIR-PPC | D-JRP10-0.5 | Minutes of project meetings ( closing project) | 24 | - | 34 | The final meeting will be held at University of Surrey, but a date has not been set yet. |
| MoMIR-PPC | D-JRP10-0.6 | Financial and scientific reports for the EJP Coordinator (Intermediated report) | 13 | 13 | - | Completed |
| MoMIR-PPC | D-JRP10-0.7 | Financial and scientific reports for the EJP Coordinator (closing report) | 24 | - | 36 | |
| MoMIR-PPC | D-JRP10-1.02 | Identification of risk factors for shedding of Salmonella in pigs and poultry farms | 12 | 24 | 32 | The task has been delayed due to difficulties in obtaining all the necessary permissions to perform animal experiments and to the modification of the partners able to perform experiments in farm condition. The large majority of animal experiments have been done. Global analyses of the data obtained are in progress. |
| MoMIR-PPC | D-JRP10-1.02 | Development of the immune-signature chips | 12 | - | - | According to the results showing that the antibody level cannot be used as a |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | biomarker, the deliverable has been cancelled. |
| MoMIR-PPC | D-JRP10-1.03 | Identification and purification of commensal bacteria present before Salmonella colonization in low shedders (first round) | 12 | 18 | - | Completed. |
| MoMIR-PPC | D-JRP10-1.03 | Identification and purification of commensal bacteria present before Salmonella colonization in low shedders (second round) | 20 | - | 30 | Work in progress. |
| MoMIR-PPC | D-JRP10-1.04 | In vitro virulence levels of different Salmonella strains recovered from high and low shedders in animals and humans (first round) | 20 | - | 30 | Work in progress.<br>UoS will also analyse the attachment and biofilm formation. This work began 17/06/19. |
| MoMIR-PPC | D-JRP10-1.05 | In vitro virulence levels of different Salmonella strains recovered from high and low shedders in animals and humans (second round) | 21 | - | 30 | Work in progress. |
| MoMIR-PPC | D-JRP10-1.05 | Microbiome analyses of human stool samples | - | - | 36 | No available results to date. The microbiome analyses of the human stool samples are planned to start after completion of the sample collection, by June 2020. |
| MoMIR-PPC | D-JRP10-1.06 | Definition of predictive immunological markers associated to the high and low shedders in chickens and in pigs. | 20 | - | 34 | Deliverable delayed<br>Analyses are ongoing. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| MoMIR-PPC | D-JRP10-1.07 | Definition of immunological markers associated to the high and low shedders in chickens and in pigs. | 20 | - | 34 | Deliverable delayed<br>Analyses are ongoing. |
| MoMIR-PPC | D-JRP10-1.08 | Characterization, evolution and comparison of the microbiome of pig and poultry with different shedding status of Salmonella | 18 | 24 | - | Chicken experiments are complete, but still in progress for pigs.<br>The majority of samplings have been done. Faecal samples have been received by UoS from the in vivo pig infection study at ANSES and DNA extraction is underway. Shipping of samples from the ISS pig in vivo study will be carried out in the near future (end of 2019). |
| MoMIR-PPC | D-JRP10-1.09 | Definition of predictive microbiota markers associated to the high and low shedders in chickens and in pigs. | 20 | - | 32 | Completed for chicken, but new experiments are now in progress.<br>Samples have been received and 16S analysis is currently underway (see D-JRP10-1.04). |
| MoMIR-PPC | D-JRP10-1.10 | Definition of microbiota markers associated to the high and low shedders in chickens and in pigs. | 20 | - | 32 | Completed for chicken, but new experiments are now in progress.<br>Samples have been received and 16S analysis is currently underway (see D-JRP10-1.04). |
| MoMIR-PPC | D-JRP10-1.11 | Recovery of all human samples | 15 | - | 32 | Sampling start was delayed by 12 months due to delayed ethical clearance that resulted in extension of sampling period until August 2020. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | Update December 2019: The sampling period is now due to end by 31st of December 2019. |
| MoMIR-PPC | D-JRP10-1.12 | Antimicrobial susceptibility testing, serotyping and whole genome sequencing of all Salmonella isolates from participants submitted to the Norwegian Reference laboratory | 17 | - | 34 | Will be finished approx. three months after the last Salmonella sample has been received. The sampling period is expected to last until the 31st of December 2019. |
| MoMIR-PPC | D-JRP10-1.13 | Microbiome analyses of human stool samples.<br>Salmonella colonization in low shedders (first round) | 24 | - | 36 | No available results to date. The microbiome analyses of the human stool samples are planned to start after completion of the sample collection, by June 2020. |
| MoMIR-PPC | D-JRP10-1.14 | Identification, from in vitro studies, of immune parameters related to high and low shedders | 20 | - | 30 | Work in progress. |
| MoMIR-PPC | D-JRP10-1.15 | Understanding whether high and low shedder phenotypes are mainly related to Salmonella strain variation, immune status, or microbiota composition. | 24 | - | 34 | NA |
| MoMIR-PPC | D-JRP10-1.16 | A predictor, based on the designed set of mimotopes, using machine learning techniques to discriminate between the diagnostic groups. | 24 | - | - | According to the results showing that the antibody level cannot be used as a biomarker, the deliverable has been cancelled. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| MoMIR-PPC | D-JRP10-2.01 | In vitro effect of already characterized probiotics on Salmonella growth and cell invasion | 10 | 24 | 30 | Some experiments have been delayed due to the modification of the participating partners. The recovery of Salmonella strains required for this task has thus been delayed. |
| MoMIR-PPC | D-JRP10-2.02 | Description of the microbiome and resistome in farms | 12 | 36 | 36 | NDRVMI has replaced Vet-DTU at month 6 of the project. Work now in progress |
| MoMIR-PPC | D-JRP10-2.03 | Characterization of protective commensal bacteria able to inhibit Salmonella colonization (two rounds) | 14 | - | 34 | Work in progress for at least two bacteria<br>Protective activity of Enterococcus faecium and L. reuteri isolates strains has been tested in pigs or chickens. |
| MoMIR-PPC | D-JRP10-2.04 | Characterization of protective commensal bacteria able to inhibit Salmonella colonization (two rounds) | 22 | - | 30 | NA |
| MoMIR-PPC | D-JRP10-2.05 | Determine the influence of defined and undefined probiotics on the microbiome signature, the immune response, gut physiology and welfare of pig and/ or chicken | 24 | - | 30 | Work done for gut microbiota, in progress for immune response. |
| MoMIR-PPC | D-JRP10-2.06 | Impact of defined and undefined probiotics on Salmonella colonization in pig and chicken | 24 | - | 26 | The experiments have been done for chicken. Analyses are ongoing. |
| MoMIR-PPC | D-JRP10-2.07 | Impact of pre-biotics or feed on the immune parameters, the | 24 | - | 30 | The experiments have been done for chickens and pigs. Analyses are ongoing. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | microbiome and resistome, gut physiology and welfare of pig and chicken. | | | | |
| MoMIR-PPC | D-JRP10-2.08 | Impact of pre-biotics or feed on Salmonella colonization in pig, chicken. Effect on the super-shedders and low-shedders. | 24 | - | 30 | The experiments have been done for chickens and pigs. Analyses are ongoing. |
| MoMIR-PPC | D-JRP10-2.09 | Dynamics of the microbiome and resistome in the different groups and conditions | 24 | - | 36 | This part will be done with the human samples. |
| MoMIR-PPC | D-JRP10-2.10 | Validation of immunological and microbiota markers, identified in WP1 and associated to high/low shedding, in the control groups of WP2 | 24 | - | 34 | NA |
| MoMIR-PPC | D-JRP10-2.11 | Evolution of the immune-signature in pig, chicken and/ or human according to the context (infection, treatment…) | 20 | - | 34 | Partly completed.<br>Work in progress. |
| MoMIR-PPC | D-JRP10-2.11 | Differences in the resistome before and after travel to high-risk areas and after pre-biotic administration | 24 | - | - | The task has been modified in the new project. This deliverable has thus been cancelled. |
| MoMIR-PPC | D-JRP10-3.01 | Models at within-host scale taking into account the interactions between infection and microbiota and mechanisms of interventions | 22 | - | 36 | Partly completed, work in progress. A first version was formulated. An improved version is under progress. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| MoMIR-PPC | D-JRP10-3.02 | Models at between-host scale, linking to the within-host modelling and taking into account the mechanisms of interventions | 22 | - | 36 | Partly completed, work in progress. To be completed in December 2020. |
| MoMIR-PPC | D-JRP10-3.03 | Intervention measures inventory | 20 | 24 | 30 | First version (Milestone) is completed; systematic inventory due in June 2020. |
| MoMIR-PPC | D-JRP10-3.04 | Economic analysis tools | 24 | 24 | 28 | Completed (manuscript) |
| MoMIR-PPC | D-JRP10-3.05 | Definition of intervention measures to target super-shedders | 24 | 24 | 28 | Completed (manuscript) |
| MoMIR-PPC | D-JRP10-3.06 | Evaluation scheme for cost effectiveness of the intervention strategies | 24 | 24 | 28 | Completed (manuscript) |
| MoMIR-PPC | D-JRP10-4.04 | Publication, and presentation at conferences | 24 | - | 36 | In progress. Several publications and presentations have been done. |
| MoMIR-PPC | D-JRP10-4.05 | Dissemination to lay-public communities, to policy-makers and regulators, farmers and companies | 24 | - | 36 | In progress. Several publications and presentations have been done. |
| MoMIR-PPC | D-JRP10-4.2 | Data management policy and strategies | 4 | 18 | 26 | Completed. |

*Milestones*

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| MoMIR-PPC | M-JRP10-7 | Recovery of samples from the first round of experimentally infected animals | 8 | yes | 24 | Some experiments have been delayed due to the modification of the participating partners.<br>UoS faecal samples have been received from the in vivo pig infection study conducted at ANSES and DNA extraction is underway. Shipping of samples from the ISS pig in vivo study has been done. |
| MoMIR-PPC | M-JRP10-8 | Four sets of NGS derived 105 mimotope sequences – positively and negatively enriched in IgM and IgA | 8 | yes | - | Resulting from the leave of SAIM (A. Pashov), this task has been deleted in the new version of the project. Part of this task has been performed by partner 18 (INRA). |
| MoMIR-PPC | M-JRP10-9 | Recovery of samples from experimentally infected animals and from farms, pretreated with pre-biotics or neutraceuticals | 8 | In part | 32 | Pig experiments have been performed. Chicken experiments have started on May 2019. All samples have been recovered. Work in progress in field condition. |
| MoMIR-PPC | M-JRP10-10 | In vitro infection of cell lines and organoids with the Salmonella strains recovered from high and low shedders in animals and humans (from the first experiments) | 10 | In part | 30 | Some experiments have been delayed due to the modification of the partner participants. Work has been done for cell invasion. Cell response is in progress. |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| MoMIR-PPC | M-JRP10-12 | Comparison of immune response of high and low shedders in chickens and pigs | 11 | In part | 26 | Animal samples have been recovered. Data are under analyse. |
| MoMIR-PPC | M-JRP10-13 | Comparison of microbiota composition of high and low shedders in chickens and pigs. | 11 | In part | 32 | Completed for chickens.<br>Three experimental pig infection studies have been performed (in ISS, IZLER and ANSES). All faecal samples from these studies will be shipped to UoS for 16S community analysis.<br>UoS faecal samples have been received from the in vivo pig infection study at ANSES and ISS and DNA extraction has been completed. The 16s sequencing of 205 samples was performed and the data are currently under analysis. |
| MoMIR-PPC | M-JRP10-15 | Comparison of the predictive markers obtained in pigs with those obtained in chickens. | 12 | No | 32 | Experiments completed for chickens.<br>Pig experiments have been performed but samples should be analysed. |
| MoMIR-PPC | M-JRP10-16 | Comparison of the transcriptomic immune response induced in vitro between different strains to identify immunological markers | 12 | No | 32 | Some experiments have been delayed due to the modification of the participating partners. The recovery of Salmonella strains required for this task has thus been delayed. |
| MoMIR-PPC | M-JRP10-17 | Recruitment of the first three sets of human participants including stool sampling and Salmonella culture | 12 | In part | 32 | Recruitment and sampling of the human subjects started on the 1st of January 2019.<br>This milestone is not applicable any more as the sampling protocol has been changed to continuous recruitment and |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | sampling of subjects. All samples are expected to be collected by March 2020. See also comments to D-JRP10-1.11. |
| MoMIR-PPC | M-JRP10-20 | First inventory of intervention measures completed | 12 | yes | 16 | Completed with delay due to MSc Student project timing. |
| MoMIR-PPC | M-JRP10-21 | First version of economic analysis tools completed | 12 | No | 24 | New planning due to better availability of research capacity in 2019 . |
| MoMIR-PPC | M-JRP10-22 | Organization of consortium meetings (intermediate and closure) | 13 | Yes | 24 | Mid-term meeting held in Feb 2019. The final meeting will be held at UoS, but a date has not been set yet. |
| MoMIR-PPC | M-JRP10-23 | Recruitment of the fourth set of human participants including stool sampling and Salmonella culture | 15 | In part | 36 | The milestone is no longer applicable. See comments to D-JRP10-11.11 and M-JRP10-17. |
| MoMIR-PPC | M-JRP10-24 | Recovery of samples from experimentally infected animals and from farms, pretreated with probiotics | 16 | In part | 32 | Suitable pre- and probiotics have now been sent by UoS to our Bulgarian partner.<br>Experimental infections are ongoing. |
| MoMIR-PPC | M-JRP10-25 | Comparison of immune response of high and low shedders in chickens and pigs | 18 | No | 32 | All experiments have been done for chickens. Analysis of data from pigs are in progress. |
| MoMIR-PPC | M-JRP10-26 | MALDI TOF Imaging processing and workflow on the paraffin wax slides of intestine wall in high and low shedder from chickens and pigs | 18 | No | - | This milestone has been cancelled. |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| MoMIR-PPC | M-JRP10-27 | Comparison of microbiota composition between high and low shedders in chickens and pigs and in humans between prolonged and short Salmonella excretion. | 18 | In part | Humans: 36 | Human microbiota composition will be analysed after all samples have been collected (month 32). |
| MoMIR-PPC | M-JRP10-28 | In vitro infection of several cell lines and organoids with the different Salmonella strains recovered from high and low shedders in animals and humans (second round) | 18 | In part | 30 | Work has been done for cell invasion. Cell response is in progress. |
| MoMIR-PPC | M-JRP10-29 | Comparison of the immune signature of high and low shedders in chicken and pig | 18 | In part | 32 | Analysis of the data is in progress. |
| MoMIR-PPC | M-JRP10-30 | Comparison of the predictive markers obtained in pigs with those obtained in chickens. | 19 | In part | 32 | Predictive markers have been obtained for chicken. In progress for pigs. |
| MoMIR-PPC | M-JRP10-31 | Comparison of the transcriptomic immune response induced in vitro between different strains to identify immunological markers | 20 | No | 32 | Analysis of the data is in progress. |
| MoMIR-PPC | M-JRP10-32 | Final inventory of intervention measures completed | 20 | No | 30 | This work is in progress. |
| MoMIR-PPC | M-JRP10-33 | Updated version of within-host models completed | 21 | No | 36 | This work is in progress. |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| MoMIR-PPC | M-JRP10-34 | Updated version of between-host models completed | 21 | No | 36 | This work is in progress. |
| MoMIR-PPC | M-JRP10-35 | Comparative genomic analyses of human Salmonella isolates in relation with their virulence level | 22 | No | 36 | NA |
| MoMIR-PPC | M-JRP10-36 | Updated version of economic analysis tools completed | 22 | yes | | Complete. |
| MoMIR-PPC | M-JRP10-37 | Potential interventions included in between-host modelling | 22 | no | 36 | NA |
| MoMIR-PPC | M-JRP10-38 | Organization of consortium meetings (closure) | 23 | no | 34 | The final meeting will be held at UoS, but a date has not been set yet. |
| MoMIR-PPC | M-JRP10-39 | Organization of the high strategy meeting | 24 | no | 34 | Currently in discussion. |

## 4. Publications and patents

Several publications were already accepted and other have been submitted: see report of June 2019.

Simon Labarthe, Béatrice Laroche, Thao Nguyen, Bastien Polizzi, Florian Patout, et al. A Multi-Scale Epidemic Model of Salmonella infection with Heterogeneous Shedding. To appear in ESAIM Proceedings (open access). A first draft was accessible on HAL repository https://hal.archives-ouvertes.fr/hal-02043742

Rebollada-Merino A., Bárcena C., Ugarte-Ruiz M., Porras-González N., Mayoral-Alegre F., Tome-Sánchez I., Domínguez L. and Rodríguez-Bertos A. Effects on Intestinal Mucosal Morphology, Productive Parameters and Microbiota Composition after Supplementation with Fermented Defatted Alperujo (FDA) in Laying Hens. Antibiotics. 8:215. 2019. (A). ISSN: 2079-6382. DOI: 10.3390/antibiotics8040215. Golden open access. Costs of the publication: 435.89 euros. Public publication (https://www.mdpi.com/2079-6382/8/4/215).

## 5. Impact & relevance

The impact and relevance of the project could be only detailed when the majority of the deliverables will be finished. Currrently, preliminary conclusions have shown that the main causes of the appearance of low- and super-shedder phenotypes are poorly related to pathogen virulence level modification but mainly to the gut microbiota composition. The role of immune response status need to be better characterized. The effect of probiotics and prebiotics to decrease Salmonella infection in experimental infection and in field condition are ongoing. Preliminary results are encouraging. A draft inventory of relevant intervention measures against Salmonella in laying hens has been developed within the framework of a HACCP analysis. It will be completed during the next year to evaluate concrete preventive measures to avoid contamination of livestock and of humans as well as the cost effectiveness (utility) of intervention strategies using probiotics to reduce pathogen prevalence in broilers. The link between animal health and public health will not be made before next year because we had an important delay in starting the experiments with human. Nevertheless, several biomarkers have been described in the project, which need to be confirmed in other animal species or in human.

The main impact of this project, in line with EFSA recommendation, should be to show that the control of Salmonella must be based upon the implementation of preventive actions throughout the whole production chain (EFSA 2006 The EFSA Journal, 341(1)). More specifically, measures should be addressed to (i) the prevention of introduction of Salmonella into the herd/ flock, (ii) the prevention of within-herd transmission, and (iii) the increase of the resistance to the infection by improving the barrier effect provided by gut microbiota and host immune response.

## 6. Interactions with other JRPs/JIPs or with external (EU or national) relevant project

Participation to the EJP meeting in Dublin and to other congresses not directly related to EJP but related to academic and private companies.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements | Measures and actions taken | Comments Ethics Advisors in January-February 2019 | Measures and actions taken by Project Leaders at the end of 2019 |
|---|---|---|---|
| The applicants must confirm that animal samples used have been collected with the appropriate ethical approvals (approval letters, etc) so complying with EU standards. | All persons living in Norway diagnosed with Salmonella during the sampling period (now 01.01.2019 – 31.08.2020) will be contacted by the Norwegian Surveillance System for Infectious Diseases (MSIS). MSIS will send an information letter about the study as well as consent forms. If the persons (or both guardians, in case of children) give their consent, then FHI will send them a self-sampling kit, a questionnaire and pre-paid return envelope. | Satisfactory reply | / |
| The applicants must confirm that ethics approvals for the conduct of the clinical study have been sought. | No insurance policy is needed, as the health risk of taking a stool sample with a self-testing kit is considered minimal. | Satisfactory reply | / |
| The applicants must confirm that ethics approvals for the use of biological samples have been sought. | All animal experiments have been approved by appropriate ethical committee before manipulations. The time necessary to obtain this approval delayed some experiments. | No details are given on the 3Rs. The team was asked to provide the licence approval number / ethical approval code. This is not provided. This information should also include the name of the approving body (e.g. site and name of the AWERB). This can be done through the provision of the approval letter.<br><br>Limited response, more information needed. | Each partner has send the documents to the appropriate ethical committee where the details on the 3R were described. |
| More detailed information must be provided on the recruitment procedure of the study participants. | No clinical study will be undertaken, as described in the changed project description approved December 2017. | Satisfactory reply | / |

| Requirements | Measures and actions taken | Comments Ethics Advisors in January-February 2019 | Measures and actions taken by Project Leaders at the end of 2019 |
|---|---|---|---|
| The applicants must confirm that an insurance policy has been established to cover the study participants. | For the human part, the ethical clearance from the Norwegian Committee for Medical and Health Ethics has been granted 15.05.2018, and updated 22.11.2018. | Satisfactory reply | / |
| The applicants must confirm the compliance with GDPR. | Data collection and treatment is in compliance with GDPR. | Satisfactory reply. As a reminder of one important part of GRPD, the beneficiaries must provide the contact address of the Data Protector Officer of the institution in charge of processing the data obtained | The Data Protector Officer of the NIPH is Erlend Bakken, however the researchers managing the study is in charge of conducting the study in accordance with GDPR and the approval from the Norwegian Committee for Medical and Health Ethics. |

## 8. List of critical risks

| Description of risk | Yes/No |
|---|---|
| Loss of key-persons (staff and / or leaders) | yes |
| Delay in work plan execution | yes |
| Conflicts within the consortium | No |
| Lack of commitment of partners | No |
| Delay in duties, tasks or reporting | yes |
| Poor intra-project (JRP) relationship | No |
| Potential entry/exit of partners | yes |
| Other risks (please describe) | No |

### *Additional information*

An exceptional extension of 12 months of the project at no extra expense has been obtained by the EJP board. This extension was motivated by:

The withdrawal of SAIM from the EJP consortium after the approval of our project.

Astrid-Louise Webster has left the FHI, just before the beginning of the project, so that we have adapted the project with Anke-Stüken. These modifications have delayed the ethics committee approvals, resulting of one year delay in the human experiments.

The fact that after the beginning of the project, Vet-DTU has been closed by its Government. To compensate for this loss, we looked for new partners in the EJP consortium and in June (6 months after the beginning of the project), H. Daskalov (NDRVMI) has joined our consortium. Consequently, part of the work related to WP2 has been delayed by one year.

Finally, several partners had had difficulties to obtain the ethics committee approval for animal experiments, which has also delayed some experiments. This was especially true for experiments with humans, where the recruitment of study participants will continue until the 31st of December 2019.

## 9. List of dissemination and communication activities

| | |
|---|---|
| ***Name of the activity:*** | Association of Veterinary Students - RVC - AMR and biofilms |
| ***Date:*** | *January 2019* |
| ***Place:*** | *Please update* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | | ***Media*** | |
| ***Industry*** | | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | | | |

| | |
|---|---|
| ***Name of the activity:*** | ***Microbiology Society 2019 Annual conference. Flash poster presentation titled "Creation and characterisation of probiotic libraries for use in pigs"*** |
| ***Date:*** | *March 2019* |
| ***Place:*** | *Please update* |

| ***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories*** | | | |
|---|---|---|---|
| | *Yes / No* | | *Yes / No* |
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

| ***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories*** | | | |
|---|---|---|---|
| | *Number* | | *Number* |
| ***Scientific Community (Higher Education, Research)*** | | ***Media*** | |
| ***Industry*** | | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | | | |

| | |
|---|---|
| ***Name of the activity:*** | ***One health EJP annual scientific meeting: oral presentation titled "Creation and characterisation of probiotic libraries for use to control zoonotic pathogens in pigs"*** |
| ***Date:*** | *21-24 May 2019* |
| ***Place:*** | *Teagasc conference centre Dublin* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | ***300*** | ***Media*** | |
| ***Industry*** | ***5*** | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | ***5*** | | |

| | |
|---|---|
| ***Name of the activity:*** | *1st Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Poster communication.* |
| ***Date:*** | *22-24 May* |
| ***Place:*** | *Dublin, Ireland.* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | ***300*** | ***Media*** | |
| ***Industry*** | ***5*** | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | ***5*** | | |

| | |
|---|---|
| ***Name of the activity:*** | Microbiote et résistance du poulet aux salmonelles. 4ème journée thématique "Je suis un écosystème : le microbiote dans tous ses états" dans le cadre du Réseau Thématique de Recherche soutenu par la Région Centre Val de Loire |
| ***Date:*** | *28 June 2019* |
| ***Place:*** | *Château de Beaulieu, Joué les Tours* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | ***30*** | ***Media*** | |
| ***Industry*** | ***5*** | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | ***10*** | ***Other*** | |
| ***Policy Makers*** | | | |

| | |
|---|---|
| ***Name of the activity:*** | *Faecal gut microbiota composition of chicks can predict resistance to Salmonella Enteritidis colonization. Presented at NEM Research Network,)* |
| ***Date:*** | *2019-05-21 - 2019-05-22* |
| ***Place:*** | *Nantes, FRA* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | ***60*** | ***Media*** | |
| ***Industry*** | | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | | | |

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|---|---|
| ***Name of the activity:*** | *Salmonella resistant vs super-shedder broilers: how can we improve birds' resistance. Presented at Phileo symposium "Animal health with less ATB and more food safety Latest technologies"* |
| ***Date:*** | *2019-04-02 - 2019-04-02* |
| ***Place:*** | *Rome, ITA* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | ***10*** | ***Media*** | |
| ***Industry*** | ***60*** | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | | | |

| | |
|---|---|
| ***Name of the activity:*** | *Faecal gut microbiota composition of chicks can predict resistance to Salmonella Enteritidis colonization. Poster communication at Gordon Research Conference "Molecular Mechanisms, Evolution and Treatment of Salmonella"* |
| ***Date:*** | *2019-06-02 - 2019-06-07* |
| ***Place:*** | *Easton, MA, US* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *Yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | ***200*** | ***Media*** | |
| ***Industry*** | | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | | | |

| | |
|---|---|
| ***Name of the activity:*** | *Faecal gut microbiota composition of chicks can predict resistance to* Salmonella *Enteritidis colonization. Oral presentation at the 6th international conference on poultry intestinal Health* |
| ***Date:*** | *2019-04-03 - 2019-04-05* |
| ***Place:*** | *Roma* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | ***400*** | ***Media*** | |
| ***Industry*** | ***360*** | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | | | |

| | |
|---|---|
| ***Name of the activity:*** | *1st Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Poster communication.*<br><br>*Rebollada-Merino A., Ugarte-Ruiz M., Mayoral-Alegre F.J.,Maasoumi-Nouha N., Tomé-Sánchez I., Rivero E., Porras-González N., García M., Domínguez L. and Rodríguez-Bertos A. Changes on Caecal Mucosa Morphology and Microbiota in Laying Hens Supplemented with a Nutraceutical Product Obtained from Olive Oil Production* |
| ***Date:*** | *22-24 May* |
| ***Place:*** | *Dublin, Ireland.* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | ***300*** | ***Media*** | |
| ***Industry*** | ***5*** | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | ***5*** | | |

| | |
|---|---|
| ***Name of the activity:*** | *Poster presentation* |

| | |
|---|---|
| | *13th International Symposium On Epidemiology and Control Of Foodborne Pathogens In Pork (Safepork)*<br><br>*Kerouanton A., Souchaud F., Houdayer C., Houard E., Nagard B., Guionnet J-M., Fougeroux A., Paboeuf F. and Denis M. Pigs infected experimentally with the same dose of monophasic variant of Salmonella Typhimurium exhibit different shedding levels.* |
| ***Date:*** | *26-29 septembre **2019*** |
| ***Place:*** | *Berlin, Germany* |

***Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories***

| | *Yes / No* | | *Yes / No* |
|---|---|---|---|
| ***Organisation of a Conference*** | | ***Participation to a Conference*** | *yes* |
| ***Organisation of a Workshop*** | | ***Participation to a Workshop*** | |
| ***Press release*** | | ***Participation to an Event other than a Conference or a Workshop*** | |
| ***Non-scientific and non-peer-reviewed publication (popularised publication)*** | | ***Video/Film*** | |
| ***Exhibition*** | | ***Brokerage Event*** | |
| ***Flyer*** | | ***Pitch Event*** | |
| ***Training*** | | ***Trade Fair*** | |
| ***Social Media*** | | ***Participation in activities organized jointly with other H2020 projects*** | |
| ***Website*** | | ***Other*** | |
| ***Communication Campaign (e.g. Radio, TV)*** | | | |

***Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories***

| | *Number* | | *Number* |
|---|---|---|---|
| ***Scientific Community (Higher Education, Research)*** | | ***Media*** | |
| ***Industry*** | | ***Investors*** | |
| ***Civil Society*** | | ***Customers*** | |
| ***General Public*** | | ***Other*** | |
| ***Policy Makers*** | | | |

## JRP11 - MEDVETKLEBS

### 1. Summary of the work carried out in year 2 (January to December 2019)

One of the main objectives of the MedVetKlebs project was the development and dissemination of novel methods to detect *Klebsiella pneumoniae* (Kp) in complex environments, such as food and soil.

For detection and isolation of Kp, we have established SCAI (Simmons Citrate with Inositol) agar as the most suitable medium, based on the productivity and specificity results. We also found that it allows selective growth across the breadth of Kp taxonomic diversity. Besides, to adapt the Kp culture strategy to food microbiology processes, the Kp isolation protocols were optimized by testing several routinely used enrichment conditions and incubation temperatures for selected types of matrix/sources: human and animal faeces, food, soil and water (task 1.1). The protocols for isolation are being updated to the online platform protocols.io (dx.doi.org/10.17504/protocols.io.662hhge). Regarding the molecular approach (task 1.2), we have developed a real-time PCR (ZKIR qPCR) for the detection of Kp (In the broad taxonomic sense, i.e., including its 7 different phylogroups or species) directly in complex samples such as soil. The ZKIR qPCR consists in the amplification of a specific intergenic region of Kp using a SYBR Green chemistry. The manuscript has been submitted for publication and is already available for the scientific community in BioRxiv (https://doi.org/10.1101/855668). The protocol was distributed and validated by the partners of the consortium (task 1.3). To render the protocol more easily available for the microbiological research and surveillance community, it was made publicly available through protocols.io (dx.doi.org/10.17504/protocols.io.7n6hmhe). Finally, we are also developing a qPCR method for the more targeted identification of Kp phylogroups (or species) directly in the samples (task 1.2). The protocol is still being developed at INRA before being distributed to the partners.

Based on broad sampling of multiple sources (3781 samples were analysed; mainly food and environmental samples, task 2.1), we selected chicken meat and ready-to-eat salads for deep sampling analyses (task 2.2). The goal was to compare qPCR and culture methods and to perform a comparative phenotypic and genomic analysis of recovered Kp isolates. A multicentric study with six MedVetKlebs partners is under way. Chicken meat and ready-to-eat salads were sampled in five countries (n=245 samples; ~25 of both sample types in each center). qPCR and culture methods were applied. Currently, whole genome sequencing (task 3.1) is being performed for isolates identified as Kp based on qPCR and MALDI-TOF mass spectrometry from chicken meat and salads. Besides, other isolates were recovered from a diversity of environmental sources, in particular soil. Part of the soil Kp genomic sequences were analyzed and included in the ZKIR qPCR development manuscript (https://doi.org/10.1101/855668).

Regarding the exploitation of results (task 4.3): the main results of these 24 months of the MedVetKlebs project were presented at the One Health ASM 2019 conference (6 posters and 1 oral presentation), Nor-Kleb-Net workshop (Sommaroy, Norway, 21st Aug 2019) and IMMEM-XII (1 oral presentation); and 2 abstracts summited to the 30th ECCMID (to be held in April 2020, Paris). In addition to the publications cited above, two publications related to the project are being prepared. Our final meeting is scheduled to take place in Paris, April 16-17th, 2020.

**Additional activity and deliverables.** In order to achieve our detection and identification goals, it was important to define the target species precisely, and delineate it from closely-related taxa. We therefore performed in addition to the initially planned activities, taxonomic updates of the genus *Klebsiella*. Two updates of the Klebsiella taxonomy were published this year. First, two novel taxa closely related to *K. pneumoniae* were discovered and described: *K. africana* and *K. variicola* subsp. *tropica* (https://doi.org/10.1016/j.resmic.2019.02.003 and https://www.sciencedirect.com/science/article/pii/S0923250819300956). Second, in collaboration with the JPIAMR-funded SpARK project (https://www.jpiamr.eu/supportedprojects/third-joint-callresult/), two novel species previously misidentified as *K. oxytoca* were described: *K. pasteurii* and *K. spallanzanii* (https://doi.org/10.3389/fmicb.2019.02360).

Also not initially planned, we evaluated the potential of MALDI-TOF mass spectrometry (MS), a fast and cost-effective technique that is well established in clinical microbiology laboratories for microbial identification, to identify the novel taxa. Specific peaks associated with the different taxa were found, allowing their identification by MALDI-TOF MS (https://www.ncbi.nlm.nih.gov/pubmed/30581423 and taxonomic references above). Therefore, reference spectra of the novel taxa should be incorporated in the future into the reference MALDI-TOF databases used in microbiology laboratories. In the meanwhile, we are intending to develop a publicly accessible web tool for the identification of *Klebsiella taxa* by MALDI-TOF MS.

## 2. Work carried out in the JRP, scientific results

### WP1. Methods for Kp detection and isolation

#### ***JRP11-WP1-T1****: Evaluation and optimization of culture-based approaches (M1-M12)*

This task has been completed, see annual report 2018.

#### ***JRP11-WP1-T2****: Detection and quantification (M1-M12)*

This task is finalized for the methodological development component: we have developed a SYBR-green based qPCR (ZKIR qPCR; IP and INRA were involved in this part) for the detection of Kp complex, which was validated in six partners/institutes (INRA, ANSES, AGES, NUIG, SSI, ISZAM). This novel assay is based on the amplification of a specific intergenic region of 78 bp between *zur* (zinc uptake regulation protein) and *khe* (coding for a haemolysin) (which we called ZKIR intergenic region) that is universally conserved in, and unique to, the Kp complex. The ZKIR qPCR assay is specific for its target group of pathogens, as all strains from other species were negative. The assay was tested for detection in soil samples and proved very sensitive, allowing to detect 0.15 CFU per gram after overnight sample enrichment. The manuscript was submitted and is available in a pre-print platform (https://doi.org/10.1101/855668), as well as the protocol (dx.doi.org/10.17504/protocols.io.7n6hmhe).

Due to its complexity, the task of quantification of Kp in samples will probably not be achievable during the project. However, the developed qPCR will allow this to be done in future dedicated studies. An approximation might be attained within the MedVetKlebs project using Ct values obtained with the qPCR results from salads and chicken screening and the calibration curve that we have derived from extracted DNA.

The novel qPCR method was implemented in five partner's institutes. A multicentric study comparing culture and qPCR methods was then performed by these five partners (ANSES, AGES, NUIG, SSI, ISZAM) by sampling chicken meat and salads (previously identified as potential sources of *K. pneumoniae* for humans). In total, 245 samples were analysed (130 from chicken meat and 115 from salads). The concordance between the two methods was high, with higher sensitivity of the qPCR. Antimicrobial susceptibility testing and whole-genome sequence is being performed at the moment to shed light on the characteristics of the recovered Kp isolates.

We are also developing a multiplex qPCR for the identification at the more precise phylogenetic level of species/phylogroups of Kp, directly in the samples (task 1.2). For this a pan-genome strategy (performed at IP) was used to define the target genes specific for phylogroups. qPCR primers/probes were designed and validated on a reference panel of strains (performed by INRA). The protocol is being finalized at INRA before being distributed to the partners and to the community.

#### ***JRP11-WP1-T3****: Harmonization and alignment (M1-M24)*

Culture methods have been optimized collectively and harmonized across partners, to detect *K. pneumoniae* from food, water, soil and faecal material (humans, animals). For better dissemination, the protocol for SCAI preparation and isolation of Kp from animal and human faeces was made available on protocols.io (dx.doi.org/10.17504/protocols.io.662hhge). The remaining protocols

(isolation of *K. pneumoniae* from food, water and soil) were distributed across all the consortium partners and will be made available for the community in protocols.io during the next weeks.

As explained above, the ZKIR qPCR method was disseminated, implemented and validated in five partners' labs and disseminated publicly (dx.doi.org/10.17504/protocols.io.7n6hmhe). The pre-print of the manuscript is also available (https://doi.org/10.1101/855668).

All the strategies adopted for the dissemination and exploitation of our results are available in the MedVetKlebs Communication Strategy Plan v1 (D-4.3).

Taxonomic updates of *K. pneumoniae* (7 phylogroups distributed among 5 species) and *K. oxytoca* (6 phylogroups corresponding to 6 different species) complexes, as well as MALDI-TOF MS identification method of *Klebsiella*, was disseminated through the respective publications (https://doi.org/10.1016/j.resmic.2019.02.003, https://doi.org/10.3389/fmicb.2019.02360; https://doi.org/10.3389/fmicb.2018.03000).

Furthermore, we are developing an open-source website for the direct identification of different taxa belonging to *K. pneumoniae* and *K. oxytoca* complexes based on MALDI-TOF using the spectra output from the different platforms (Bruker, BioMérieux). Although these tasks were not initially planned as deliverables of our project (because the novel species were not discovered yet, and because the successful application of MALDI-TOF was not anticipated), we believe that they are extremely important for its development, for the scientific community and for the harmonization of methods to identify *Klebsiella*.

## *WP2. Sampling*

### ***JRP11-WP2-T1**: Broad sampling of potential reservoirs and sources of Kp (M1-M12)*

Broad sampling of varied sources, to be checked for the presence of Kp, was performed. In total, 3781 samples from different sources were analysed. Among the food sources tested chicken meat and vegetables (mainly ready-to-eat salads and carrots) presented the higher recovery rate of Kp, whereas among the animals, swine and rabbits showed the higher prevalence (but so far, based on a low number of samples tested).

### ***JRP11-WP2-T2**: Deep sampling of selected sources (M13-M14)*

In accordance with the results of the broad sampling, and based on the relevance regarding transmission of Kp from food to humans, we have decided to perform a deep sampling of chicken meat and salads. For this purpose, 245 samples were analysed (130 from chicken meat and 115 from salads). Chicken meat showed a prevalence of *K. pneumoniae* twice as high as salads. The antibiotic susceptibility tests revealed that 75% of the isolates (74% in salads and 40% in chicken meat) presented a wild-type phenotype, being susceptible to all the antibiotics tested (except ampicillin, for which all Kp are resistant and which is used to isolate them).

The next step may be to sample more types of meat (pork, beef) and ready-to-eat food products (other type of vegetables, sushi, street food); this will be decided among partners through a TC. Besides that, some partners are also screening wastewater and seawater, and companion animals (cats and dogs) for the presence of *K. pneumoniae*.

## *WP3. Genomics and Modelling*

### ***JRP11-WP3-T1:** Analyses of genomic sequences (M13-M24)*

Whole-genome sequencing is currently being performed for all isolates of the chicken and salad study. Isolates from animals (n= 208), human carriage (n=59) and soil/irrigation water (n=214) were already sequenced and the results are currently being analysed. A preliminary analysis reveals a distinct epidemiology from the clinical setting, with high-risk clones and antibiotic resistance and virulence

genes being detected in a low level, making it difficult to establish the link with the nosocomial scenario.

Part of the genomic analysis performed for soil isolates was already included in the pre-print manuscript of the ZKIR qPCR development (https://doi.org/10.1101/855668).

***JRP11-WP3-T2:*** *Modelling and source attribution (M1-M24)*

Modelling (task 3.2) analyses are starting. The post-doc responsible for performing this work (Audrey Duval) was recently recruited (9 December 2019) at Institut Pasteur.

## *WP4: Management, dissemination, exploitation*

***JRP11-WP4-T1****: Implementation of the project management structure (M1-M24)*

Nothing specific to report here except that we have asked for the 6-months extension. The second version of the data management plan was sent to the One Health EJP coordination on November 20th, 2019.

***JRP11-WP4-T2:*** *Administrative, legal, financial and ethical support to the consortium (M1-M24)*

Nothing specific to report here. Financial reports have been submitted as scheduled.

***JRP11-WP4-T3:*** *Exploitation of results and Intellectual Property rights management (M1-M24)*

Five publications citing the MedVetKlebs project and One Health EJP funding were issued

- Wisgrill L, Lepuschitz S, Blaschitz M, Rittenschober-Böhm J, Diab-El Schahawi M, Schubert S, *et al.* Outbreak of Yersiniabactin-producing *Klebsiella pneumoniae* in a Neonatal Intensive Care Unit. *Pediatr Infect Dis J* 2019;38:638–42. https://doi.org/10.1097/INF.0000000000002258
- Rodrigues C, Passet V, Rakotondrasoa A, Diallo TA, Criscuolo A, Brisse S. Description of *Klebsiella africanensis* sp. nov., *Klebsiella variicola* subsp. *tropicalensis* subsp. nov. and *Klebsiella variicola* subsp. *variicola* subsp. nov. *Res Microbiol* 2019;170:165-170. https://doi.org/10.1016/j.resmic.2019.02.003 .
- Rodrigues C, Passet V, Rakotondrasoa A, Brisse S. Identification of *Klebsiella pneumoniae*, Klebsiella quasipneumoniae, Klebsiella variicola and Related Phylogroups by MALDI-TOF Mass Spectrometry. *Front Microbiol* 2018;9:3000. https://doi.org/10.3389/fmicb.2018.03000.
- Merla C, Rodrigues C, Passet V, Corbella M, Thorpe HA, Kallonen TVS, Zong Z, Marone P, Bandi C, Sassera D, Corander J, Feil EJ and Brisse S. Description of *Klebsiella spallanzanii* sp. nov. and of *Klebsiella pasteurii* sp. nov.. *Front. Microbiol.* 2019;10:2360. https://doi.org/10.3389/fmicb.2019.02360
- Barbier E, Rodrigues C, Depret G, Passet V, Gal L, Piveteau P. The ZKIR Assay, a novel Real-Time PCR Method for the Detection of *Klebsiella pneumoniae* and Closely Related Species in Environmental Samples. 2019. BioRxiv. https://doi.org/10.1101/855668 (submitted to *Applied and Environmental Microbiology*)

**Two more publications are being prepared:** the results of the multicentric study comparing the ZKIR qPCR and culture methods from salads and chicken; and a study on the prevalence, antimicrobial susceptibility phenotypes and genomic characterization of *K. pneumoniae* from food/producing animals (poultry and bovine).

Several posters and one oral presentation from the MedVetKlebs consortium were presented at Dublin One Health EJP ASM and IMMEM-XII:

- Carla Rodrigues, Sylvain Brisse on the behalf of MedVetKlebs consortium. The MedVetKlebs project: *Klebsiella pneumoniae* from Ecology to Source Attribution and Transmission Control (poster).
- Carla Rodrigues, Virginie Passet, Andriniaina Rakotondrasoa, Sylvain Brisse. Suitability of MALDI-TOF Mass Spectrometry to Discriminate Species within the *Klebsiella pneumoniae* Complex (poster).
- Carla Rodrigues, Marisa Haenni, Maxime Bour, Cécile Ponsin, Jean-Louis Pinsard, Virginie Passet, Jean-Yves Madec, Sylvain Brisse. Genomic Diversity, Antimicrobial Resistance and Virulence of *Klebsiella pneumoniae* from Healthy Food-producing Animals and Horses (oral).
- Elodie Barbier, Carla Rodrigues, Géraldine Depret, Virginie Passet, Laurent Gal, Pascal Piveteau and Sylvain Brisse. Design, Development and Validation of a Real-Time PCR Assay for Detection of *Klebsiella pneumoniae* Complex in Environmental Matrixes (poster).
- Elodie Barbier, Juan Sebastian Lopez-Fernandez, Carla Rodrigues, Virginie Passet, Laurent Gal, Sylvain Brisse, Pascal Piveteau. Development of Phylogroup-Specific Taqman Real-Time Assays for Identification of Members of *Klebsiella pneumoniae* Complex (poster).
- Małgorzata Ligowska-Marzęta, Katrine Grimstrup Joensen, Carla Rodrigues, Sylvain Brisse and Eva Møller Nielsen. Broad Sampling for Presence of *Klebsiella pneumoniae* in Different Sources from Denmark (poster).
- Violeta Di Marzio, Gabriella Centorotola, Cristina Marfoglia, Alessandra Cornacchia, Maria Antonietta Saletti, Carla Rodrigues, Sylvain Brisse, Francesco Pomilio. A Comparative Study of Productivity, Selectivity and Specificity of Three Selective Culture Media for *Klebsiella* spp. Detection (poster).
- Carla Rodrigues, Marisa Haenni, Maxime Bour, Cécile Ponsin, Jean-Louis Pinsard, Virginie Passet, Jean-Yves Madec, Sylvain Brisse. Genomic Diversity, Antimicrobial Resistance and Virulence of *Klebsiella pneumoniae* from Animal Carriage: a Comprehensive Analysis in an One Health Perspective (oral).

## 3. Progress of the research project: milestones and deliverables

### Deliverables

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| MedVetKlebs | D-JRP11-2.1 | List of high-Kp occurrence sources | 12 | 24 | | Multiple (3781 in total) samples were screened by the consortium. This task gained impetus due to the availability of the qPCR detection method. Some sources where Kp is prevalent were already identified including wastewater, seawater, soil, chicken meat, vegetables and ready-to-eat food. |
| MedVetKlebs | D-JRP11-2.2 | Prevalence in selected sources | 18 | 24 | | Chicken meat and salads were selected as important potential Kp transmission sources; 245 samples, 115 from salads and 130 from chicken meat, were screened using the harmonized protocol within the consortium. |
| MedVetKlebs | D-JRP11-2.3 | Quantification of Kp in selected sources | 18 | | | This task will probably not be achievable during the project due to its complexity. However, the developed qPCR will allow this to be done in future dedicated studies. An approximation might be attained within the MedVetKlebs project using Ct values obtained with the qPCR |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | | | | | results from salads and chicken screening and the calibration curve that we have derived from extracted DNA. |
| MedVetKlebs | D-JRP11-2.4 | Genome sequence data | 18 | | 28 | Genome sequencing has finished for animals, soil and healthy humans (n=481 genomes).<br>Genomes from chicken meat and salads are being sequenced. |
| MedVetKlebs | D-JRP11-3.1 | Source distribution of clonal groups, plasmids and genes | 18 | | 30 | First results from the 481 genomes sequenced are being analysed.<br>Some of the results were included in the pre-print publication of ZKIR qPCR manuscript (https://doi.org/10.1101/855668) |
| MedVetKlebs | D-JRP11-3.2 | Source attribution models by microbial subtyping and comparative exposure assessment | 24 | | 30 | Hiring of the post-doc in charge has been done (Dec 9th, 2019). Work is starting. |
| MedVetKlebs | D-JRP11-3.3 | Computer program for dynamic models simulation | 18 | | 30 | Postdoc hired started in December 2019. This task can now progress. |
| MedVetKlebs | D-JRP11-3.4 | Estimates of the attribution proportion for different food, animal and environmental sources | 24 | | 30 | This task will start in January 2020. |

| JRP name | Project deliverable number | Deliverable name | Delivery date from AWP | Actual delivery date | If deliverable not submitted on time: Forecast delivery date | Comments |
|---|---|---|---|---|---|---|
| | | for the countries included. | | | | |
| MedVetKlebs | D-JRP11-3.5 | Estimates of the relative contribution of different transmission routes for exposure to Kp in the different countries included. | 24 | | 30 | This task will start in January 2020. |
| MedVetKlebs | D-JRP11-4.1 | Project Periodic Reports | 9,12,18,24 | | 24 | As scheduled (9, 12, 18 months reports were delivered) |
| MedVetKlebs | D-JRP11-4.2 | Consortium meetings – Review of work done/progress made and definition of priorities for next period | 1,12,24 | 1,12,16 | | Minutes of the previous consortium meeting were uploaded on the portal. Next and final meeting is scheduled April 16th, 2020 |
| MedVetKlebs | D-JRP11-4.3 | Communication strategy plan | 12 | 23 | | A first version has been produced, validated by all partners and sent to the One Health EJP responsible on Nov 20th, 2019. |
| MedVetKlebs | D-JRP11-4.4 | Plan for the dissemination and exploitation of results (data management plan) | 18 | 23 | | A second version has been produced, validated by all partners and sent to the One Health EJP responsible on Nov 20th, 2019. |
| MedVetKlebs | D-JRP11-4.5 | Final Report | 24 | | 30 | The project was extended for 6-months, so the final report is re-scheduled for June 2020. |

***Additional output that is not a deliverable (non-scientific publication, video, …)***

We have delivered additional actions/results not initially scheduled: 3 publications (2 taxonomic publications; one publication on MALDI-TOF MS). See above (WP1 and abstract) for details.

*Milestones*

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| MedVetKlebs | M-JRP11-2 | Preparation of draft of the strategic communication plan | 10 | Yes | | A first version has been produced, validated by all partners and sent to the One Health EJP responsible. |
| MedVetKlebs | M-JRP11-6 | Broad survey of Kp in multiple sources complete | 12 | No | M28 | This task has started recently in a large-scale, but will be continued. |
| MedVetKlebs | M-JRP11-7 | Development of model frameworks for dynamic modelling and source attribution | 12 | No | M29 | Not started yet, although the strategy is defined; the postdoc hired for this task started only in December 2019 (modelling PI was on maternity leave). |
| MedVetKlebs | M-JRP11-8 | Initial prevalence, quantification and genomic data for model refining | 18 | No | M28 | In process, except for quantification. |
| MedVetKlebs | M-JRP11-9 | 1st batch of clonal groups, plasmids and genes defined, for refinement of models | 18 | Yes | M24 | Finished. Results for soil and human carriage genomes were analysed. Part of the results included in the pre-print manuscript of ZKIR qPCR (https://doi.org/10.1101/855668) |
| MedVetKlebs | M-JRP11-10 | Compilation and integration of the data produced in WP1 and WP2 to be used in the dynamic and source attribution models | 18 | No | M30 | To be discussed with the hired modelling postdoc. |
| MedVetKlebs | M-JRP11-11 | Identification of a list of scenarios for control measures to be assessed through model simulations | 18 | No | M30 | To be discussed with the hired modelling postdoc. |

| JRP name | Milestone number | Milestone name | Delivery date from AWP | Achieved (Yes / No) | If not achieved: Forecast achievement date | Comments |
|---|---|---|---|---|---|---|
| MedVetKlebs | M-JRP11-12 | Consolidated prevalence, quantification and genomic data for modeling of Kp transmission | 24 | No | M28 | This task will be complete once we have the final genome dataset. |
| MedVetKlebs | M-JRP11-13 | Application of dynamic and source attribution models to data collected from different countries | 24 | No | M30 | To be performed with the hired modelling postdoc. |
| MedVetKlebs | M-JRP11-14 | Reporting of transmission and source attribution estimates | 24 | No | M30 | This task will be complete based on the final genome dataset. |

## 5. Publications and patents

Barbier, Elodie, Carla Rodrigues, Geraldine Depret, Virginie Passet, Laurent Gal, Pascal Piveteau, and Sylvain Brisse. "The ZKIR Assay, a Real-Time PCR Method for the Detection of *Klebsiella Pneumoniae* and Closely Related Species in Environmental Samples." Edited by Edward G. Dudley. *Applied and Environmental Microbiology* 86, no. 7 (January 31, 2020): e02711-19, /aem/86/7/AEM.02711-19.atom. https://doi.org/10.1128/AEM.02711-19.

Merla, Cristina, Carla Rodrigues, Virginie Passet, Marta Corbella, Harry A. Thorpe, Teemu V. S. Kallonen, Zhiyong Zong, et al. "Description of Klebsiella Spallanzanii Sp. Nov. and of Klebsiella Pasteurii Sp. Nov." *Frontiers in Microbiology* 10 (October 25, 2019): 2360. https://doi.org/10.3389/fmicb.2019.02360.

Rebollada-Merino, Agustín, Carmen Bárcena, María Ugarte-Ruiz, Néstor Porras, Francisco J. Mayoral-Alegre, Irene Tomé-Sánchez, Lucas Domínguez, and Antonio Rodríguez-Bertos. "Effects on Intestinal Mucosal Morphology, Productive Parameters and Microbiota Composition after Supplementation with Fermented Defatted Alperujo (FDA) in Laying Hens." *Antibiotics* 8, no. 4 (November 9, 2019): 215. https://doi.org/10.3390/antibiotics8040215.

Rodrigues, Carla, Virginie Passet, Andriniaina Rakotondrasoa, and Sylvain Brisse. "Identification of Klebsiella Pneumoniae, Klebsiella Quasipneumoniae, Klebsiella Variicola and Related Phylogroups by MALDI-TOF Mass Spectrometry." *Frontiers in Microbiology* 9 (December 7, 2018): 3000. https://doi.org/10.3389/fmicb.2018.03000.

Rodrigues, Carla, Virginie Passet, Andriniaina Rakotondrasoa, Thierno Abdoulaye Diallo, Alexis Criscuolo, and Sylvain Brisse. "Description of Klebsiella Africanensis Sp. Nov., Klebsiella Variicola Subsp. Tropicalensis Subsp. Nov. and Klebsiella Variicola Subsp. Variicola Subsp. Nov." *Research in Microbiology* 170, no. 3 (April 2019): 165–70. https://doi.org/10.1016/j.resmic.2019.02.003.

Wisgrill, Lukas, Sarah Lepuschitz, Marion Blaschitz, Judith Rittenschober-Böhm, Magda Diab-El Schahawi, Sören Schubert, Alexander Indra, and Angelika Berger. "Outbreak of Yersiniabactin-Producing Klebsiella Pneumoniae in a Neonatal Intensive Care Unit:" *The Pediatric Infectious Disease Journal* 38, no. 6 (June 2019): 638–42. https://doi.org/10.1097/INF.0000000000002258.

## 5. Impact & relevance

The MedVetKlebs project:

- Has delivered innovative and widely applicable methods to isolate, identify and detect strains of the pathogen *Klebsiella pneumoniae* (Kp), currently one of the most worrisome emerging threats to public health. This will translate into enhanced surveillance of this pathogen in the clinical setting (through easier and more precise identification), as well as enhanced capacity for Kp surveillance in other sectors including food, veterinary and environmental microbiology;
- Is contributing to harmonizing methods for naming *Klebsiella* species, identifying them and detecting them in complex matrices (food, soil), across sectors of activity (animal health, public health), thus contributing to promote One Health knowledge on this pathogen;
- Is collecting large genomic datasets that will inform on the genomic and antimicrobial-susceptibility of Kp isolates in food and animals; these will be compared to similar data from human clinical settings (collected elsewhere) and will enable a better understanding of reservoirs and routes of transmission of Kp from the environment, food, animals into humans, and vice-versa.
- Is contributing to integrate its partner institutions through the sharing of common experimental protocols, publications and knowledge about Kp.

## 6. *Interactions with other JRPs/JIPs or with external (EU or national) relevant project*

**Kleb-NET** – H2020 EU JPIAMR 7th call on surveillance - https://research.pasteur.fr/en/project/klebnet-a-one-health-network-bridging-science-and-surveillance-on-antimicrobial-resistant-klebsiella/ (PI: Sylvain Brisse). The KlebNET project was successfully built upon initial networking activities within the MedVetKlebs project.

**SpARK** – JPIAMR 3rd Joint Call on Transmission Dynamics - https://www.jpiamr.eu/wp-content/uploads/2016/11/SpARK.pdf (PI: Edward Feil). This project includes S Brisse as partner, and benefitted from our MedVetKlebs validated SCAI culture method – more than 4000 samples from soil, animals, humans and food were searched for Kp presence by culture and metagenomics methods.

**KLEB-GAP** - Bergen Research Foundation grants - https://www.vetinst.no/en/research-and-innovation/ongoing-research-projects/kleb-gap-klebsiella-pneumoniae-a-key-driver-in-the-global-spread-of-antimicrobial-resistance-and-a-target-for-new-approaches-in-diagnostics-surveillance-and-alternative-therapeutics (PI: Arnfinn Sundsfjord and Co-PI Iren Høyland Löhr). S Brisse is a partner in this project, and provided expertise, culture and qPCR protocols developed in the MedVetKlebs project, ahead of public dissemination.

**NOR-KLEB-NET** – funded by the Norwegian Research Council - http://www.nor-kleb.net . Sylvain Brisse is a partner in this project, and provided expertise, culture and qPCR protocols developed in the MedVetKlebs project, ahead of public dissemination. The Kp sampling performed through this project has allowed to compare preliminary results on Kp prevalence in distinct sources, with the MedVetKlebs results.

## 7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

| Requirements | Measures and actions taken by Project Leaders at the end of 2018 | Comments Ethics Advisors in January-February 2019 | Measures and actions taken by Project Leaders at the end of 2019 |
|---|---|---|---|
| The applicants must confirm that animal samples used have been collected with the appropriate ethical approvals (approval letters, etc) so complying with EU standards | (See below) | EA did not comment | All animal samples were faeces, collected outside the animals, and therefore not invasive for animals. |
| The applicants must confirm that ethics approvals for the use of isolates from human origin have been sought | "This study received ethics approval from the Medical Research Ethics Committee of Utrecht University (WAG/om/14/012490). Informed consent was obtained from all participants. All participants gave consent and in the case of children, parents gave consent." | No details. More information needed | Please let us know what additional details you would need. |
| Isolates of Human Origin are used therefore resubmit the Ethics Checklist based on the requirements noted above | "This study received ethics approval from the Medical Research Ethics Committee of Utrecht University (WAG/om/14/012490). Informed consent was obtained from all participants. All participants gave consent and in the case of children, parents gave consent." | Satisfactory reply although some aspect of the response should be clarified. Considering the nature of the work being done it is surprising to see such a limited response. | Please let us know what additional details you would need. |

## 8. List of critical risks

| Description of risk | Yes/No |
|---|---|
| Loss of key-persons (staff and / or leaders) | No |
| Delay in work plan execution | No |
| Conflicts within the consortium | No |
| Lack of commitment of partners | No |
| Delay in duties, tasks or reporting | No |
| Poor intra-project (JRP) relationship | No |
| Potential entry/exit of partners | No |
| Other risks (please describe) | No |

## 9. List of dissemination and communication activities

| | | | |
|---|---|---|---|
| Name of the activity: | Poster at OHEJP ASM: Carla Rodrigues, Sylvain Brisse on the behalf of MedVetKlebs consortium. The MedVetKlebs project: *Klebsiella pneumoniae* from Ecology to Source Attribution and Transmission Control. | | |
| Date: | 21-24 May 2019 | | |
| Place: | Teagasc conference centre Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | |
|---|---|
| Name of the activity: | Poster at OHEJP ASM: Carla Rodrigues, Virginie Passet, Andriniaina Rakotondrasoa, Sylvain Brisse. Suitability of MALDI-TOF Mass Spectrometry to Discriminate Species within the Klebsiella pneumoniae Complex |
| Date: | 21-24 May 2019 |
| Place: | Teagasc conference centre Dublin |

| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |

| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
|---|---|---|---|
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | OHEJP ASM, oral presentation: Carla Rodrigues, Marisa Haenni, Maxime Bour, Cécile Ponsin, Jean-Louis Pinsard, Virginie Passet, Jean-Yves Madec, Sylvain Brisse. Genomic Diversity, Antimicrobial Resistance and Virulence of Klebsiella pneumoniae from Healthy Food-producing Animals and Horses | | |
| Date: | 21-24 May 2019 | | |
| Place: | Teagasc conference center Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Poster at OHEJP ASM: Elodie Barbier, Carla Rodrigues, Géraldine Depret, Virginie Passet, Laurent Gal, Pascal Piveteau and Sylvain Brisse. Design, Development and Validation of a Real-Time PCR Assay for Detection of Klebsiella pneumoniae Complex in Environmental Matrixes | | |
| Date: | 21-24 May 2019 | | |
| Place: | Teagasc conference centre Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | |
|---|---|
| Name of the activity: | Poster at OHEJP ASM: Carla Rodrigues, Virginie Passet, Andriniaina Rakotondrasoa, Sylvain Brisse. Suitability of MALDI-TOF Mass Spectrometry to Discriminate Species within the Klebsiella pneumoniae Complex |
| Date: | 21-24 May 2019 |
| Place: | Teagasc conference centre Dublin |

| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |

| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
|---|---|---|---|
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Poster at OHEJP ASM: Elodie Barbier, Juan Sebastian Lopez-Fernandez, Carla Rodrigues, Virginie Passet, Laurent Gal, Sylvain Brisse, Pascal Piveteau. Development of Phylogroup-Specific Taqman Real-Time Assays for Identification of Members of Klebsiella pneumoniae Complex | | |
| Date: | 21-24 May 2019 | | |
| Place: | Teagasc conference centre Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | |
|---|---|
| Name of the activity: | Poster at OHEJP ASM: Violeta Di Marzio, Gabriella Centorotola, Cristina Marfoglia, Alessandra Cornacchia, Maria Antonietta Saletti, Carla Rodrigues, Sylvain Brisse, Francesco Pomilio. A Comparative Study of Productivity, Selectivity and Specificity of Three Selective Culture Media for Klebsiella spp. Detection |
| Date: | 21-24 May 2019 |
| Place: | Teagasc conference centre Dublin |

| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |

| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
|---|---|---|---|
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | |
|---|---|
| Name of the activity: | Poster at OHEJP ASM: Małgorzata Ligowska-Marzęta, Katrine Grimstrup Joensen, Carla Rodrigues, Sylvain Brisse and Eva Møller Nielsen. Broad Sampling for |

| | | | |
|---|---|---|---|
| | Presence of Klebsiella pneumoniae in Different Sources from Denmark | | |
| Date: | 21-24 May 2019 | | |
| Place: | Teagasc conference centre Dublin | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | yes |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 300 | Media | |
| Industry | 5 | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 5 | | |

| | | | |
|---|---|---|---|
| Name of the activity: | Sylvain BRISSE has presented the MedVetKlebs project optimized culture methods and qPCR protocol at the Nor-Kleb-Net meeting. | | |
| Date: | August 21st, 2019 | | |
| Place: | Sommaroy, Norway | | |
| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | |
| Organisation of a Workshop | | Participation to a Workshop | |
| Press release | | Participation to an Event other than a Conference or a Workshop | yes |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |
| **Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories** | | | |
| | **Number** | | **Number** |
| Scientific Community (Higher Education, Research) | 40 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | | | |

| | |
|---|---|
| Name of the activity: | Oral presentation at IMMEM-XII: Carla Rodrigues, Marisa Haenni, Maxime Bour, Cécile Ponsin, Jean-Louis Pinsard , Virginie Passet, Jean-Yves Madec, Sylvain Brisse. Genomic Diversity, Antimicrobial Resistance and Virulence of *Klebsiella pneumoniae* from Animal Carriage: a Comprehensive Analysis in an One Health Perspective |
| Date: | 18th- 21st September 2019 |
| Place: | Dubrovnik, Croatia |

| Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories | | | |
|---|---|---|---|
| | Yes / No | | Yes / No |
| Organisation of a Conference | | Participation to a Conference | Yes |
| Organisation of a Workshop | | Participation to a Workshop | Yes |
| Press release | | Participation to an Event other than a Conference or a Workshop | |
| Non-scientific and non-peer-reviewed publication (popularised publication) | | Video/Film | |
| Exhibition | | Brokerage Event | |
| Flyer | | Pitch Event | |
| Training | | Trade Fair | |
| Social Media | Yes | Participation in activities organized jointly with other H2020 projects | |
| Website | | Other | |
| Communication Campaign (e.g. Radio, TV) | | | |

| Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories | | | |
|---|---|---|---|
| | Number | | Number |
| Scientific Community (Higher Education, Research) | 275 | Media | |
| Industry | | Investors | |
| Civil Society | | Customers | |
| General Public | | Other | |
| Policy Makers | 10 | | |

## *10. List of planned tele- or video conferences, face to face meetings in the next year*

We do TC meetings as felt necessary, with no particular planning but with regularity (every three months approx.)

We are planning our final face-to-face meeting in Paris, Institut Pasteur, on April 16-17th, 2020.