



D1.10 Summary Progress Report Year 3

WP1 Coordination

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1 Glossary

AH: Animal Health

ASM: Annual Scientific Meeting

AWP: Annual Work Plan

CaM: Communication workshop and Media training

CPD: Continuing Professional Development

CT: Coordination Team

ESAB: External Scientific Advisory Board

FS: Food Safety

JIP: Joint Integrative Projects

JRP: Joint Research Project

MS: Member State

OHEJP: One Health European Joint Programme

PH: Public Health

PMC: Programme Managers Committee

PMT: Project Management Team

POC: Programme Owners Committee

REA: Research Executive Agency

SPR: Summary Progress Report

SS: Summer School

SSB: Scientific Steering Board

ST: Support Team

STM: Short Term Mission

WS: Workshop

2 Publishable summary

2.1 Summary of the context and overall objectives of the project

2.1.1 What is the problem/issue being addressed?

The One Health EJP is a policy driven research network addressing issues related to needs identified in the food safety area.

- Need to strengthen the links between human health, animal health and environmental aspects: One Health approach
- Need to further integrate surveillance and response capacities, preventive approaches, detection systems as well as preparedness and response to disease outbreaks



- Need of collaboration in Joint Research and Joint Integrative Projects, as well as Training and Education activities throughout a consortium of national public mission organisations
- Need to foster interaction between European, national authorities and stakeholders
- Need to update policy makers on these achievements and, built on this knowledge, to take appropriate action

2.1.2 Why is it important for society?

The integrated health approach, known as 'One Health', is based on strengthening collaboration between human health, animal health and environmental management. It focuses on developing surveillance and response capacities, strengthening early-warning and detection systems; reinforcing the capacities of public health and veterinary authorities as regards prevention, preparedness and response to disease outbreaks; evaluating the social and economic impact of diseases; promoting inter-sector collaboration for the health of the livestock, wildlife and ecosystems concerned; research on the conditions under which diseases emerge and spread. Thus coordination between the different health systems, which are generally run separately, must enable economies of scale by encouraging synergies, and guarantee improved health security. Particular attention is paid to the communication of risks at all levels of action.

2.1.3 What are the overall objectives?

The overall objective of the One Health EJP is to develop a European network of research institutes, mainly with reference laboratory functions, integrating medical, veterinary and food scientists in the field of food and feed safety in order to improve research on antimicrobial resistance and on the prevention and control of mainly foodborne zoonoses, while taking into account the public health concerns of consumers and other stakeholders throughout the food chain.

2.2 Work performed during the reporting period (M25-M33) and main results achieved

2.2.1 WP1

The Coordination Team continues with the management of the One Health EJP by having weekly conference calls for the day-to-day management of the project. Regular teleconferences with PMT were organised (30 January, 4 March, 1 April and 5 June), as well as one TC with SSB (19 March) and another planned for September 2020). The planned PMC and POC meeting will be jointly organised on 13 October, if possible as a face-to-face meeting.

The management of the OHEJP has been strongly impacted by the Covid-19 outbreak, making impossible a number of activities and physical meetings, which have been cancelled consequently. In this context, the use of the force majeure clause has been requested and managed by the Support Team in order to assure the eligibility of costs incurred for the activities cancelled or postponed.

The meetings affected by the COVID-19 crisis were the following:

- DG SANTE meeting Brussels, 17 March, 2020 (cancelled)
- SSB meeting Lisbon, 19 March 2020 (converted into TC)
- PMT meetings: 30 January (Brussels), 4 March, 1 April, 5 June and 27 August 2020 (virtual meetings).
- ASM Prague, 27-29 May, 2020 (went on as webinar)



- Stakeholders meeting Prague, 26 May, 2020 (converted into TC)
- ESAB meeting Prague, 28 May, 2020) (converted into TC on 19 June, 2020)
- REA and Steering Group meeting, 06 July, 2020 (TC planned)
- SSB meeting Surrey, 18 September, 2020 (converted into TC)
- Joint PMC/POC meeting, 13 October, 2020 (converted into TC).

The Support Team has managed finances and the financial report of year 2 as well as the second amendment to the Grant Agreement.

The Coordination Team together with the Project Management Team has prepared the periodic report and submitted it to REA on 25 March 2020.

In addition, the CT and the PMT have implemented together the OHEJP Consortium enlargement procedure.

As for the follow-up of possible ethical issues, the Ethics Advisors evaluated both the ongoing first round projects, the projects that started in January 2020 and all the PhD studies. The ethics report (D1.24) was delivered as planned.

2.2.2 WP2

In WP2, relevant scientific developments, including the emergence of zoonotic threats and opportunities for scientific innovations were monitored. For this, the output of OHEJP projects (e.g. deliverables, scientific publications) were screened, as well as relevant information from external sources. In addition, the repository of EU projects/initiatives has been updated in the OHEJP website and distributed to all partners and OHEJP project leaders. Strategic interactions with a number of related EU projects and initiatives were established. WP2 has collaborated with WP4 to select the projects/initiatives to organize the cogwheel workshops during 2020. WP2 has also worked closely with WP7 towards the elaboration of the OHEJP Strategic Research and Innovative Agenda.

2.2.3 WP3

In the third year of the One Health EJP, no new official procedures have to be delivered.

A main task of WP3 is the monitoring of ongoing joint research projects, both those that started in January 2018 (first call) and those launched in January 2020 (second call). A full report (D3.11) on the progress of the 11 first call JRP was delivered in spring 2020, and a brief report on the start of the second call projects was uploaded as D3.14 in August. Since only one JRP has ended in December 2019 (i.e. MAD-VIR), WP3 chose to combine its evaluation together with one or more projects that would end in June 2020. Since all project leaders however requested a 6-month extension of their projects, the evaluation process for MAD-VIR was nevertheless launched before the summer 2020.

Due to the COVID-19 crisis and the consecutive travel restrictions, WP3 decided to turn the Annual Scientific Meeting (ASM) initially planned for May 2020 in Prague into an online event, during the same 3 days. The conference was a tremendous success, with more than 700 registrations from 38 countries all over the world! Also, the team selected the host for ASM2021, i.e. Copenhagen, Denmark.

2.2.4 WP4

During Y3, much of the focus in WP4 has been on the upstart of the three new JIPs, integrative activities and planning for the dissemination of results and outcomes. The three new JIPs, CARE, OH-HARMONY-CAP and MATRIX all had their kick-off meetings during the first months of the year. Already in March



they had to deal with the COVID-19 crisis and make plans for how to proceed. COVID-19 has caused some delays, but all JIPs plan to deliver according to their amended work plans. There was also a successful shift of project leaders in two of the new JIPs. To avoid overlaps with other projects and learn from each other a thematic integrative meeting as well as two cogwheel workshops have been arranged. The digital form of meetings has allowed a high number of participants and many new contacts between projects within the OHEJP and to other H2020 projects have been made possible. Further, a new OHEJP-JIP, COVRIN, which is targeted to the one health aspects of SARS-CoV2, has been planned for and a proposal is currently under evaluation.

WP4 has also explored different opportunities for further dissemination of outputs including additional integrative initiatives. After a discussion in the PMT, a list of opportunities was presented to the Scientific Steering Board. The proposal to arrange a simulation exercise was highest ranked and this is now the activity we are planning for. Financing is under discussion. The earlier suggested data sharing initiative will be reconsidered later if resources are available. The data management plans have also been in focus to contribute to the dissemination of results from the OHEJP JIPs/JIPs. A new software tool has been adapted to the OHEJP structure and made available for the newly started projects. The nomenclature used in the DMPs build on the One Health EJP Glossary, which was earlier developed.

2.2.5 WP5

The purpose of the activities was to consolidate the dialogue with ECDC and EFSA as the Key EU stakeholders of the One Health EJP and to establish contacts with other international stakeholders, both European (EEA, EMA) and global (FAO, OIE, WHO). The fifth Stakeholder Committee meeting was held in connection to ASM 2020 as an online meeting, and saw participation from EFSA, EEA, EMA, FAO, and WHO-EURO. The sixth will take place in October 2020 the day before the joint POC/PMT meeting in Berlin.

Stakeholders' needs were collected by the regular "scanning of stakeholders' documents" activity, as well as by direct contacts with organisations' representatives. The summary reports were presented on the consortium webpage accessible for all consortium members.

The dissemination activities of WP5 were further developed to disseminate new scientific data and results to the different stakeholder groups in a targeted manner. These include regular targeted reports to Key EU stakeholders, as well as thematic reports where stakeholders' needs are analysed and related to OHEJP activities.

The One Health Outcome Inventory (OHOI) was deployed and content updated to support interaction and collaboration between consortium members and stakeholders.

2.2.6 WP6

In Y3, several WP6 training events and educational activities were significantly affected by COVID-19:

- Of the five Short Term Missions (STMs) funded to take place this year, two STMs were successfully completed, although the planned outcomes from these missions were also impacted. Of the remaining three STMs, two were postponed to the end of Y3 and one was cancelled indefinitely as the 'new' timing of the training no longer fits in with the planned work.
- WP6 evaluated and reported an inventory of non-refundable costs and delays due to the impact of COVID-19 on the 17 OHEJP PhDs taking place (WP6 and WP7). Of 17 projects, two suffered non-refundable costs, and 11 projects have confirmed that their projects will suffer



delays in completion. These details are discussed in more detail in the individual PhD reports later in this deliverable.

- The first Continuing Professional Development (CPD) module was impacted during the final organisation stages. This event was postponed, and a virtual interactive workshop will be delivered in M35 by RIVM. This event will be offered to those who were originally selected for participation. Depending on the final numbers confirmed, the application call may be launched once more.

The first CPD deliverable report D6.5 was been delayed (an extension was requested in advance). During the call for the first CPD module, WP6 did not receive any applications, and so the call was extended. RIVM's successful application planned to deliver the event in M27, whereas the deliverable was due M24, hence an extension was requested to M28. This was further delayed due to the COVID-19 pandemic, and a new deadline has been requested for D6.5 for M36.

- The second CPD module organiser was successfully selected. This event will be organised by BfR (Germany) and is planned to be delivered in M38.

Like the first CPD call, WP6 did not initially receive any applications for the second CPD module. Therefore, this call was also extended. BfR's successful application planned to deliver the event in M38. Therefore, a request has been submitted to delay the deadline of deliverable report D6.9 to M40.

- The ASM Satellite workshop 2020 module was impacted during the final organisation stages. This event was postponed. The nature and interactions of the satellite workshop means that it is very challenging to deliver the workshop online. Alternative dates and options to deliver this event are being. This event will be offered to those who were originally selected for participation. Depending on the final numbers confirmed, the application call may be launched once more. This deliverable will be delayed, and an extension will be requested.

The Summer School 2020 was also impacted by the COVID-19 pandemic as it could not be delivered as a physical event. This event was planned and re-organised as a virtual event, and was delivered in M32. Deliverable report D6.8 will be delivered on time in M36. See section 7.1.4 for more details.

- The Communication and Media workshop has also been impacted by COVID-19 as it could not be delivered as a physical event. This event was planned and re-organised by NDVRMI (Bulgaria) as a virtual event and will be delivered in M34. Deliverable report D6.2 will be delivered as planned in M36. WP6 and local organisers have planned a high-quality interactive programme to be delivered on the Google Meets online platform.
- The calls for the summer school, CPD module, satellite workshop and STMs to take place in Y4 were successfully launched in M25.
 - o Four applications were received through the STM call, and these will all be co-funded in Y4. The validated procedure to select the STMs was followed.
 - o The third Summer School organiser was successfully selected. This event will be organised by ISS (Italy) and is planned to be delivered in M43.
 - o No applications were received to organise the CPD and Satellite Workshop in Y4. These calls have been extended, and will be promoted more during governance meetings and through internal communication channels.

All selection procedures and protocols will be reviewed, modified where necessary and validated for the organisation of events and participation in missions taking place in 2022.

2.2.7 WP7



A SWOT analysis has been completed and delivered: the analysis of strengths, weaknesses, opportunities and threats of the present and future of the OHEJP is considered as a main support to the OHEJP sustainability. The PhD project SUSTAIN was launched to assess the constraints to a OH approach arising in different EU contexts. At this first stage, interviews were collected from experts of the Swedish Veterinary Agency, the Swedish National Food Agency and the Swedish Public Health Agency.

2.3 Progress beyond the state of the art, expected results until the end of the project and potential impacts.

Consistent with the “Prevent-Detect-Respond” concept, integrative activities will feed the approach of evidence based risk assessment and therefore risk management by the competent authorities.

Intensive collaboration between the most relevant partners in Europe in the field of foodborne zoonoses and antimicrobial resistance contribute to help to reduce unnecessarily duplication of work on these topics.

It is of importance to efficiently organize knowledge dissemination to the appropriate stakeholders (ECDC, EFSA, DG AGRI, DG Santé, the national authorities and beyond); these tasks were and will be taken forward by WP2 (Strategic Research Agenda), WP5 (Science to Policy) and WP6 (Education & Training).

The EJP aims at enhancing harmonization, alignment and integration of activities in these domains, but this process may not be finalised at the end of the 5-year programme. To make sure that the integrative activities will last beyond the lifespan of the One Health EJP, a specific WP (WP7) is dedicated to create a significant long-term capacity building and alignment among all EJP partners.

3 WP1 - Coordination and Management

3.1 Work carried out to date

3.1.1 Task 1.1: Management of EC contractual obligations

Regarding contractual procedures, the One Health EJP Support Team (ST) has ensured a strict monitoring of the deliverables and milestones, which has allowed the submission of the deliverables due in the period as well as the notification of the milestones achieved. The WP1 deliverables (D1.8 + D1.9 + Ethical review report for year 2, Summary Progress Report and Annual Work Plan) have been prepared by the Coordination Team in order to report to the REA. The ST has also prepared the second amendment to the Grant Agreement including budget adjustments, clarification of articles 11, 12 and 15 of the Grant Agreement used by some Beneficiaries, withdrawal of one of Consortium partners and update of the work programme. The second Amendment to the Grant Agreement (AMD-773830-40) was submitted on 23 January 2020 and approved by the European Commission on 23 March 2020.

3.1.2 Task 1.2: Project management

The CT, consisting of the Coordinator, Scientific Coordinator and Support Team, provided effective management support to ensure the quality of the work both in terms of results and timing and to manage the relationships between partners and to ensure an effective internal communication. The CT has frequently organised teleconferences to monitor the project's progress and to ensure the timely implementation of the AWP year 3. When any important issue has arisen, the Coordination team has liaised with the Research Executive Agency (REA) to inform the Project Officers (PO) in the first place



and request a delay in the submission of deliverables or a change of content of Annex 1 of the Grant Agreement whenever needed and relevant.

The CT and the Project Management Team (PMT) had a face-to-face meeting on 30 January at Sciensano's premises in Brussels and held regular teleconferences to monitor the progress of the activities per work package (WP). The PMT reviewed, commented and provided relevant guidance and input on important WP documents, and also validated the deliverables, which have been prepared and submitted during this period. Two specific tasks were undertaken during the considered period.

3.1.2.1 Initiation to response to COVID pandemics

Most importantly, PMT decided to react on the COVID-19 crisis in two major ways:

- By communicating on the efforts ongoing and planned in the partner institutes regarding COVID-19: animal health institutes that supported the clinical diagnostics laboratories, development of new or improved techniques, corona related studies, etc.
- By proposing an additional Joint Integrative Project on COVID-19, namely COVRIN

When in January 2020 the news about the COVID-19 threat in Wuhan, China, its global spread and its probable animal origin reached Europe, some Project Leaders and all One Health EJP beneficiaries (Scientific Steering Board members and institute representatives) were contacted (mail 24 January 2020). The objective of the contact was to define a OHEJP-approach to this coronavirus emergency in order to be credible for our national stakeholders, ECDC, EFSA and the EC. The One Health EJP consortium has a preferential contact with the national and international authorities and could thus offer cooperation of its network of public and animal health laboratories in new initiatives related to COVID-19. Suggested actions were:

- Test development for detection and characterization; validation of these tests; drafting a harmonized protocol;
- Propose surveillance strategy (screening, confirmation; animal and human population)
- Risk assessment;
- Intervention measures.

After a discussion in its meeting of 30 January, the PMT decided to communicate through the One Health EJP website and social media on the efforts that are ongoing and planned in the partner institutes regarding COVID-19: animal health institutes that supported the clinical diagnostics laboratories, development of new or improved techniques, corona related studies, etc. In addition, PMT agreed on launching the procedure for a joint integrative project (JIP) on the novel coronavirus SARS-CoV-2 among its partners (see WP2 and WP4).

3.1.2.2 Start of the consortium enlargement campaign

One of the recommendations made in 2019 by the REA Steering Group members, the EC external independent reviewers, and the ESAB members all together, was to enlarge the consortium in two priority ways: to complete the twinning Public Health/Animal Health in participating countries where it was not achieved yet and to expand to new European countries, the EU Member States in priority. To that end a procedure was set up including a detailed way of identification of putative new organisation to be invited to join.

There are currently 17 MS on board the OHEJP consortium out of which 12 have completed the twinning PH/AH. The two additional participating EU countries, namely Norway and the UK, have already completed the PH/AH twinning.



Invitations to join the consortium were sent in June. Those organisations which had not responded were reminded in July.

To date:

- 7 new MS would be interested to join out of which
 - Cyprus-AH
 - Finland-AH/PH
 - Croatia-PH
 - Lithuania-AH
 - Latvia-AH
 - Luxembourg-PH
 - Malta-AH
- 2 would achieve their twinning
 - Hungary-AH
 - Spain-PH

That would bring on board 6 new organisations on Animal Health and 4 on Public Health, provided negotiations with their Line Ministries can lead to an agreement.

The table below summarizes the progress of achievement of the enlargement procedure



Country	AH/PH		Accept/reject invitation	Negotiation with line Ministries and preparation of official documents	Examination by OHEJP governance	Examination by REA & REA steering group	Accession to the GA
BU	PH	National Centre of Infectious and Parasitic Diseases (NCIPD)	Not invited as they previously resigned from the OHEJP	N/A	N/A	N/A	N/A
CY	AH	Director of Veterinary Services: Laboratory for the Control of Foods of Animal Origin (LCFAO), Laboratory of Animal Health (LAH) and State Veterinary Laboratories (SVL) (MOA)	Accept	In progress			
	PH	Medical and Public Health Services (MPHS) Department of the Ministry of Health (MoH)	No answer	N/A	N/A	N/A	N/A
ES	PH	Instituto de Salud Carlos III (ISCIII)	Accept	In progress			
FI	AH	Finnish Food Authority (RUOKA, formerly EVIRA)	Accept	In progress			
	PH	National Institute for Health and Welfare (THL)					
GR	AH	Veterinary Laboratory of Chalkis		N/A	N/A	N/A	N/A



Country	AH/PH		Accept/reject invitation	Negotiation with line Ministries and preparation of official documents	Examination by OHEJP governance	Examination by REA & REA steering group	Accession to the GA
	PH	National School of Public Health (Εθνική Σχολή Δημόσιας Υγείας, ESDY) University of West Attica Department of Public Health Policy	Interested but not enough resources (financial human time)				
HR	AH	Croatian Veterinary Institute	No answer	N/A	N/A	N/A	N/A
	PH	Croatian National Institute of Public Health (CNIPH/HZJZ)	Accept	In progress			
HU	AH	National Food Chain Safety Office (Nébih/NFCO) (successor of the Central Agricultural Office (CAO) and the Hungarian Food Safety Office)	Accept	In progress			
LT	AH	National Food and Veterinary Risk Assessment Institute (NMVRVI)	Accept	In progress			
	PH	Lithuania National Public Health Surveillance Laboratory (NVSPL)	No answer	N/A	N/A	N/A	N/A



Country	AH/PH		Accept/reject invitation	Negotiation with line Ministries and preparation of official documents	Examination by OHEJP governance	Examination by REA & REA steering group	Accession to the GA
LV	AH	Scientific Institute of Food Safety, Animal Health and Environment BIOR	Accept	In progress			
	PH	The Centre for Disease Prevention and Control (CDPC) (SPKC)	No answer	N/A	N/A	N/A	N/A
LU	AH	Laboratoire de Médecine Vétérinaire de l'État (LMVE) - subordinated to ASV	No answer	N/A	N/A	N/A	N/A
	PH	Laboratoire National de Santé (LNS)	Accept	In progress			
MT	AH	Veterinary and Phytosanitary Regulation Division (VPRD) (coordinates the Veterinary Regulation Directorate (VRD) and the National Veterinary Laboratory (NVL))	Accept	In progress			
	PH	Public Health Laboratory (coordinated by the Environmental Health Directorate)	No answer	N/A	N/A	N/A	N/A
PO	PH	National Institute of Public Health – National Institute of Hygiene (Narodowy Instytut Zdrowia Publicznego - Państwowy Zakład Higieny)	No answer	N/A	N/A	N/A	N/A



Country	AH/PH		Accept/reject invitation	Negotiation with line Ministries and preparation of official documents	Examination by OHEJP governance	Examination by REA & REA steering group	Accession to the GA
RO	PH	National Institute of Public Health (Institutul National De Sanatate Publica, INSP) (hosts the Centre for Communicable Disease Surveillance and Control (NICSC))	No answer	N/A	N/A	N/A	N/A
SI	AH	National Veterinary Institute (NVI, within the University of Ljubljana, Veterinary Faculty)	No answer	N/A	N/A	N/A	N/A
	PH	National institute of Public Health (NIJZ)	No answer	N/A	N/A	N/A	N/A
SL	AH	State Veterinary and Food Institute Dolný Kubín (VPI- Dolný Kubín)	No answer	N/A	N/A	N/A	N/A
	PH	Public Health Authority of the Slovak Republic (PHA or UVZ-SR)	No answer	N/A	N/A	N/A	N/A



3.1.3 Task 1.3: Organisation of EJP management and governance meetings

All consortium governance bodies (Coordination Team, Project Management Team, Scientific Steering Board, Programme Managers Committee) have actively cooperated in supporting the efficient implementation of the One Health EJP activities.

The Scientific Steering Board (SSB) members has been regularly informed of the One Health EJP progress and their input has been requested on several occasions such as when implementing the Consortium enlargement procedure, setting up additional integrative activities, allocating the unspent budget to the new activities, approving the extension of scientific projects.

The first 2020 SSB meeting was initially planned as a face-to-face meeting to be held in Lisbon on 19 March. Due to Covid-19 outbreak, it was implemented as an online meeting that successfully meets its objectives including:

- review and validation of the periodic technical and financial report of year 2 (2019);
- discussion on the progress of the consortium enlargement campaign according to the consortium enlargement procedure;
- reallocation of the unspent budget for other activities, notably considering additional integrative activities;
- discussion on the progress of the JRPs and JIPs of the first and second internal call and on the sustainability of the OHEJP, notably within the future EU Partnerships.

The second 2020 SSB meeting was held as a teleconference on 18 September with main purpose to validate the OHEJP Annual Workplan Y4 and the Summary Progress Report Y3 and to discuss the implementation of the JIP COVRIN, OHEJP Consortium enlargement procedure and sustainability issues.

The Programme Manager Committee (PMC), the Governing Board of the OHEJP and the Programme Owner Committee (POC), composed of the Beneficiaries' line Ministries Representatives, were expected to have their combined meeting on 13 October 2020 in the premises of the BfR - German Federal Institute for Risk Assessment in Berlin. Due to COVID-19 situation, this meeting has been converted into an online meeting. The Representatives of ECDC and EFSA, as major international stakeholders of the OHEJP are also invited to join this meeting pursuing the following objectives:

- To get informed about the progress of the OHEJP overarching activities (periodic report, evaluation, grant management, etc.);
- To find out practical outcomes/results of the One Health EJP and its encompassing projects after two years of research and integrative activities (JRPs, JIP, PhDs, training activities);
- To have EFSA and ECDC representatives testify from their experience with the One Health EJP;
- To invite participants (POC members, ECDC and EFSA) to assess the deliverables as regards to their organisations' needs and to consider further interactions between ECDC, EFSA and the national Authorities;
- To discuss extension of activities of the One Health EJP in EU Partnerships under Horizon Europe.

Furthermore, an online meeting with the External Scientific Advisory Board (ESAB) was held on 19 June 2020 and discussed the progress One Health EJP made since the last ESAB meeting in May 2019.



3.1.4 Task 1.4 Communication tools

The Communications Team based at the University of Surrey has carried out the following work:

- We have recruited a further Communications Officer to focus on the visual aspects of the OHEJP brand and Communication Strategy. Our new Communications Officer brings a wealth of creative communications and marketing experience to the Team and will help to elevate all aspects of the OHEJP brand and further develop the Communications Strategy.
- The website has been developed and improved according to feedback from the consortium. The front end of the website has been edited and expanded to include more important documentation. In addition to a "Latest News" page which reports on the recent COVID-19 outbreak and the OHEJP's response to the outbreak. Furthermore, important OHEJP news and examples of our success are also posted on this page. A publication page and deliverables page have also been added to highlight all of the OHEJP success.
- The Communications Team have worked closely with the PMT to further develop the OHEJP Communications Strategy which determines how the OHEJP Communication Team will proceed with communication tasks until the end of the OHEJP. The Communications Strategy is a comprehensive document which has been disseminated within the PMT. This document will be widely available to consortium members, however, a simplified version of this document will be created and disseminated to the OHEJP consortium in a bundle of information being prepared to support communication and dissemination activities.
- The Communications Team have supported all OHEJP events. This included advertising on social media, [posting on the OHEJP website](#), providing videoconference links and logos etc to event organisers. Furthermore, for the [CPD](#) and [ASM Satellite Workshop](#) the Communications Team supported the application process to the local organiser. Continued support for the remaining WP6 events that are able to proceed this year will be provided, including the [Summer School](#) and [Communication and Media workshop](#). The team have also created case study pdf documents for all the [2019](#) and [2020 Short Term Missions](#) (and [Air Sample](#) and will continue to grow the number of case studies) These have been disseminated in the WP6 bulletin, OHEJP newsletter and on the OHEJP website.
- The deliverable 1.8: Annual report on the internal and external newsletter produced during the second year has been completed (31.01.20) and disseminated. This document detailed the internal and external newsletters produced during the second year.
- The deliverable D1.9: Complete version of annual report for stakeholders n°2 has been completed (25.05.20) and widely disseminated to all OHEJP audiences, including the general public. It details the key objectives of the OHEJP and the progress made by all WPs, JRPs, JIPs and other activities, including but not limited to dissemination and communications.
- A more substantial role in dissemination of deliverables, outcomes and data has been undertaken by the communications team this year. The team have worked with WP3 to address how to illustrate the dissemination process using an infographic. The Communications Team are also responsible for curating the open access repository Zenodo and uploading deliverables and publications to the OHEJP website. Additionally, a representative from the Communications Team has joined the Data Management Plan Committee established in March 2020. The involvement of the Communication Team enables the links between all of the dissemination activities to be made and to work with other WPs to address Project Leader's needs.
- The Communications Team played a significant role in the OHEJP ASM 2020 and this year were on the organising committee of the event. Due to the COVID-19 pandemic the Team took responsibility for finding, commissioning and subsequently managing a specialist production



company to ensure the event took place online. The University of Surrey were subsequently responsible for the finances and online delivery of the 2020 ASM. Furthermore, the team undertook a significant role in the administration and promotion leading up to the event. In addition, the Team created interactive and static programmes, awards certificates, and many of the visual elements used during the event. Finally, the team ensured that the event had active social media accounts, focussing primary on Twitter. The success on Twitter and LinkedIn increased that of the previous year.

- OHEJP merchandise, an important part of OHEJP branding and awareness, was sent prior to all events as requested by the event organiser. Additionally, members of the PMT that requested merchandise also received boxes of pens, notepads, USBs, post-it notes and lanyards directly to their institutes.

3.1.5 Task 1.5 Ethics

The follow-up of possible ethical issues consisted of three parts: the ongoing first round projects based on the replies of the project leaders to the recommendations from the Ethics Advisors, the projects that started in January 2020 and all the PhD studies. The two latter parts were based on the self-assessments that the project leaders and PhD supervisors sent in together with the project and PhD proposals, respectively.

The ethics report (D1.24) was finalised by the Ethics Advisors in due time. It states:

“Following the initial comments by the Ethics Advisory Board (EAB) which have been feedback to the Project Leaders some of the replies received were not adequate and so repeated questions needed to be answered. After two rounds of comments and replies, most questions from the EAB have been answered. Two enquires from the EAB still require clarification but neither of this issues present anything above moderate significance in ethical terms and therefore as long as the Project Leaders address these with through the One Health EJP Coordinator, that is appropriate.”

The report details all recommendations from the Ethics Advisors and how the Project Leaders have dealt with those, per JRP or JIP. As stated in the report, no significant issues were identified.

In contrast to 2019, the Coordination Team decided to feedback the ethics recommendations both by mail in the beginning of March 2020 and through the 9M-reporting, thus encouraging Project Leaders to timely take appropriate actions.

At the time of drafting this report, the replies to the recommendations of the ethics advisors were available for the first call JRP and JIP, for all but one project of the second call, and for all PhD. As soon as possible, the project leaders' comments will be handed over to the ethics advisors for further comments.



3.1.5.1 Follow-up of the recommendations and comments by the Ethics Advisors

3.1.5.1.1 JRP01-AMR1-IMPART

IMPART					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must clarify the <u>safety mitigation measures</u> 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		information still pending	All thirteen partner institutes have confirmed by email to the project leader of IMPART that they had the correct safety mitigations measures in place to protect the environment and the staff. All permits are available in the individual partner institutes upon simple request.



3.1.5.1.2 JRP02-AMR2-ARDIG

ARDIG					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
As mentioned p10 'Patient information such ... will be provided once the appropriate ethics approval is in place (in the process of submission to IRAS)'. Copy of the approval must be presented.	no reply provided	question proposed again	no reply provided	this issue must be addressed	Isolates of human origin are collected from reference laboratories in hospitals, therefore ethical approval is not required.
The applicants must confirm that ethics approvals for the <u>use of biological samples</u> 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)	No comment	Again, the Ethics Advisors ask: Is 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)?	Isolates of human origin are collected from reference laboratories in hospitals, therefore ethical approval is not required.



ARDIG					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm the <u>application of 3Rs</u> and the ethical approvals (approval letters, etc) for <u>animal work</u> 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 (eg in-vivo mouse work, etc). Please elaborate.	The applicants can confirm the application of 3Rs. Partners who will undertake any animal work eg in vivo mouse work, will do so in a justifiable way with full ethical approval, applying the principles of 3Rs.	<p>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 (eg site and name of the AWERB). This can be done through the provision of the approval letter.</p> <p>Limited response, more information needed</p>	PL has requested additional information within the consortium	information still pending	No vivo work has been performed to date. This work has been delayed and may be hampered due to the impact of COVID19.



ARDIG					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm the compliance with <u>GDPR</u>	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.	satisfactory answer - Closed	



ARDIG					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must document the <u>safety mitigation measures</u> 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.	What are the safety measure, BSL? working conditions or standard protocols required? Please provide some details	As already mentioned detailed RAs, which comply with all local/institute H&S requirements and approved by H&S teams, are already in place for all laboratory work that has been undertaken in ARDIG (e.g. bacterial manipulation, DNA extraction, whole genome sequencing, etc). Laboratories in each institute regularly participate in H&S inspections to ensure compliance.



ARDIG					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must specify whether the <u>samples used for genetic analysis</u> permit to <u>identify the sample donors</u> . 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	No comment	satisfactory answer - Closed	
The applicants must specify why the <u>misuse issue</u> 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	No comment	satisfactory answer - Closed	

3.1.5.1.3 JRP03-AMR3-RADAR

N/A



3.1.5.1.4 JRP05-ET1-TOXdetect

TOXDETECT					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm the <u>source of tissues</u>	Cell culture needs administrative authorization	Satisfactory reply	No comment	Satisfactory reply - Closed	No comment
The applicants must document the <u>safety mitigation measures</u> in place to protect the staff	BSL2 working conditions	Satisfactory reply	No comment	Satisfactory reply - Closed	No comment



3.1.5.1.5 JRP06-FBZ1-NOVA

NOVA					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm that ethics approvals for the use of <u>isolates from human origin</u> 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. Ethical approvals for the use these data will not be needed.	Satisfactory reply	No comment	Satisfactory reply - Closed	-



NOVA					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm the compliance with <u>GDPR</u>	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. 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.	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.	Satisfactory reply - Closed	-



3.1.5.1.6 JRP07-FBZ2-LISTADAPT

LISTADAPT					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
'Animal samples' are collected, please re-confirm no animal experimentation approvals are required for the <u>animal sampling protocols</u> (eg do to interventions, restrictions, etc)	No animal experimentation are done. Samples corresponds to fecal samples	EA did not comment	No comment	Satisfactory reply – Closed	
The applicants must confirm that ethics approvals for the <u>use of isolates from human origin</u> 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.	Satisfactory reply	No comment	Satisfactory reply – Closed	



LISTADAPT					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must document the <u>safety mitigation measures</u> in place to protect the staff	Every partners have the biosafety level associated with the handling of Listeria	Satisfactory reply	No comment	Satisfactory reply - Closed	
The applicants must document there are no <u>environmental safety issue</u> from the sampling etc and protocols are in place	A video has been proposed to partners that samples environment (soil, water)	EA did not comment	No comment	Please can the Project Leader explain what is meant by "A video has been proposed to partners that samples environment (soil, water)". Does this mean there is harmonised training meeting the EU standard {ADD please}. Please clarify	All soil and water samples were taken from agricultural or wilderness areas according to a common procedure provided to the samplers based on existing guidelines reported in the literature. The integration of the information provided by the samplers resulted in a continuous improvement of the sampling protocol. This is not harmonised training corresponding to the European standard.



3.1.5.1.7 JRP08-FBZ2-METASTAVA

METASTAVA					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm that ethics approvals for the <u>use of biological samples</u> , 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 from the JRP leader.	Satisfactory reply	No comments	Satisfactory reply - Closed	



METASTAVA					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm the compliance with <u>GDPR</u>	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 from 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: <ul style="list-style-type: none">· Sciensano InformationSecurity@sciensano.be· FLI datenschutz@fli.de· ANSES data protection officer : Director of Legal Affairs (saisine-daj@anses.fr)· SVA registrator@sva.se· WBVR functionarisgegevensbescherming@wur.nl.· EMC functionaris.gegevensbescherming@erasmusmc.nl	Satisfactory reply - Closed	



METASTAVA					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must document the <u>safety mitigation measures</u> 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 from the JRP leader.	Satisfactory reply	No comments	Satisfactory reply - Closed	



3.1.5.1.8 JRP09-FBZ3-AIR-SAMPLE

AIRSAMPLE					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
'Samples' are collected in an animal unit, please confirm no animal experimentation approvals are required for the <u>animal sampling protocols</u> (eg do to interventions, restrictions, etc)	This project does not have animal experimentations	<p>More info needed</p> <p>The answer is not adequate as the ethics question was not asking if there are animal experiments but was asking since: "Samples' are collected in an animal unit, please confirm no animal experimentation approvals are required for the animal sampling protocols (eg do to interventions, restrictions, etc)"</p> <p>Some of these procedures can require approval (for example from an AWERB under Schedule 1 requirements)</p>	No manipulation or contact to animals are done in this project. We just sample the air in chicken houses.	Satisfactory reply - Closed	



AIRSAMPLE					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
Due to the sampling protocol, the programme team may be collecting personal and business data Please confirm compliance with <u>GDPR</u>	This project does not make use of animals and will not impact any societal or ethical aspects. The identity of farms participating in the sampling plans will not be revealed. 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.	Satisfactory reply - Closed	



3.1.5.1.9 JRP10-FBZ3-MoMIR-PPC

MOMIRPPC					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
More detailed information must be provided on the <u>recruitment procedure of the study participants</u>	All persons living in Norway diagnosed with Salmonella during the sampling period (now 01012019 – 31082020) 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	No comments	Satisfactory reply	



MOMIRPPC					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm that an <u>insurance policy</u> has been established to cover the study participants	No insurance policy is needed, as the health risk of taking a stool sample with a self-testing kit is considered minimal	Satisfactory reply	No comments	Satisfactory reply	
The applicants must confirm that <u>animal samples</u> used have been collected with the appropriate ethical approvals (approval letters, etc) so complying with EU standards	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 (eg site and name of the AWERB). This can be done through the provision of the approval letter. Limited response, more information needed	Each partner has sent the documents to the appropriate ethical committee where the details on the 3R were described.	The team was asked to provide the licence approval number / ethical approval code. This is not provided. Please provide	All partners have now provide their license approval number. Licence approval numbers and ethical approval codes are available in partner institutes.



MOMIRPPC					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm that ethics approvals for the <u>conduct of the clinical study</u> have been sought	No clinical study will be undertaken, as described in the changed project description approved December 2017	Satisfactory reply	No comments	Satisfactory reply - Closed	
The applicants must confirm that ethics approvals for the use of <u>biological samples</u> have been sought	For the human part, the ethical clearance from the Norwegian Committee for Medical and Health Ethics has been granted 15052018, and updated 22112018	Satisfactory reply	No comments	Satisfactory reply - Closed	



MOMIRPPC					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm the compliance with <u>GDPR</u>	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.	Satisfactory reply - Closed	



3.1.5.1.10 JRP11-FBZ4-MedVetKlebs

MEDVETKLEBS					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm that <u>animal samples</u> used have been <u>collected</u> with the appropriate ethical approvals (approval letters, etc) so complying with EU standards	"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 is needed.	All animal samples were faeces, collected outside the animals, and therefore not invasive for animals.	Satisfactory reply - Closed	



MEDVETKLEBS					
Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm that ethics approvals for the <u>use of isolates from human origin</u> 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"	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.	The validity dates of the ethics approval should be precised. Please supply.	We have provided the documents supporting the ethical aspects.

3.1.5.1.11 JRP12-AMRSH5-FARMED

FARMED	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(1) Human biological samples The beneficiaries must confirm that relevant approvals have been obtained to collect and process Human biological samples.	Currently, there is no use of human samples. However, when we start on human samples, these will be analysed and retrieved from existing national surveillance program, from where we already have the appropriate permissions to do so. Sample numbers, patient names, social security numbers, origin, etc. will be made anonymous with no possibility to trace back. If this would change, proper measures will be taken.



FARMED	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(2) personal data processing</p> <p>The beneficiaries must confirm that the personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.</p>	<p>All personal data will be processed according to GDPR (EU 2016/679). SSI DPO is: Helle Ginnerup-Nielsen, Ministry of Health, email: hgn@sum.dk.</p> <p>Currently, no use of human samples is being undertaken within this project. If this would change, proper measures will be taken.</p>
<p>(3) Animals</p> <p>Further details are needed on the interaction with the legal animals (e.g. the pigs). Although they are not experimental animals as defined in Directive 2010/63/EU, please state the 3Rs aspects of this work. Please describe how the animals welfare is protected and considered (e.g. the pigs affected when taking samples).</p>	<p>Faecal samples will be collected directly from the ground and farm environment; therefore, no direct animal handling is required and is outside of the Directive 2010/63/EU (protection of animals used for scientific purposes).</p> <p>Animal welfare on farm is covered by the animal welfare legislation in the EU and legislation within member states.</p> <p>Faecal samples collected from other scientific projects may be reused in FARMED provided that ethical approval from local regulatory authorities are in place.</p>
<p>(4) Other – Overall Ethics Management</p> <p>Considering the nature of the ethical issue raised, we would encourage the beneficiaries to include an Ethics element as part of the project management to ensure that the various ethics issues, raised by this work as it is done, are properly handled.</p>	<p>The FARMED consortium will continuously review and consider the ethical use of samples included in the project Ethics will be discussed during the face-to-face meeting (postponed to October).</p>



3.1.5.1.12 JRP13-AMRSH5-WORLDCOM

WORLD COM	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
Satisfactory ethics self-assessment Satisfactory ethics self-assessment. Good to see the wider reflection on issues in the WorldCom self-assessment form	
(1) Human biological samples Copy of the ethical approval from the NUI's REC must be presented	NUI Galway has no plans currently to analyse human biological samples as part of the WorldCOM project, hence no Ethics REC approval has been requested to date.
(2) personal data processing The beneficiaries must provide the contact address of the Data Protector Officer of the institution in charge of processing the data obtained	Data Protector Officer contacts are available from the WorldCOM partners upon request

3.1.5.1.13 JRP14-AMR2.1-FULL-FORCE

FULL FORCE	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
Human biological samples As 'spread of AMR will be investigated in people living in proximity to farms' (p14/26), the beneficiaries must confirm that, if relevant, appropriate authorizations will be sought to collect human samples.	No human samples will be collected in the context of this study. We will only re-analyse DNA extracted from bacterial strains in our stocks.



3.1.5.1.14 JRP15-AMR2.1-FED-AMR

FEDAMR	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(1) Human biological samples The beneficiaries must confirm that appropriate authorizations will be sought to collect the Human samples.	The ethical self-assessment was sent out to all partners of the FED-AMR project to ensure besides visibility and awareness of the high demand and principles of ethical conduct, a basic feedback on the mentioned issues relevant for OHEJP and this project. As no critical paths were identified and no current risks for the further progress of FED-AMR became obvious by this first assessment, the digestion and iterative update of the process are running in regular terms. An amended and clean version of the ethics section will be presented in the regular reporting periods. The first re-evaluation (end date 10 th September) revealed no further critical paths and can be found in the annex of this report.
(2) personal data processing The beneficiaries must confirm that the personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.	
(3) Animals Further details are need on the use of animals which are legal animals and any experimental animals (i.e. pigs). Please clearly state the 3Rs aspects of this work. Please describe how the beneficiaries are complying with access to animal material requirements and animal welfare laws.	



3.1.5.1.15 JRP16-ET2.2-TELE-Vir

TELEVIR	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(1) Human biological samples</p> <p>The beneficiaries must confirm that appropriate authorizations will be sought to collect the Human samples.</p>	<p>Human samples used for this project have been obtained with the due signed consent of the owner permitting the use of the collected material for research purposes.</p> <p>Also many of the human samples are collected for diagnostic purposes and exemption for review by the ethical committee system and informed consent has been given by the Committee on Biomedical Research Ethics - Capital region in accordance with Danish law on assay development projects.</p>
<p>(2) Health and Safety</p> <p>The beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.</p>	<p>The group works in a high-biosecurity (BSL-3) laboratory compliant with the biosafety and biosecurity rules of the INIA-CISA (Biosafety Reference Laboratory for FAO). Bio risk assessment of the different pathogens used in the project has been evaluated by INIA-CISA Biosafety & Biocontainment service.</p> <p>Our unit Quality, (bio)safety and environment aims to guaranteeing compliance with quality, (bio)safety and environmental standards and the health and welfare of all employees.</p> <p>All research conducted at Surrey will follow the health and safety guidelines: https://surreynet.surrey.ac.uk/sites/default/files/2019-09/2169-0917%20A5%20Health%20and%20Safety%20Booklet%20v2.pdf</p>



TELEVIR	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(2) Health and Safety (2)</p> <p>The beneficiaries must confirm that authorisations for relevant facilities (e.g. security classification of laboratory) have been obtained.</p>	<p>INIA-CISA contains a large high-biosecurity facilities (BSL-3 and BSL-3+) with the due authorization to work with animal and zoonotic pathogens requiring this facilities, such as foot-and-mouth disease virus, African swine fever virus, SARS-CoV and SARS-CoV-2, Rift valley fever virus, West Nile virus, Avian influenza virus, among others. Moreover, INIA-CISA is Biosafety Reference Laboratory for FAO.</p> <p>Sciensano has obtained the authorisations for facilities up to risk classification of 3 by the competent authorities (Decision number LABO-415117).</p> <p>SSI has a large high-biosecurity facilities (BSL-3) with the due authorization to work with human and animal zoonotic pathogens requiring this facilities, such as African swine fever virus, SARS-CoV, HIV, Vaccinia, influenza virus, among others.</p>
<p>(3) Animals</p> <p>Further details are needed on the use of animals which are legal animals and any experimentation animals (e.g. from wild boar to horses). Please clearly state the 3Rs aspects of this work. Please describe how the beneficiaries are complying with access to animal material requirements and animal welfare laws.</p>	<p>Animal samples used for this project have been obtained from field cases and have been provided to INIA-CISA as EU/FAO reference laboratory for African swine fever or as project collaborators.</p> <p>Many of the animal specimens are collected for diagnostic purposes from sick or dead animals.</p>



3.1.5.1.16 JRP17-ET2.2-IDEMBRU

IDEMBRU	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(1) Human biological samples The beneficiaries must confirm that appropriate authorizations will be sought to collect the Human samples.	Samples collected in Portugal already have approval from ethical commission of National Institute of Health and the anonymity of the patients is maintained.
(2) Human macrophage cell lines Details on cells/tissues type and provider must be given.	THP-1 – Immortalized human macrophages from peripheral blood sought from ATCC.
(3) personal data processing The beneficiaries must confirm that the personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.	Regardless of sample origin, each animal sample will be coded with a unique IDEMBRU identification number and the database will not include personal data according to GDPR (EU 2016/679). Access to the database will be restricted to allocated staff (with individual login and password; and signed confidentiality statement).
(4) Animals Further details are need on the use of legal animals and experimentation licenses. Please clearly state the 3Rs aspects of this work. Please describe how the beneficiaries are complying with material transfer agreed and EU standards in animal use and related welfare aspects.	WP-1 to 4: Animal samples will be collected through partner networks from surveillance programs and/or from dead animals and no live animals will be captured in this project so no ethical approval will be required. WP-5: ANSES will submit animal infection protocol to the local Ethics comity (ANSES/Ecole nationale vétérinaire d'Alfort Ethics comity) for approval in Year 4. All pathogenicity experiments, that can be performed <i>in vitro</i> will not require animal experimentation.



IDEMBRU	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(5) Health and Safety - The beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	All staff involved in this project comply with health and safety rules and regulations of their respective institution. Each institute is in charge of biosecurity / biosafety training of allocated staff involved in the project.
(5) Health and Safety (2) - The beneficiaries must confirm that authorisations for relevant facilities (e.g. security classification of laboratory) have been obtained	Manipulation with <i>Brucella</i> spp. potentially infected samples will be conducted exclusively in BSL-3 laboratories, respecting institutional and national rules and guidelines and standards.
(6) dual-use On the ethics checklist you have ticked that this research involves some “dual-use items in the sense of Regulations 428/2009, or other items for which an authorisation is required”, therefore please provide a risk assessment and details on measures to prevent misuse of research findings must be provided.	Specific individual sample data will not be publicly accessible. Anonymity of each sample will be protected by assigning specific individual code for analyses and results dissemination between consortium partners. Both data sharing platforms security will be maintained by ANSES informatics department.

3.1.5.1.17 JRP18-ET1.1-MEmE

MEME	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
Ethical issues will be considered as part of WP5 activities	



MEME	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(1) Human</p> <p>In case of Human participation, the beneficiaries must confirm that relevant authorizations have been obtained.</p>	<p>Authorizations for the submission of questionnaires will be requested to the ethics committee of participating centres.</p>
<p>(2) personal data processing</p> <p>The beneficiaries must confirm that the personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.</p>	<p>It will be taken into account.</p>
<p>(3) Animals</p> <p>Further details are needed on the use of animals which are legal animals and / or experimentation animals (e.g. red foxes). Please clearly state the 3Rs aspects of this work. Please describe how the beneficiaries are complying with access to animal material requirements and animal welfare laws.</p>	<p>ANSES: According to the animal welfare and the experimentation rule, we have the authorization to work on wildlife in our animals facilities in experimentation (decree n° 2013-118 dated 1 February 2013 relating to the protection of animals used for scientific purpose; Order of April 18, 2016 setting the general operating rules for animal breeding facilities for non-domestic animals D54-431-1).</p> <p>Concerning the infection of mice and foxes by Echinococcus (Em and Eg), we have obtained the authorization for experimental infection from the from the ethics committee (N° du Dossier: 16-073 N° APAFiS: 2016091313348095 N° de l'Avis : 11/10/16-3).</p> <p>Concerning the rule of 3R the number of mice to use for maintaining the strain is reduced to 10 mice every 2 months. For as concern foxes, when the protocol do not need euthanasia they are kept in animal facility for the collection of infected faeces and reused after deworming.</p>



MEME	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(4) Health and Safety The beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	All participants are complying on health and safety procedures according to EU legislation.
(4) Health and Safety (2) The beneficiaries must confirm that authorisations for relevant facilities (e.g. security classification of laboratory) have been obtained.	Authorization for fox and mice model obtained from ANSES, France. Authorization for sheep model pending for INIAV, Portugal.
(5) non EU countries (China) The beneficiaries must confirm that the research conducted outside the EU is in compliance with H2020 rules.	Not applicable. China will no longer participate to MEME OHEJP. Any collaboration during the project will be in compliance with H2020 rules.
(5) non EU countries (China) (2) The beneficiaries must confirm that the adequate authorisations have been obtained to import/export data.	Not applicable. China will no longer participate to MEME OHEJP. Any collaboration during the project will be in compliance with H2020 rules.

3.1.5.1.18 JRP19-ET1.1-PARADISE

PARADISE	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(1) Human Participation For the human participation in this project, the beneficiaries must confirm that relevant authorisations have been obtained.	Partners involved in the collection of human samples will provide evidence to the project coordinator that the relevant authorisations have been requested and obtained



PARADISE	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(2) personal data processing The beneficiaries must confirm that any personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.	Partners involved in handling personal data will provide evidence to the project coordinator of adherence to the GDPR (EU 2016/679), as well as the contact address of the Data Protection Officer, if available.
(3) Health and Safety The beneficiaries must confirm that authorisations for relevant facilities (e.g. security classification of laboratory) have been obtained.	The project involves handling/processing of class 2 pathogens. Partners will provide evidence to the project coordinator that their facilities are authorised for the specific use.
(4) Animals Further details are need on the use of animals, which are legal animals although not experimentation animals. Please describe how the beneficiaries are complying with access to animal material requirements and animal welfare laws.	Animal work, i.e., immunization and blood sampling of new world camelids, outsourced to a dedicated company (Preclinics, Potsdam, Germany). The work has been approved by the local authorities (licence 17A210).
(5) non EU countries (China, Australia, Middle-East) The beneficiaries must confirm that the research conducted outside the EU is in compliance with H2020 rules.	It is unclear whether non-EU countries are still to be considered associate partners of the project.
(5) non EU countries (China, Australia, Middle-East) (2) The beneficiaries must confirm that the adequate authorisations have been obtained to import/export materials.	It is unclear whether non-EU countries are still to be considered associate partners of the project.



PARADISE	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(5) non EU countries (China, Australia, Middle-East) (3) The statement at the end of the checklist is helpful but it does not provide all of the relevant information as mentioned above	It is unclear whether non-EU countries are still to be considered associate partners of the project.

3.1.5.1.19 JRP20-FBZSH3-DiSCoVeR

DISCOVER	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(1) Human biological samples The beneficiaries must confirm that appropriate authorizations will be sought to collect the Human samples.	This project is not collecting human samples, but uses available results from the partners' countries' laboratory surveillance. Results of human samples e.g. diagnoses and WGS information are obtained from the participating countries' reference laboratories, which the project partners are either a part of or collaborating with. This means that all necessary authorizations are in place and is irrelevant to apply for these specifically for this project.
(2) non EU countries (African countries) The beneficiaries must confirm that the research conducted outside the EU is in compliance with H2020 rules.	The non-EU obtained data that we plan to use in DiSCoVeR originates from another project (FOCAL) co-funded by BMGF and FCDO . This project has obtained all ethical approvals necessary from all involved countries and since the project's PI is of DTU, the project is conducted also in compliance with H2020 rules. We plan only to use a small proportion of the samples (sewage samples) collected in this project for DiSCoVeR.



DISCOVER	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(2) non EU countries (African countries)</p> <p>The beneficiaries must confirm that the adequate authorisations have been obtained to import/export materials / appropriate Material Transfer Agreements. More specifically, the beneficiary must provide details on the material which will be imported to/exported from EU and confirm that the adequate authorisations have been obtained.</p>	<p>As mentioned above, the activities involving using sample results from African countries originates from another project. All necessary material and data transfer agreements between partners/countries in this project have been obtained and is available from the PI, who is also PI of DiSCoVeR. It is irrelevant to obtain separate authorisations for DiSCoVeR.</p>
<p>(2) non EU countries (African countries) (2)</p> <p>As low / middle income countries are participating in the study, the beneficiaries must confirm that fair benefit-sharing arrangements with local stakeholders are ensured during the project (cf the Global Code of Conduct for research in resource-poor settings – www.globalcodeofconduct.org). The research team needs to state and submit their policies and practice for ensuring good practice when doing research in resource-poor countries.</p>	<p>Again, we will only use a subset of the sample results from the FOCAL project in DiSCoVeR, and the FOCAL project, co-funded by BMGF and FCDO, adheres to the policies of these two foundations, which also include a policy for conducting research in LMICs. To this end, any research output in DiSCoVeR based on samples from the FOCAL project will also be a FOCAL output (co-financing DiSCoVeR) and therefore comply with this policy. Practically, this means that any LMIC data collector and/or data analyst will contribute to the outputs and be included as co-author etc. in relevant publications and deliverables.</p>
<p>(3) Health and Safety</p> <p>The beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.</p>	<p>This is confirmed by the fact that all involved partners are employed by public governmental institutions (universities or research institutions), which must be expected to conform to all relevant local/national guidelines/legislation.</p>



DISCOVER	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(4) Other – Overall Ethics Management</p> <p>Considering the nature of the ethical issue raised, we would encourage the beneficiaries to include an Ethics element as part of the project management to ensure that the various ethics issues, raised by this work as it is done, are properly handled.</p>	<p>An ethic element, more specifically a project-level ethical committee, is included in the FOCAL project, and is, therefore, considered irrelevant for DiSCoVeR.</p> <p>In addition, it can be mentioned that the FOCAL project is conducted in accordance with the Danish Act on scientific ethical treatment of health research, as administrated and confirmed by the Research Ethics Committees of the Capital Region of Denmark (www.regionh.dk), Journal nr.: H-14013582. and fulfils the requirements of the Nagoya Protocol.</p>

3.1.5.1.20 JRP21-FBZ3.1-BIOPIGEE

BIOPIGEE	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(1) Human biological samples</p> <p>In case of use of human cells/tissues available commercially, details on cells/tissues type and provider must be provided.</p>	<p>There was an error in the ethics form. The project does not use any human biological samples. A new form is being prepared and sent.</p>
<p>(1) Human biological samples (2)</p> <p>In case human cells/tissues are obtained within the project, details on cells/tissues type must be provided. The beneficiary must also confirm that relevant approval has been obtained.</p>	<p>See above.</p>



BIOPIGEE	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(2) Environmental Harms The ethical checklist has not been completed, please tick all boxes. You have ticked the “harm to environment” box. Please confirm the types of environmental harms and that the work will be compliant with good practice and EU legislation	Has been done and form is available.

3.1.5.1.21 JRP22-FBZ4.1-TOXOSOURCES

TOXOSOURCES	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
Ethical aspects have been carefully considered , but the grant beneficiaries still need to clarify a few aspects:	Ethical considerations are integrated into planned work and discussed regularly within the TOXOSOURCES consortium. We thank the Ethics Advisors for the further comments, which were carefully considered.
(1) Human biological samples The beneficiaries must confirm that appropriate authorizations will be sought to collect the Human samples.	Appropriate authorizations will be sought for any collection of human samples. We largely build on previously performed work and take use of existing parasite isolates that have been collected earlier, following appropriate authorizations.



TOXOSOURCES	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(2) personal data processing</p> <p>The beneficiaries must confirm that a Data Protection Officer (DPO) has been appointed, and the contact details of the DPO are made available to all data subjects involved in the research.</p>	<p>Data protection aspects are carefully considered in all work done in TOXOSOURCES. In particular, we focus on limiting personal data collection to what is necessary in relation to the purpose. For all activities where it is needed, the contact details of DPO are made available to all data subjects involved.</p> <p>Example: The questionnaire survey of TOXOSOURCES WP3-T2, gathering grey literature on <i>T. gondii</i> oocyst contamination of fresh produce, bivalves and environmental samples in Europe, collects name and email address of the participants, because this is necessary to enable follow up. The University of Surrey Self-Assessment for Ethics and Governance was filled to confirm that ethical review was not required for this survey. The contact email address of DPO of University of Surrey is provided in the introduction to the questionnaire, together with other information about data protection. The online survey platform is GDPR-compliant and recommended by the University of Surrey for the purpose. The personal data will be stored on secure University of Surrey servers, and is accessible only to members of the research team.</p> <p>The email list of TOXOSOURCES Consortium for dissemination of relevant One Health EJP messages is managed by the One Health EJP Communications Team at the University of Surrey. Email addresses of Consortium members are used internally within the Consortium for communication.</p> <p>Information on fresh produce trade and consumption will be treated as Food Related Research Equivalent to Medical Research according to the Guidance Note-Ethics and Food-Related Research of the EC (http://ec.europa.eu/research/participants/data/ref/fp7/89847/research-food_en.pdf).</p>
<p>(3) non EU country (China)</p> <p>The beneficiaries must confirm that the research conducted outside the EU is in compliance with H2020 rules.</p>	<p>No research actively planned with the potential non-EU external partners; any collaboration during the project will be in compliance with H2020 rules.</p>



TOXOSOURCES	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(4) Health and Safety</p> <p>The beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.</p>	<p>Appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in TOXOSOURCES project.</p> <p>For all the new laboratory methods developed, relevant health protection and safety aspects will be emphasized.</p>

3.1.5.1.22 JRP23-FBZSH5-ADONIS

ADONIS	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>(1) Human biological samples</p> <p>The beneficiaries must confirm that appropriate authorizations will be sought to collect the Human samples.</p>	<p>In this project no human samples other than bacterial isolates from patients will be used. This is done within existing national surveillance programs of the public health institutes and covered by existing regulations.</p>
<p>(2) Health and Safety</p> <p>As you have ticked yes on the checklist, please can you the beneficiaries confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.</p>	<p>Laboratory work with Salmonella isolates require BSL2 safety levels. Institutes/labs involved in this project all regular work with Salmonella and all of them comply to the safety regulations.</p>



3.1.5.1.23 JRP24-FBZSH9-BeONE

BEONE	
Requirements of ethical reviewers in 2020	What measures and actions do you propose?
<p>Personal data processing</p> <p>The beneficiaries must confirm that the personal data will be processed according to GDPR (EU 2016/679), and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.</p>	<p>We confirm that personal data will be processed according to GDPR (EU 2016/679), in that all data will be anonymized by the providing institutions before being shared with the project. An anonymization instruction has been written and distributed to the partners. DPO for SSI: Helle Ginnerup-Nielsen (HGN@sum.dk)</p>



3.1.6 Task 1.6 Declaration of Cofund

N/A

3.2 Deliverables and Milestones

3.2.1 Deliverables

Del. Ref.	Deliverable title	Submission
D1.8	Annual report on the internal and external newsletter produced during the second year	M25
D1.9	Complete version of annual report for stakeholders n°2	M29
D1.10	Summary progress report of the third year	M33
D1.11	Annual work plan of the fourth year	M33
D1.24	Ethical review report of the second year	M26

3.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS5	SSB Meeting n°4	M26	SSB TC was held on 19 March 2020
MS6	SSB Meeting n°5	M32	SSB TC planned on 18 September 2020
MS14	PMC/POC/ESAB & SC Annual meeting n°3	M33	PMC/POC meeting planned in Berlin, 13 October 2020

4 WP2 – Integrative strategic research agenda

4.1 Work carried out to date

4.1.1 Task 2.1: Development of the SRA

At the start of OHEJP, in January 2018, a provisional Strategic Research Agenda (SRA) has been delivered. In 2018, the SRA was updated and in the first month of 2019 the updated SRA was delivered (D2.7). The SRA is the product of a structured prioritisation process in which priority research and integrative topics have been identified. In this process both research and integrative needs of the EU member states participating in OHEJP as well as EU stakeholders (ECDC and EFSA) have been taken



into account. The lists and descriptions of the priority topics have provided the basis for the internal calls for Joint Research Projects (WP3), Joint Integrative Projects (WP4) and PhD projects (WP6). In addition to the updated SRA, which was delivered as a confidential document (D2.7), a more concise, public version of the SRA was developed to be used for external dissemination purposes. This public SRA was delivered as an extra deliverable (D2.10). Furthermore, in task 2.1 relevant scientific developments, including the emergence of zoonotic threats and opportunities for scientific innovations are monitored. For this, the output from OHEJP projects (e.g. deliverables, scientific publications) is screened as well as relevant information from external sources. This information will be used as input for the development of the strategic research and innovation agenda (SRIA) in WP7.

4.1.2 Task 2.2: Strategic interactions with EU projects and initiatives

The main objective of this task is to foster strategic interactions with related EU projects and initiatives. For this, an analysis was made of the relevant EU-projects/initiatives and potential strategic interactions. The information available in the CORDIS (Community Research and Development Information Service) database and the OHEJP partners regarding relevant EU-projects/initiatives was compiled and centralized in a repository of EU projects/initiatives that is available to all partners through the OHEJP website. This inventory (Milestone 22) and the reports on the identification of relevant EU-projects and initiatives and the procedure to identify potential strategic interactions (D2.3) and potential strategic interactions with EU projects and initiatives (D2.4) was delivered during year 1. During the third year, as defined in the deliverables, the repository has been updated and downloaded on the website including new relevant EU-projects/initiatives in the three main domains of foodborne zoonoses (i.e. ICRAD-International Coordination of Research on Infectious Animal Diseases), antimicrobial resistance (i.e. AVANT-Alternatives to Veterinary Antimicrobials) and emerging threats (i.e. VetBioNet-Veterinary Biocontained facility Network for excellence in animal infectious disease research and experimentation). Moreover, the 9th JPIAMR Joint calls were also included for OHEJP consortium consultation. Regarding potential strategic interactions, contact has been established through e-mails, teleconferences or in congresses/meetings to look for synergies with other projects and initiatives. All WP and project leaders have also participated actively in looking for interactions with other relevant EU projects to avoid duplications. WP2 has been in contact with WP4 for the selection of EU projects (INFAC and JPIAMR) before the organization of the Cogwheel workshops of 2020.

4.2 Deliverables and Milestones

4.2.1 Deliverables

N/A

4.2.2 Milestones

N/A

5 WP3 - Joint research projects

5.1 Work carried out to date

5.1.1 Task 3.1: Drawing up of guidelines for submission, selection and evaluation of JRP proposals as well as request of extension of accepted JRPs.

The activities related to this task and the corresponding deliverables have been finalised in 2019. However, guidelines were drafted that served Project Leaders from the first call to ask for a no cost extension of maximum 6 months. Included in the procedure was a template for harmonized communication to the PMT.

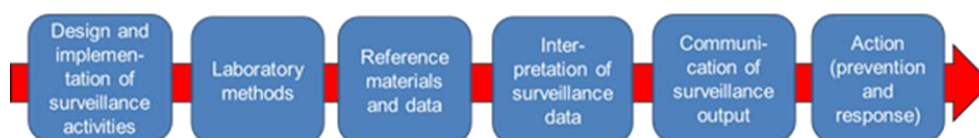
As reported in the SPR Y2 , a procedure for the inclusion of new One Health EJP beneficiaries in on-going projects with clear integrative activities was under discussion. Integrative activities are the core business of the One Health EJP, supporting the harmonization and alignment of the consortium's capacity, protocols, databases, biobanks, surveillance strategies etc. Therefore, an additional budget could endorse and encourage the enlargement of existing projects. However, since the budget that could be made available is not yet known in detail in the spring of Y3 and since priority has been given to launch an additional JIP related to the COVID-19 crisis, no further effort was put into the enlargement of existing JRP.

5.1.2 Task 3.2: Supervision of the JRP in the first round of projects.

The annual, periodic report D3.11 that describes the monitoring of all 11 JRP has been submitted in time. Only one project, MAD-VIR has finalized its activities in December 2019; all other 2y-JRP have applied for an extension. As a consequence, about half of the JRP deliverables are delayed and will only be delivered in 2020 at the earliest.

The template for the 9M-report has been modified as compared to Y2, in order to improve the monitoring of the projects. The new template is valuable for both JRP and JIP, and for first and second call projects. The main new elements are the following:

- As integrative activities take a crucial role in the One Health EJP, Project Leaders have been requested to categorize the project deliverables according to the 'Integrative Strategy Matrix' that is described in the One Health EJP Strategic Research Agenda. This matrix follows essential steps in the surveillance programmes:



Therefore, deliverables should be classified in one of the following categories:

1. Design and implementation of surveillance and control activities;
2. Harmonised protocols and applied best practice;
3. Databases of reference materials and data, incl. metadata;
4. Standardised data formats, aligned data analysis for interpretation of surveillance data;
5. Sharing and communication of surveillance data;
6. Sharing of best intervention activities (response);
7. Prevention: aligned use of facilities and models;
8. Other (please specify);



9. This is supportive to an integrative activity;
10. This is not an integrative activity.

This exercise encourages the project leaders to identify the added value a deliverable may have for the reference laboratories, risk assessors or risk managers, and thus to promote the impact the project (and the OHEJP) may have.

- The follow up of the ethics recommendations has been included to allow for a more efficient monitoring in time.
- To assess the impact that the COVID-19 crisis will have on the project, deliverables touched by the measures taken in the various member states and the related cost per (sub)task should be described. This overview will be important for the Project Management Team to take appropriate action.
- Information on the Data Management Plan (see WP4).

The information gathered through this 9M-report is included in this SPR Y3, see paragraph 'Detailed follow up of JRP'.

Since in June 2020 no additional project has been terminated, the project evaluation process was started for MAD-VIR. Briefly, the evaluation procedure D.9 (Guidelines for WP3 on the evaluation of final reports) was clarified to describe the evaluation by two scientific external experts (one of whom had previously evaluated the project proposal) and one 'end user', i.e. a representative of the Programme Owner Committee. This approach allows the evaluation of both the scientific and integrative outcomes. An online registration form was developed to facilitate the final selection of the evaluators. The evaluation forms are expected by the end of August 2020 and will be discussed by the Scientific Steering Board in September 2020. The report describing the evaluation of JRP by external experts is deliverable D3.13 (Report n°1 on evaluation of finalised JRP) and will become available by the end of 2020.

5.1.3 Task 3.3: Organisation of a second round of projects and their supervision.

Together with three new JIP, thirteen new Joint Research Projects started in January 2020:

- JRP12-AMRSH5-FARMED, Fast Antimicrobial Resistance and Mobile-Element Detection using metagenomics for animal and human on-site tests; Project Leader Manal AbuOun, APHA, UK
- JRP13-AMRSH5-WORLDCOM, Development of new tools for real-time detection of zoonotic bacteria and antimicrobial resistance in veterinary, human and environmental sources; Project Leader Terry Smith, NUI Galway, EIRE
- JRP14-AMR2.1-FULL-FORCE, Full-length sequencing for an enhanced EFFORT to map and understand drivers and reservoirs of antimicrobial resistance; Project Leader Pieter-Jan Ceyssens, Sciensano, BE
- JRP15-AMR2.1-FED-AMR, The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain; Project Leaders Werner Ruppitsch and Adriana Cabal-Rosel, AGES, AT
- JRP16-ET2.2-TELE-Vir, Point-of-incidence toolbox for emerging virus threats; Project Leaders Anders Fomsgaard and Maiken Worsøe Rosenstjerne, SSI, DK
- JRP17-ET2.2-IDEMBRU, Identification of emerging Brucella species: new threats for human and animals; Project Leader Claire Ponsart ANSES, FR
- JRP18-ET1.1-MEmE, Multi-centre study on Echinococcus multilocularis and Echinococcus granulosus s.l. in Europe: development and harmonization of diagnostic methods in the food chain; Project Leader ADRIANO CASULLI, ISS, IT



- JRP19-ET1.1-PARADISE, Parasite Detection, Isolation and Evaluation; Project Leader Simone M. Cacciò, ISS, IT
- JRP20-FBZSH3-DISCoVeR, Discovering the sources of Salmonella, Campylobacter, VTEC and antimicrobial Resistance; Project Leader Hald Tine, DTU, DK
- JRP21-FBZ3.1-BIOPIGEE, Biosecurity practices for pig farming across Europe; Project Leaders Chris Kollas, BfR, DE
- JRP22-FBZ4.1-TOXOSOURCES, Toxoplasma gondii sources quantified; Project Leader Pikka Jokelainen, SSI, DK
- JRP23-FBZSH5-ADONIS, Assessing Determinants Of the Non-decreasing Incidence of Salmonella; Project Leader Eelco Franz, RIVM, NL
- JRP24-FBZSH9-BeONE, Building integrative tools for One Health Surveillance; Project Leader Kristoffer Kiil, SSI, DK

As was done in Y1, the Project Leaders of these newly launched projects were contacted on 25 February 2020 and requested to fill in an online questionnaire. The results showed that the projects had started as planned, indicated good information flow of relevant aspects, and provided constructive feedback that can inform further support activities.

- Project leaders were well informed about the need for a Data Management Plan, and that they had taken action to fulfil this obligation.
- There seemed to be a need for support concerning the communication and dissemination of the outcomes of the projects.
- The survey also highlighted the need to bring project leaders into contact with the international stakeholders, not only ECDC and EFSA but also with those organisations that joined the Stakeholders Committee in the spring of 2020, i.e. EEA, EMA, FAO, WHO-EU and OIE.
- As for the mirror groups that are ongoing in the various countries, it was not possible to better inform project leaders, as this information is far from complete.
- Many project leaders noted that their relevant contacts regarding their project are the SSB members.
- Finally, the project leaders made useful suggestions to the One Health EJP management that may support their collaboration.

This second report on recently started projects was uploaded as deliverable D3.14.



5.1.4 Impact of COVID-19 crisis on the project

5.1.4.1 JRP01-AMR1-IMPART

IMPART								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP1-WP1-T6	M21	M33	D-1-6		M33	Delay in analysing data during the lock-down		
JRP1-WP1-T7	M30	M36	D-1-7		M36	Dependent of JRP1-WP1-T6		
JRP1-WP1-T8	M20	M36	D-1-8		M36	Dependent of JRP1-WP1-T6 and JRP1-WP1-T7		
JRP1-WP2-T2.6	M26	M33	D-JRP1-2.6		M33	Delay in analysing data and redoing analysis in lab complicated during the lock-down		
JRP1- WP2-T2.7	M30	M36	D-JRP1-2.7		M36	Dependent of JRP1-WP2-T2.6		



IMPART								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP1- WP2-T2.8	M30	M36	D-JRP1-2.8		M36	Dependent of JRP1-WP2-T2.6 and JRP1-WP2-T2.7		
JRP1-WP3-T3	M18	M31	M-JRP1-9		M31	Further delay in production and collection of MIC data during the lock-down		
JRP1-WP3-T4	M30	M35	D-JRP1-3.2		M35	Delay in analysing data during the lock-down (dependent on D-JRP1-1.9)		
Travel costs of all partners to attend closing meeting	M30				M36	Delay in results, physical closing meeting possible in 2021?		



IMPART								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
End meeting, costs for rent meeting room, lunch and drinks	M30				M36	Delay in results, physical closing meeting possible in 2021?		
JRP1-WP4-T3	M27	M32	D-JRP1-4.3	M27	M33	Problems in delivery of test samples, availability of participating partners		
JRP1-WP4-T4	M30	M32	D-JRP1-4.1, M-JRP1-10	M21, M26	M33	Shortage of staff, limited access to laboratories		

It is impossible for us to specify exact budgets to the delayed tasks mentioned in the table. As stated above all delayed deliverables will be finished before the end of the project (December 2020). All partners will fulfil their tasks according to the revised budgets of Y3 as provide earlier.

5.1.4.2 JRP02-AMR2-ARDIG

Many partner institutes are/have been involved in the COVID-19 response, as well as having to observe closure of their laboratories due to the nationwide lockdown imposed on countries. This has meant that almost all partners have had to stop ARDIG related laboratory and other work, such as sample collection, further delaying some components. Moreover, due to the resulting uncertainties from COVID-19, partners are not clear when they can return to “business as



usual” and social distancing measures will need to continue to be implemented, so slowing down the pace of work in general. Therefore, we believe that all outstanding Milestones and Deliverables will require to be delayed by 6 months, and we are grateful that a 6 months extension has been granted to ARDIG to enable greater depth and higher quality delivery of all outstanding Deliverables and Outputs.

5.1.4.3 JRP03-AMR3-RADAR

The project as a whole is affected by the COVID situation. Therefore, the project requested an extension till the end of 2020, which was granted. In the Table under chapter 3 the newly planned delivery dates of deliverable and milestones is indicated. In addition, we delivered on request a financial planning of the RADAR project to the EJP management at ANSES where it was indicated that due to the extensions the budget will be spent in delay but will be spent entirely.

5.1.4.4 JRP05-ET1-TOXdetect

TOXDETECT								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP5-WP0-T1: General coordination and management of the project (administrative and financial)	M36	M42					0€	The remaining budget
JRP5-WP0-T2 to JRP5-WP0-T5: Organisation of four face-to-face meetings with all partners			D0.4	25	/		0€	The remaining budget
			D0.5	36	36		0€	The remaining budget



TOXDETECT								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			D0.6	36	42	Face-to-face meeting postponed in 2021 (project extended until June 2021)	0€	The remaining budget
JRP5-WP1-T1: Constitution of <i>S. aureus</i> strains collection		M30	D1.1	3	/		0€	The remaining budget
JRP5-WP1-T2: Constitution of <i>B. cereus</i> strains collection			D1.1	3	/		0€	The remaining budget
JRP5-WP1-T3: Constitution of <i>C. perfringens</i> strains collection			D1.1	3	/		0€	The remaining budget
JRP5-WP1-T4: Transfer of libraries of MALDI-ToF reference spectra			D1.2	24	30 34	Expected in M27, had been postponed in M34 due to Covid 19 crisis	0€	The remaining budget



TOXDETECT								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP5-WP2-T1: Characterization of candidate toxin and/or virulence genes using toxicity tests	M30		D2.1	32	36	Due to technical problems, and significant differences in cytotoxicity of certain strains of <i>Bacillus cereus</i> compared to results generated from a previous project, ANSES and Institute Pasteur have to prepare new <i>Bacillus cereus</i> culture supernatants.	0€	The remaining budget
JRP5-WP2-T2: Assessment of virulence and toxin gene expression using RT-PCR and transcriptomic assays			D2.2	32	36	Data are currently being analysed.	0€	The remaining budget



TOXDETECT								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP5-WP2-T3: Correlation of specific toxicity profiles with expression patterns of bacterial toxins/virulence factors			D2.3	30	36	Toxicity profiles and expression patterns of bacterial toxins will not be available until all B. cereus and C. perfringens strains have been completely characterized in terms of cytotoxicity profiles	0€	The remaining budget



TOXDETECT								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP5-WP3-T1: development of Mass Spectrometry-based methods for the detection of new enterotoxins (eg SEG, SEH, SEI) from S. aureus	M30	M39	D3.1	27	36	First, a delay for D3.1 has occurred due to the limited access to the standards. Toxins standards foreseen for 2019 were delivered in May/June 2020 (see WP4). Before that, tested strains didn't gave positive answer for the toxins, only based on theory. Moreover, due to the Covid 19 crisis and the very limited experimental work during last months, a finalized and optimized method for spiked supernatant analysis should be expected before end of 2020.	0€	The remaining budget



TOXDETECT								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP5-WP3-T2: development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from <i>B. cereus</i>			D3.2	27	36	The method has been applied to one sample to finalize the optimization, but due to the Covid 19 crisis, we could not go further.	0€	The remaining budget
JRP5-WP3-T3: Development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from <i>C. perfringens</i>			D3.3	27	36	The results of the final method developments/optimization and supernatant analyses are in progress and should be expected to be released at the third quarter of 2020, taking into account the delay due to Covid 19 sanitary crisis and the complete interruption of experimental work during three months.	0€	The remaining budget



TOXDETECT								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP5-WP3-T4: Transfer of LC-MS/MS methods			D3.4	30	39		0€	The remaining budget
JRP5-WP4-T1: Development of quantitative immunoassays for five known S. aureus and B. cereus toxins and virulence factors	M32	M36	D4.1	33	36		0€	The remaining budget
JRP5-WP4-T2: Development of a quantitative immunoassay on a new B cereus toxin or virulence factor						Toxins standards foreseen for 2019 were delivered in May/June 2020. Rabbits immunization procedure has been disrupted due to the Covid-19 lockdown. A new batch of proteins is in production	0€	The remaining budget
JRP5-WP5	M36	M42	D5.1	25	33			



TOXDETECT								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP5-WP5-T1: Inter-lab test on Maldi-ToF for species identification	M36	M40	D5.2	34	42	COVID 19 crisis has induced partner laboratories closure, stopping method development during more than 4 months. In consequence, ILC organisation has been postponed to 2021.	0€	The remaining budget
JRP5-WP5-T2: Inter-lab test on LC-MS/MS								
JRP5-WP5-T3: Inter-lab test on immuno-enzymatic assays								



5.1.4.5 JRP06-FBZ1-NOVA

NOVA								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Mapping of surveillance: data, regulatory framework, key stakeholders, opportunities and barriers	M36	M42	JRP6-WP1-T2	M36	M42	Reallocation of staff		150,741.25

We had no costs due to organisation of meetings because we managed to cancel our face-to-face annual meeting in Madrid just after it was concluded that CoViD-19 was spreading in Spain and most part of Europe.

5.1.4.6 JRP07-FBZ2-LISTADAPT

No tasks or sub-tasks were affected by the Covid-19, but activities were shifted slightly in order to adapt the work to which laboratory facilities that were accessible during the lockdown. Exchange of data and discussions were also slightly delayed.



5.1.4.7 JRP08-FBZ2-METASTAVA

METASTAVA								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP8-WP1-T4	30	36	D-JRP8-1.4	30	36	Staff availability		
			D-JRP8-1.5	30	36	Staff availability		
JRP8-WP2-T1:	30	36	D-JRP8-2.1	30	36	Staff availability		
JRP8-WP3-T1	30	36	D-JRP8-3.3	30	36	Staff availability		
				30	36	Staff availability		
JRP8-WP3-T2	30	36	D-JRP8-3.4	30	36	Staff availability		
JRP8-WP3-T5	30	36	D-JRP8-3.7	30	36	Staff availability		
All tasks related to dissemin. And reporting in WP4 &5	30	36	several	30	36	Dependency on above		

Several partners have actively been involved in diagnostic or research activities in response to the SARS-CoV-2 pandemic. Budgetary impact on task expenditure was not possible to evaluate within the reporting deadline. A general overview per partner of budget planned to be used will be provided to OHEJPcoord. The originally foreseen live final meeting (Brussels, spring 2020) was cancelled due to travel restrictions. Budgetary impact of this cancellation needs to be assessed specifically by each partner (as this was already in a no-cost project extension, some travel costs were not covered by the Metastava



budgeted). Due to poor availability of key staff & thanks to the obtained 6 month extension to mediate the impact of COVID-19, this meeting will be replaced by an online event in November or December 2020.

5.1.4.8 JRP09-FBZ3-AIR-SAMPLE

AIRSAMPLE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
2.2	June 2020	Dec 2020	D-JRP9-2.4.	June 2020	Dec 2020	Pandemic	0	<u>DKK</u> 60000

The demonstration workshop was a physical workshop that cannot be hold this year due to the pandemic. Instead, we have directed our effort to communication via public magazines and publications. In addition, we will prepare a video film to publish online for those with interest in taking up the protocol. This video film is in preparation.



5.1.4.9 JRP10-FBZ3-MoMIR-PPC

MOMIRPPC								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP10-WP0-T3:		June 2021				Delay due to COVID		
JRP10-WP0-T4:		June 2021				Delay due to COVID		yes
JRP10-WP1-T3:		June 2021	D-JRP10-1.05	30	36	The sample recovery has been delayed		yes
JRP10-WP2-T1:		February 2021	D-JRP10-2.06	26	30	The first in vivo experiments have been cancelled and planned 5 months later		yes
JRP10-WP2-T1:		June 2021	D-JRP10-2.01	30	36	The in vitro analyses has been delayed		yes
JRP10-WP2-T2:		March 2021	D-JRP10-2.07 ; D-JRP10-2.08	30	33	The in vivo analyses has been delayed		yes
JRP10-WP3-T2-ST2		March 2021				The development of the model has been delayed due to the Covid19		
JRP10-WP4-T2:						The High Strategic Meeting has been cancelled	Yes (reimburse)	



5.1.4.10 JRP11-FBZ4-MedVetKlebs

Tasks linked to sampling and experimental work (qPCR developments, genomic sequencing) have been delayed. Modelling has not.

5.1.4.11 JRP12-AMRSH5-FARMED

FARMED								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP16-WP1-T1	M40	M43	M-JRP16-02 & M-JRP16-03	M40	M46	All institutes were affected by national lockdowns (to various extents) and some have been directly involved in their national COVID-19 testing. The planned first face-to-face meeting had to be delayed to travel bans initiated by institutes due to COVID-19. A call was planned which had to be postponed due to the rapidly changing situation in all institutes. Due to this, lab access was restricted, which effected distribution of spiked wastewater and animal faecal samples. Deadlines will be re-evaluated in 12 month report (M34-M36)		
JRP16-WP1-T2	M42	M45	M-JRP16-05	M42	M45			
JRP16-WP1-T3	M44	M47	M-JRP16-08	M44	M47			
JRP16-WP1-T1	M40	M43	D-JRP16-WP1.1	M44	M47			



FARMED								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JRP16-WP1-T4	M42	M46	M-JRP16-06	M42	M46	Results & samples needed from JRP16-WP1-T1 delayed which delays this task.		
JRP16-WP1-T4	M42	M46	D-JRP16-WP1.2	M42	M46			
JRP16-WP3-T1	M29	M36	D-JRP16-WP3.1	M30	M36	Delay due to national lockdowns and reprioritisation of essential work.		
JRP16-WP3-T2	M41	M43	D-JRP16-WP3.2	M41	M43			

Sciensano (4) was put into lockdown on 17th March. Only essential activities could be continued on site (business continuity plan was activated). All research activities, including the OHEJP project were considered as non-essential and could not be continued, i.e. no activity in the R&D labs was allowed. As of the beginning of June, slowly the exit phase has started, allowing again some R&D activities on site; however, homework stays the general rule. Also, at all-times social distancing needs to be assured, meaning only a limited number of people can work in the R&D labs. This has caused a delay for the FARMED project, and activities could not yet be restarted in the lab. However we will try to send the B. subtilis strain to BfR, so that the spiking for WP1 is no longer blocked. For the FARMED experiments, it will need to be evaluated when they can be undertaken, it is expected that the soonest will be July, so a further delay for FARMED. In the beginning of the project, the recruitment of a scientist to work on the FARMED project turned out to be difficult, and could only be accomplished half of February. As from the start of the lockdown, this scientist was asked to contribute to the COVID-19 related activities.

BfR (9) was not completely lockdown; however, the BfR FARMED team was limited in its activities due to missing childcare and thus being not fully effective in work since the beginning of March. Project activities were reduced to a minimum because official duties had to be done first and there were limited amounts of disinfectants. However, they were able to introduce the FARMED PhD and do some preliminary work. That resulted in a delay of three months for WP1-T1,



2, 3 until the current time point. Furthermore, *Bacillus subtilis* strain for the defined microbial community was not available due to Sciensano lockdown and could not be exchanged. Thus, the preparation of the defined microbial community could not be carried out. This work is now in progress. Furthermore, shipment of inoculated simple and complex matrices to project partners due to lockdown of institutes was impossible.

DTU (12) were in complete lockdown from 11th of March until mid-May, after then we started a slow-phase opening. Only SARS-CoV-2-related work was carried out in our labs, and none of the remaining projects, including FARMED, was in progress during the lockdown period. We were also not able to send or receive any samples/isolates to be used, for example, in WP1 for the mock community analyses, which will also be employed in WP2. This delayed all the deadlines of our current projects by 2-3 months. We are currently open and all our projects are starting up including the One Health EJP ones.

SSI (13) have been affected by COVID-19 as many employees have been directly involved in the work and laboratory activities have been limited to only essential work. Even though we did not plan any FARMED laboratory work during this period, many other research projects have been delayed and new plans for their execution will take place that may affect the time line of FARMED laboratory work.

APHA (21) entered complete lockdown in 23rd March, where only essential statutory laboratory work could be undertaken. June 2020 APHA is still not able to perform non-essential laboratory although the agency is working to start opening labs in the summer. However, due to COVID-19 testing commencing at APHA, lab staff will have to support this activity on a part time basis, so progress on the FARMED project will further be delayed. Not helped by COVID-19, recruitment of post-doc was delayed by 3 months; they arrived in post on 1st June, however they will be required to undertake some hours for COVID-19 testing.

ISS (27) from the end of February is the national coordinator of the epidemiological and molecular surveillance of covid-19 infection; ISS gives indications for the public health service and is involved in covid-19 diagnostic and research. In lock down phase only essential and covid-19 emergency laboratory work could be undertaken. During the current period, ISS recommends smart working and turnover of the personnel, to avoid overcrowding of laboratories and offices. The decreasing of the number of the personnel leads to delay for all activities including FARMED project. The ISS unit for FARMED project can commits in shipment of characterized bacteria to be spiked in complex matrices.

IZSAM (P28) is involved in the COVID-19 diagnostic in Italy since the epidemic started on late February and beginning of March. Moreover, to contain the spread of the COVID-19 outbreak, the IZSAM has had several measures to maintain the social distancing and avoiding the crowding of laboratory rooms like smart working and personnel time schedule split in two turns. During the current period, IZSAM experienced shortage of staff working at the laboratory units thus all the activities have slowed down including also the project Farmed.

WBVR (31) has continued work at reduced capacity and mostly for statutory tasks since the beginning of March. From the start of July, it is expected that most work will be able to continue as planned. However, due to diversion of some capacity to human and animal COVID-19 testing, the rate at which all research activities can start are not completely clear yet



5.1.4.12 JRP13-AMRSH5-WORLDCOM

WORLDCOM								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP1-T1	28	30	D-JPR15-AMR2.1-WP1.1	M30	M30	No delay	N/A	N/A
WP1-T2	30	36	M- JPR15-AMR2.1-01	M30	M30	No delay	N/A	N/A
WP1-T3	36	40	D-JPR15-AMR2.1-WP1.2 M- JPR15-AMR2.1-02	M36	M43	Recruitment of new staff	N/A	N/A

COVID-19 has affected all partners in different ways, with some continuing to be directly involved in laboratory activities, but others had their laboratories access removed for varying periods of time. Thus, laboratory work and results have been delayed during this period e.g. NGS sequence determination and analysis. The recruitment of fellows for the project was also delayed, which has also delayed progress of aspects of the project. However, all partners' laboratories have reopened, and partners have returned to laboratory research. As a result, the project is progressing well.



5.1.4.13 JRP14-AMR2.1-FULL-FORCE

FULL FORCE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Task 0.1	M54	M54	D-JRP19-WP0.D1	M27	M33	Travel restrictions	-	-
Task 1.1	M27	M32	D-JRP19-WP1.D3	M27	M32	Lab closures	-	-
Task 1.2	M30	M34	D-JRP19-WP1.D5	M29	M33	Travel restrictions	-	-
			M-JRP19-M6	M30	M33	Travel restrictions	-	6,200 €
Task 1.3	M36	M40	D-JRP19-WP1.D6	M32	M34	Travel restrictions	-	1,000 €
			D-JRP19-WP1.D7	M33	M39	Travel restrictions	-	-
			D-JRP19-WP1.D8	M36	M40	Travel restrictions	-	-
			M-JRP19-M7	M32	M34	Travel restrictions	-	-
			M-JRP19-M15	M35	M38	Travel restrictions	-	-
			M-JRP19-M16	M36	M40	Travel restrictions	-	-
Task 2.1	M54	M54	D-JRP19-WP2.D1	M33	M36	Lab closures	-	-
			M-JRP19-M10	M34	M36	Lab closures	-	-



FULL FORCE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Task 2.2	M54	M54	D-JRP19-WP2.D2	M33	M36	Lab closures	-	-
			M-JRP19-M11	M34	M36	Lab closures	-	-
Task 2.3	M54	M54	D-JRP19-WP2.D3	M33	M36	Lab closures	-	-
			M-JRP19-M12	M34	M36	Lab closures	-	-
Task 2.4	M54	M54	D-JRP19-WP2.D4	M33	M36	Lab closures	-	-
			M-JRP19-M13	M34	M36	Lab closures	-	-
Task 2.5	M54	M54	D-JRP19-WP2.D5	M33	M36	Lab closures	-	-
			M-JRP19-M14	M34	M36	Lab closures	-	-
Task 4.1	M42	M45	D-JRP19-WP4.D1	M30	M34	Workshop postponed	-	-
			M-JRP19-M3	M28	M34	Workshop postponed	-	-
Task 4.2	M54	M54	M-JRP19-M8	M33	M36	MTA required	-	-

The basis of this project was supposed to be an on-site, three day workshop on SMRT sequencing, held at the State Serum Institute (SSI, DK), followed by a proficiency test to analyse each partner's capacity to perform SMRT sequencing. Due to restrictions imposed by all EU governments, we first had to **postpone**, and then to **reorient this workshop to an online course held from September 7-9**. Given the intensive workload during the course, we hope that our consortium partners with limited SMRT skills will still be able to reach a sufficient technical level in plasmid sequencing. Likewise, the physical kick-off meeting,



planned during ECCMID 2020 in Paris, was cancelled and replaced by a meeting in Brussels on October 8, unless the progress of the pandemic decides otherwise.

Given lab closures during M28-30, all WP2 deliverable and milestones due dates are shifted back 3 months. Given the dependency on WP2 results, WP3 and WP4 are also delayed 3 months. Currently (June 2020), we are still hopeful to catch up all the delays and to complete the entire work plan by mid-2022.

5.1.4.14 JRP15-AMR2.1-FED-AMR

FEDAMR								
Tasks or Subtasks	Milestones and Deliverables						Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP1							--	--
WP2						Laboratories closed Staff and resources allocated to COVID testing sample dispatch impaired	--	--
WP3							--	--
WP4							--	--



FEDAMR								
Tasks or Subtasks	Milestones and Deliverables						Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP5 WP5-T1	M36	M45	D-JRP17-FED-AMR-WP5.1	M26	M36	Laboratories closed plus additional technical challenges	-	67,288
WP5-T1-ST1	M27	M36	D-JRP17-FED-AMR-WP5.2	M27	M39			
WP5-T1-ST2	M30	M39	D-JRP17-FED-AMR-WP5.3	M28	M37			
WP5-T1-ST3	M33	M45	D-JRP17-FED-AMR-WP5.4	M30	M43			
WP5-T1-ST4	M36	M5	D-JRP17-FED-AMR-WP5.5	M31	M45			
			D-JRP17-FED-AMR-WP5.6	M33	M45			



FEDAMR								
Tasks or Subtasks	Milestones and Deliverables						Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			D-JRP17-FED-AMR-WP5.7	M36	M51			
			D-JRP17-FED-AMR-WP5.8	M36	M51			
			M-JRP17-FED-AMR-37	M25	M34			
			M-JRP17-FED-AMR-38	M26	M40			
			M-JRP17-FED-AMR-39	M30	M41			
			M-JRP17-FED-AMR-40	M31	M42			



FEDAMR								
Tasks or Subtasks	Milestones and Deliverables						Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			M-JRP17-FED-AMR-41	M33	M43			
			M-JRP17-FED-AMR-42	M33	M44			
			M-JRP17-FED-AMR-43	M36	M51			
WP6							--	--

All partners have been affected by COVID-19 as many have been directly involved in the work and laboratory activities have been shut down to handle only essential work. Additionally, in many cases, collection, laboratory work, and result analysis of samples have not been performed during this period (e.g. in WP4: impossibility of sending samples to the laboratory performing the analysis, due to national COVID-19 restrictions). The recruitment of postdocs, PhD and MSc students and technicians for the project needed to be postponed, which considerably delayed the start of various WPs and tasks, and the progress of the project. Finally, many other projects and tasks from OHEJP projects have been delayed and the new plans for their execution will take place differently for the initially planned, which may affect the time line of FED-AMR work (laboratory, data analysis, and results dissemination). Budget can be expected to exceed the initial plan due to these reasons, which will be more correctly evaluated after reviewing the new fellows recruited. The budget not spent during the COVID lockdown and restriction period will be needed at a later date in order to perform the necessary analyses and recruitments. This in turn might lead



to higher costs than anticipated, because of the higher administrative complexity and because some sampling rounds could not be performed and had to be postponed.

WP1 as an exception as it was nearly not hampered by the shut down due to mainly desktop work and had on the other hand additional effort by facilitating and finding alternative working ways and resources.

5.1.4.15 JRP16-ET2.2-TELE-Vir

The COVID-19 pandemic has had a positive impact on the TELE-Vir project and many of the experiences and problems encountered during the crisis can be used or translated to the development of the TELE-Vir poi-tool box. Because of this the overall TELE-Vir project is, at this current moment (august 2020) not significantly delayed by the COVID-19 crisis.

The IZSLER laboratory was involved in the diagnosis of COVID-19 and consequently the project activity was influenced by the COVID-19 crisis. This crisis also had an impact on the availability of the project budget. This budget was only made available in June 2020, therefore the activities and purchase of reagents had to be postponed. However, no tasks, milestones or deliverables are affected by this.

The INIA-CISA laboratory was involved in the diagnosis of COVID-19 and consequently the project activity was influenced by the COVID-19 crisis. However, no tasks, milestones or deliverables are affected by this.

The SSI laboratory was involved in the diagnosis of COVID-19 and consequently the project activity was influenced by the COVID-19 crisis. However, no tasks, milestones or deliverables are affected by this.

NVI Laboratory was closed from March to and including May due to Covid-19 making it unavailable for performing any kind of experiments. However, no tasks, milestones or deliverables are affected by this.



5.1.4.16 JRP17-ET2.2-IDEMBRU

IDEMBRU								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP1-T1	30	36	Del1; M6	30; 30	36; 36	COVID-19	0	Total 18 061 (4 155 ANSES; 7 722 FLI; 6 184 IZSAM)
WP1-T2	44	50	M7	30	36	COVID-19	0	Total 62 600 (7 155 ANSES; 1 841 APHA; 22 560 INIAV; 23 144 IZSAM; 7 900 NDRVMI-BVSA)
WP2-T1	30	36	Del1; M1; M5	30; 28; 29	36; 34; 35	COVID-19	0	Total 9 155 (4 155 ANSES; 5 000 INSA)
WP2-T2	44	50	M8	30	36	COVID-19	0	Total 19 155 (4 155 ANSES; 15 000 INSA)
WP3-T1	46	52	Del1; M16	36; 36	42; 42	COVID-19	0	Total 13 495.5 (2 071.5 ANSES; 4 708 APHA; 2 440 INIAV; 4 276 IZSAM)
WP3-T2	36	42	Del2; M15	36; 35	42; 41	COVID-19	0	Total 17 340.5 (2 071.5 ANSES; 7 993 APHA; 3 000 INIAV; 4 276 IZSAM)



IDEMBRU								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP3-T3	48	52				COVID-19	0	Total 38 941.5 (7 830.5 ANSES; 3 154 APHA; 8 122 FLI; 3 000 INIAV; 10 000 INSA; 6 835 IZSAM)
WP4-T1	48	54				COVID-19	0	Total 16 362 (3 862 ANSES; 2 000 INIAV; 10 000 INSA; 500 IZSAM)
WP4-T3	54	60	M9	30	36	COVID-19	0	Total 46179.7 (1 362 ANSES; 2 175 APHA; 18 895.7 BfR; 23 747 FLI)
WP5-T1	42	48				COVID-19	0	Total 10 347 (1 362 ANSES; 960 APHA; 8 025 WBVR)
WP5-T2	48	54				COVID-19	0	Total 1362 (1362 ANSES)
WP7-T1	54	60	Del4; Del5; Del6; M4; M10; M11; M12;	36; 46; 54; 28; 30; 32; 34;	42; 52; 60; 34; ;36; 38; 40	COVID-19	0	Total 3 375 (2 775 APHA; 600 NDRVMI-BVSA)



IDEMBRU								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP7-T2	54	60	Del1; Del2; Del3; M2; M3; M4	30; 32; 34; 28; 28; 28	36; 38; 40; 34; 34; 34	COVID-19	0	600 NDRVMI-BVSA
WP7-T3	54	60				COVID-19	0	
WP7-T4	54	60	M13; M14	34; 34	40; 40	COVID-19	0	6 822 FLI

Comments:

IZSAM (P28) is currently involved in the COVID-19 diagnostic in Italy since the epidemic started on late February and beginning of March. Only essential activities could be continued on site. Moreover, to contain the spread of the COVID-19 outbreak, the IZSAM has had several measures to maintain the social distancing and avoiding the crowding of laboratory rooms like smart working and personnel time schedule split in two turns. During the current period, IZSAM experienced shortage of staff working at the laboratory units thus all the activities have slowed down including also the project IDEMBRU.



5.1.4.17 JRP18-ET1.1-MEmE

MEME								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP1-T3	M48	Unknown	M-JRP18-03	M28	Depending on ethics committee feedback at INIAV, Portugal	No problems with ST1 (Mouse/fox model of Em). Problems with ST2 (Sheep model of Eg). Infact, <u>COVID-19 pandemic</u> delayed the ethic approval for the sheep model at INIAV, Portugal. Waiting Ethics approval. This is the most relevant delay with domino effects on proteomic study. Serious concerns on feasibility of this task in due time because of the huge amount of time needed for the growth of parasites after animal infection (>1 year)		



MEME								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP2-T3	M44	Unknown	M-JRP18-05	M30	M34	A 1st version already available but needs to be put in the required format. NVI has sent the mc DNA extraction with manual washing steps SOP to SVA to put in the correct format (June). The NVI PCR SOP has been delayed due to changing the PCR machine and that we need to validate method with the new machine – this is being done but probably not finalised until end of October – <u>COVID 19</u> restrictions to who can get access to the lab and the prioritisation of analysing diagnostic samples over research projects have delayed this work by about 3 months so far		



MEME								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP3-T4	M52	Unknown	M-JRP18-08	M30	Depending on ethics committee at INIAV, Portugal	<u>COVID-19 pandemic</u> delayed the ethic approval for the sheep model at INIAV, Portugal. This is the most relevant delay with domino effects on proteomic study (WP3-T4)		
WP3-T7	M48	M48	M-JRP18-10	M30	M32	<u>Covid-19 pandemic</u> delayed the beginning of collection of samples	0	all
WP3-T8	M48	M52	M-JRP18-11	M30	M36	<u>Covid-19 pandemic</u> delayed networking at hospital level for the European centres involved		

The COVID-19 pandemic has had an effect on the planned activities. The timeline has flexibility to minor delays, but some milestones and deliverables were delayed and some in near future are expected to be delayed. The consortium has shown ability to adapt, and the overall progress has been good, taken the situation. Some of the budget may need to be moved to Y4 (especially budget for travels and laboratory work). European Multicolloquium of Parasitology (EMOP) was postponed to 2021.



5.1.4.18 JRP19-ET1.1-PARADISE

PARADISE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP2.T3 Experimental amplicon-based and shotgun metagenomics for detection of foodborne parasites	M52	M52	D-JRP-PARADISE-WP2.1 Protocol for 18S rDNA-based amplicon sequencing for detection of relevant FBPs	M30	M36	Delay due to limited access to working places	Undeterminable at present	Undeterminable at present



PARADISE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP3.T1 In silico selection of informative loci from comparative genomics data	M42	M42	D-JRP-PARADISE-WP3.1 Report on the <i>in silico</i> selection of highly polymorphic sequences in <i>C. parvum</i> and <i>G. duodenalis</i> genomes	M30	M36	Delay in WP2-T1	Undeterminable at present	Undeterminable at present
WP4-T1 Development of pre-DNA extraction enrichment strategies	M54	M54	M-JRP-PARADISE-2 Study visit (ISS-ANSES) for optimizing aptamer selection strategy	M28	Cancelled	International travel limitations	No budget allocated	None



PARADISE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP2-T1 NGS-based genome study of selected isolates of <i>C. parvum</i> and <i>G. duodenalis</i>	M36	M36	M-JRP-PARADISE-3 Key isolates of <i>C. parvum</i> collected	M30	M33	Delay in the collection/shipment of samples due to the COVID-19 epidemics	Undeterminable at present	Undeterminable at present
WP2-T1 NGS-based genome study of selected isolates of <i>C. parvum</i> and <i>G. duodenalis</i>	M36	M36	M-JRP-PARADISE-4 Key isolates of <i>G. duodenalis</i> collected	M30	M33	Delay in the collection/shipment of samples due to the COVID-19 epidemics	Undeterminable at present	Undeterminable at present



PARADISE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP2-T3 Experimental amplicon-based and shotgun metagenomics for detection of foodborne parasites	M52	M52	M-JRP-PARADISE-5 Referenced database of foodborne parasite genomes established	M30	M36	Delay due to limited access to working places	Undeterminable at present	Undeterminable at present
WP2-T3 Experimental amplicon-based and shotgun metagenomics for detection of foodborne parasites	M52	M52	M-JRP-PARADISE-6 Pipeline for metagenome data analysis for FBPs optimized	M30	M36	Delay due to limited access to working places	Undeterminable at present	Undeterminable at present



PARADISE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP3-T1 In silico selection of informative loci from comparative genomics data.	M42	M42	M-JRP-PARADISE-7 First set of candidate markers for MLST development available	M30	M36	Impact of delay in WP2-T1 activities	Undeterminable at present	Undeterminable at present
WP4-T1 Development of pre-DNA extraction enrichment strategies	M54	M54	M-JRP-PARADISE-8 Animal immunization and cDNA library of nanobody sequences for <i>C. parvum</i> and for <i>G. duodenalis</i> completed	M30	M33	Animal immunization completed, cDNA library under construction	Undeterminable at present	Undeterminable at present



The COVID-19 pandemic has had an effect on the planned activities. The timeline has flexibility to minor delays, but some milestones and deliverables were delayed and some in near future are expected to be delayed. The consortium has shown ability to adapt, and the overall progress has been good, taken the situation. Some of the budget may need to be moved to Y4 (especially budget for laboratory work).

5.1.4.19 JRP20-FBZSH3-DISCoVer

DISCOVER								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP2-T3	42	Will not be done	D-JRPFBZ-1-WP4.2	42	42 (but without these data)	See a) below	No budget allocated to this tasks in UCM budget	NA
WP2-T2, T3, T4, T5	48	52	D-JRPFBZ-1-WP4.1, WP4.2, WP4.3, WP4.4	40, 42, 49, 49	44, 46, 53, 53	See b) below		INSA: 9.000€ for sampling/isolation/phenotypic characterization 11.000€ for sequencing used until M39



DISCOVER								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP2-T4	48	52	D-JRPFZ-1-WP4.3	49	53	See c), d), and e) below		As for now, affected partners plan to spend all of the allocated budget, but with delay

General considerations:

Several partners have experienced delays in collecting new data, as all field work was cancelled due to COVID-19.

Some partners also experienced delays (some still are) in laboratory activities (e.g. sequencing) for already existing samples/isolates, due to lock down of the laboratories and/or reallocating of resources including staff to handle the COVID-19 crisis.

It is at this point difficult to estimate the impact and resulting delays for the project as a whole, since not all activities are resumed yet. We expect that all lab activities and most of the planned sampling will be completed, but possible with 3-4 months delay that may be caught up later in the project. However, a few sampling activities may be cancelled all together.

Specific for STEC

Several partners had planned sampling of STEC during 2020 as STEC data for source attribution are sparse. In general, many partners in task 2.4 have a delay in sampling for STEC. This is including, but not limited to difficulties getting samples in to the lab, capacity to handle research samples in the lab and difficulties in getting STEC isolates and information about STEC isolates from national collaborators due to the COVID-19 crisis.



Specific delays or cancellation of activities per partner:

- a) UCM: Unfortunately due to the pandemic the partner that would be sharing human *Campylobacter* isolates is no longer able to do so (since they have no resources to divert to this activity). This was done as part of our ongoing collaboration and therefore we had no budget allocated to this. There is uncertainty about other tasks due to the situation but in the current scenario we should deliver in all the other fronts.
- b) INSA: Many employees have been directly involved in the COVID-19 diagnosis and laboratory activities have been shut down to handle only essential work. Therefore, all research activity was stopped from March to June, including new sampling and NGS-related activities for DiSCoVeR.
- c) APHA: We had planned to collect O157 STEC from cattle farms in the UK between March 2020 and December 2021. The farm sampling has been postponed due to the pandemic and we are unsure when it will restart. As a contingency we are spending more time looking for suitable STECs to study in our archives, so progress is still being made.
- d) NVI: We have difficulties in performing screening and isolation of STEC isolates from dogs and wild animal samples due to prioritization of diagnostics and surveillance programmes over research projects during the COVID-19 crisis. Many employees have been working in smaller teams focusing on diagnostics and routine analysis especially during spring 2020 (March-June) and others were then forced to have home offices and not perform laboratory activities.
- e) INIAV: Sampling of wildlife and pets delayed. Will commence sampling in March 2021, if the COVID situation allows.



5.1.4.20 JRP21-FBZ3.1-BIOPIGEE

BIOPIGEE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis
JRP21-WP2-T2 Application of the biosecurity protocol (M27-M42)	M42	M48	# see below	M40	M46	Impact by Covid19 outbreak	NA	No change
JRP21-WP2-T4 Field studies (M25-M48)	M48	M54	# see below	M40	M46	Impact by Covid19 outbreak	NA	No change
JRP21-WP3-T1 Comparison of methods for testing the effect of disinfectants	M40	M48	# see below	M40	M48	Covid19 work	NA	Not known yet



BIOPIGEE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis
JRP21-WP3-T2 Effect of disinfectants on biofilm-associated wild type Salmonella	M50	M54	D-JRP.21-WP3.9	M50	M54	Covid19 lockdown in laboratory. Not possible to conduct laboratory experiments for a period during lockdown/restrictions. M-JRP.21-10 is not affected.	NA	Se below
JRP21-WP3-T3 Study of HEV stability in relation to disinfection approaches	M50	M60	D-JRP#-WP3.9	M36	M42	People have worked on Covid19 instead for a large part of their time, lab has been used for Covid19 work for a large part of the capacity, work on HEV has started again	NA	No change



BIOPIGEE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis
JRP21-WP4-T2 Stochastic simulations on the effectiveness of biosecurity measures	M49	NA	D-JRP#-WP4.11	M40	NA	Depends on the delay from other WPs	NA	No change
JRP21-WP6-T3-ST2 and ST3	M26 M30	unknown	D-JRP21-WP6.3, M-JRP21-03, M-JRP21-07	30, 26,30	unknown	Physical conferences cannot be hold and stakeholders cannot be invited due to the CoVID-19 outbreak.	Not known yet	Not known yet

WP2: The field studies within this project are currently being delayed due to COVID-19 restrictions on farm visits and capability for lab testing (due to redeployment of staff to COVID-19 testing). The field studies are delayed by around six months. We expect being able to start them in most partner countries in autumn instead of springtime. There is some hope that the consortium institutes may be able to recover and increase the rate of the farm visits later in the year but it is still likely that the number of farms visited will need to be reduced which would lead to reduced power and limit opportunities to statistically analyse the collected data. These trials are the main data gathering objectives of the project and these data will be used in work packages 2, 4, 5 and 6, and so these work packages will suffer from reduced statistical validity and power to detect significant associations. During the first half year, we set up our biosecurity questionnaire (T2.1/T2.2) in a technical tool and are checking and editing incoming data parallel to the running survey, which may save us some time when preparing data for evaluation later in the project.

If no extension is provided to enable the full number of planned farm visits to take place, then we will reduce the number of farms covered by task 2 (application of biosecurity questionnaire) and 4 (longitudinal studies) and we have to accept a lower statistical power to detect associations. Consequently, we would need to utilise expert panels to estimate the effects of biosecurity measures on pathogen prevalence as supporting evidence to be joined with results supplied



through the field data. The project team will monitor field work progress and will set a deadline (around M39) in the second project year in which to estimate the likely reduction in statistical power and need to utilise an expert panel.

WP3: This WP is delayed by 3 to 6 months due to Covid-19 activities: T3.1 is going as planned. T3.2 is delayed but can still perform all the planned tasks but the finish date is a bit later than originally planned. Similarly in T3.3, the new date for delivery is scheduled for M39.

NORWEGIAN VETERINARY INSTITUTE (Participating in WP3)

Original and revised budget for 2020 (status 09.09.2020)

BUDGET IN EURO	ORIGINAL	REVISED	TRANSFER TO 2022
Total direct personell costs	47.595,70	18.168,52	29.427,18
Total other costs	7.779,34	3.721,66	4.057,68
Indirect costs	13.844,00	5.472,55	8.371,45
Total	69.219,04	27.362,73	41.856,31
EU reimbursement 44%	30.456,80	12.039,60	18.417,20

Wageningen Bioveterinary Research/NCOH/UU

Original and revised budget for 2020 (status 16.09.2020)

BUDGET IN EURO	ORIGINAL	REVISED	TRANSFER TO 2022
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Summary Progress Report
Third Year - 2020
M25-M36



Total direct personell costs	63.020,00	41.593,00	21.427,00
Total other costs	12.100,00	7.986,00	4.114,00
Indirect costs	18.780,00	12.394,00	6.386,00
Total	93.900,00	61.973,00	31.927,00
EU reimbursement 44%	41.099,56	27.125,70	13.973,86

German Robert Koch Institute ((Participating in WP3)

Original and revised budget for 2020 (status 16.09.2020)

BUDGET IN EURO	ORIGINAL	REVISED	TRANSFER TO 2022
Total direct personell costs	74.365,00	17.632,00	56.733,00
Total other costs	5.600,00	0,00	5.600,00
Indirect costs	19.991,25	4.408,00	15.583,25
Total	99.956,25	22.040,00	77.916,25
EU reimbursement 44%	43.750,35	9.646,80	34.103,55



WP 4: Task 4.2 depends on the data of the effectiveness of biosecurity measures on the reduction of prevalence provided from WP 2, 3 and 5. Thus, if these WPs 2, 3 and 5 are affected by the CoViD-19 crisis, it will also affect WP4 because the other WPs should provide data inputs for the models in WP 4. WP4 will fill its models as much as possible with information from an expanded literature review in order to be more independent and be able to start working on the modelling before finalisation of the other WPs.

WP5: We are planning to drop 5.3 (machine learning) to save time and since data is limited for this task. Instead, we will extend the T5.2 literature review and support WP4.

WP6: Workshops were postponed to 2021 and will be held either online or national on a small scale. We will check the situation and options at the beginning of next year (M38). We are planning to transfer the budget for the workshop from 2020 to 2021 (2100€).

We will postpone physical conference participation and budget (1400€) to 2021.



5.1.4.21 JRP22-FBZ4.1-TOXOSOURCES

TOXOSOURCES								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
TOXOSOURCES-WP2, TOXOSOURCES-WP3, TOXOSOURCES-WP4, TOXOSOURCES-WP5					Measurable outcomes expected to be reached as planned	COVID-19 pandemic		Some personnel cost budget due to recruiting delays, time allocation challenges of consortium members working on COVID-19 response, and postponement of some laboratory work

COVID-19-situation is a challenge, but no measurable outcomes of TOXOSOURCES have been delayed. TOXOSOURCES Consortium has shown impressive resourcefulness and adaptability to the situation, and the careful risks-and-dependencies planning has proven useful. All Milestones, Deliverables and reports have been reached and submitted by their planned deadlines, and no delays are expected in reaching the future measurable outcomes by their planned deadlines. Follow-up continues.

- No planned meetings had to be cancelled by M33.
- We placed more focus on some literature review tasks earlier than planned, while some lab work had to be postponed.



- The location of the technical workshops of TOXOSOURCES-WP3 was changed from Rome (IT) to Brno (CZ), and the dates were set to be late in the year. If this updated plan is affected by travel restrictions, adjusting the time of the workshops as well as options to have the workshop virtually will be investigated.
- Recruiting was slower because administrative processes were slower. We did not lack any key personnel, thus the negative impact of this delay was limited. Delay in recruiting affected TOXOSOURCES-WP5 and was followed-up during the spring.
- Lab work for projects was put to hold in several countries, including in the key laboratories in Italy, Spain, France, Germany, and Denmark. This affected mainly TOXOSOURCES-WP3 and TOXOSOURCES-WP4, and some delays are expected, and to a lesser extent TOXOSOURCES-WP5.
- Due to COVID-19-related duties, some TOXOSOURCES Consortium members could not allocate the time planned for TOXOSOURCES especially during the spring. This affected for example TOXOSOURCES-WP2-T5, but the synergy with TOXOSOURCES-WP2-T2 allowed planning the task to start with minor delays, and we expect its measurable outcomes to be reached as planned.
- Sending materials and samples was challenging. This affected mainly TOXOSOURCES-WP4 and TOXOSOURCES-WP5.
- Purchasing and delivery of reagents was challenging. This affected mainly TOXOSOURCES-WP3.
- Access to grey literature was limited due to remote working.
- Practical and emotional challenges related to working from home were acknowledged as well.

Whether and how the points above affected the budget use during the first six months of the project was preliminarily evaluated during M32. All partner institutes are encouraged to check the budget use situation again at M34.

5.1.4.22 JRP23-FBZSH5-ADONIS

The COVID situation has not led to substantial and/or critical delays yet.

At this point is foreseen that all milestones and deliverables will reach on time or with minimal delay and then all allocated budget will be spent according to the proposal / overall budget plan.



5.1.4.23 JRP24-FBZSH9-BeONE

BEONE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
BeONE-WP1-T2	32	36	M-BeONE.1.2	30	33	Staff reallocated to COVID-19 response, limited responsiveness to surveys due to COVID-19		
			M-BeONE.1.3	32	35	Dependent on M-BeONE.1.2		
			D-BeONE.1.3	32	36	Dependent on M-BeONE.1.2 and M-BeONE.1.3		
BeONE-WP2-T1	34	36	D-BeONE.2.1	34	36			
			M-BeONE.2.1	32	34			



BEONE								
Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
BeONE-WP5-T2	54	54	M-BeONE.5.3	28	31			

Budget changes are awaiting feedback from partners



5.1.5 Publications

JRP02-AMR2-ARDIG: Extensive antimicrobial resistance mobilization via Multicopy Plasmid Encapsidation mediated by temperate phages. Lorena RODRÍGUEZ-RUBIO and Carlos SERNA, Manuel ARES-ARROYO, Bosco R. MATAMOROS, Jose F. DELGADO-BLAS, Natalia MONTERO, Cristina BERNABE-BALAS, Emilia F. WEDEL, Irene S. MENDEZ, Maite MUNIESA and Bruno GONZALEZ-ZORN. Journal Antimicrobial Chemotherapy, in press.

JRP02-AMR2-ARDIG: Monitoring Antimicrobial Resistance and Drug Usage in the Human and Livestock Sector and Foodborne Antimicrobial Resistance in Six European Countries. Octavio Mesa Varona, Katerina Chaintarli, Berit Muller-Pebody, Muna F Anjum, Tim Eckmanns, Madelaine Norström, Ides Boone, Bernd-Alois Tenhagen. Infection and Drug Resistance, 2020; 13: 957—993.

JRP02-AMR2-ARDIG: Stepwise evolution and convergent recombination underlie the global dissemination of carbapenemase-producing Escherichia coli. Rafael Patiño-Navarrete, Isabelle Rosinski-Chupin, Nicolas Cabanel, Lauraine Gauthier, Julie Takissian, Jean-Yves Madec, Monzer Hamze, Remy A. Bonnin, Thierry Naas, Philippe Glaser. Genome Medicine 2020, 12(10).

JRP02-AMR2-ARDIG: Brouwer, Michael S.M., Stephanie D. Jurburg, Frank Harders, Arie Kant, Dik J. Mevius, Adam P. Roberts, and Alex Bossers. “The Shuffling 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>.

JRP03-R1-AMR3-RADAR: Mughini-Gras, Lapo, Alejandro Dorado-García, Engeline van Duijken, 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](https://doi.org/10.1016/S2542-5196(19)30130-5).

JRP03-R1-AMR3-RADAR: Douarre, Pierre-Emmanuel, Ludovic Mallet, Nicolas Radomski, Arnaud Felten, and Michel-Yves Mistou. “Analysis of COMPASS, a New Comprehensive Plasmid Database Revealed Prevalence of Multireplicon and Extensive Diversity of IncF Plasmids.” Frontiers in Microbiology 11 (March 24, 2020): 483. <https://doi.org/10.3389/fmicb.2020.00483>.

JRP06-FBZ1-NOVA: Alvarez J, Lopez G, Muellner P, de Frutos C, Ahlstrom C, Serrano T, Moreno MA, Duran M, Saez JL, Dominguez L, Ugarte-Ruiz M. 2020. Identifying emerging trends in antimicrobial resistance using Salmonella surveillance data in poultry in Spain. TBDE, 67(1):250-262. DOI: 10.1111/tbed.13346.

JRP06-FBZ1-NOVA: Nova Bosch J, Iglesias I, Martínez M, de la Torre A. 2020. Climatic and topographic tolerance limits of wild boar in Eurasia: Implications for their expansion. Geography, Environment, Sustainability, 13(1):107-114. <https://doi.org/10.24057/2071-9388-2019-52>.

JRP06-FBZ1-NOVA: Nova Teng KT., Martinez-Aviles M., Ugarte-Ruiz M., Barcena C., de la Torre A., Lopez G., Moreno MA., Dominguez L. y Alvarez J. Spatial trends in Salmonella infection in pigs in Spain. Frontiers in veterinary science. In Press. (A). ISSN: 2297-1769. DOI: 10.3389/fvets.2020.00345

JRP06-FBZ1-NOVA: Martínez-Avilés, Marta, Macarena Garrido-Esteva, 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>.

JRP06-FBZ1-NOVA: 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>.



JRP09-FBZ3-AIRSAMPLE : Johannessen GS, Garofolo G, Serafino GD, Kolackova I, Karpiskova R, Wieczorek K, Osek J, Christensen J, Torp M, and J (2020). Campylobacter in chicken – critical parameters for international, multicentre evaluation of air sampling and detection methods. Food Microbiology 90, 03455. <https://doi.org/10.1016/j.fm.2020.103455>

JRP09-FBZ3-AIRSAMPLE: Hoorfar J, Kolackova I, Johannessen GS, Garofolo G, Marotta F, Wieczorek G, Osek J, Torp M, Spilsberg B, Sekse C, Thornval NR, Karpiskova R. Foodborne Campylobacter in chicken: A multi-centre proposal for a fast screening tool in biosecured chicken flocks. Applied and Environmental Microbiology (submitted on May 5, 2020). 10.1128/AEM.01051-20

JRP09-FBZ3-AIRSAMPLE : 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>.

JRP07-FBZ2-LISTADAPT : Félix B, Sévellec Y, et al. A European-wide dataset to decipher adaptation mechanisms of Listeria monocytogenes to diverse ecological niches. Will be submitted to the review Scientific data. (Ongoing)

JRP07-FBZ2-LISTADAPT : Palma F, Radomski N, et al. Genomics elements located in the accessory repertoire drive the adaptation to biocides in Listeria monocytogenes strains from different ecological niches. Will be submitted to Molecular Ecology in June 2020. (Ongoing)

JRP07-FBZ2-LISTADAPT : Guérin A, Bridier A et al. Exposure to quaternary ammonium compounds selects resistance to ciprofloxacin in Listeria monocytogenes. Will be submitted in Frontiers in Microbiology in September 2020. (Ongoing)

JRP07-FBZ2-LISTADAPT : Sévellec Y, Torresi M et al., First report on the occurrence of Listeria monocytogenes ST121 strain in a dolphin brain. Will be submitted in July 2020; (ongoing).

JRP08-FBZ2-METASTAVA: Steven Van Borm, Qiang Fu, Raf Winand, Kevin Vanneste, Mikhayil Hakhverdyan, Dirk Höper , Frank Vandenbussche.2020. Evaluation of a commercial exogenous internal process control for diagnostic RNA virus metagenomics from different animal clinical samples. Journal of Virological Methods. In Press. Gold open access (2540€). <https://doi.org/10.1016/j.jviromet.2020.113916>

JRP08-FBZ2-METASTAVA: Steven Van Borm, Kevin Vanneste, Qiang Fu, Dominiek Maes, Alexandra Schoos, Eline Vallaey, Frank Vandenbussche. 2020. Increased viral read counts and metagenomic full genome characterization of porcine astrovirus 4 and Posavirus 1 in sows in a swine farm with unexplained neonatal piglet diarrhea. Virus Genes. Green open access. <https://pubmed.ncbi.nlm.nih.gov/32880793/>

JRP10-FBZ3-MOMIRPPC: Labarthe, S., Laroche, B., Nguyen, T. N. T., Polizzi, B., Patout, F., Ribot, M., & Stegmaier, T. (2020). A multi-scale epidemic model of Salmonella infection with heterogeneous shedding. ESAIM: Proceedings and Surveys, 67, 261-284." 10.1051/proc/202067015

JRP10-FBZ3-MOMIRPPC: Kempf F, P. Menanteau, I. Rychlik, T. Kubasova, J. Trotereau, I. Virlogeux-Payant, S. Schaeffer, C. Scouler, R. Drumo, E. Guitton and P. Velge. (2020) Gut microbiota composition before infection determines the Salmonella super- and low-shedder phenotypes in chicken. Microbial Biotechnology (in press); <https://doi.org/10.1111/1751-7915.13621>

JRP10-FBZ3-MOMIRPPC: Rebollada-Merino, Agustín, María Ugarte-Ruiz, Marta Hernández, Pedro Miguela-Villoldo, David Abad, Pedro Cuesta-Álvarez, David Rodríguez-Lázaro, Lucía de Juan, Lucas Domínguez, and Antonio Rodríguez-Bertos. “Dietary Supplementation with Fermented Defatted ‘Alperujo’ Induces Modifications of the Intestinal Mucosa and Cecal Microbiota of Broiler Chickens.” Poultry Science, August 2020, S0032579120304843. <https://doi.org/10.1016/j.psj.2020.07.015>.



JRP10-FBZ3-MOMIRPPC: Van Wagenberg, C.P.A. van Horne P.L.M. and van Asseldonk M.A.P.M. Cost-effectiveness analysis of using probiotics to control *Campylobacter* in broilers (submitted).

JRP11-FBZ4-MEDVETKLEBS: Barbier E, Rodrigues C, Depret G, et al. The ZKIR Assay, a Real-Time PCR Method for the Detection of *Klebsiella pneumoniae* and Closely Related Species in Environmental Samples. *Appl Environ Microbiol.* 2020;86(7):e02711-19. Published 2020 Mar 18. doi:10.1128/AEM.02711-19.

JRP11-FBZ4-MEDVETKLEBS: Igor Loncaric, Adriana Cabal Rosel, Michael P. Szostak, Theresia Franziska Licka, Franz Allerberger, Werner Ruppitsch, Joachim Spergser. Broad-spectrum cephalosporin-resistant *Klebsiella* spp. isolated from diseased horses in Austria. *Animals* 2020;20(2):332. doi: 10.3390/ani10020332.

JRP11-FBZ4-MEDVETKLEBS: Huynh BT, Passet V, Rakotondrasoa A, Diallo T, Kerleguer A, Hennart M, Lauzanne A, Herindrainy P, Seck A, Bercion R, Borand L, Pardos de la Gandara M, Delarocque-Astagneau E, Guillemot D, Vray M, Garin B, Collard JM, Rodrigues C, Brisse S, *Klebsiella pneumoniae* carriage in low-income countries: antimicrobial resistance, genomic diversity and risk factors. *Gut Microbes* 2020, 11(5): 1287-1299.

JRP11-FBZ4-MEDVETKLEBS: 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>.

JRP11-FBZ4-MEDVETKLEBS: Rodrigues, Carla, Virginie Passet, Andrianiaina 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>.

JRP11-FBZ4-MEDVETKLEBS: Rodrigues, Carla, Virginie Passet, Andrianiaina 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>.

JRP11-FBZ4-MEDVETKLEBS: 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>.

JRP11-FBZ4-MEDVETKLEBS: 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>.

JRP16 -9M Report 2020-TELEVIR: Fomsgaard, Anna S., and Maiken Worsøe Rosenstjerne. "An Alternative Workflow for Molecular Detection of SARS-CoV-2 – Escape from the NA Extraction Kit-Shortage, Copenhagen, Denmark, March 2020." *Eurosurveillance* 25, no. 14 (April 9, 2020). <https://doi.org/10.2807/1560-7917.ES.2020.25.14.2000398>.

JRP18-ET1.1-MEME: Casulli A. Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis. *Lancet Glob Health.* 2020;8(4):e470-e471. doi:10.1016/S2214-109X(20)30066-8



JRP18-ET1.1-MEME: Selim M'rad, Myriam Oudni-M'rad, Vanessa Bastid, Laure Bournez, Sana Mosbahi, Abdelallatif Nouri, Hamouda Babba, Frédéric Grenouillet, Franck Boué, Gérald Umhang. Microsatellite Investigations of Multiple *Echinococcus Granulosus Ssensu Stricto* Cysts in Single Hosts Reveal Different Patterns of Infection Events between Livestock and Humans. *Pathogens*. 2020;9(6):E444. Published 2020 Jun 5. doi:10.3390/pathogens9060444

JRP18-ET1.1-MEME: Pavlo Maksimov, Hannes Bergmann, Marion Wassermann, Thomas Romig, Bruno Gottstein, Adriano Casulli, Franz J. Conraths. **Species detection within the *Echinococcus granulosus sensu lato* complex by novel probe based Real-Time PCRs.** *Pathogens*. <https://doi.org/10.1101/2020.07.24.220756>

JRP18-ET1.1-MEME: (submitted) **Bayesian analysis to evaluate three diagnostic methods for Cystic Echinococcosis in sheep.** *Acta tropica*.

JRP22-FBZ4.1-TOXOSOURCES: Sandra Klein, Daniel Stern, Frank Seeber. Expression of in vivo biotinylated recombinant antigens SAG1 and SAG2A from *Toxoplasma gondii* for improved seroepidemiological bead-based multiplex assays, 09 June 2020, PREPRINT (Version 1) available at Research Square [<https://doi.org/10.21203/rs.3.rs-33963/v1>]. Submitted to BMC Biotechnology (Gold open access).

JRP22-FBZ4.1-TOXOSOURCES: Benedikt T. Fabian, Fatima Hedar, Martin Koethe, Berit Bangoura, Pavlo Maksimov, Franz J. Conraths, Isabelle Villena, Dominique Aubert, Frank Seeber, Gereon Schares. Fluorescent bead-based serological detection of *Toxoplasma gondii* infection in chickens, 11 June 2020, PREPRINT (Version 1) available at Research Square [<https://doi.org/10.21203/rs.3.rs-34121/v1>]. Accepted for publication on 25 June 2020, *Parasites & Vectors* (Gold open access).

JRP22-FBZ4.1-TOXOSOURCES Fernández-Escobar, M., Calero-Bernal, R., Benavides, J., Regidor-Cerrillo, J., Guerrero-Molina, M. C., Gutiérrez-Expósito, D., Collantes-Fernández, E., & Ortega-Mora, L. M. (2020). Isolation and genetic characterization of *Toxoplasma gondii* in Spanish sheep flocks. *Parasites & Vectors*, 13(1), 396. <https://doi.org/10.1186/s13071-020-04275-z>. Gold Open Access, article processing fee 1990 EUR.

5.1.6 Oral communications:

JRP06-FBZ1-NOVA: Cha, W., Dórea, F., Grøneng, G.M., Rø, G., Hopp, P., Jonsson, M., Dryselius, R. Development of One Health syndromic surveillance for *Campylobacter* in Norway and Sweden. In: Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats; May 27-29, 2020; Online meeting.

JRP06-FBZ1-NOVA: Gustafsson, W., Andersson, M.G. Designing multivariate syndromic surveillance for animal diseases in Sweden. In: Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats; May 27-29, 2020; Online meeting.

JRP07-FBZ2-LISTADAPT: Skjerdal T, Fagereng T, Osland AM, Lagesen K, Fiskerbeck E, Nesse L, Sévellec Y, Felix B, Roussel S. 2020. Phenotypical responses to stress of in *Listeria monocytogenes* strains of different Clonal Complexes isolated along the nature-to-farm-to-fork chain. 27-29 May. Second annual meeting EJP (virtual meeting)

JRP07-FBZ2-LISTADAPT: Sévellec Y, Ascencio Schuttz E, Félix B, Guillier L, Roussel S, Piveteau P. Comparative Analysis of the genomic diversity of *Listeria monocytogenes* in soil and water and food processing through pan Genome Wide Association Study. 27-29 May. Second annual meeting EJP (virtual meeting)



JRP07-FBZ2-LISTADAPT : Palma F, Guérin A, Radomski N, Bridier A, Sévellec Y, Félix B, Soumet C, Guillier L, Roussel S. Deciphering the Biocide-Resistance of *Listeria monocytogenes* Strains from Europe through Genome-Wide Associations at the pangenomic scale. 27-29 May. Second annual meeting EJP (virtual meeting)

JRP07-FBZ2-LISTADAPT : Guérin A, Palma F Le Grandois P, Bridier A, Soumet C, Sevellec Y, Roussel S. Exposure to quaternary ammonium compounds show resistance to ciprofloxacin for *Listeria monocytogenes* from diverse ecological niches. 27-29 May. Second annual meeting EJP (virtual meeting)

JRP07-FBZ2-LISTADAPT : 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.

JRP07-FBZ2-LISTADAPT : Félix B, Feurer C, Maillet A, Desmonts M H, Hickey B, Jankuloski D, Karpíšková R, Skjerdal T, Denis M, Gareis M, Zdobych 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.

JRP07-FBZ2-LISTADAPT : 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

JRP07-FBZ2-LISTADAPT : 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

JRP07-FBZ2-LISTADAPT : Felix, B., 2018. Population genetic structure of *Listeria monocytogenes* strains isolated from the pig and pork production chain in France, presented at Food Micro September 2018, Berlin (Germany).

JRP07-FBZ2-LISTADAPT : S. Antoci, V. A. Acciari, V. Di Marzio, I. Del Matto, G. Centorotola, M. Torresi, C. Marfoggia, G. Iannitto, A. Ruolo, G. A. Santarelli, G. Migliorati, F. Pomilio. Preliminary results on prevalence and persistence of *Listeria monocytogenes* in different dairy and meat processing plants in Central Italy, presented at “International meeting on emerging diseases and surveillance” November 2018, Vienna (Austria).

JRP08-FBZ2-METASTAVA : 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.

JRP08-FBZ2-METASTAVA : Sander van Boheemen. Sample Pretreatment: Challenges in Virology. Workshop: ESCV Next Generations Sequencing in Clinical Virology. 20-21 November, 2018

JRP08-FBZ2-METASTAVA : 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

JRP08-FBZ2-METASTAVA : Van Borm S. Next Generation Sequencing from sample to result : how does it work, what is the current status, and what is the added diagnostic value? World Veterinary Poultry Association, Belgian Branch. Study day 27/02/2020.

JRP11-FBZ4-MEDVETKLEBS : 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).



JRP11-FBZ4-MEDVETKLEBS: 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).

JRP16-9M Report 2020-TELEVIR: Following the INSaFLU usability for whole genome sequencing analysis of SARS-CoV-2, INSA's team has been recently invited to present this platform to broad audiences on behalf of ECDC COVID-19 Laboratory Networks Influenza (20 May 2020) and the WHO Europe Laboratory Workshop (1 June 2020).

JRP22-FBZ4.1-TOXOSOURCES: Oral presentation at OHEJPASM2020, May 27-29, 2020:

Source attribution for *Toxoplasma gondii* infections in Europe

Marieke Opsteegh (1), Hannah Morgan (1), Huifang Deng (1), Gereon Schares (2), Sandra Stelzer (2), Sara Monteiro Pires (3), Helga Waap (5), Jacek Sroka (6), Heidi Enemark (7), Jelena Srbljanovic (8), Olgica Djurkovic-Djakovic (8), Chiara Trevisan (9), Agnetha Hofhuis (1), Lasse S. Vestergaard (4), Pikka Jokelainen (4), Joke van der Giessen (1), Euro-FBP (COST Action FA1408), TOXOSOURCES Consortium RIVM, The Netherlands (1); FLI, Germany (2); DTU, Denmark (3); SSI, Denmark (4); INIAV, Portugal (5); PIWET, Poland (6); NVI, Norway (7); UoB, Serbia (8); ITG, Belgium (9)

JRP22-FBZ4.1-TOXOSOURCES: Poster at OHEJPASM2020, May 27-29, 2020:

TOXOSOURCES – *Toxoplasma gondii* sources quantified

Pikka Jokelainen (1), Marieke Opsteegh (2), Marco Lalle (3), Furio Spano (3), Gereon Schares (4), Sara Monteiro Pires (5), Anne Mayer-Scholl (6), Frank Seeber (7), Simone M. Cacciò (3), Joke van der Giessen (2), TOXOSOURCES Consortium (Joint Research Project of the One Health European Joint Programme) SSI, Denmark (1); RIVM, The Netherlands (2); ISS, Italy (3); FLI, Germany (4); DTU Food, Denmark (5); BfR, Germany (6); RKI, Germany (7) [10.5281/zenodo.3924467](https://doi.org/10.5281/zenodo.3924467)

JRP22-FBZ4.1-TOXOSOURCES: Poster and talk at 3-Minute-Thesis competition at OHEJPASM2020, May 27-29, 2020: Tropism and persistence of *Toxoplasma gondii*: from pork carcass to sausage and dry ham, a quantitative risk assessment

Filip DAMEK¹, Bastien FREMAUX², Dominique AUBERT³, Marieke OPSTEEGH⁴, Sandra VUILLERMET¹, Pikka JOKELAINEN⁵, Joke VAN DER GIESSEN⁴, Pascal BOIREAU¹, Isabelle VILLENA³, Radu BLAGA¹

¹ UMR BIPAR, Ecole Nationale Vétérinaire d'Alfort, ANSES, France ² IFIP - Institut du Porc, France ³ National Reference Center on Toxoplasmosis, Toxoplasma Biological Resources Center, CHU Reims and EA7510, SFR CAP-Santé, University of Reims Champagne-Ardenne, USC EpiToxo ANSES, France ⁴ National Institute for Public Health and the Environment, The Netherlands ⁵ Statens Serum Institut, Denmark.

JRP22-FBZ4.1-TOXOSOURCES: Short oral presentation at One Health EJP Cogwheel workshop with JPIAMR, April 28, 2020:

#TOXOSOURCES *Toxoplasma gondii* sources quantified

Pikka Jokelainen, SSI, Denmark

JRP22-FBZ4.1-TOXOSOURCES: Poster and short oral presentation in a webinar 'Toxoplasma gondii e toxoplasmosis in una prorspettiva One Health' organized by Italian Society of Parasitology (SOIPA), June 30, 2020:

TOXOSOURCES – TOXO*plasma gondii* SOURCES quantified



P JOKELAINEN¹, M OPSTEEGH², M LALLE³, F SPANO³, G SCHARES⁴, S MONTEIRO PIRES⁵, A MAYER-SCHOLL⁶, F SEEBER⁷, S M CACCIÒ³, J VAN DER GIESSEN², TOXOSOURCES CONSORTIUM (JOINT RESEARCH PROJECT OF THE ONE HEALTH EUROPEAN JOINT PROGRAMME)

1 Statens Serum Institut, Copenhagen, Denmark, 2 National Institute for Public Health and the Environment, Bilthoven, The Netherlands, 3 Istituto Superiore di Sanità, Rome, Italy, 4 Friedrich Loeffler Institute, Insel Riems, Germany, 5 Technical University of Denmark, Kongens Lyngby, Denmark, 6 German Federal Institute for Risk Assessment, Berlin, Germany, 7 Robert Koch Institute, Berlin, Germany

JRP22-FBZ4.1-TOXOSOURCES: Two talks at One Health EJP Summer School 2020:

Parasites in the food chain: global One Health risks

Wildlife and Public Health

Joke van der Giessen, RIVM, The Netherlands

5.1.7 Posters

JRP05-ET1-TOXDETECT: D. Clermont submitted a poster at OHEJP ASM entitled “Establishment of a shared MALDI-ToF reference spectra base, covering three pathogens of interest”

JRP07-FBZ2-LISTADAPT: 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)

JRP07-FBZ2-LISTADAPT: 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, Pomilio 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)

JRP07-FBZ2-LISTADAPT: 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., JRP07-FBZ2-LISTADAPT: 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)

JRP07-FBZ2-LISTADAPT: 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 *Listeria monocytogenes* CC121. ISOPOL XX (24 – 27 September 2019 Toronto)

JRP07-FBZ2-LISTADAPT: 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)

JRP07-FBZ2-LISTADAPT: 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. ISOPOL XX (24 – 27 September 2019 Toronto)

JRP07-FBZ2-LISTADAPT: 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



JRP07-FBZ2-LISTADAPT : 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

JRP07-FBZ2-LISTADAPT : Felix B, Feurer C, Maillet A, Guillier L, Boscher E, Kerouanton A, Denis M, Roussel S. 2018. Population genetic structure of *Listeria monocytogenes* strains isolated from the pig and pork production chain in France. IAFP EU Stockholm 25-27th April 2018;

JRP11-FBZ4-MEDVETKLEBS: Carla Rodrigues, Sylvain Brisse on the behalf of MedVetKlebs consortium. The MedVetKlebs project: *Klebsiella pneumoniae* from Ecology to Source Attribution and Transmission Control (poster).

JRP11-FBZ4-MEDVETKLEBS: Carla Rodrigues, Virginie Passet, Andrianiaina Rakotondrasoa, Sylvain Brisse. Suitability of MALDI-TOF Mass Spectrometry to Discriminate Species within the *Klebsiella pneumoniae* Complex (poster).

JRP11-FBZ4-MEDVETKLEBS: 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).

JRP11-FBZ4-MEDVETKLEBS: 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).

JRP11-FBZ4-MEDVETKLEBS: 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).

JRP11-FBZ4-MEDVETKLEBS: Violeta Di Marzio, Gabriella Centorotola, Cristina Marfoggia, 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).

JRP11-FBZ4-MEDVETKLEBS: Alessandra Cornacchia, Maria Antonietta Saletti, Violeta Di Marzio, Aurora Ciarrocchi, Gabriella Centorotola, Cristina Marfoggia, Carla Rodrigues, Sylvain Brisse, Francesco Pomilio. Detection of *Klebsiella* spp. in chicken meat: methods performance study (poster).

JRP11-FBZ4-MEDVETKLEBS: Violeta Di Marzio, Gabriella Centorotola, Aurora Ciarrocchi, Cornacchia Alessandra, Cristina Marfoggia, Maria Antonietta Saletti, Carla Rodrigues, Sylvain Brisse, Francesco Pomilio. Evaluation of antimicrobial resistance of *Klebsiella pneumoniae* strains in foods (poster).

JRP12-AMRSH5-FARMED: Poster of project “FARMED: Fast Antimicrobial Resistance and Mobile-Element Detection using metagenomics for animal and human on-site tests” presented at the One Health EJP Meeting, held in virtually in Prague 27-29th May.

JRP17-ET2.1-IDEMBRU: Ponsart C, Al Dahouk S, Ashford R, Daskalov H, De Massis F, Freddi L, Garofolo G, Melzer F., Pelerito A, Umanets A, Whatmore A, Ferreira AC. “Identification of emerging *Brucella* species: new threats for human and animals (IDEMBRU)”. Poster presentation at One Health EJP Annual Scientific Meeting 2020, Prague (CZ), 27-29 May.

JRP18-ET1.1-MEME : Adriano Casulli on behalf of MEME consortium (Franz J. Conraths, Franck Boue, Joke van der Giessen, Jacek Karamon, Mats Isaksson, Heidi L. Enemark, Pikka Jokelainen, Jacinto Gomes, Maria João Gargate, Urmas Saarma, Epp Moks, William Byrne, Gunita Deksnė, Wenbao Zhang, Jing Ding, Zhuangzhi Zhang, Junwei WANG). [Multi-centre study on *Echinococcus multilocularis* and *Echinococcus granulosus* s.l. in Europe: development and harmonization of diagnostic methods in the food chain](#). XXVIII World Congress on Echinococcosis, Lima, Peru, October 29-31, 2019. Abstract book, pp 101.



JRP18-ET1.1-MEME : Gerald Umhang, Franck Boue, Pavlo Maksimov, Franz J. Conraths, Joke Van Der Giessen, Jacek Karamon, Mats Isaksson, Rebecca Davidson, Øivind Øines, Pikka Jokelainen, Jacinto Gomes, Helga Waap, Maria João Gargate, Urmas Saarma, Epp Moks, Age Kärssin, William Byrne, Giovanna Masala, Gunita Deksne, Laura Rinaldi, Marion Wassermann, Peter Deplazes, Eran Dvir, Francesca Tamarozzi, Adriano Casulli. [Multi-centre study on *Echinococcus multilocularis* and *Echinococcus granulosus* s.l. in Europe: development and harmonization of diagnostic methods in the food chain \(MEME project\).](#)

OHEJP Abstract book for 2nd Annual Scientific Meeting, pp 152.

JRP22-FBZ4.1-TOXOSOURCES: [#TOXOSOURCES](#) has been used on social media: <https://twitter.com/hashtag/toxosources?f=live>

Involved partner institutes have mentioned TOXOSOURCES in their communications:

https://www.rki.de/DE/Content/Forsch/EJP_OH2020.html
<https://www.ssi.dk/aktuelt/nyhedsbreve/epi-nyt/2020/uge-4---2020>

JRP21-FBZ3.1-BIOPIGEE: Burow E., Prigge C., Smith R., Meester M., Santucci G., Young B., Rose N., Käsbohrer A., Kollas C. (2020): Questionnaire on best biosecurity practices to limit *Salmonella* & HEV occurrence in European pig farms. Poster at OHEJP ASM 2020, web conference, 27.-29.05.2020.

5.1.8 Overview of the JRP deliverables and milestones

In September 2020, there were 253 due deliverables and 269 milestones. 53% of the deliverables and 65% of the milestones were achieved. The delays are mainly due to the Covid-19 situation which consequences are explained in this report.

However, the dissemination procedure, with guidance on the flow of project deliverables, is now adopted by the project leaders who use the correct templates and make sure that the deliverables are uploaded to the OHEJP website and to Zenodo once the deliverables are “public”.

5.1.8.1 Deliverables

PROJECT	DELIVERABLES DUE FOR SEPTEMBER 2020	ACHIEVED DELIVERABLES	% OF ACHIEVED DELIVERABLES	DELAYED DELIVERABLES
JRP01-IMPART	24	15	63%	9
JRP02-ARDIG	10	7	70%	3
JRP03-RADAR	27	10	37%	17
JRP05-TOXDETECT	14	9	64%	5
JRP06-NOVA	24	20	83%	4
JRP07-LISTADAPT	24	20	83%	4



PROJECT	DELIVERABLES DUE FOR SEPTEMBER 2020	ACHIEVED DELIVERABLES	% OF ACHIEVED DELIVERABLES	DELAYED DELIVERABLES
JRP08-METASTAVA	22	9	41%	13
JRP09-AIRSAMPLE	5	5	100%	0
JRP10-MOMIRPPC	43	15	35%	28
JRP11-MEDVETKLEBS	17	8	47%	9
JRP12-FARMED	1	0	0%	1
JRP13-WORLDCOM	1	0	0%	1
JRP14-FULLFORCE	8	3	38%	5
JRP15-FEDAMR	10	2	20%	8
JRP16-TELEVIR	2	1	50%	1
JRP17-IDEMBRU	4	0	0%	4
JRP18-MEME	3	3	100%	0
JRP19-PARADISE	3	1	33%	2
JRP20-DISCOVER	2	1	50%	1
21-BIOPIGEE	4	2	50%	2
JRP22-TOXOSOURCES	2	2	100%	0
JRP23-ADONIS	3	1	33%	2
TOTAL	253	134	53%	119



5.1.8.2 Milestones

PROJECT	MILESTONES DUE FOR SEPTEMBER 2020	ACHIEVED MILESTONES	% OF ACHIEVED MILESTONES	DELAYED MILESTONES
JRP01-IMPART	17	15	88%	2
JRP02-ARDIG	10	6	60%	2
JRP03-RADAR	36	30	83%	6
JRP05-TOXDETECT	16	6	38%	10
JRP06-NOVA	25	21	84%	4
JRP07-LISTADAPT	26	22	85%	2
JRP08-METASTAVA	9	5	56%	4
JRP09-AIRSAMPLE	4	4	100%	0
JRP10-MOMIRPPC	32	26	81%	6
JRP11-MEDVETKLEBS	13	7	54%	6
JRP12-FARMED	1	0	0%	1
JRP13-WORLDCOM	1	0	0%	1
JRP14-FULLFORCE	7	2	29%	5
JRP15-FEDAMR	19	4	21%	15
JRP16-TELEVIR	0	0	0%	0
JRP17-IDEMBRU	11	3	27%	
JRP18-MEME	12	5	42%	
JRP19-PARADISE	8	1	13%	7
JRP20-DISCOVER	4	3	75%	1
21-BIOPIGEE	8	6	75%	2
JRP22-TOXOSOURCES	3	3	100%	0
JRP23-ADONIS	7	5	71%	2
TOTAL	269	174	65%	76



5.1.9 Overview of the JRPs DMP update

Project	Have you uploaded a first version of the project's DMP to the DMP group on the OHEJP website?	Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.
JRP01-IMPART	The main difficulty encountered is due to our lack of experience in DMP writing. The Excel sheet provided was a good way to try not to forget any item. The difficult part was to navigate between the boxes. Maybe in the future a web-based questionnaire would be more user friendly	We will be ready to implement the changes and take into account advice from the OH-EJP DMP team to improve our current version of the DMP as soon as we get them.
JRP02-ARDIG	We have had some problems with the format and adding information but the EJP management have been very helpful in providing support.	ARDIG has another year left, therefore this will be done nearer to the time.
JRP03-RADAR	Yes, lots of detail needed.	No not yet.
JRP05-TOXDETECT	No	No, this work will be initiated in January 2021 as the project was extended until June 2021
JRP06-NOVA	None of the project members in NOVA had experience from setting up DMPs before this project, so initially we were unsure what approach to use. After some training, and guidance provided by the OHEJP management, we chose to use an overarching plan/template with detailed Excel-files for each WP and task to keep track of how data is handled. Once we had this in place, updates have been easy.	No
JRP07-LISTADAPT	The paper, submitted to the review Scientific Data in July 2020, listed all the strains, metadata and genomes produced during LISTADAPT.	All the produced genomes will be available to the scientific community (umbrella Bioproject in European Nucleotide Archive (ENA) as soon as the paper is accepted.
JRP08-METASTAVA	Complex procedure encompassing the complete H2020-compliant DMP description for a project with very limited resources.	No



Project	Have you uploaded a first version of the project's DMP to the DMP group on the OHEJP website?	Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.
JRP09-AIRSAMPLE	No.	Yes, provided.
JRP10-MOMIRPPC	Too numerous metagenomics data to detail a DMP plan per database	Yes
JRP11-MEDVETKLEBS	No	No
JRP12-FARMED	We are awaiting instructions from Dr Geraldine BOSERET at Sciensano to use the DMP use-friendly tool, originally planned for release in June 2020	No
JRP13-WORLDCOM	The DMP V1.1 is ready to submit, but has not yet been submitted due to instruction from OHEJP DMP contact Dr Boseret (Sciensano). Thus, the deadline has been postponed in order that the first DMP can be created using a "specific intuitive use-friendly tool", which was recently acquired by the OHEJP WP4 team.	Yes, the DMP coordinator (Dr Liam Burke) was unable to create a DMP using the originally indicated online tool at https://dmponline.be/ . However, he was able to access the DMPs of other consortia through the DMP group on the OHEJP website. He used these DMPs, plus the D4.7 Guidelines for Data Management Plan implementation for guidance in developing the WorldCOM DMP. Dr Burke has completed CDP training and is ready to update CDP platform with information on WorldCOM data from task leaders.
JRP14-FULLFORCE	No. This will be compiled and uploaded by September 2020, in collaboration with Geraldine Boseret.	A lack of clear instructions. However, these were solved in July 2020, allowing ample time to compose the DMP by September.



Project	Have you uploaded a first version of the project's DMP to the DMP group on the OHEJP website?	Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.
JRP15-FEDAMR	Not yet, but it is expected by M34. At the Project Leader's Forum we were informed about a tool that will help Project partners to develop easily their DMP, which we expected to be delivered before the end of M31. However, the training was provided by the OHEJP WP4 team on August 5th 2020 for the new OHEJP data management platform CDP; the application is now being adapted with details of FED-AMR data for a first version of DMP, which will be also done throughout all the project, with information provided to the leader and deputy leader by task leaders on their datasets. This task is ongoing.	N/A
JRP16-TELEVIR	Initially the OHEJP project management team postponed the deadline of first DMP delivery in order to find a specific intuitive use-friendly platform that can help us create our DMP. After the DMP leader attended the training session (5th of august 2020), an excel sheet will be distributed to all TELE-Vir partners for them to fill in the required information based on the Lisam platform (https://apps.lisam.com/app/#Apps/CDP)	It has been difficult to simplify the DMP, but after the training session for the Lisam platform, the generation of the DMP is much easier.
JRP17-IDEMBRU	No, since after communication with EJP DMP contact point person we were informed that new guidelines and new platform will be communicated to all projects. After finishing training session, we are adapting our DMPs and will upload them as soon as possible.	Very broad descriptions and no indication of information importance. Redundancy of several fields. Some example would be useful.
JRP18-MEME	After OHEJP training in September 2020, we will start working on the delivery of DMP.	N/A
JRP19-PARADISE	Not yet, but the Consortium already stated discussion on this.	N/A



Project	Have you uploaded a first version of the project's DMP to the DMP group on the OHEJP website?	Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.
JRP20-DISCOVER	No, not yet. Considering the structure of the new DMP tool, we will upload information about our selected datasets, when we have a 'finalised' dataset ready for analysis. Currently, we have just finalised selecting appropriate dataset and decided on how to improve these.	Although, we haven't yet uploaded information about our data, we have explored the tool, and it appears not to be so well suited for describing datasets for source attribution, which typically consist of data from different sources/species and therefore also consist of different sampling material (matrix). So strictly following the data-table structure when reporting will in principle not allow us to upload information on a specific and delimited SA dataset under a single "Dataset-ID", which we consider the most appropriate approach – especially, for informing other potential researcher of the usefulness of the datasets for SA. Still, we will try to explain as best as we can.
JRP21-BIOPIGEE	No, we have not. After preparing all the WP-specific xls-sheets to be useful for DMPonline, that will not be needed now (as a new tool is being proposed), we stopped working on that. We attended a training session for the new tool on 9th September 2020 and have started to fill information in CDP.	Yes. We were not able build a new DMP at DMPonline as the BfR is not member institution of DMPonline.
JRP22-TOXOSOURCES	Yes, it was uploaded M30 as planned.	No.
JRP23-ADONIS	No this has not been done yet.	N/A
JRP24-BEONE	Yes (by M33)	Not so far

5.1.10 Detailed follow-up of JRP

5.1.10.1 JRP01-R1-AMR1-IMPART

5.1.10.1.1 Summary of the work carried out in the JRP

The IMPART project received an extension from M30 to M36 and new due date is December 2020. The final ring trials of WP1 and WP2 have been performed respectively in May and September 2019 with



eleven partners involved in both trials. The results of these ring trials are currently being analysed and evaluated. Within WP2, all participants of the ring trial have received a report containing their own reported results compared to what was expected. Preliminary results of the WP2 ring trial were presented at the 2nd OHEJP-ASM. All participants of WP1 and WP2 will receive a final report in September 2020. Within WP3, the collection of MIC distributions from all partners was finished in April 2020. Quality control and uploading of the MIC distributions on the EUCAST website will be finished in September 2020 followed by the calculation of Epidemiological Cut Off (ECOFFs) values for all drug/bug combinations for which we collected the sufficient quality and quantity of MIC data available. Within WP4, the assembly and characterization of *Clostridium difficile* strain collection was finished in December 2019. All 525 strains were completely characterized according to the proposal and the report 2019. Inhibition zone diameter distributions have been determined for eight antimicrobials analysing 525 strains of the *C. difficile* strain collection. Preliminary results and cut-off values were presented at the 2nd OHEJP ASM and uploaded to the OHEJP website. This task will be finalized in September 2020 (M33). The ring trial to finalize the validation of the proposed disk diffusion method was conducted in June 2020 with seven participating laboratories. The results were reported until 30th June 2020. Reports were sent out to the participants on 25th August 2020 and uploaded to the group on the OHEJP website. Within WP5 emails were sent out by the WP leaders 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. The closing meeting was initially planned in June 2020, but postponed to the end of 2020 because of COVID-19. Hopefully the meeting can still be held physically at Schiphol airport. Alternatively, an online meeting will be organised. Furthermore, three abstracts comprising results from WP2, WP3 and WP4 were presented as poster at the OHEJP ASM online meeting in May 2020.

5.1.10.1.2 Progress of the project: description of activities

WP1: Selective isolation, detection and characterization of colistin-resistant Enterobacteriaceae (M1-M30)

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)

Task was finalized in November 2019, see annual report 2019.

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

Task was finalized in June 2019, see annual report 2019.

JRP1-WP1-T5: Performing the final ring trial (M17)

Final ring trial was organized in May 2019. (see 9M report from 2019 for details).

JRP1-WP1-T6: Analysis of the results and reporting (M18-M19)

This task has been accomplished from September 2019 to May 2020. Final report to the 11 participants should be sent out at the end of summer 2020 (M33).

JRP1-WP1-T7: Publication in peer-reviewed journal (M25-M30)

This task is planned for the second half of 2020.

JRP1-WP1-T8: Plan joint implementation (M25-M30)



This task is planned for the second half of 2020.

WP2: Selective isolation, detection and characterization of carbapenemase-producing Enterobacteriaceae (M1-M30)

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)

Task was finalized in July 2019, see annual report 2019.

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

Task was finalized in September 2019, see annual report 2019.

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

The final ring trial was performed among eleven laboratories participating in IMPART. The samples (n=8) were prepared at Anses Fougères Laboratory on Friday September 6th 2019 and shipped to the eleven participants on Monday September 9th 2019. Analysis started immediately at arrival of the samples. Prior to the sample shipment, the ring trial protocol was distributed per email to each participant. The six selective agar plates to be tested, were shipped with the samples.

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

Each participating lab has received their own report containing what was reported and what was expected, M27 March 2020. The analysis and drafting of the report from the entire ring trial was delayed due to the CoViD-19 situation, but a draft was sent to the participants before the summer holidays (M30) and the report will be finalized by M33, September 2020.

JRP1-WP2-T7: Publication in peer-reviewed journal (M25-M30)

The project received a last minute extension from M30 to M36 and due date is December 2020. This task has been delayed and is expected to be performed during the last months of the project.

JRP1-WP2-T8: Plan joint implementation (M25-M30)

The project received a last minute extension from M30 to M36 and due date is December 2020. This task has been delayed and is expected to be performed during the last months of the project.

WP3. Establishing epidemiological cut-off values (ECOFFs) (M1-M30)

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

Task was finalized in July 2019, see annual report 2019.

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

Task was finalised in May 2020 (collection of MIC distributions completed)

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

Nine partner institutes performed AST of 2,831 bacterial isolates involving 19 different veterinary pathogenic bacteria including staphylococci (*Staphylococcus pseudintermedius*, *S. hyicus*), streptococci (*Streptococcus agalactiae*, *S. dysgalactiae*, *S. uberis*, *S. suis*, *S. canis*, *S. equi* subsp. *zooepidemicus*, *S.*



equi subsp. *equi*, *S. equisimilis*), *Pasteurella multocida*, *Mannheimia haemolytica*, *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, *Haemophilus parasuis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Klebsiella variicola*. This resulted in 1,310 MIC-distributions consisting 47,640 MIC-values of 34 different antimicrobials. All QC data have been collected by the WP leader. Quality control will be finished in September 2020.

JRP1-WP3-T4: Analysis of the data and publication of ECOFFs (M25-M30)

All MIC distributions will be uploaded in the EUCAST database in September 2020. Analysis of data and publication and new ECOFFs will be published in December 2020.

WP4: Developing and optimizing a disk diffusion method for antimicrobial susceptibility testing of *Clostridium difficile* (M4-M30)

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

This task was finalized in December 2019, see annual report 2019.

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

This task was finalized in December 2019, see annual report 2019. A few more strains were added in the third year, so that the final number of strains in the strain collection is 525. All strains were completely characterized according to the proposal and the report 2019. JRP1-WP4-T3: Performance of a ring trial study (M22-M27)

The ring trial was conducted in June 2020 with seven participating laboratories. The results were reported by 30th June 2020. Reports were sent out to the participants on 25th August 2020 and uploaded to the group on the OHEJP website.

JRP1-WP4-T4: Producing inhibition zone diameter distributions and proposing cut-off values for *C. difficile* (M17-M30)

Inhibition zone diameter distributions have been determined for eight antimicrobials analyzing 525 strains of the *C. difficile* strain collection (see JRP1-WP4-T2). Preliminary results and cut-off values were presented at the 2nd OHEJP-ASM and uploaded to the OHEJP website. This task will be finalized in September 2020 (M33).

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-M30)

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-M30)

Emails were sent out by the WP leaders 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. The closing meeting was initially planned in June 2020, but postponed to the end of 2020 because of COVID-19. Hopefully the meeting can still be held physically at Schiphol airport. Alternatively, an online meeting will be organized.

JRP1-WP5-T3: Communication beyond IMPART (M1-M30)



Three abstracts comprising results from WP2, WP3 and WP4 were accepted as poster presentations at the online OHEJP ASM meeting in May 2020.



5.1.10.1.3 Progress of the research project: deliverables and milestones

5.1.10.1.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
01	D-JRP1-1.6	Evaluation of the final ring trial	M21		M33	Confidential until paper is published (D-JRP1-1.7)	9
01	D-JRP1-1.7	Publication in an open-access peer-reviewed journal	M30		M36	Public	8
01	D-JRP1-1.8	Proposal(s) for epidemiological study to monitor resistance to colistin	M30		M36	Public	1
01	D-JRP1-2.6	Evaluation of the final ring trial	M26		M33	Confidential until paper is published (D-JRP1-2.7)	9
01	D-JRP1-2.7	Publication in an open-access peer-reviewed journal	M30		M36	Public	8
01	D-JRP1-2.8	Proposal(s) for epidemiological study to monitor resistance to carbapenems	M30		M36	Public	1



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
01	D-JRP1-3.2	Analysis of the data and publication of ECOFFs	M30		M36	Public on EUCAST website	9
01	D-JRP1-4.1	Collection of inhibition zone diameter distributions	M21	M30		Confidential until paper is published (D-JRP1-4.2)	9
01	D-JRP1-4.2	Publication in an open-access peer-reviewed journal	M30		M36	Public	8
01	D-JRP1-4.3	Performance of a ring trial study	M27		M33	Public on EUCAST website	2, 9
01	D-JRP1-5.3	IMPART News online on One Health EJP website	M30		M36	Public	8
01	D-JRP1-5.1	Invitation to the final meeting sent to participants	M27		M33	Public	10
01	D-JRP1-5.6	Final meeting notes sent to participants	M30		M36	Public	10

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of



surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity

5.1.10.1.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
01	M-JRP1-9	MIC data collection complete (WP3)	M24	Yes		Achieved in M29
01	M-JRP1-10	Proposal of cut-off values based on inhibition zone diameter distributions (WP4)	M26	No	M35	
01	M-JRP1-11	Final meeting (notes of the meeting)	M30	No	M33	Due to the COVID-19 issues, the final meeting had to be postponed until a better epidemiologic situation might allow us to organize a physical meeting between IMPART partners. Hopefully, this meeting is planned to month 35-36



5.1.10.1.4 Ongoing collaborations

WP1: in collaboration with EFSA and EURL-AR, we will be able to review together the output of this work package to determine if a future surveillance programme is either feasible and relevant at a European level.

WP2: In collaboration with EURL-AR on the draft of the protocol. After the report is finished, both EURL-AR and EFSA would have great benefit of the outcome of the multicentre study to modify the protocol for the harmonized surveillance of carbapenemase producing *Enterobacteriaceae* at a European level.

WP3: In close collaboration with EUCAST/VetCAST in setting new ECOFFs (PL of IMPART is member of the steering committee of VetCAST). In addition, MIC distributions produced by the IMPART consortium have been presented by the PL leader at the first meeting of COST ACTION (number CA18217) called ENOVAT held on 24 February 2020 in Tirana (Albania). PL leader is member of the management committee of ENOVAT. The IMPART results will be used as starting point for the collection of more MIC distributions in order to set additional ECOFFs for veterinary antimicrobials.

WP4: OHEJP project FED-AMR aims to use the proposed protocol and cut-off values for the agar disk diffusion in IMPART to characterize and compare *C. difficile* strains with regard to their antimicrobial resistance.

5.1.10.2 JRP02-AMR2-ARDIG

5.1.10.2.1 Summary of the work carried out in the JRP

The ARDIG project has continued to progress, and after 30 months there have been substantial achievements made by 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. Several work package specific meetings have also taken place over the past six months and there have also been regular communications within the consortium by email. Both oral and poster presentations made by ARDIG partners were well received at the OH-EJP Annual Scientific Meeting held virtually in May 2020.

WP1 (Comparison of AMR and antibiotic sales/usage data collected through existing national surveillance and research programs and assessment of risk factors). Antimicrobial resistance (AMR) and antimicrobial usage data has been obtained from healthy and diseased animals from different partners and countries. From preliminary analysis of the data it has become apparent that due to differences in methodology and interpretation criteria, it will be difficult to compare MIC or disc diffusion data obtained for AMR from *Escherichia coli* isolated from diseased animals. Therefore, ARDIG partners have been exploring statistical methods to set epidemiological cut-off (ECOFF) values for each antimicrobial. It is expected that by using a harmonised method to calculate ECOFF for each antimicrobial, a more detailed analysis of trends in the clinical data set can be made across countries, as well as enabling comparisons between clinical and non-clinical *E. coli* datasets.

WP2 (**Longitudinal studies of AMR persistence**). Both Med and Vet partners have been collecting *E. coli* isolates from both retrospective as well as prospective studies, including *E. coli* isolated from urinary tract infections from local hospitals and General Practitioner over 12 months. Several partners have been characterising *E. coli* isolated through national surveillance. In addition, all partners who participated in a whole genome sequencing (WGS) AMR workshop have submitted ~50 WGS each (~450 in total) for analysis by five pipelines: APHA SeqFinder/Abricate, PHE GeneFinder, WBVR, Ariba, ResFinder/PointFinder. MIC determinations to the EFSA panel of antimicrobials for these isolates have also been performed to help compare the AMR genotypes with the corresponding phenotypes.



WP3 (AMR characterization, transmission of plasmids and fitness of MDR isolates). All WP3 partners have continued AMR gene, plasmid and mobile genetic element characterization in isolates collected in WP2 by WGS (short and long reads), as well as other molecular techniques. Phylogenetic analysis performed by partners on their dataset have also helped identify transmission events or epidemiological links between isolates of particular sequence types or clones of *E. coli* collected from different hospital or farm settings. In addition, all WP3 partners have participated in the AMR pipeline comparison work to help harmonise *in silico* AMR gene prediction. The results of running WGS data from ~450 isolates, through five different AMR pipelines, have been compared by partners with their corresponding MIC phenotypes. These findings are currently being compiled by APHA for further analysis to assess the impact of methodology on AMR gene prediction.

5.1.10.2.2 *Progress of the project: description of activities*

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 (M1-M36)

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

Task completed. See second annual report, 2019.

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

BfR

Univariate analyses were performed for each animal species and antimicrobial/antimicrobial family, assessing each explanatory variable (data type, country and year). Further multivariate analyses assessing only relevant variables were carried out when more than one variable per antimicrobial/antimicrobial family and animal species showed significant associations in univariate logistic regression. Data type variable was analysed as a binary factor (clinical vs. non-clinical), year as a numeric factor and country as a binary (comparing two by two) and/or a categorical factor (comparing all three countries). Odd ratios were used to determine which populations showed higher resistance proportions. Analyses were carried out on national level and across countries. On the national level, more antimicrobials could be included and there are fewer limitations with respect to non-harmonized use data. Across countries, there were limitations with respect to a necessary overlap of tested antimicrobials and investigated animal populations on the clinical side.

In relation to AMU in livestock, they are being assessed in order to try to overcome the lack of harmonization. In case of successfully solving the harmonization issue, they will be included in the analyses as a risk factor.

Data on clinical isolates from food producing animals in France were obtained in collaboration with the EU-project JAMRAI that strives to establish a European data collection on AMR data from clinical isolates and is therefore confronted with the same issues of lack of harmonization. With the help of the colleagues from ANSES involved in JAMRAI, ARDIG now has access to quantitative data on AMR in clinical isolates of *E. coli* from pigs and poultry in France in addition to the data on cattle that were previously available.

APHA

For this task, APHA has been responsible for the 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 to identify trends and associations between clinical AMR data and antimicrobial usage data. The analyses are currently on-going. Only three countries provided relevant data; 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. Obtaining individual data from France is being discussed. As previously reported, considerable differences in laboratory methods, interpretation criteria and standards used by each country were identified. To overcome these obstacles, APHA together with BfR and ANSES has carried out analysis (using each country's own data) of epidemiological cut offs for the clinical AMR based on the NRI method. Several teleconferences have been carried out in order to discuss this work. The use of a harmonised method will allow more detailed analysis of trends in clinical AMR between the three countries and analysis of associations between AMR and antimicrobial usage.

In addition to the analyses carried out, APHA also delivered an oral presentation during the OH EJP ASM in May entitled as: "Antimicrobial resistance of *Escherichia coli* isolates originating from diagnostic submissions from veterinary scanning surveillance in UK, Germany and France from 2014 to 2017".

NVI

A meeting (digital) was held together with the partners of WP1 in April 2020, where it was decided to try to explore and use different statistical methods (NRI Kronvall and Turnidge) to set epidemiological cut-off values for each partners own distributions on both data types (clinical and non-clinical isolates) from each animal species, separately. NVI has carried out analysis on its own data of epidemiological cut offs for the clinical AMR based on the NRI method.. Final results will be compared among the partners to assess the usability of the methods and ECOFFs set. Data from 2015, 2017 and 2019 (cattle and pig) are being further investigated for trends.

RKI

The RKI has transferred the extracted AMR and AMU data (2014-2017) from its surveillance system to the joint database after the transfer/sharing agreement had finally been approved. RKI- activities of joint investigation of trends have been delayed due to the ongoing Coronavirus-outbreak. We are dependent on the data managers, who are all obliged to work for COVID-19. To allow for joint analysis of risk factors using comparable human AMR-data (Germany and UK), we will explore which variables can be added to the database (e.g. geographical data to the AMR community data).

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

This objective is ongoing. Recommendations for improved surveillance strategies and harmonized data collection systems will be drawn following the results of analysis of clinical, non-clinical and human AMR data and their associations with antimicrobial usage performed in collaboration between APHA, BfR and ANSES.

The final recommendations will be based on challenges encountered when attempting to analyse the data across countries and the potential solutions developed to overcome the challenges.

WP2. Longitudinal studies of persistence ESBL/AmpC/carbapenem/mcr-1 and -2/PMQR producing Enterobacteriaceae on farms or hospitals (M1-M36)

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



NVI

Isolates from a previous study focusing on cephalosporin resistant Enterobacteriaceae 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 batches of animals on the same farm or broiler house. In total, 11 different *E. coli* Sequence Types were identified. Data on serotypes will be extracted from WGS data. 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 in ongoing and a publication is in planned.

IP

In order to address the dissemination of MDR *E. coli*, we are working on two strain collections. For the first we are performing a retrospective study using the i-bird strain collection in collaboration with Lulla Opatowski and Didier Guillemot (Institut Pasteur). The second isolate collection that we are working with are from a prospective study (see below).

The i-bird study (Individual-Based Investigation of Resistance Dissemination coordinated by D. Guillemot) is a 4-month study which took place in 2009 in a rehabilitation hospital and followed up more than 600 patients and Health Care Workers with weekly rectal swabs performed in patients and human-human proximities recorded from wireless captors. 604 ESBL-Enterobacteriales were isolated from 84 patients. Our objectives are (i) to characterize strains shared by different patients and to infer transmissions; (ii) to identify cases of ESBL-plasmid transfers and (iii) to identify factors contributing to ESBL-*E. coli* transmission in this hospital environment. In order to characterize the within host diversity we have selected several *E. coli* isolates (up to eight) in some patients. We have first analyzed 113 *E. coli* and 61 *Klebsiella pneumoniae* isolates. The 113 *E. coli* isolates were from 65 patients (we have selected at least one isolate per colonized patient). All the isolates have been sequenced using the Illumina technology (see WP3). Based on results from the analysis of those isolates 142 additional isolates have been selected to better characterize the within-host diversity (94 *E. coli* and 48 *K. pneumoniae*). However, due to the COVID-19 crisis these isolates have not been sequenced yet.

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

UoS

Task completed. See second annual report, 2019.

WBVR

In 2019 and 2020, a group of approximately 700 veal calves in the Netherlands was individually followed from birth to slaughter on 5 to 6 sampling moments. The animals were born on 13 dairy farms



spread throughout the country and transported between 14 and 28 days of age to 8 veal farms for fattening. Rectal swabs were taken at each sampling moment for selective culturing on cefotaxime containing media to determine the prevalence of ESBL/AmpC producing *E. coli*. At the dairy farms the prevalence of *E. coli* ranged from 0-86% (average 26.4%). At all veal farms the prevalence of ESBL/AmpC producing *E. coli* amongst the animals went up to >50% at least one sampling moment. In 6 farms, prevalence significantly decreased over time.

Extensive records were recorded on farm management, hygiene, antimicrobial usage and health parameters of the animals and statistical analysis is currently carried out to determine correlation with ESBL carriage. Molecular analysis of ESBL genes and MLST typing is currently determined to allow defining selection criteria for whole-genome sequencing. These data will be used to determine transmission of ESBL/AmpC *E. coli* and plasmids between individual animals on the farms and colonisation time of a specific *E. coli* strain.

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. In the spring of 2020 it was decided not to perform a longitudinal study in pigs, but instead participate in a study with longitudinally sampled human bacterial isolates (task3/WP2).

APHA

We have completed the collection of samples for a longitudinal study which focused on two sites of the same UK pig farm which are separated geographically; a non-clinical farm site that houses five age classes of healthy pigs and has ceased group antimicrobial treatments for at least five years, and a clinical farm site that is comprised of three age classes of pigs sent from healthy sites following disease, that have subsequently undergone group and individual antimicrobial treatment. Faecal samples were obtained from both sites from pigs at four time-points at 6 month intervals over 18 months, alongside seagull faecal samples from two time points. Representative *E. coli* were purified from all time points from non-selective and antibiotic selective agar plates (cefotaxime and ciprofloxacin), followed by Illumina whole-genome sequencing (WGS). The WGS data was analysed by reconstructing phylogeny of the *E. coli* isolates, determining presence of AMR genes, plasmid replicon types, in silico Multilocus Sequence Type and mobile genetic elements (WP3).

MIC determination for the isolates collected during visits 4 and 5 has also been completed. An overall analysis of the MIC data across 5 visits has been carried out. Temporal trends were evaluated, as well as a comparison between antibiotic treated and non-antibiotic treated groups of pigs was carried out. A draft publication is in preparation to report these results.

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

UoS

The UoS has finalised the collection of *E. coli* isolates from human urinary tract infections in collaboration with a local hospital. A total of 272 isolates were collected during a longitudinal study taking place from January to December of 2019. Out of 272 isolates, 134 were collected from GP patients (representing community-acquired infections) and 138 from patients admitted to hospital (representing healthcare-acquired infections). The phenotypic resistance profile of the collection



showed a higher percentage of resistance in isolates from hospitals when compared to GPs. The set of isolates will be sent to PHE for sequencing.

Another local hospital has been collecting an additional set of uropathogenic *E. coli* isolates from June 2019 until May 2020. The new set of isolates collected in this longitudinal study will be shortly sent to the UoS for sequencing. The antibiotic resistance profile of the isolates and any relevant clinical data from the corresponding patients will also be collected to evaluate the risks of resistance.

NVI

E. coli isolates from humans with UVI in a large centrally located hospital in Norway, and from GP in the same area were collected (the 20 first isolates of each category, from each month in 2019). The isolates were sent us monthly and they are stored at NVI, together with relevant/requested data. The isolates are currently sequenced at NVI and MIC determination will be carried out.

IP

The second isolate collection that we are working with are from a prospective study. For this we performed a longitudinal sampling of *E. coli* responsible for urinary tract infections (UTI). The objective of this study is to compare the diversity and the ARG profiles of *E. coli* strains responsible for UTI in four ARDIG participating countries, who are using a similar protocol. The work program was to collect each month during 12 months, 10 isolates from community infections and 10 isolates from hospital patients. This work was performed in collaboration with Thierry Naas at the Bicêtre Hospital. Due to difficulties to have access to a private microbiology laboratory, we have selected each month 10 isolates from outpatients (from the emergency of the Bicêtre Hospital) and 10 isolates from hospital patients. Isolates were picked randomly from the isolate collection from the Bicêtre Hospital Clinical Microbiology laboratory. In total 250 isolates have been obtained and all isolates have been sequenced by using the Illumina technology (WP3).

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

BfR

Since the beginning of the ARDIG project, we have characterized *Escherichia coli* isolates recovered from livestock and food in 2017 for their phenotypic and genotypic resistance profile. In one part of the ARDIG project we had focused on (fluoro-)quinolone-resistant isolates, while the second part deals with mobilizable colistin resistances (*mcr*-genes).

WBVR

Between 2014-2019 in the Netherlands, blaCTX-M-14 and blaCTX-M15 harbouring isolates have increased in prevalence in both veal calves and dairy cattle. In-depth molecular characterisation is being performed and currently ongoing but preliminary results show that these are often encoded on phage-like plasmids which cannot be detected by the widely used PBRT method.

IP

In collaboration with Thierry Naas, we are analysing the genomic diversity of carbapenemase producing *E. coli* received by the National Reference Centre laboratory until 2015 (manuscript is in preparation).



NVI

Analysis of data collected in WP2/task1 is ongoing.

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

APHA, ANSES, BfR, IP, NVI, PHE, UCM, UoS, WBVR

ARDIG partners have submitted WGS of up to 50 *Escherichia coli* isolates per institute to a repository and five partners (APHA, WBVR, PHE, UCM, and NVI), representing the diversity of pipelines (APHA SeqFinder/Abricate, PHE GeneFinder, WBVR, Ariba, ResFinder/PointFinder), have analysed ~450 WGS data through the pipelines.

All partners have performed MICs on the EFSA panel of antimicrobials on their isolates from this panel of 450 isolates, so phenotypic data could be obtained for comparison with the AMR genotypes resulting from each of the 5 bioinformatic pipelines.

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:

Plasmid comparison:

Incl1 & CTX-M-1

InclK/I & CMY-2

Any Inc & MCR 1&4

InclL/M & Incl NDM1 plasmids

CTX-M-14/15 in non-typed plasmids

Isolate comparison :

E. coli ST744

E. coli ST38

E. coli ST1196

UoS

The UoS has agreed to carry out a comparative analysis of isolates harbouring *bla*CMY-2 gene. Short and long read sequences have been requested from the partners involved in WP3, representing 6 different European countries (UK, France, Norway, Germany, Spain and The Netherlands).

APHA

APHA has collected WGS from *E. coli* ST744 isolates from partners in the consortium for further characterisation. The aim is to compare the WGS data by reconstructing phylogeny of the *E. coli* isolates, determine presence of AMR genes and mobile genetic elements present in the isolates.

BfR

In order to strength the collaboration of the ARDIG partners, individual projects of the partners on specific *E. coli* lineages, plasmid types or resistance genes were supported by the exchange of WGS



data. Based on the prevailing genome data of the NRL-AR (BfR), we had chosen to support several projects (i.e. ST-744-, ST-1196-, ST-38- or mcr-1-project) by providing short- and/or long-read sequencing data.

WP3. AMR characterization, transmission of plasmids and fitness of MDR isolates (M6-M36)

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

APHA, ANSES, BfR, IP, NVI, PHE, UCM, UoS, WBVR

Five partners (APHA/PHE/WBVR/NVI/UCM) have run WGS data from 450 *E. coli* isolates submitted by ARDIG partners (APHA, ANSES, BfR, IP, NVI, PHE, UCM, UoS, WBVR) through their pipelines (APHA SeqFinder/Abricate, PHE GeneFinder, WBVR, Ariba, ResFinder/PointFinder) to assess the impact of similarities and differences in methodologies commonly used for AMR genotyping within European Institutes on AMR gene predictions. Results from the pipelines analysis have been shared with the consortium, and partners have compared the AMR genotype prediction for each isolate with the corresponding antimicrobial tested by MIC (EFSA panel), using the database/gene catalogue available to them through their Institute. The results of the genotype/ phenotype comparison are being compiled by APHA for further analysis to identify agreements and discrepancies between each approach. Such comparisons are of extreme importance to EFSA and ECDC as they are moving to reporting of AMR data by genotyping, and we expect our study to make a valuable contribution to harmonisation of current approaches between animal and human sectors in this context of One Health.

UoS

The UoS has completed the molecular characterisation by whole genome sequencing of 337 *E. coli* isolates from different sources. The set includes 94 isolates from human urinary tract infections, 111 from human blood bacteraemia, 20 from healthy human faeces, 47 from healthy pig faeces, 10 from healthy chicken faeces, 28 from healthy cattle faeces and 27 from avian colibacillosis. The reminder of the isolates from the animal and human longitudinal studies will be sequenced in the coming months.

Bioinformatic analysis of the sequenced isolates has been performed, including phylogenetic analysis, pangenome analysis and database searches 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 underway to look for patterns that maybe useful for understanding AMR prevalence in humans and animals.

BfR

Investigations on (fluoro-)quinolone-resistant *E. coli*: Isolates with MIC values of ≥ 8 mg/L and/or ≥ 0.25 mg/L against nalidixic acid and ciprofloxacin, respectively, were chosen for further analysis. In total, 452 *E. coli* of the National Reference Laboratory for Antimicrobial Resistances (NRL-AR) were screened for the presence of qnrA, qnrB, qnrC, qnrD, qnrS and qnrVC. The most frequent qnr-variant was qnrS (16.1%). Despite of qnrS, the occurrence of other tested qnr genes was rather low. For phylogenetic analysis, qnr-positive *E. coli* isolates were subjected to pulsed-field-gel-electrophoresis (XbaI-PFGE). In general, the phylogenetic dendrogram showed a high heterogeneity but a few isolates did show certain relationship. By S1-PFGE, the plasmidal state of the 103 qnr-positive *E. coli* isolates was determined and the plasmidal localization of the qnr-gene was confirmed by DNA-DNA hybridization. For determination of the genetic basis, all 103 *E. coli* were subjected to illumina (NextSeq) sequencing. For all isolates, the ST-type distribution, matrix belongings and occurrence of known resistance genes, distinct to qnr are available. To determine the diversity of prevailing plasmid types carrying qnr-genes,



in-silico plasmid finishing was conducted. For this, we had used the refSNPer workflow (https://gitlab.com/bfr_bioinformatics/refsnper/-/tree/master). The pipeline elects the closest reference, by mapping the input sample to a chosen reference set and identifies the coverage by using bedtools. With this, we had classified several qnr-carrying plasmids to known reference plasmids and track putative plasmid paths. Moreover, we discovered novel qnr-plasmids, which are not described so far. Altogether, with those comprehensive investigations of qnr-positiv *E.coli* isolates a thorough and complex picture will be generated for the mobile genetic elements and their dissemination as well as their commonalities in commensal *E. coli* within the isolates from the German zoonoses monitoring program in 2017. Currently, two publications were prepared. One is focusing on the performance of different sequencing platforms for AMR prediction in *E. coli*, while the second will deal with the diversity of qnr-carrying *E. coli* and the diversity of qnr-plasmids.

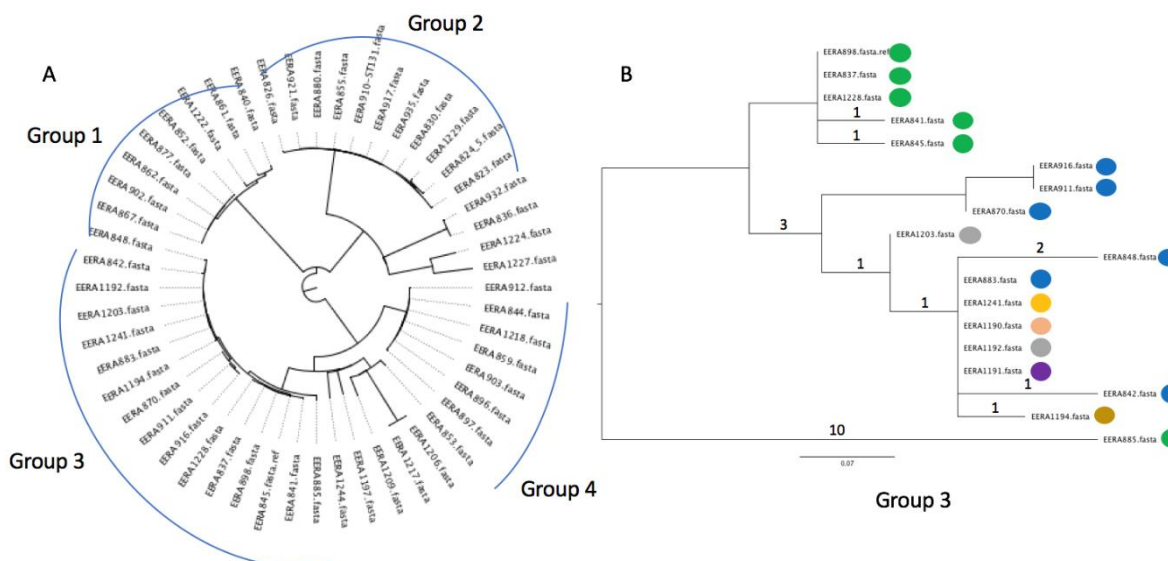
WBVR

In the Netherlands, over 350 caecal samples from broilers were collected over a 10 month period on 5 farms in 2018-2019. Using selective culturing on cefotaxim containing media, 23.8% of these samples were positive for ESBL/AmpC producing *E. coli*, very similar to the 23% prevalence of ESBL/AmpC producing *E. coli* found by the national surveillance in 2018. Molecular analysis indicated that of these isolates, 85% produced blaCTX-M-1, blaCMY-2 or blaSHV-12, similar to results of the national surveillance.

As only 1 farm contained ESBL/AmpC producing *E. coli* on more than 3 consecutive sampling moments, 120 *E. coli* isolates from this farm were selected for whole genome sequencing. Phylogenetic analysis indicates that several lineages of *E. coli* are present in the farm which were found in all three barns that were monitored over several production rounds, while each production round was mostly dominated by a specific lineage. On the farm, blaSHV-12 encoded on an IncI1 plasmid was most prevalent, followed by blaCTX-M15 on an IncFIB plasmid and blaCMY-2 on an IncI1 plasmid. While blaCMY-2 was contained to a specific lineage, blaSHV-12 and blaCTX-M-15 were present in more diverse backgrounds and identical *E. coli* that do not contain the plasmids were also present indicating that the resistance genes are disseminated through the farm by horizontal gene transfer.

IP

Analysis of the i-bird collection. The 94 isolates belong to 28 different STs revealing a broad diversity of ESBL-Ec carried by the patient. However, 59 belong to ST131 (62%). They are carriage isolates recovered from systematic screening and not clinical isolates, showing that, in this environment, ST131 is dominant even among colonizing ESBL isolates. In-depth phylogenetic analysis of the 91 isolates revealed cases of transmission or of a common source only for ST131 isolates. These isolates clustered in four groups (Figure 1) with exchanges between patients, as exemplified for group 3 identified in seven patients. Furthermore, we observed a within host diversity we aim to further analyze. For this purpose, additional isolates will be sequenced to better characterize the within-host diversity and to infer transmission and directionality. The genomic data will be confronted with temporal and contact data collected during the i-bird project. We also identified three cases of plasmid transmission from *K. pneumoniae* to *E. coli*.



Carbapenemase producing *E. coli* are very diverse and belong to 162 different STs. However, 45% of the isolates belong to three dominant lineages: CC10 (including ST167), CC23 (including ST410) and ST38. By an in-depth analysis of ST410 isolates from the NRC and publicly available *E. coli* sequences, we have proposed a model for the dissemination of specific lineages enriched in CP-Ec. These lineages show a reduced susceptibility to β -lactams due to variants in the porin genes *ompC* and *ompF* and to mutation in *ftsI* coding for the penicillin binding protein 3. These lineages are also frequently carrying ESBL genes from the CTX-M family (Patino Navarrete et al. Genome Medicine 2020). We are currently focusing our analyses on isolates belonging to the ST38 which represents the dominant CP-Ec ST in France but also in the Netherlands. By integrating the analysis of ST38 isolates from animal origin collected by the ARDIG consortium and human isolates we aim to identify additional factors contributing to the acquisition of carbapenemase genes, in particular of those of the *bla*OXA48 family and to their dissemination.

Analysis of UTI isolates.

The 250 isolates from UTI have been sequenced by the Illumina technology. Preliminary analyses have been performed. The isolates belong to 81 different ST revealing a broad diversity. The four most frequent STs are also frequently reported in other studies as responsible for UTI (ST73, 31 isolates; ST131, 20 isolates; ST69, 19 isolates and ST95, 18 isolates). Susceptibility to β -lactams has been determined by disc diffusion assays for the first 120 isolates. MICs of 25 isolates for 14 antibiotics were determined by serial microdilution by ANSES for benchmarking pipelines for ARG identification and for genotype phenotype correlation. The sequences of all isolates were only recently obtained and the analyses are only preliminary. The next step will be to combine the analysis of UTI *E. coli* from the ARDIG teams participating to the longitudinal study.

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.



A request for long and short read sequence data for strains with blaCTX-M-1/IncI plasmids have been sent to the consortium members. The outcome will be a comparative study on IncI/blaCTX-M-1 plasmids with data from several European countries (coordinated by NVI).

Sequence data for ST744 isolates have been sent to APHA for inclusion on a study focusing on this ST.

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 (isolates with blaCTX-M-1 located on IncI1-ly plasmids). The blaCTX-M-1/ IncI1-ly plasmids studied grouped into two main plasmid lineages ;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. The study was published in Frontiers in Microbiology.

APHA

The farm isolates were characterised further by analysis of the WGS data. Levels of AMR genes present within indicator *E. coli* from non-selective media varied significantly between sites, with 84% identified as multi-drug resistant (3 or more AMR genes) on the clinical site in comparison to 4% on the non-clinical, with a corresponding difference in Sequence Types (ST) identified. In contrast, *E. coli* isolated on both sites from antibiotic selective media were mostly identical STs, with ST744 being the dominant *E. coli* isolated from ciprofloxacin containing media and ST88 the dominant from cefotaxime media. Persistence of ST744 clones with <10 SNP differences were identified across time-points, age classes of pigs and seagull samples in both sites. Both STs have previously been reported from animals and humans globally.

The presence of *E. coli* of the same ST with few SNP differences across time points, pigs and gulls indicates persistence and transmission of *E. coli* subtypes on and between sites. Further work is planned to identify factors that may be selecting these clones on site and maintaining AMR in the absence/low use of antimicrobials. A manuscript is being prepared on this work.

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

UoS

Transfer of IncL/M plasmids carrying NDM-1 and OXA-48 genes found in local hospitals has been analysed *in vitro*. The results of these experiments have been presented at the OHEJP ASM 2020 in a poster entitled "A broad-host-range plasmid outbreak: dynamics of IncL/M plasmids transferring carbapenemase genes". As a summary, IncL/M plasmids were found to be inter-species vehicles of NDM-1 and OXA-48 and some *E. coli* phylogroups seemed less permissive to the acquisition of these plasmids via conjugation. Additional experiments will be carried out to complete the study (e.g. transfer to a larger set of sequenced isolates to find common features impeding plasmid acquisition).

BfR

Investigations on mcr-carrying *E. coli*: Investigations on the occurrence of the mobilizable colistin resistances mcr-4 and mcr-5 among *E. coli* from livestock and food were finished. Overall, more than 900 colistin-resistant *E. coli* isolates from the annual German monitoring programs were screened for the presence of mcr-1 to mcr-9. With the focus on mcr-4- and mcr-5-positive *E. coli*, all information like the MIC, short-/long-read WGS data, XbaI-macrorestriction pattern, plasmid profiles, plasmid transmission were successively determined. We are currently preparing two manuscripts, one the diversity of mcr-4-carrying *E. coli* and one on the description of a novel mcr-5 encoding plasmid. Some initial information on both datasets were recently presented at the ASM EJP conference in Prague. Further in vitro investigations were conducted to determine the influence of different mcr-1 carrying



plasmids (>15 plasmid types) on the fitness of *E. coli*. The influence of the different mcr-4 and mcr-5 plasmids were also determined and compared to the results of the mcr-1 plasmids.

WBVR

In the Netherlands, over 350 caecal samples from broilers were collected over a 10 month period on 5 farms in 2018-2019. Using selective culturing on cefotaxim containing media, 23.8% of these samples were positive for ESBL/AmpC producing *E. coli*, very similar to the 23% prevalence of ESBL/AmpC producing *E. coli* found by the national surveillance in 2018. Molecular analysis indicated that of these isolates, 85% produced blaCTX-M-1, blaCMY-2 or blaSHV-12, similar to results of the national surveillance.

As only 1 farm contained ESBL/AmpC producing *E. coli* on more than 3 consecutive sampling moments, 120 *E. coli* isolates from this farm were selected for whole genome sequencing. Phylogenetic analysis indicates that several lineages of *E. coli* are present in the farm which were found in all three barns that were monitored over several production rounds, while each production round was mostly dominated by a specific lineage. On the farm, blaSHV-12 encoded on an IncI1 plasmid was most prevalent, followed by blaCTX-M15 on an IncFIB plasmid and blaCMY-2 on an IncI1 plasmid. While blaCMY-2 was contained to a specific lineage, blaSHV-12 and blaCTX-M-15 were present in more diverse backgrounds and identical *E. coli* that do not contain the plasmids were also present indicating that the resistance genes are disseminated through the farm by horizontal gene transfer.

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-ly plasmids from broilers in Norway showed that a plasmid from an ST57 isolate harboured both IncI1-ly and IncFIB replicons. Further characterization implied that this was an IncI1-ly/IncFIB co-integrated plasmid that consisted of a complete IncI1-ly 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-ly plasmid. Co-integrated IncI1-ly/IncFIB plasmids was found to be present exclusively in ST57 and where detected from a total of five different farms during the six months sampling periode in 2016, this could indicate a successful plasmid-host combination. The IncI1-ly/IncFIB co-integrated plasmid and additional IncI1-ly plasmids with genes encoding cephalosporin resistance have been subjected to conjugation experiments. Recipient strains used are laboratory strains as well as wild type strains from broilers. Most plasmids were successfully conjugated, with some exceptions, into various *E. coli* STs, however conjugation into *Klebsiella pneumonia* was not successful.

APHA

We have used short and long-read whole-genome sequencing (WGS) techniques to track *Escherichia coli* isolates and identify their associated AMR genes over the duration of 1 year on a UK outdoor pig farm with low antimicrobial usage. Our results showed low levels of AMR *E. coli* presence on this farm but those that were present were likely to be multi-drug resistant (MDR) *E. coli* of particular sequence types (STs), with clones showing epidemiological linkage between pig and wild bird populations. Possible transmission and recycling of the MDR *E. coli* clones within these animal groups drove on-farm persistence for the duration of the study. Most notable was the identification of a large population of sequence type (ST) 744 isolates harbouring up to 14 AMR genes localised on their chromosome within an IS1 flanked transposon region that was variable in AMR gene content but



persisted throughout the study within samples obtained from pigs and environmental gull samples. While previous studies have highlighted the importance of horizontal transmission of plasmids as a driver of AMR spread, within our study there was limited evidence of plasmid spread between *E. coli* STs, with plasmids identified instead as remaining associated with individual ST populations, often integrated within the chromosome.

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

UoS

The UoS has carried out preliminary fitness experiments of the IncL/M plasmids harbouring NDM-1 in different bacterial hosts. Further studies with a broader range of hosts and phylogroups is planned to be performed in the coming weeks. Stability experiments with selected hosts will also be performed.

NVI

Fitness cost and competitive growth of IncI plasmids with ESBL/pAmpC genes from Norwegian broiler production have been performed. Data analysis is currently ongoing.

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

Due to COVID19 and restrictions of working in the laboratory progress of this task has been hampered.

WP4: Project coordination and management (M1-M36)

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)

Due to COVID19 posing restrictions on travel the ARDIG consortium was unable to meet physically for the annual ARDIG meeting, which had been organised to correspond with ECCMID 2020 in Paris, where at least one member from each partner organization was expecting to attend. However, an online Zoom meeting, hosted by UoS, was successfully held between partners. It provided an opportunity for partners from all WPs to interact and discuss the work being performed in ARDIG.

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

Several work package associated subgroup meetings have been held online to provide time for more in-depth discussion between partners.

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 and Year 2.

There have been a number of publications, presentation (both oral and poster) from ARDIG partners which has included work performed within ARDIG.



5.1.10.2.3 Progress of the research project: deliverables and milestones

5.1.10.2.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
02	D-JRP2-1.2	Description of the specified AMR prevalence/frequency and AMU at population/country/regional level.	24	M25		Confidential	Report; 9
02	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	M25		Confidential	Report; 9



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
02	D-JRP2-2.6	Comparative analysis of strains persistence in farms and hospital through longitudinal studies	30		42	Confidential This is still ongoing as data is still being gathered for comparison across partners.	Report; 9
02	D-JRP2-3.3	Predictive modelling of plasmid spread	30		42	Confidential This is still ongoing as data is still being gathered for comparison across partners.	Report; 9
02	D-JRP2-4.2	Annual communication to stakeholders	25			Completed	communication

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.2.3.2 Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
02	M-JRP2-8	Assessment of ecological and management factors associated with AMR and Antimicrobial usage (from WP1)	30		36	This work is still ongoing.
02	M-JRP2-10	Collecting of samples and veterinary data, phenotypical testing of resistant isolates from farms and slaughterhouses	30		36	This work is still ongoing.
02	M-JRP2-11	Collecting of samples and clinical data, phenotypical testing of resistant isolates from hospitals and care homes.	30		36	This work is still ongoing.



5.1.10.2.4 Ongoing collaborations

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. We are planning to follow up communication with EFSA in the near future.

ARDIG WP1 has a collaboration with the Joint Action Antimicrobial Resistance and Healthcare-Associated Infections (JAMRAI) project. The main objective of the collaboration is to develop a new method that will allow comparing not harmonized AMR data.

Partner BfR (Dr. B.-A. Tenhagen) is member of the JIACRA working group of EFSA, EMA and ECDC. The group analyses the association of antimicrobial use and antimicrobial resistance on based on data submitted to the European Agencies, i.e. it utilizes the national aggregated data, while in ARDIG concerning AMR there is a focus on individual isolate data. Therefore the two approaches are complementary.

5.1.10.3 JRP03-AMR3-RADAR

5.1.10.3.1 Summary of the work carried out in the JRP

The RADAR project is in its final phase. Due to the corona-crisis the RADAR project suffered from lack in capacity and/or shift in institutional priorities. Therefore a budget neutral extension has been request to the end of 2020. This request was granted. Progress of the project deliverables is closely monitored by the project management team and this team is confident about finishing all tasks within the extended deadline.

WP1 characterized a large amount of *Salmonella* plasmids and concluded that resistance plasmids (in contrast to virulence plasmids) are shared among many different serovars. In addition, genome-wide association tools were benchmarked. Both aspects are important for future risk assessments that will incorporate genetic data. WP2 reaches its final phase with the completion of pharmaco-kinetic and on-farm transmission models. These models were used to start simulating on-farm intervention strategies. WP3 completed the development and implementation of the RADAR model inventory. The development of food-chain specific and a general risk assessment framework for AMR is reaching its final state. WP4 finished most of the analysis but is still working on the sensitivity and testing of model robustness regarding machine learning methods. WP5 finished the task of proposing a methodology for computing the burden of AMR that calculates AMR-attributable BoD as excess BoD, which is currently being written down in a scientific publication with urinary tract infections as a case study. In addition, WP5 performed analysis showing that using a metagenomics approach for AMR surveillance in most situations achieves the same as using phenotypic resistance in indicator *E. coli*. In addition, the metagenomics approach makes it possible to identify specific genes causing the observed trends in AMR development. WP6 established a protocol for linking the primary production models outputs of WP2 to the evidence synthesis input. A draft scientific paper on the evidence synthesis is being written and will be updated with the latest results of the different WPs.



5.1.10.3.2 Progress of the project: description of activities

WP0: Coordination and communication (M1-M30)

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

Extension has been requested and granted for six additional months to December 2020.

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

No physical meetings were held and planned. A videocall with the RADAR project management team is planned in June to discuss the planning of the last six months.

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

Completed. See second annual report, 2019.

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

Completed. See second annual report, 2019.

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

This will be discussed at the next videocall with the RADAR project management team in June. Due to the corona crisis it is highly uncertain whether this can be a physical meeting. We will discuss the options for a virtual scientific meeting jointly with ARDIG.

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

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

Completed, see first annual report.

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

Completed, see second annual report.

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

In progress.

WP1. New genomic information to feed AMR transmission models (M1-M30)

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

Completed. See second annual report, 2019.

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

Completed. See second annual report, 2019.

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

This task has been completed, findings & deliverable will be added to the final report. We used the pipeline we developed in **JRP3-WP1-T2** to characterize the plasmidome of 3109 genomes of *Salmonella enterica* strains isolated from all food, animal and environmental sectors (anses collection). We successfully identified and reconstructed 2776 plasmids, which were categorized into 242 clusters. Replicon and MOB types identified a diverse population of plasmids. The resistome and the virulome were characterized in all the plasmids and compared within different serovars. Our results suggest that resistant plasmids are shared among different serovars while virulent plasmid (like the pSLT) were restricted to few serovars.

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



Benchmarking of several Whole-genome association studies (WGAS) tools have been performed and preliminary test using Roary/Scoary programs have been carried out. The design of the final dataset that will be tested to identify SNP's and Indels statistically associated to AMR traits is still ongoing.

WP2. Pharmacodynamics and transmission models (M1-M24)

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

Completed. See second annual report, 2019.

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

Simulations were carried-out to study the influence of bacteriological and pharmacodynamic parameters describing 2 bacterial population from a same clone with and without a plasmid coding for a reduced susceptibility. Pattern analysis and clustering have also been performed and will help to identify key influential parameters.

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

Development of the first draft of the on-farm model is complete and currently in internal review. Unfortunately COVID-19 has led to a lot of APHA staff being diverted to work on matters related to the pandemic which has delayed his review process.

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

The first draft of the model includes three farm-based interventions, to investigate possible options for reduction of ESBL transmission between pigs. Firstly, sick pigs were simulated to move to a quarantined sick pen during the duration of their antibiotic treatment course. It was hypothesised that this could isolate the pigs which are shedding the highest levels of resistant bacteria, and therefore reduce pen contamination levels. A further intervention was modelled to understand the effect of an enhanced cleaning and disinfection protocol. Finally, to allow for variability around the antimicrobial usage practices on farm, we simulated scenarios with different proportions of pigs starting a course of antibiotics per day and antibiotic treatment durations.

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

A first draft of the on-farm model report is currently in internal review, but COVID-19 is delaying progress.

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

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

Completed (see deliverable 2.3). A systematic review and meta-analysis was conducted in order to assess relevance of transmission routes of antibiotic resistant bacteria calculated using different methodologies and the relevance of routes per pathogen. This is being transformed in to a scientific paper. PubMed and EMBASE were searched, resulting in 6017 articles published up until December 20th, 2018. Full text screening was performed on 518 articles and 275 are included.

We extracted 741 estimates, 716 were for one bacteria species/group, mostly produced with statistical methods (556), of which risk (242) and ORs (239) were most common, followed by genetic overlap (87), modelling (62) and bacterial intake (17). *S. aureus* (273), *E. coli* (156) and Enterobacteriaceae (99) were mostly studied. Occupational exposure (157) was the most studied route followed by travelling (110) and contacting a colonised person (93). The United States (141), the Netherlands (87) and Germany (60) were the most studied countries. Comparing methods was difficult as not all studied the same routes and due to study heterogeneity not all estimates could be pooled. A missing link exists between routes and their occurrence, which disables estimation of transmission routes' importance.



To create effective policies reducing ARB, estimates of transmission should be weighed by the frequency of exposure occurrence.

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

Ongoing.

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

Ongoing. A poster was presented at the Epidemics Conference in December 2019 (P1.069) . D2.3 I being transformed into a scientific paper.

WP3. Transmission through the food chain (M1-M30)

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)

We have completed the development and implementation of the RADAR model inventory. This includes the provision of a proper infrastructure for exchanging and annotating models in an exchangeable and reproducible file format called FSK and key or desirable features that facilitate access and usability of the inventory. The features were defined in the annual report 2018. However, the inventory currently runs on a firebase server hosted by Google (<http://ejp-radar.eu>). It is planned to migrate the web application to a stand-alone BfR server as soon as possible. We have uploaded three models to the inventory, two showcase models and the primary production model from JRP3-WP3-T1-ST2.

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)

The primary production model for chicken has been annotated according to the FSK standard.

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

An update for each of the models tackling different animal species is given below.

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

We have been working on the generic model which means to translate the existing model into generic modules which can be combined by future users to a customized chicken production chain. We have already modularized the primary production model and are currently modularizing the processing model. Since our model is implemented in R the modules are user-defined functions and as generic model elements should be used by combining these user-defined functions in order to create a customized production chain model. **JRP3-WP3-T2-ST2:** Exposure assessment model for the pork production chain (M13-M24)

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

Development is finalized 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 second sub-model is the pork slaughterhouse model. We have amended the existing EFSA Salmonella in Pork model. Since also this model was integrated in WP6, we greatly simplified the model



structure, and replace Salmonella specific parameter values with a parametrisation more appropriate for ESBL *E. coli*. The model structure was implemented in R, in such a way as to be easily used in WP6. Appropriate data and parameter distributions have been collected. 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)

Experiments have been conducted to assess the effect of heat treatment on *E. coli* contaminated blue mussels, and whether the presence of ESBL producing genotypes have a higher or lower probability of transmission. The data from the treatment series have been analysed statistically separately and are being integrated in a consistent framework by developing a machine learning optimization algorithm that is nearing finalization. Writing up results for publication has commenced.

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

Among the gathered microbial isolate data, we selected sequences of ESBL-*E. coli* collected from broilers and from chicken meat at retail. We are currently running machine-learning classification algorithms in order to identify genes that are transferred from farm to retail, in the broiler production chain.

WP4: Machine learning methods for quantification of risk and health effects (M1-M30)

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

Please update

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.

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

Completed. See second annual report, 2019.

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

Completed. See second annual report, 2019.

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

Completed. See second annual report, 2019.

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

Ongoing: Model analysis has been completed but sensitivity and testing of model robustness ongoing

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



Completed. See second annual report, 2019.

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

Completed. See second annual report, 2019.

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

Ongoing: Criteria for answering the question will be elaborated further

WP5: The burden of disease caused by AMR exposure (M1-M30)

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)

Completed by delivering deliverable D5.1 Defining methodological framework Burden of Disease.

In this document, we have proposed a methodology for computing the burden of AMR that calculates AMR-attributable BoD as excess BoD. We have outlined the data needs for testing this method for a single site (UTI) and single resistant bacteria (ESBL-producing *E. coli*). The proposed approach has advantages (the burden of susceptible infection is treated as a counterfactual, so true excess BoD – the portion of observed burden that is attributable to resistance per se – is estimated) and disadvantages (requires data on the incidence of resistant infection and model parameters for both resistant and susceptible infections). The ECDC approach could also be extended to compute excess burden instead of total burden; thus if the assumptions underlying both approaches are valid and correctly specified, they should lead to the same result. This is currently being written as a scientific paper.

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)

Completed by delivering deliverable **D-JRP3-5.3 Publication (submitted) on source attribution ESBL *E. coli* general population (July 4 2019) and publication in the Lancet Planetary Health (Mughini-Gras et al. 2019 3:357-369).**

Although complex dynamics are involved in ESBL-EC and pAmpC-EC transmission, our results provide quantitative links between specific ESBL-EC and pAmpC-EC genes in the open community and their probable human and non-human sources of direct transmission. Approximately two-thirds of community-acquired ESBL-EC and pAmpC-EC carriers are attributable to human-to-human transmission, with the considered non-human sources accounting for the other third. While anthroponotic sources prevail, our findings underpin the need for longitudinal studies and warrant continuous monitoring in both human and non-human populations, because intracommunity ESBL-EC and pAmpC-EC spread alone seems unlikely to be self-maintaining without transmission to and from non-human sources. Transmission routes of antibiotic resistance are complex, with numerous interconnected cycles and subcycles involving different hosts and environments. Because resistant bacteria might pass into humans from animals and food, and via environment-mediated and human-to-human transmission, a One Health approach is needed that values interdisciplinarity and stresses



the connections between public, animal, and environmental health, and provides an integrated framework for improving our understanding of the global threat of antimicrobial resistance.

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

Ongoing.

We gathered data on the annual 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 collected for AMR surveillance in the pig production in Denmark throughout the period 2001-2018. We recovered faecal samples collected from pigs at slaughter in two time periods (1999-2004 and 2015-2018), sequenced them with shotgun metagenomics and mapped them to the database ResFinder, to determine their resistome.

First, we compared phenotypic resistance in different bacteria with antimicrobial use at the farm in sows/piglets, weaners and fatteners, in a univariate analysis using Spearman rank correlation tests. Phenotypic resistance in indicator bacteria presented generally positive, but with low correlation with the estimated AMU. AMU in weaners described as treatment incidence yielded positive and high correlation to resistance for more antimicrobial classes than sow/piglets and fatteners. In contrast, correlation of resistance at slaughter and AMU in sows/piglets was generally low. Overall, tetracycline was the antimicrobial class with highest correlation between AMU and phenotypic resistance.

Second, we adopted a multivariate approach and performed Procrustes rotation analysis to assess the significance of the correlation between the annual estimated AMU and the annual estimated phenotypic resistance in the indicator species *E. coli*, and compare it to the correlation between the annual estimated AMU and the annual estimated genotypic resistance determined by shotgun sequencing. When AMU was described as treatment incidence (period 2015-2018), genotypic resistance correlated positively, and better than *E. coli* phenotypic resistance, against AMU in all age classes. The multivariate Procrustes analysis showed higher correlation values for phenotypic resistance in *E. coli* vs. AMU than the univariate Spearman correlation analysis, showing that AMU and AMR should be treated as multivariate variables in surveillance.

Third, we performed clustering and ordination analysis (PCA) of the resistome data, relative to year of sampling and antimicrobial class. This analysis of genotypic resistance by means of relative abundance showed useful for ranking antimicrobial classes as a general “high” or “low” abundance class over time, and to identify increase or decrease of the relative abundance of a class, individually and in combination with other classes. It also showed that samples from the two sampling periods (1999-2004 and 2015-2018) formed two separate clusters (with a degree of overlap of samples from 2015), with a strong influence of the year of sampling in clustering.

Fourth, we performed MANOVA tests to identify antimicrobial classes with a significant change in their relative abundance in the resistome between the periods 2001-2004 and 2015-2018. The results showed that apart from glycopeptide and macrolide-lincosamide-streptogramin resistance, relative abundance of genetic determinants of resistance to most antimicrobial classes has overall significantly increased in 2015-2018. Furthermore, we could identify which individual (predicted) phenotypes within each antimicrobial class contributed to an overall significant change at class level, and which specific resistance genes have significantly contributed to the estimated significant changes in any given phenotype. This analysis of genotypic resistance data thus allowed the identification of significant changes in the abundance of resistance genetic determinants at several levels of classification – individual resistance genes, (predicted) resistance phenotypes and antimicrobial resistance classes.

In summary, the results of the above analyses show that using a metagenomics approach for AMR surveillance in most situations achieves the same as using phenotypic resistance in indicator *E. coli*. In



addition, the metagenomics approach makes it possible to identify specific genes causing the observed trends in AMR development.

As the next step, we will perform time-series analysis of the trends in antibiotic use, proportion of resistant isolates and relative abundance of resistant genes.

WP6: Integration of information by Bayesian evidence synthesis (M1-M30)

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)

The results from JRP3-WP3-T2-ST2 will be used for the evidence synthesis network. The other exposure assessments from WP3 will undoubtedly be valuable for future work. The evidence synthesis framework links with **JRP3-WP2-T1-ST3** (development of an on-farm transmission model for ESBL *E coli* in pigs) and **JRP3-WP3-T2-ST2** (development of a model for the pig production chain and consumption phase). With regards to the farm phase, we have established a protocol for linking the farm model output to the evidence synthesis input, but a final parametrised farm model for the Dutch situation is not yet available (due to delays introduced by the covid-crisis). The integration of the pig processing model and consumer phase model is nearly completed, with some final improvements being implemented. Epidemiological inputs to the model were finalized.

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

The model is currently in a state where further major improvements are not feasible in the remainder of the current project, and not expected to yield great rewards. Therefore, upon finalizing the implementation of the pig production and consumer phase, the model development will be frozen. A draft scientific paper has been written, and will be updated when results of the finalized model are produced.



5.1.10.3.3 Progress of the research project: deliverables and milestones

5.1.10.3.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
03	D-JRP3-0.3.2	Third annual report	30		36	Public COVID delay and granted extension	9
03	D-JRP3-1.6	WGAS-based method for genomic data analysis	30		36	Public COVID delay and granted extension	2
03	D-JRP3-1.7	Development of regression model for genomic data analysis	30		36	Public COVID delay and granted extension	9
03	D-JRP3-1.8	Integration of genetic traits associated to AMR and plasmid content information into models (WP6)	30		36	Public COVID delay and granted extension	9
03	D-JRP3-2.1	Report/draft paper on generic PK/PD model	30		36	Public COVID delay and granted extension	8



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
03	D-JRP3-2.2	Report/draft paper on on-farm model	30		36	Public COVID delay and granted extension	8
03	D-JRP3-2.3	Report/draft paper on transmission model	30	30	36	Public “D2.3 report/draft paper on transmission routes”	8
03	D-JRP3-2.4	Report/draft paper on intervention strategies	30		36	Public COVID delay and granted extension	8
03	D-JRP3-3.1	Inventory with models and related data in FSK standard	30		36	Public COVID delay and granted extension	3
03	D-JRP3-3.2	Scientific report on a generic model for the chicken production chain	30		36	Public COVID delay and granted extension	8 or 9
03	D-JRP3-3.3	Scientific report on an adapted model for the pork production chain developed	30		36	Public COVID delay and granted extension	8 or 9



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
03	D-JRP3-3.4	Scientific report on an model for the exposure assessment of AMR through mussels	30		36	Public COVID delay and granted extension	8 or 9
03	D-JRP3-3.5	Scientific report on a generic comparative exposure assessment model	30		36	Public COVID delay and granted extension	8 or 9
03	D-JRP3-4.2	Recommended methods for risk profiling in investigations on antibiotic resistance available	30		36	Public COVID delay and granted extension	2
03	D-JRP3-5.1	The 'excess burden' approach for computing the burden of disease attributable to AMR: Application to urinary tract infection	30	30		Public	9
03	D-JRP3-5.2	A proposal for a new paradigm for AMR surveillance (NEW)	30		36	Public COVID delay and granted extension	9



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
03	D-JRP3-6.1	Publication on final evidence network	30		36	Public COVID delay and granted extension	8
03	D-JRP3-6.2	Policy-targeted report	30		36	Public COVID delay and granted extension	9

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.3.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved : Forecast achievement date	Comments
03	M 0.2.2	Final meeting	30	NO	36	COVID delay and granted extension
03	M JRP3.10	Data gaps in PK/PD modelling and propositions of experimental studies with a defined methodology (ANSES)	30	YES	30	
03	M-JRP3-20	Simulations of the PK/PD model for green AMDs (ANSES)	28	YES	30	
03	M-JRP3-25	Analysis of field genomic data with WGAS-based method	30	YES	30	
03	M-JRP3-26	Analysis of field genomic data with regression model	30	YES	30	
03	M-JRP3-28	Formulate/investigate interventions (RIVM, NCOH, CVI, BfR43)	28	NO	34	COVID delay and granted extension



JRP Co de	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved : Forecast achievement date	Comments
03	M-JRP3-29	Structural adaptations of the PK/PD model according to AMR mechanisms (ANSES)	28	YES	30	
03	M-JRP3-30	Concept on a generic comparative exposure assessment model	28	YES	30	
03	M-JRP3-32	Assimilate project-generated data (NCOH)	28	NO	34	COVID delay and granted extension
03	M-JRP3-33	Assess hypothetical intervention measures using on-farm model (APHA)	28	NO	34	COVID delay and granted extension
03	M-JRP3-34	Final meeting and report	30	NO	36	COVID delay and granted extension
03	M-JRP3-35	Make recommendations to fill data gaps (RIVM, NCOH, CVI)	30	NO	36	COVID delay and granted extension



5.1.10.3.4 Ongoing collaborations

Regarding the disease burden model discussions took place with the authors of the ECDC AMR burden paper. Several partners communicated with programme owners regarding RADAR work in the light of cofinancing projects (for example RIVM regarding disease burden, and DTU regarding AMR metagenomic surveillance).

5.1.10.4 JRP04-ET1-MADVIR

The MAD-VIR project has ended in December 2019 and a final report was issued.

Following the OHEJP guidelines, “D3.1 Procedure for Project Leaders to report on JRP”, “Guidelines for the evaluation of the final Joint Research Project reports, based on D3.9” and “D3.5 Guidelines for WP3/WP4 to monitor projects”, the final report should be first sent to external evaluators, then submitted to SSB for discussion. After the SSB review, the report will be presented to the External Scientific Advisory Board (ESAB). The report will serve as an input for WP5 (Science to Policy) and WP7 (Sustainability).

In July 2020, the final report was submitted to 3 evaluators (one external reviewer who had already reviewed the MAD-VIR full proposal in 2016, another external reviewer and a POC member). The reviewers filled in the final report review forms and these forms were collected and presented to SSB members during the last SSB meeting that occurred on the 18th of September 2020. The final report and evaluations will be communicated to the ESAB.

5.1.10.5 JRP05-ET1-TOXdetect

5.1.10.5.1 Summary of the work carried out in the JRP

The third meeting of the Tox-Detect project has been organized on January 15-16, 2020. After general information dedicated to architecture of the EJP, elements of reporting and budget, a focus on the work package progress has been discussed.

Among hot topics, the consortium decided to launch MALDI-ToF library implementation at NWI (Norway). Moreover, the WP5 and inter-laboratory comparison tests organisation were discussed according to the progress of technical work packages, WP1, WP3 and WP4.

Also, expected times of WP2, WP3 and WP4 deliverables were updated due to technical issues. Consequently, the impact on the expected time of WP5 deliverables has been discussed as this WP is directly related to WP3 and WP4 outcomes.

Finally, 6-months extension period for Tox-Detect project was discussed and proposed in order to achieve the deliverables with high quality.

Due to COVID-19 crisis, Tox-Detect consortium studied the impact on WP and especially cases of short contract and technical WP as laboratory activities were strongly limited.

Even if some delays had been observed in the project, the managerial structure remains confident in the positive outcomes of this project in the near future.

5.1.10.5.2 Progress of the project: description of activities

WP0. Coordination, management and communication (M0-M36)

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.

TC WP1 on 28 January 2020

Face to Face meeting on WP2 14 February 2020

TC WP3 on organisation 24 February 2020

TC WP2 on 20 March 2020

TC WP0 with all partners about the work program impact due to COVID-19 crisis on 10 June 2020

TC WP4 on 16 June 2020

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

23 participants representing all Tox-Detect partners were present during the 2020 general meeting (on 15 and 16 January 2020). C Cordevant and A Callegari were invited by the coordination of Tox-Detect Project as SSB member and general coordination, respectively. All participants presented their institutions, activities and involvement in the Tox-Detect project.

The meeting was split into 1.5 days. General discussion dealing with EJP projects took place after the presentation of A Callegari from EJP general coordination. Briefly, he presented Guidelines, specific rules, budget, communication tools and spoke about the possible 6-month extension. A Callegari also highlighted the need to be present in the ASM meeting which would take place in May in Prague (CZ).

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

This task is in progress, expected in September 2021 as the project was extended until June 2021.

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)

Done. Exchange of strains from the collection between partners is planned. A due Diligence process regarding Nagoya protocol is underway. Focal points of countries, parties of Nagoya protocol, have been contacted to request a PIC and a MAT for the access and use of the strains. Strain transfer will be done after this process.

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

Done. Some *Bacillus cereus* strains proposed by INRAE have been added to the strain collection previously established by partners. Exchange of strains from the collection between partners is planned. A due Diligence process regarding Nagoya protocol is underway. Focal points of countries, parties of Nagoya protocol, have been contacted to request a PIC and a MAT for the access and use of the strains. Strain transfer will be done after this process.

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

Done. Exchange of strains from the collection between partners is planned. A due Diligence process regarding Nagoya protocol is underway. Focal points of countries, parties of Nagoya protocol, have been contacted to request a PIC and a MAT for the access and use of the strains. Strain transfer will be done after this process.

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

Due time of this task was revised, see report of year 2

The MALDI-ToF reference spectra library has been completed on December 2019, see deliverable D1.2.



Technical and data processing transfer has been discussed with partners for an export planned on March 2020 before organization of a dedicated PT trial (cf WP5). Due to COVID-19, the transfer could not be done and it was rescheduled during october2020.

WP2 Characterization of toxins/virulence factors

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

Bacillus cereus

All *Bacillus cereus* supernatants were tested for toxicity by MTT analysis on Caco2 cells.

Due to technical problems, and significant differences in cytotoxicity of certain strains of *Bacillus cereus* compared to results generated from a previous project, it has been asked that ANSES and Institut Pasteur prepare new productions of bacterial culture supernatants. These supernatants will be tested for toxin production before shipment to ANSES-Fougères for cytotoxicity assays. As a result of the COVID-19 crisis, ANSES and Institut Pasteur laboratories were closed from March to June of 2020 delaying the evaluation of toxicity by 4 months. Toxicity testing and HCA assays will only begin once all samples have been received at ANSES-Fougères. Upon reception of the samples, three to four months will be required to complete the toxicity assays.

Clostridium perfringens

Supernatants for the 3 CPE+ selected strains from the collection of ANSES (to be sequenced for RNA-Seq study) have sent to Anses Fougères to be tested for cytotoxicity. Three independent productions of supernatants from vegetative and sporulating phases have been tested for cytotoxicity and IL-8 production. High Content Analysis assays on these supernatants are currently underway, and will investigate apoptosis, DNA Damage, mitochondrial membrane potential and the pro-inflammatory response.

Institut Pasteur will send supernatants from their collection to ANSES-Fougères for toxicity testing and HCA assays.

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

Culture conditions have been tested for the expression profile of the *nhe* gene by qRT-PCR. The RNA extraction protocol has been optimized in these culture conditions (microaerophily, pH 7) to obtain sufficient RNA to send for RNA depletion and sequencing. Bioinformatic analysis was performed at INRAE in collaboration with Maiaage. Differential gene expression analyses have been performed.

In addition, other culture conditions have also been tested to mimic a cellular environment (immune stress). The RNA extraction protocol has been optimized in these culture conditions (various concentrations and time) to obtain sufficient RNA to send for RNA depletion and sequencing. Bioinformatic analysis was performed at INRAE in collaboration with Maiaage. Differential gene expression analyses have been performed. We obtained very promising data that are currently being analysed.

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

Data concerning the correlation of toxicity profiles and expression patterns of bacterial toxins will not be available until all *B. cereus* and *C. perfringens* strains have been completely characterized in terms



of cytotoxicity profiles. Toxicity testing can only be completed once ANSES-Fougères has received all of the strains from ANSES and Institut Pasteur.

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

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

A scientific collaborator has been hired to carry out analytical development and analyses of Staphylococcal enterotoxins M, N & O. A global method based on an “on filter” digestion is being finalized (based on SEB enterotoxin analysis). The selection of peptides for SEM, SEN & SEO has been done based on the theoretical protein sequence and a LC-MS method has also been implemented. In June 2020 the SEM, SEN & SEO proteins were produced and provided from BfR (P9 – WP4 task 4.1) and the method will be extend on these toxins in the next months. Due to the COVID-19 crisis and the very limited experimental work during last months, a finalized and optimized method for spiked supernatant analysis should be expected before end of 2020. Then this method will also be apply on strains selected in WP1.

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

A bottom-up LC-MS/MS approach has been set up for the detection of three *B. cereus* toxins: Cytotoxin K2, Hemolysin II, and Sphingomyelinase using on samples enriched with recombinant toxins. The selection of proteotypic peptides has been done and the fragmentation conditions optimized to obtain the best signal/noise ratio for all selected transitions. The method has been applied to one sample to finalize the optimization, but due to the COVID-19 crisis, we could not go further. Next steps will be to make sure that the final method allows the appropriate detection and quantification of selected toxins in real samples.

A SOP for this method is also in preparation.

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 has been implemented during summer 2019 on the Proteomic and Metabolomic Exploration Facility (PFEM) and 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 third quarter of 2020, taking



into account the delay due to COVID-19 sanitary crisis and the complete interruption of experimental work during three months. In parallel, a SOP method is also in preparation for the end of 2020.

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

Transfer of LC-MS/MS methods is postponed due to COVID-19 crisis

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)

Toxin production procedures for SEM, SEN and SEO have been successful and purified toxins have been sent to the WP3 partners (SEN and SEO, 0.5 mg each, on 25.05.2020; SEM, 0.5 mg on 22.06.2020). Due to the delays in the project, the coordinators have requested that polyclonal antisera for two SEs be generated for the development of the immuno-enzymatic assays (instead of the originally planned monoclonal antibodies). We have complied and purified, recombinant SEN and SEO have been sent (on 22.06.2020) to the antibody company Covalab (arranged for the BfR by Michel Gohar) for immunization of rabbits and generation of the respective polyclonal antisera. According to Covalab, their procedure requires 67 days from first immunization until delivery of the antisera is possible. We therefore expect to receive the antisera (if the immunizations, etc., are successful) by the middle of September 2020. For *B. cereus*, the three toxins have been produced and samples were sent to Institut Pasteur for WP3 task 2. For one of the toxin (sphingomyelinase), the antiserum have already been received and characterized, and an LFA (lateral flow assay) is currently in development. The two other toxins were sent in July to Covalab for antibodies production, and were expected to be received in mid-September. However, one of the toxins was delivered 5 days after shipment (due to a transporter failure) and was received unfrozen (and was therefore lost). Another batch was prepared and was sent, but because Covalab do not start immunisation procedures in August, the antiserum will not be available before the end of September.

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

Antibodies against sphingomyelinase are available and have been characterized. A lateral flow assay is currently in development. For the two other toxins, HlyII and CytK2, the rabbits immunization procedure has been **disrupted** due to the COVID-19 lockdown. A new batch of proteins is in production and will be sent to Covalab to reinitiate rabbits immunization.

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

The 5.1 deliverable "Inter-lab tests documents according to ISO/IEC 17043 regulation, adapted to each method" had been shared with task leaders in January 2020 for validation. This document presents the objectives and a detailed plan for each ILC (Inter-lab comparison test) organisation.

It has been presented to the partners during the general meeting on 15-16 January 2020. The detailed timeline of ILC organisation was presented as a reminder for WP leaders that had to update their deliverable planning. A sample composition suggestion was presented for each ILC and discussed during the meeting.

Validation of the 5.1 deliverable (drafted and shared with the consortium in January 2020) was suspended because Pasteur Institute was not able to implement the expected task as described in the deliverable. The deliverable D5.1 has been validated by the consortium in August 2020.

COVID-19 crisis has induced partner laboratories closure, stopping method development during more than 4 months. In consequence, ILC organisation has been postponed to the end of 2020.

JRP5-WP5-T1: Inter-lab test on Maldi-ToF for species identification



A TC was organised on 28th January 2020 involving Anses and Institut Pasteur. The aim of this TC was to determine strain distribution between WP1 and NVI (Norway) as Maldi ToF ILC organiser. The idea was to test MALDI ToF libraries in the organiser and future participants laboratories to MALDI ToF ILC.

Library transfer, initially expected in the 2nd trimester of 2020, has been postponed due to COVID-19 crisis deferring MALDI ToF ILC, initially expected in the 3rd trimester of 2020.

JRP5-WP5-T2: Inter-lab test on LC-MS/MS

A TC was organised on 24th February 2020 between Sciensano (Task 5.2 leader) and WP3 partners in order to update deliverables schedule, taking into account the necessary period (10 to 12 months) to organise LC-MS/MS ILC.

JRP5-WP5-T3: Inter-lab test on immuno-enzymatic assays

This task was postponed as development of immune enzymatic assays has been extended due to COVID-19 crisis.

WP6. Dissemination, protection and exploitation of results

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

A Abdelrahim (WP2) participated to the online OH-EJP TIM “Practical use of NGS”

D Clermont submitted a poster at OHEJP ASM entitled “Establishment of a shared MALDI-ToF reference spectra base, covering three pathogens of interest”

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

Tox-Detect coordination submitted

one abstracts at the IAFP symposium “Characterisation of Food Born Outbreaks Due to Emerging Staphylococcal Enterotoxins”,

one abstract for IAFP round table “Tox-Detect: Development and Harmonization of Innovative Methods for Comprehensive Analysis of Food-Borne Toxigenic Bacteria, ie. Staphylococci, Bacillus Cereus and Clostridium Perfringens. How can standardization help in validation non-standardized/alternative methods ?”.

The two abstracts were accepted, but the IAFP symposium was cancelled due to COVID-19 crisis.



5.1.10.5.3 Progress of the research project: deliverables and milestones

5.1.10.5.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
05	D0.4	Report of the meeting 2	M25	M26	M26		10
05	D-JRP5-1.1	Data Management Plan	M30	N/A		Confidential	8
05	D-JRP5-1.2	Libraries of MALDI-ToF reference spectra	M24		M30	Confidential, this concern reference strains under MTA and will be published	3
05	D-JRP5-2.1	Report on results from toxicity assays (classical toxicity tests and High Content Analysis)	M32		M36	Confidential, technical data under publication	9
05	D-JRP5-2.2	Report on TR-PCR and RNAseq data analysis	M32		M36	Confidential, technical data under publication	9



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
05	D2.3.	Report on correlation between RNAseq data analysis and toxicity assays (including toolbox for toxicity prediction)	M30		M36	Confidential, technical data under publication	9
05	D3.1	Report on Mass Spectrometry-based methods for the detection of new enterotoxins (eg SEG, SEH, SEI) from S. aureus	M27		M36	Confidential, new developed method, will be published by the end of the project	9
05	D3.2	Report on Mass Spectrometry-based methods for the detection of cereulide analogs and enterotoxins from B. cereus	M27		M36	Confidential, new developed method, will be published by the end of the project	9
05	D3.3	Report on Mass Spectrometry-based methods for the detection of CPE and virulence factors from C. perfringens	M27		M36	Confidential, new developed method, will be published by the end of the project	9



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
05	D3.4	Report on the performance criteria for method harmonisation	M30		M39	Confidential, new developed method, will be published by the end of the project	9
05	D4.1.	Report on the immuno-enzymatic assays	M33		M36	Confidential, new developed method, will be published by the end of the project	9
05	D5.1.	Inter-lab tests documents according to ISO/IEC 17043 regulation, adapted to each method	M25		M33		9

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.5.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
05	M-JRP5-04	Exchange of libraries of MALDI-ToF reference spectra	M27	No	M33	In progress
05	M-JRP5-05	Reference materials available	M18	Yes		
05	MS2.2	RT-PCR assays developed	M32		M36	
05	MS2.3	High content analysis methods developed	M32		M36	
05	MS2.4	RNAseq data analysed	M32		M36	
05	MS2.5	Correlation between toxicity profiles and expression patterns assessed.	M30		M36	
05	MS3.2	Methods developed and assessed	M24	No	M33	
05	MS3.3	Methods transferred to partners	M30	No	M34	



5.1.10.5.4 Ongoing collaborations

Progress of ToxDeetct Project was presented during the EURL staphylococci annual workshop (April 2020). In fact, LC-MS (WP3) and ELISA (WP4) methods, under development, dedicated to staphylococcal enterotoxins should be used for the Food Born Outbreak characterization at EURL network level.

Also, in 2019, Tox-Detect coordination was invited by the Australia (AG) in order to present our expertise opinion on staphylococcal enterotoxins as biological agent. Tox-Detect project objectives, and specifically enterotoxins tools were presented during this meeting. For information Australia group is an informal forum of countries which, through the harmonisation of export controls, seeks to ensure that exports do not contribute to the development of chemical or biological weapons.

M Michaut presented Tox-detect project during EFSA days dedicated to emerging Toxins.

Finally, Tox-detect project was presented to the Italian National Reference network dedicated to staphylococci, and will be presented in December 2020 during meeting dedicated to French National Reference Laboratories

5.1.10.6 JRP06-FBZ1-NOVA

5.1.10.6.1 Summary of the work carried out in the JRP

On the project management level, several tasks have been influenced by the COVID-19 pandemic. The annual assembly was cancelled just a month before the planned date and there have been needs and requests to investigate how the outbreak have affected and will affect work in NOVA. Several project members have had to turn their attention to surveillance and management of the outbreak, both on professional and private level. However, based on our inventories, we conclude that with the extension of NOVA into 2021, we will be able to finish our remaining tasks and deliverables.

In WP1 work has been focussed on the studies of potential barriers or opportunities for surveillance across the food chain. After careful preparations, including considering advantages and disadvantages of slightly different methodological approaches, interviews of disease surveillance experts are now ongoing.

The studies in WP2 are led and performed mainly by public health institutes and have this been heavily affected by the COVID-19 pandemic. However, there has still been some progress. For example, a large dataset with food purchase data has been obtained, which will enable analyses of simulated outbreaks. An electronic web module in which consumers can give consent for their purchase data to be used has also been prepared. The work related to electronic food purchase data at the institutional level for investigation of nosocomial foodborne outbreaks is on-going, including submission of a literature review manuscript and a questionnaire study. Work to develop improved tools for food risk mapping and integrate them into the state-of-the-art tracing tool software FoodChain-Lab also continues.

In WP3, we are working on the methods to integrate signals from different univariate syndromic surveillance systems to improve outbreak detection in humans. The approach is to use an explanatory Bayesian analysis to calculate the value of evidence for any detected outbreak.

WP4 continues the spatial and temporal analyses with a one health perspective, mainly focussing on *Salmonella*, and also AMR. In studies of the role of the environment on disease occurrence, a machine learning algorithm has been developed to correlate infection data with environmental drivers. The plan is to apply this on different scenarios to identify environmental risk factors.



In WP5, the work with different transmission models to investigate potential disease spread and compare surveillance strategies is in some parts finished and continues in others, and two manuscripts have been submitted for publication. In order to obtain a better measure of the occurrence of AMR in animal production, a simulation model for how to utilize metagenomics on samples from very large pools is also under development. Another model is adapted to estimate cost-effectiveness of retail sampling.

5.1.10.6.2 Progress of the project: description of activities

WP0: Coordination and project management (M1-M36)

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

Monthly meetings with WP leaders have been held. The project leader and the deputy project leader also joined the Project Leaders' Forum at the OHEJP Annual Scientific Meeting online.

A request to extend the project was sent to OHEJP management in May. Due to slow recruiting processes in several partner institutes, we decided already in 2019 that we would ask for an extension. This year, the COVID-19 pandemic has also influenced the work in several partner institutes/countries. The request was endorsed and accepted by the PMT and the members of the SSB in June.

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

Organisation of a third annual assembly was started at the end of 2019 and was planned to be held in Madrid, 23-24 April 2020, hosted by UCM. The meeting was cancelled in March due to the COVID-19 pandemic. Instead, a shorter online meeting was held on the 23rd of April, with short scientific presentations from the WPs and general information.

We have not yet made a final decision on if and when we will have our next face-to-face meeting and assembly, but we hope that it will be possible to travel and meet in spring 2021, and use the plans already set up for a meeting in Madrid.

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

In response to a request by the OHEJP coordination in April, the project partners were asked to report any costs that had been wasted due to the COVID-19 pandemic. Only two partner institutes reported such costs.

Later, the OHEJP coordination also requested that the budget for the third year of the project were updated by each partner and specific budget files were filled in and sent to OHEJP centrally in June. The conclusion from this update was that partners plan to not underspend but to use potentially remaining resources during the extension period in the fourth year.

WP1: Food chain surveillance mapping (M1-M36)

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 the integrative ORION project in which members of NOVA project WP1 also participated in the first year, in order to complete the deliverable of this WP, a collaboration has been established with the respective ORION team, as well as COHESIVE.

WP1-T1 has collected 274 terms from partners leading all WPs. The NOVA Glossary has been delivered to ORION project leader, BfR, in order to incorporate the NOVA Glossary into the common OH Glossary. After this there has been several exchanges with BfR in order to adjust the NOVA Glossary into the common version. The final result can be found at:

<https://ckan-aginfra.d4science.org/organization/about/orionknowledgehub>

The OHEJP Glossary has 3 main functionalities: First the collection of One Health (OH) related terms and definitions in the sectors public health, animal health and food safety. Second, highlighting



similarities and differences of terms and definitions between the sectors. Finally, an infrastructure to reference, search and filter terms and definitions.

As an outcome of this cooperation (of all three projects) is a common scientific paper that is currently being drafted (June 2020). This publication work is coordinated by BfR.

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

A methodology has been developed and is currently put in practice to identify barriers and opportunities in food-borne disease surveillance from a One Health perspective. This information is collected through interviews with professionals with selected profiles from four countries (Belgium, France, Sweden, Norway). For this, a number of tools has been developed; i) map of existing food chain (used as a visual tool for cueing), ii) interview guide document, iii) demographic questionnaire, iv) excel files for collection of data, v) training on performing interviews and qualitative research, and vi) interview try-outs.

The interviews are performed by three WP1 participants who have received the same training on performing interviews. So far (20/6/2020) 17 out of 20 interviews planned are performed and the information collected by the interviewers is double-checked and validated from the interviewees to ensure credibility.

WP2: Analysis of food purchase data (M1-M36)

General comment: A number of scientists involved in WP2 have been working full time on the COVID-19 response during spring 2020. Therefore, there has been little progress in some of the tasks (see comments below).

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

Completed; see second annual report (2019).

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

This aim of this task is to describe the use of consumer purchase data (CPD) as an outbreak investigation methodology. This encompasses the parts described below.

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.

Task completed; see second annual report (2019).

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

A manuscript describing the results has been drafted and gone through two successive rounds in the international group – it is currently awaiting a final round which has been put on hold due to COVID-19.

JRP6-WP2-T2-ST3: Describe potential for use as an analytical tool

The part continues particularly as a cooperation between FHI and SSI. Following a series of meetings with Norwegian supermarket chains, a large dataset with purchase data from more than 900,000 households has been obtained. This will form the basis for analyses of simulated outbreaks.

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 which has not previously been done. An electronic web module in which Danish users can be securely invited to sign up and give consent for their purchase data to be used has been built but use has been stalled due to COVID-19.



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.

For this, we aim to use the large dataset described above. Work has been initiated but put on hold due to COVID-19.

JRP6-WP2-T3-ST2: Case control study of foods posing a risk for sporadic campylobacter infections.

We will not do a study on sporadic campylobacter infections, but plan to do one on sporadic salmonella infections. However, work has been stalled due to COVID-19 and we are currently not sure if it will be continued this year.

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 manuscript "Healthcare-associated foodborne outbreaks in high-income countries: a literature review and surveillance study" was submitted to Eurosurveillance last week. Further, a questionnaire study has been performed within health institutions in Germany and Italy, and a manuscript describing the results is under production.

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, tasks 1-3.

The likelihood model has been implemented and validated on Norwegian outbreak data. Current work focuses on integration with FoodChain-Lab and simulation experiments to explore sensitivity and what if any additional improvements on model structure and/or realistically attainable data quality would be sufficient to make the approach of more practical use.

JRP6-WP2-T5-ST2: Develop the likelihood method so that it handles data on time together with GTIN.

The handover of the likelihood method R script for integration in FoodChain-Lab took place in June 2020, a bit later than planned. The integration of the likelihood method into the analysis of the FoodChain-Lab as a cloud service is the next step. It is planned to provide a downgraded implementation of the method by October 2020.

WP3. Syndromic surveillance (M1-M36)

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

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

This task has been completed, see annual report 2018.

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

This task has been completed, see annual report 2018.

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

This task is completed for two partners (SVA, NHI) and still on-going for Anses. When developing univariate syndromic surveillance systems, several points of discussion about methodologies have been raised, as temporal and spatial scales. The methodological discussion is presented in D.3.4.

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

SVA and NHI are working on the methods to integrate signals from different univariate syndromic surveillance systems to improve outbreak detection in humans. SVA is developing a system integrating animal signals (detection of *Campylobacter* in poultry flocks) and environmental data (rainfall,



temperature) to detect sanitary alerts in humans (gastro-intestinal outbreaks). We use an Explanatory Bayesian analysis to calculate the value of evidence for any detected outbreak. The Norwegian veterinary and public health institutes are working on adding veterinary data (*Campylobacter* in poultry) to the current syndromic surveillance system for human gastro-intestinal outbreaks. We compare several methods for outbreak prediction. The pilot study was planned for summer 2020 but had to be postponed to Summer 2021 due to COVID 19 crisis. There will not be any use in setting up the pilot during Autumn as there are no general testing of *Campylobacter* in poultry in Norway during winter. Nonetheless, we aim at analysing the data from Spring and Summer 2020 retrospectively.

WP4: Spatial risk mapping (M1-M36)

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)

Task completed. See second annual report 2019.

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

Task completed. See second annual report 2019.

Additional efforts have been developed under T1, e.g.: Spatial distribution and temporal trends for *Salmonella* in pork and humans (INIA-UCM); Explaining the spatial distribution of *Salmonella* positive farms in Spain (UCM-RIVM); Spatio-temporal models for detection of outbreaks of human salmonellosis using state-level data (USA) (UCM); Spatio-temporal model to determine association between *Salmonella* in dairy cow and environment in Sweden (SVA).

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

This deliverable needs to be changed because formaldehyde is forbidden in the EU since 2017. An alternative is under evaluation: Use of surveillance AMR data for monitoring emergence of *Salmonella* strains in swine (UCM).

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

A machine learning algorithm has been developed to correlate infection data with environmental drivers (accuracy of 93%). It will be applied on different scenarios (e.g. extensive pig production, wild boar) to identify environmental risk factors for salmonella.

WP5: Evaluation of surveillance programs & cost efficiency (M1-M36)

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)

An existing transmission model for *Salmonella* in pigs (part of a previously developed quantitative risk assessment) has been adapted to model different surveillance strategies on the prevalence of infected pigs entering the food chain. The model represents the different productions stages, and simulates the impact of surveillance on farm and subsequent control measures on the farms that exceed a threshold number of positive tests. The model has been parameterized for *Salmonella* Typhimurium in GB.

To estimate the effect of current and alternative sampling schedules for detecting *Salmonella* infected grand-parent and parent flocks in the broiler production, a stochastic dynamic modelling of transmission of *Salmonella* in parent flocks and combined that with the relation between flock prevalence and test sensitivity for environmental samples in the flock has been developed. Also, a model to assess the effect of alternative sampling schedules has been developed. The model is parametrized for Danish broiler production. A manuscript entitle "*Salmonella* in broiler production – Using stochastic dynamic modelling to estimate the likelihood to detect infected parent flocks in



current and alternative sampling schedules" was submitted to the journal Scientific Reports and is, as of June 2020, under review.

A manuscript entitled "Modelling spread and surveillance of *Mycobacterium avium* subsp. paratuberculosis in the Swedish cattle trade network" was submitted to the journal of preventive veterinary medicine and is, as of June 2020, under review. This work defines a method for the modelling of surveillance performance in the primary production using an infectious disease spread model. Further work in this task includes the planned delivery of a workshop on this method as part of the 4th International Conference on Animal Health Surveillance, which was postponed to November 2020, due to the ongoing pandemic.

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

Current surveillance of antimicrobial resistance (AMR) in DK is done using minimum inhibitory concentration (MIC) values as an indicator for bacteria isolated from individual animals and isolates of pathogens. To obtain a more robust and representative measure of the occurrence of AMR in animal production, a simulation model for how to utilize metagenomics on samples from very large pools is under development.

As a model for this, we utilize existing metagenomics data of AMR in the Danish pig production. These data are utilized in a stochastic model, wherein the effect of different pooling strategies in different sites of the production (farms, slaughterhouses) can be estimated. The effect is measuring both on likelihood to detect very uncommon genes, as well as to detect significant changes in the abundance of common AMR genes. The stochasticity of the model takes into account both uncertainty in knowledge about abundance as well as the sampling error arising from randomness at sampling.

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

The work in this task is based on Salmonella in pigs and pork.

The estimation of cost-effectiveness of (pre-) retail sampling, was done using the formula sampling cost/DALYs evaded.

Sampling cost are estimated as the number of samples taken in a country multiplied with the cost per sample, or the overall cost of a sampling program in a country. DALYs evaded is estimated as number of product recalls * size of recall total DALYs due to Salmonella in consumed pork / total pig meat consumption.

The effect is estimated as the direct effect of product recall (DALYs evaded). To put the estimate in a socio-economic context, the estimated cost/DALY evaded, is compared to the "WHO-estimate" that interventions are cost-effective when costs are less than USD 50,000 per DALY gained.

The modelling and calculations have been performed for NL. At the moment, data from FR, NL, SE, and UK is being gathered. This approach can be applied to all other countries, but due to variation in data availability between countries, adjustments of calculations is needed.



5.1.10.6.3 Progress of the research project: deliverables and milestones

5.1.10.6.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
06	D-JRP6-2.4	Assessment of the use of the method as an analytical tool, rather than merely hypothesis-generation.	M30		M40	Due to the COVID-19 outbreak and the impact that has had on the possibilities of the SSI to engage in large population studies, this project may need to be redefined. Also, work has been paused throughout the spring of 2020, because the researchers involved have been moved to work on the COVID-19 response exclusively. We would like to propose to move the deliverable and the corresponding milestone (they are part of the same project) to the end of the new project period, i.e. Month 40	4, 7, 9
06	D-JRP6-3.4	Recommendations about the quality standardization of data produced across the food chain for their use in Sys	M30	2020-06-30		CO	1, 4



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
06	D-JRP6-5.1	Report comparing performance of surveillance strategies	M30		M42	D-JRP6-5.1 - Report comparing performance of surveillance strategies" and "D-JRP6-5.2 - Recommendations for metrics to evaluate surveillance performance, will be joined into one deliverable. Deadline after extension of the project is M42 (36+6).	1, 9
06	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.	M32		M36	Due to lag in recruitment this activity started later than first planned	2, 5, 9

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.6.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
06	M-FBZ1-18	Result reporting on hot spot areas for Salmonella transmission between wild boars and low biosecurity systems.	M30	Yes		
06	M-FBZ1.22	Case control study of food risk factors for sporadic salmonella infections.	M30		M40	Due to the COVID-19 outbreak and the impact that has had on the possibilities of the SSI to engage in large population studies, this project may need to be redefined. Also, work has been paused throughout the spring of 2020, because the researchers involved have been moved to work on the COVID-19 response exclusively. We would like to propose to move the deliverable and the corresponding milestone (they are part of the same project) to the end of the new project period, i.e. Month 40
06	M-FBZ1.NOVA.23	surveillance layer added to models	M30	Yes		



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JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
06	M-FBZ1.NOVA.24	A dynamic modelling layer of surveillance is overlaid the submodels	M30	Yes		
06	M-FBZ1.NOVA.25	initial set of results circulated to project team	M32	No (partly)	M36	



5.1.10.6.4 Ongoing collaborations

OHEJP MATRIX

A communication for potential collaboration has been initiated between WP1 and the OHEJP Matrix Consortium. Focus is on a questionnaire to ask countries about essential variables in a successful OH surveillance system.

Intermediate results of WP1 (i.e. adapted map of food-chain developed for the purposes of the WP) have also been shared with the Matrix WP2 ("Best-practices and multi-sectorial collaboration").

OHEJP DISCOVER

This is a project looking at source attribution. The Salmonella modelling carried out in the NOVA project WP5, and in particular the parameter estimation (estimation of prevalence of *S. Typhimurium* at slaughter) will provide useful inputs for the DISCOVER project.

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.

Other collaborations

Within NOVA, Anses has explored several data sources regarding Salmonella detection in farm animals, food and humans in France. We developed a combination of several algorithms and tools for result visualization that will be transmitted to data owners (National Authorities, National Laboratories and private stakeholders) in order to improve their surveillance systems. Specific detection algorithms that can process temporal signals from correlated time series simultaneously are under development. The interest of such algorithms will be discussed with the National Platform for Animal Health Surveillance (ESA) and the Health monitoring platform for the Food Supply Chain (SCA).

Methods developed in NOVA WP3 are also used in a national Campylobacter project in Sweden. One of the aims for this project is to assess the temporal correlation between Campylobacter surveillance data in broilers and human campylobacteriosis incidence. The work carried out in WP3 on the univariate analysis of broiler data in Sweden and the methodological points discussed (D-JRP6-3.4) will provide a good basis to achieve the aim.

The output from NOVA WP5 (T1) has been used as support for the industry and authorities in revision of the legislation for surveillance of Salmonella in the Danish poultry and egg industry. This legislation has to become approved by the EU commission, and thereby the approach is transferred to stakeholders at many different levels. Also in Sweden, output from NOVA has been used to support the Swedish Board of Agriculture and the animal health organisations in their work to adapt surveillance activities and Swedish legislation to the Animal Health Law within the EU. The models have been used to support decisions about the target confidence level of future surveillance and to compare surveillance alternatives (e.g. different sample size, sample type, frequency of testing).

The work conducted in WP5 T2 is a part of the ongoing revision of surveillance of AMR in the Danish animal and food production (DANMAP). In the future, this surveillance will be based on genomics



compared to cultivation that is used today, and thereby the approach and opportunities for collection of samples will be changed.

5.1.10.7 JRP07-FBZ2-LISTADAPT

5.1.10.7.1 Summary of the work carried out in the JRP

The EJP project management team and the EJP Scientific Steering Committee have both validated the extension of LISTADAPT for one year (January-December 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 (January- June 2020).

We have closed the WP1 dedicated to the collection of 1575 *Listeria monocytogenes* (Lm) strains. All the strains collected by the different partners are centralized, long-term-maintained and stored within the microbial collection of the Unit SEL of the Laboratory of Food Safety (Maisons-Alfort). The LISTADAPT collection, unique in Europe, is composed of two compartments, one of which (First compartment C1) very original, as it regroups 847 strains isolated in the environment (soil, river, farm environment) along with strains from wild and farm animals (both healthy and animal presenting clinical symptoms). The second compartment (C2) is composed of 728 strains from five ready-to-eat (RTE) food categories and also genomes from LISTADAPT partners from previous studies or from sequencing activities carried out in the context of NRL's activities.

The last batch of sequencing (WP2) was done in February 2020. The total genome collection comprises 2557 genomes with 1508 new genomes generated for this project. All the genomes are *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. We wrote a paper describing the whole LISTADAPT genome collection. This paper entitled "A European-wide dataset to decipher adaptation mechanisms of *Listeria monocytogenes* to diverse ecological niches" was submitted at the review "Scientific data" in July 2020 of the Nature Journal. All the produced genomes will be available to the scientific community (umbrella *Bioproject* submitted to European Nucleotide Archive (ENA) as soon as the paper is accepted.

In WP3, all the studies are now completed except for the phenotypic tests on biofilm formation and survival during in-vitro digestion. These task will be completed in October 2020. Linking the WP3 and WP4, we also completed 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. The results were summarized in a paper entitled "Genomics elements located in the accessory repertoire drive the adaptation to biocides in *Listeria monocytogenes* strains from different ecological niches" submitted in the review Molecular Ecology.

Regarding the WP4, we analyzed in depth the *Lm* clonal complexes (CCs) genetic diversity between the compartments. The results including those describing the whole collection will be summarized in a paper submitted during autumn 2020.

The results obtained in 2019 on LISTADAPT were summarized in four oral communication during the second annual EJP meeting (May 2020). We displayed all the results during two face-to-face meetings: (i) the first one day -17th January 2020- meeting ANSES-INRA specifically dedicated to WP3-WP4 (ii) the second of half a day -24th January 2020- with all the partners, just before the annual workshop EURL / NRL meeting. The closure meeting is planned in November 2020 and will be located at Anses-Maisons-Alfort in November 2020.



5.1.10.7.2 Progress of the project: description of activities

WP0: Coordination (M1-M30)

As LISTADAPT leader, Sophie Roussel coordinated and organised two face-to-face meetings (M25): (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. Sophie Roussel was invited to present the project during the annual workshop EURL / NRL *Listeria* meeting in January 2020 (see JRP7-WP5-T4: Dissemination).

WP1: Constitution of a strains collection representative of the different reservoirs of *Listeria monocytogenes* (M1-M12)

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

Completed ; see second annual report, 2019.

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

Completed ; see second annual report, 2019.

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

Completed ; see second annual report, 2019.

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

Completed ; see second annual report, 2019.

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

Completed ; see second annual report, 2019.

WP2: Whole genome sequencing of *Listeria monocytogenes* strains (M2-M16)

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)

Completed

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

Completed

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

Completed

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

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

Completed

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

Completed

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

Completed. All the genomes were centralized at ANSES.

JRP7-WP2-T3: Genome Assembling and Annotation (M5-M16)

Completed



WP3 Phenotypic characterisation of *Listeria monocytogenes* strains (M1-M29)

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

Completed, see second annual report, 2019.

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)

Completed, see second annual report, 2019.

JRP7-WP3-T2-ST2: Adaptation to biocides and cross-resistance development to antibiotics of relevant *Lm* strains (M12-M22)

Completed

JRP7-WP3-T2-ST3: The effect of biocides on *Lm* strains in biofilm (M12-M29)

Completed

JRP7-WP3-T3: Bacterial adhesion and biofilm formation of *Lm* strains (M3-M29)

Ongoing. The results will be obtained in November 2020.

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)

Ongoing. The experimental studies with preservatives are completed. We see different responses between strains during growth in presence of preservatives at low pH, but hardly at neutral pH. The report will be available mid October, and the bioinformatic study for searching genetic markers for strains with similar responses start within September 2020.

The in-vitro digestion studies has started and the results obtained so far for the 24 first strains are promising in terms of detecting different phenotypic responses among the strains. The studies will continue and are expected completed in October 2020.

JRP7-WP3-T4-ST2: Survival of *Lm* in soil microcosm (M3-M16)

Completed, see second annual report, 2019.

WP4: Identification of genetic traits in *Listeria monocytogenes* underlying adaptation to the ecological niches (M1-M30)

JRP7-WP4-T1: Analyze the distribution / prevalence of clonal complexes among the reservoirs (M1-M14)

Completed, see second annual report 2019.

JRP7-WP4-T2: Literature search of genes or genetic mechanisms responsible for virulence, adaptation and survival (M9-M12)

Completed, see second annual report 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)

Completed, see second annual report 2019.

JRP7-WP4-T3-ST2: Processing of all isolates (M22-M27)

Completed

JRP7-WP4-T4: Comparative analysis of phenotypic data / genotypic data (M24-M29)



Ongoing.

WP5 : Trainings and dissemination (M1-M30)

JRP7-WP5-T1: Implementation of a workshop (M1-M2)

Completed, see second annual report 2019.

JRP7-WP5-T2: Trainings (M3-M6)

Completed, see second annual report 2019.

JRP7-WP5-T3: Proficiency Testing Trials (M19-M22)

Cancelled, see second annual report 2019.

JRP7-WP5-T4: Dissemination (M1-M36)

Publications –see section “Publications”:

2020 / Congresses-Workshops:

29 January 2020 : workshop LRUE/LNR Listeria, Anses, Maisons-Alfort

One oral communication (S. Roussel)

May 2020 : Virtual Annual Scientific meeting EJP

Four oral communications (F. Palma, Y. Sévellec, T. Skjerdal, A. Guérin)

Sept 2020: Food Micro Next Generation Challenges in Food Microbiology –Athènes. Reported in September 2021

Three abstracts submitted (Y. Sévellec, S. Roussel)

November 2020 : 6th World One Health Congress –Edinburg

One oral communication (S Roussel)



5.1.10.7.3 Progress of the research project: deliverables and milestones

5.1.10.7.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
07	D-JRP7-2.1	Annotation of <i>Lm</i> genomes already sequenced (genomes available before the start of the project).	26	31		Confidential until publication in Scientific data is accepted.	3
07	D-JRP7-2.2	Annotation of the <i>Lm</i> assembled genomes from 1st batch sequencing.	26	26		Confidential until publication in Scientific data is accepted.	3
07	D-JRP7-2.3	Annotation of the <i>Lm</i> assembled genomes from 2nd batch sequencing.	26	26		Confidential until publication in Scientific data is accepted.	3
07	D-JRP7-2.4	Annotation of the <i>Lm</i> assembled genomes from <i>ad hoc</i> WGS	26	31		Confidential until publication in Scientific data is accepted.	3



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
07	D-JRP7-3.2	Assessment of the ability to adapt to biocides and develop cross-resistance to antibiotics for some illustrative <i>Lm</i> strains.	25	26		Public	9
07	D-JRP7-3.3	Data on the effect of biocides on <i>Lm</i> strains in biofilm.	29	26		Confidential until publication in Scientific data is accepted.	9
07	D-JRP7-3.4	Biofilms phenotypes for the 200 <i>Lm</i> strains.	33	34		Public	9
07	D-JRP7-3.5	Collection of data on survival of <i>Lm</i> as planktonic cells in various ecological niches.		27		Confidential until publication in Scientific data is accepted. Public	9
07	D-JRP7-4.2	Report on prevalence and distribution of clonal complexes among the reservoirs.	26	26		Public	9



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
07	D-JRP7-5.3	Publications and communications	36			Public	8

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity

5.1.10.7.3.2 Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
07	M-JRP7-15	WGS raw data produced.	25	Yes		
07	M-JRP7-16	Second batch <i>Lm</i> genomes assembly completed.	26	Yes		
07	M-JRP7-18	Second batch of <i>Lm</i> genomes annotation completed.	26	Yes		



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JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
07	M-JRP7-19	<i>Ad hoc</i> batch of <i>Lm</i> genomes assembly completed	25	Yes		
07	M-JRP7-20	Bioinformatic analysis done for all the strains	28	Yes		
07	M-JRP7-22	Ad hoc batch <i>Lm</i> genomes annotation completed	20	Yes		
07	M-JRP7-23	Face-to face meeting -2020	25	Yes		
07	M-JRP7-24	WGS Proficiency Testing trial (PTtrial) done	26	No		Cancelled (see annual report 2019)



5.1.10.7.4 Ongoing collaborations

LISTADAPT has resulted in (i) a very well characterized collection of *Lm* strains representative of different ecological niches and (ii) 1700 new *Lm* genomes available to the scientific community. These strains and genomes will be useful in relation with the EU-RL Listeria for the EJP One Health Project “CARE” (2020-2023) (Cross-sectoral framework for quality Assurance Resources for countries in the European Union).

Moreover, LISTADAPT was set up then carried out, in close collaboration with the LRUE Listeria. The coordinator made a presentation of the project and its progress each year at the annual workshop NRL/LRUE.

5.1.10.8 JRP08-FB22-METASTAVA

5.1.10.8.1 Summary of the work carried out in the JRP

Metastava’s AWP 2020 focused on the finalization of the datasets needed in WP3, their analysis, and translation into valorisations like guidelines and publications.

Due to the COVID-19 pandemic, most partners have been affected during the reporting period by shutdown of research activities and even involvement in unforeseen essential diagnostic and research activities in response to the pandemic. The impact on partner’s research planning is expected to last for several months. Accordingly, Metastava has applied for an additional 6 month no-cost extension to make sure we have the necessary time for thorough data analysis, and valorisation of the results. This extension was approved by the OHEJP PMC in June 2020, and will focus on finishing data analysis, reporting, dissemination, and reviewing available guidelines for diagnostic metagenomics.

WP1: Tasks 1.1 and 1.2 have been previously delivered. Task 1.3. (documentation of publicly available datasets) datasets regarding the implementation of exogenous controls were submitted in Sequence Read Archive (SRA), as will the datasets related to the PT, and selected WP3 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. Metastava plans to deliver a guidance document for labs considering diagnostic use of metagenomic workflows (joining the original intention of deliverables 1.4, 1.5, and 2.1) referring to the richness of published studies, norms, and guidelines published since the start of the project.

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: deliverable finished and uploaded on 15/06/2020. Publication accepted in J.Vir.Methods. 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. Analysis finished. Deliverable report in final stage of reviewing.

WP3: task 3.1 all samples listed and documented (D3.1 finalised). Task 3.6. procedure for analysing the data has been agreed (D3.2 finalised). Tasks 3.2-3.5 ongoing (most sequencing data finalized and analysis ongoing). Finished for T3.3 and T3.4.

WP4: no additional activities during the reporting period.

WP5: focus on management of COVID-19 impact on project planning with obtained cost-free extension until M30.



5.1.10.8.2 Progress of the project: description of activities

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 (M1-M30)

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-M36)

Focus will be to include with the final reporting a list of shared datasets that were generated by Metastava, and for which we have high quality metadata. Currently, these include the data related to D-JRP8-2.2 (publicly available in the Sequence Read Archive (SRA) under BioProject accession number PRJNA615303). Reproducibility data from the Metastava proficiency test will be included with a manuscript that is currently being written, as will selected datasets from WP3 and WP2. Currently, this already includes all raw datasets related to task 2.2 regarding the use of endogenous internal process controls (NCBI SRA BioProject accession number PRJNA615303).

JRP8-WP1-T4: Propose a standardised framework for the description of the application scope and analytical properties of a metagenomics assay (M18-M36)

Scheduled for the last 6 months of the project (prolongation), and is heavily linked to task 2.1. Metastava plans to deliver a guidance document (D-JRP8-1.6)for labs considering diagnostic use of metagenomic workflows (joining the original intention of deliverables 1.4, 1.5, and 2.1) referring to the richness of recently published studies, norms, and guidelines since the start of the project. Availability of key experts is affected by COVID-19 in most partner labs.

WP2. Quality assurance tools for the validation and interpretation of metagenomics (M1-M30)

JRP8-WP2-T1: The development of quality metrics to evaluate the significance of the outcome of a metagenomics experiment (M10-M36)

Scheduled for the last 6 months of the project (prolongation), and is heavily linked to task 1.4. Metastava plans to deliver a guidance document for (D-JRP8-1.6) labs considering diagnostic use of metagenomic workflows (joining the original intention of deliverables 1.4, 1.5, and 2.1) referring to the richness of recently published studies, norms, and guidelines since the start of the project. Availability of key experts is affected by COVID-19 in most partner labs.

JRP8-WP2-T2: development and evaluation of external controls for metagenomics (M1-M18)

Deliverable report uploaded on 15.06.2020 and publication accepted in J.Vir.Meth. PMID: 32574649
DOI: 10.1016/j.jviromet.2020.113916

JRP8-WP2-T3: reproducibility and batch effect evaluation (M1-M12)

Completed, see second annual report 2019.

JRP8-WP2-T4: evaluation of QC metrics on additional parallel datasets (M13-M24)

Exogenous IC (cf. D-JRP8-2.2) was used for the study of a porcine virome and will be referred to (publication accepted in Virus Genes). In response to the COVID-19 pandemic, partner Sciensano set out to validate the Metastava metagenomic protocols on clinical samples and isolates of animal coronaviruses (avian infectious bronchitis virus, genus Gammacoronavirus), where we proved the



modules for RNA virome analysis set out in D-JRP8-1.1 (Trizol/Rneasy – SSIV/NEBNext – NexteraXT – Kraken) allowed the determination of complete Gammacoronavirus (Infectious Bronchitis virus) genomes from chicken clinical samples (tissue pools and swabs), while at the same time allowing robust characterisation of co-infecting chicken Astrovirus, Sicinivirus, and avian leucosis virus (Alpharetrovirus). Similar coronavirus protocol validation was done on porcine epidemic diarrhea virus (PEDV, Alphacoronavirus) and avian coronaviruses from Guinea fowl (by partner Anses).

JRP8-WP2-T5: Metagenomic proficiency test (M13-36)

Analysis finished. Deliverable report on the proficiency test in final review round with all participants and will be uploaded in September. Publication drafted.

WP3. evaluation of the analytical properties of metagenomics workflows (M1-M30)

JRP8-WP3-T1: analytical sensitivity, HEV (M1-M36)

Some delays in generation of final dataset (COVID19). Data generated, analysis done. Deliverable report ongoing (integration from multiple partners). Final deliverable report foreseen by 31/10/2020

JRP8-WP3-T2: analytical sensitivity, norovirus (M1-M36)

Some delays in generation of final dataset (COVID19). Majority of data generated. Additional samples came available at Anses, and a final dataset will be added. Report foreseen by 30/11/2020.

JRP8-WP3-T3: analytical sensitivity, large DNA viruses (M1-M36)

Datasets generated and analysed. Deliverable report in final round of review with participants.

JRP8-WP3-T4: analytical sensitivity, STEC (M1-M24)

Datasets generated and analysed. Deliverable report finished and uploaded.

JRP8-WP3-T5: analytical sensitivity, detection of ABR genes (M1-M36)

Some delays in generation of final dataset (COVID19). Datasets are now generated, analysis is ongoing and requires updating of the bioinformatic workflow (specific tools to detect ABR markers). Reporting delayed until M36

JRP8-WP3-T6: bioinformatics and statistical analysis of analytical performance experiments (M1-M24)

The task focused on the standardisation of bioinformatics methods in WP3. Finished.

WP4: Concertation with ongoing efforts and dissemination (M1-M36)

JRP8-WP4-T1: concertation with ongoing initiatives (M1-M24)

Completed, see second annual report, 2019.

JRP8-WP4-T2: formal dissemination (M1-M36)

Completed, see second annual report, 2019. The 6 month extension of the project will be needed to finalise peer reviewed publications and other dissemination documents relating to the work in WP1-WP3. The final report will list all peer reviewed publications.

JRP8-WP4-T3: dissemination of recommendations to stakeholders (M1-M24)

Completed, see second annual report, 2019.

In addition, Metastava output was disseminated to the following stakeholders at scientific meetings:

Presentation at the International Association for Biological Standardisation(IABS)) conference on next generation sequencing for adventitious virus detection in biologics for humans and animals, Ghent, 13-14 Nov. 2019



Presentation at the Belgian branch of the World Veterinary Poultry Association (WVPA). Ghent , 27/02/2020.

JRP8-WP4-T4: Organization of a scientific meeting (M20-M24)

We focus on maximum participation in the OHEJP Annual Scientific meetings to take advantage of a wider forum. D 4.3 will review the scientific discussion during our annual scientific meetings and the presentations at the OHEJP ASM's.

WP5: Project management (M1-M36)

JRP8-WP5-T1: Consortium agreement (M1-M6)

This task has been completed, see annual report 2018

JRP8-WP5-T2: Internal communication (M1-M24)

Completed, see second annual report, 2019.

JRP8-WP5-T3: reporting and liaising with the EU (M20-M24)

Completed, see first and second annual reports (2018 and 2019).



5.1.10.8.3 Progress of the research project: deliverables and milestones

5.1.10.8.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
08	D-JRP8-1.3	List of sequence datasets	27		36	Live deliv. Public datasets OK. Metastava datasets to be added.	3
08	D-JRP8-1.4	SOP: guidelines for the description of scope and analytical properties of a metagenomic method in a diagnostic context	30		36 as D-JRP8-1.6	D1.4, D1.5, D2.1 will be joined in a single guidance document for labs envisaging mNGS as a diagnostic method	2
08	D-JRP8-1.5	Review paper	30		36	D1.4, D1.5, D2.1 will be joined in a single guidance document for labs envisaging mNGS as a diagnostic method	2
08	D-JRP8-1.6	Review document with guidelines on diagnostic applications of metagenomics	New D		36 as D-JRP8-1.6	Given the load of published material to date: D1.4, D1.5, D2.1 will be joined in a single guidance document referring readers to relevant publications, guidelines, norms etc.	2



Summary Progress Report
Third Year - 2020
M25-M36



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
08	D-JRP8-2.1	SOP: use of quality metrics for metagenomics dataset evaluation	30		36 as D-JRP8-1.6	D1.4, D1.5, D2.1 will be joined in a single guidance document for labs envisaging mNGS as a diagnostic method	2
08	D-JRP8-2.2	Report and guidelines for the use of exogenous process controls in metagenomics	26	30		Public 10.5281/zenodo.3894248	2&3
08	D-JRP8-2.4	Report on proficiency test	27		34	Final reviewing round	2&3
08	D-JRP8-3.3	Report: analytical sensitivity and robustness , hepE	30		35		2 &3
08	D-JRP8-3.4	Report: analytical sensitivity and robustness , NoV	30		35		2&3
08	D-JRP8-3.5	Report: analytical sensitivity and robustness , pox	30		34		2&3
08	D-JRP8-3.6	Report: analytical sensitivity and robustness , STEC	30	30		Confidential	2



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
08	D-JRP8-3.7	Report: analytical sensitivity and robustness , ABR genes detection	30		35		2&3
08	D-JRP8-4.2	SOP's, guidelines, scientific papers, presentations	30		36		2
08	D-JRP8-4.3	Minutes of scientific meeting	30		36		10
08	D-JRP8-5.2	Progress and final meeting minutes	30		36		10
08	D-JRP8-5.4	Final report	30		36		9

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.8.3.2 *Milestones*

JRP Code	Mileston one numbe r	Milestone name	Delivery date from AWP 2020	Achieve d (Yes/No)	If not achieved: Forecast achievement date	Comments
08	M-JRP8-M2	Public and own dataset identified	30	NO	36	Final list of Metastava generated datasets will be produced at the latest with the end reporting
08	M-JRP8-M3	Proficiency test panel ready for shipping	20	YES		
08	M-JRP8-M7	Scientific meeting	30	YES		3x Metastava annual meeting + 2x participation OHEJP ASM
08	M-JRP8-M8	Dissemination to various stakeholders including joint communication with ongoing initiatives	30	YES		Various presentations to national and international stakeholder's meetings
08	M-JRP8-M9	Final meeting	30	NO	35	Probably an online meeting to discuss final results and reporting.



5.1.10.8.4 Ongoing collaborations

OHEJP-TeleVIR: insights from Metastava can flow into this second call JRP due to shared partners

Ongoing discussions about participation in a COVID-19 response consortium within EJP-OH.

Cf. previous reports: COMPARE, EFFORT, IRIDA, INNUENDO cogwheel workshops.

Stakeholder dissemination included presentations at the OHEJP ASM's, as well as meetings of the International Association for Biological Standardisation, European Society for Clinical Virology, and The International Congress for Veterinary Virology.

5.1.10.9 JRP09-FBZ3-AIR-SAMPLE

5.1.10.9.1 Summary of the work carried out in the JRP

The third year has focussed on producing publications, SOPs, guidelines and draft standards. Substantial work has been put on writing the second publication that has been submitted to AEM in May 2020. The final airsampling protocol has been formulated as an easy-to-follow guideline and is planned for communication with the relevant working groups under ISO and CEN. Finally, a demonstration workshop has been planned as part of the Prague meeting but due to the Covid-19 outbreak has been postponed to Fall 2020. The final guideline has been also disseminated to all relevant authorities in the EU, because the results showed much improved detection. The project has conducted comprehensive field testing in four EU member states in Northern, Southern, Central and Western Europe. The researchers used Norwegian chicken flocks as negative control, as chicken faeces from Norwegian flocks are normally campylobacter-negative. The tests found no infections in Norway. The results also show that the likelihood of detecting *Campylobacter* in a chicken flock has quadrupled with the new method. That is, up to four times more chicken flocks show signs of *Campylobacter* being present when the new method is used compared to sock samples.

5.1.10.9.2 Progress of the project: description of activities

WP1. Method Development (M1-M13)

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)

Completed. See second annual report, 2019.

WP2: Validation and Standardization (M13-M30)

JRP9-WP2-T1: Validation of air sampling and DNA extraction methods (M13-M21)

Completed. See second annual report, 2019.

JRP9-WP2-T2: Statistical analysis, Standardization and dissemination (M21-M30)

The work in 2020 departed from the achievements of the first annual period and took place over the second annual period and extended in 2020. Validation is important to regulatory approvals before the methods can be used. Hence, the air sampling protocol from WP1 was validated. Following statistical data analysis of the results obtained, the best performing protocol was drafted as a Standard Operating Procedure (SOP). This was planned to be demonstrated among the EJP partner laboratories involved in testing of *Campylobacter*, but due to the Covid-19 pandemic and cancellation of the



physical meeting in Prague, the physical demonstration was cancelled. This will be attempted during Fall 2020 as part of other physical workshops, in the case the pandemic situation permits travelling.

The SOP will be also proposed in Fall 2020 as a new working item to microbiology working groups of International Organization for Standardization (ISO) and Committee of European Normalization (CEN). A manuscript describing the final results have been submitted to AEM:

The main aim of this task was to disseminate the outcome. Results of air samples used in qPCR and culturing was statistically validated against sock samples.

Project results were communicated through various relevant channels as follows:

A manuscript submitted to AEM: Hoorfar J, Kolackova I, Johannessen GS, Garofolo G, Marotta F, Wieczorek G, Osek J, Torp M, Spilsberg B, Sekse C, Thornval NR, Karpiskova R. Foodborne *Campylobacter* in chicken: A multi-centre proposal for a fast screening tool in biosecured chicken flocks. *Applied and Environmental Microbiology* (In press).

Preparation of an SOP to be submitted in October 2020 by Gro to ISO and CEN for consideration as a future guideline or standard

Communication of the project outcome to the national food safety authorities

Online video animation material for the education of broiler industry:
<https://www.youtube.com/watch?v=S9mapXSM8tw&t=95s>

Possible dissemination through a hands-on, wet-lab workshop, in the case the pandemic is eased.



5.1.10.9.3 Progress of the research project: deliverables and milestones

5.1.10.9.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
09	D-JRP9-2.3	Manuscript in press in AEM.	M30	May 2020		Public (Green Access with 6 months embargo) TD	8
09	D-JRP9-2.4	Hands-on, wet-lab workshop for relevant EJP partners.	M29			This deliverable was cancelled – the workshop in Prague was not held because of Covid-19	5

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.9.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
09	M-JRP9-4	Statistical analysis completed.	M29	Yes		Delivered as part of the manuscript submitted.



5.1.10.9.4 Ongoing collaborations

Collaboration with ISO and CEN, and dissemination to national food authorities, EFSA and EC (enclosed).

5.1.10.10 JRP10-FBZ3-MoMIR-PPC

5.1.10.10.1 Summary of the work carried out in the JRP

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.

Defining **predictive markers** that will signal the risk of both animals and *Salmonella* isolates becoming a super-shedder of *Salmonella*.

Immune and microbiota **biomarkers of excretion** to detect animal super-shedders and/ or human prolonged carriers.

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

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.

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. Numerous experiments in field conditions as well as experiments with humans have been delayed, particularly because of the Covid19 crisis. Nevertheless, up to now, these studies have enabled the consortia to identify predictive biomarkers based on gut microbiota composition in the chicken. An article has been accepted on this topic. Results in progress for pigs, are very promising. To identify immunological biomarkers all experiments have been performed and the analyses are in progress both in pigs and in chickens. First results showed that the number of circulating blood immune cells could not be a predictive marker for the appearance of the low and super-shedder phenotypes as well as the non-specific antibody levels. Unfinished experiments have suggested that the low and super-shedder phenotypes could not be explained by a modification of the virulence levels of *Salmonella* in vivo. Recruitment of participants in the human aspect of the project is also now ongoing, with analysis being carried out on a rolling basis.

Numerous putative probiotic strains have been isolated by the Partners. Four have been sent to Bulgaria for testing in chickens and pigs. However, the experiments have been delayed. The experiment with the 4 groups of day-old chicks that received the tested probiotics and prebiotic has been finished in August. The samples will be analysed in part by other partners. The experiment with pigs will start in September and will be finished in October. The comparison of the gut microbiota compositions and the immune parameters of the *probiotic*-inoculated and control groups revealed, in experimental inoculations, an overall impact of the inoculated strains. A mix of four commensal bacteria can, in part, protect chickens from *Salmonella* colonization. Similarly, chickens fed with fermented defatted 'alperujo' were in part protected against *Salmonella* colonization when they were infected at 7 or 21 days of age. At this moment the metagenomics data are under statistical analysis.

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 has been calculated.

The extension has been accepted by the EJP Project Management Team and the Scientific Steering Board. The Partner 22 (University of Surrey) will host the final project meeting.

5.1.10.10.2 Progress of the project: description of activities

WP0: Management (M1-M30)

JRP10-WP0-T1: Draft and agree Consortium Agreement (M1-M6)

Completed.

JRP10-WP0-T2: Produce project-planning, control documentation and Data Management Plan (M1-M12)

Completed. See second annual report, 2019.

JRP10-WP0-T3: Control and manage activity progresses, the timely delivery of project tasks and outputs (M1-M36)

This task is ongoing. Two exceptional extensions of the project of 12 months and then of 6 months at no extra expense has been obtained by the EJP board. The new extension was motivated by the delay in several experiments due to the Covid19 crisis:

Concerning the data obtained in human: Until now, no scientific results are available. Sample collection was started on the 1st of January 2019. The recruitment of study participants continued until the 31st of December 2019, to complete a full year of sampling. Laboratory analyses of the collected stool samples will be carried out following the completion of collection. Metagenome sequencing and analysis of the results will be conducted from June 2020 to December 2020.

JRP10-WP0-T4: Control and manage the project closure and outputs (ends: M30)

A final meeting has been planned in Surrey, the date and the condition of this meeting will depend of the evolution of the Covid19 crisis.

WP1. Risk prediction for Super-shedder animals and human asymptomatic carriers through the use of gut microbiota and immune status analyses (M1-M12)

JRP10-WP1-T1: Predictive immunological markers associated to the high and low-shedders in chickens and pigs (M1-M12)

Partner 1 (Anses) has performed a trial as already described in previous report. All the faecal samples recovered before and after pig infection have been sent to **Partner 22** (UoS) for 16S microbiota analysis and to Partner 18 (INRAE) for immune response analysis. Similarly, as indicated in the previous report, **Partner 27** (ISS) and **Partner 29** (IZLER) have performed experiments in pigs. In these studies blood and faeces sampling were performed over a period of weeks/days. For the ISS study, tissue samples were also taken at post-mortem examination. The blood was analysed by ANSES and ISS to determine if immunological markers could be linked to the *Salmonella* shedding status of the pigs. The analysis is ongoing.

In order to shed some light on the differences in the immune response of low and super-shedders, **Partner 18** (INRA) experimentally infected four groups of chickens with *S. Enteritidis*. Two groups (Group-1, 3) were inoculated at one days of age with a mix of four commensal bacteria described in previous work; Group-2 and 4 were inoculated at one days of age with buffer solution. At 7 days of age Group-1 and 2 were infected with *S. Enteritidis*. Hierarchical clustering performed on the level of



Salmonella in faecal and caecal samples identified super and low-shedder phenotypes. Kinetic of the number of blood immune cells was determined by Flow cytometry before and after infection.

The results showed that the number of blood immune cells circulating in the blood could not be a predictive marker for the appearance of the low and super-shedder phenotypes. Moreover, the analysis has shown no differences, after infection, when low and super-shedders are compared with the control group. In the same way, the immuno-histological work performed by VISAVET-UCM (**Partner 19**) on caecal samples did not show major histopathological modifications between low and super-shedders. However, the monocytes/macrophages and the heterophils numbers are significantly increased at 7 dpi in low-shedders and super-shedders, respectively, when animals were inoculated with the probiotic flora. These results suggested that inoculation of the 4 commensal bacteria has modified the number of immune cells circulating in the blood during Salmonella infection and thus has probably modified the immune response. To analyse the immune response before and after infection, RNA from blood samples and from internal organs from super and low-shedders have been extracted and analysed with the Biomark. All the experiments have been performed, we are still completing the analysis.

JRP10-WP1-T2: Predictive microbiota markers associated to the high and low-shedders in chickens and pigs (M1-M12)

The previous works performed by **Partner 18** (INRA) and **Partner 8** (VRI) demonstrated the role of gut microbiota in the susceptibility to *S. Enteritidis* infection and in the appearance of the low and super-shedder phenotypes. We especially demonstrated that (1) axenic and antibiotic-treated chicks are more prone to become super-shedders; (2) super or low-shedder phenotypes can be acquired through microbiota transfer; (3) specific gut microbiota taxonomic features determine whether the chicks develop a low- and super-shedder phenotype after Salmonella infection in isolator. This study demonstrates the key role played by gut microbiota composition in the heterogeneity of infection. An article describing this work has been accepted for publication in Microbial Biotechnol. These results have been presented at the OHEJP-ASM (virtual) conference (2020).

Partner 1 (Anses) has performed a trial as described in previous report. All the faecal samples recovered before and after pig infection have been sent to partner 22 for 16S microbiota analysis. Similarly, as indicated in the previous report, **Partner 27** (ISS) and **Partner 29** (IZLER) have performed experiments in pigs and sent the samples to **Partner 22**. Partner 22 have extracted DNA, amplified the V3-V4 region of the 16S and sent off for next generation sequencing. Raw sequencing data have been returned in Nov 2019. The data have been analysed in conjunction with metadata related to the animal's overall health and shedding status, to test hypotheses regarding the association of the gut microbiome with Salmonella shedding status. It should be noted that both the studies were carried out using pigs with different genetic backgrounds and Salmonella strains, therefore an opportunity to test the association between the microbiome and salmonella shedding in two different scenarios.

Figure 1 shows the bioinformatic pipeline used to analyse the gut microbiota composition. An overview analysis highlighted a difference in microbial diversity (measured as the distribution of operational taxonomic units, OTUs in different parts of the intestinal tract (showing a lower diversity in ileum compared to the colon and caecum Fig.2) and small, but statistically significant differences in the microbiome of animals classified as high, intermediate and low Salmonella shedders Fig.3.

The results of this analysis have been reported in a poster presented at the OHEJP-ASM (virtual) conference (2020) and the abstract is in the conference proceedings.

The biological meaning of these small differences is the next stage of the analysis, including a machine learning longitudinal study.

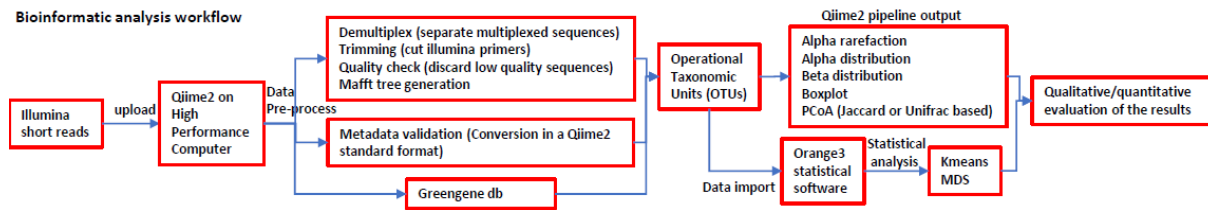


Fig.1: Bioinformatic pipeline used at UoS for the metagenomic analysis of pig gut microbiota.

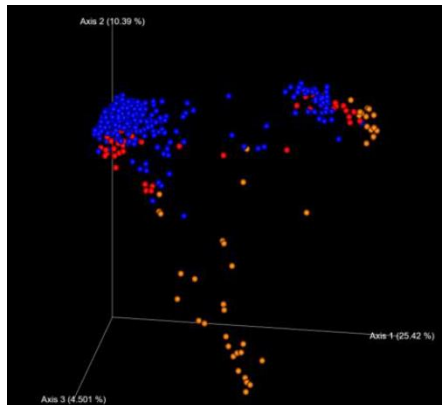
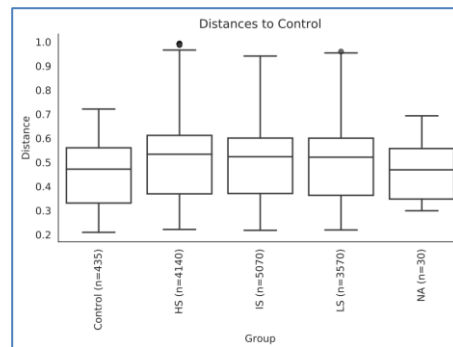


Fig.2: Unweighted Unifrac PCoA considering the samples type. Blue= caecum, red= colon, brown= ileum.



Pairwise permanova results

Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
Control	HS	100	999	6.391540	0.001	0.000333
	IS	100	999	7.542104	0.001	0.000333
	LS	100	999	5.810066	0.001	0.000333

Fig.3: β -distribution of samples according to salmonella shedding class. Small, but statistically significant differences (highlighted in red in the table) can be noted.

JRP10-WP1-T3: Risk factors associated with prolonged convalescent Salmonella shedding in humans (M1-M12)

There are no scientific results available yet. Sample collection was started on the 1st of January 2019. The recruitment of study participants continued until the 31st of December 2019, to complete a full year of sampling. Currently, stool samples from 338 study participants in total have been collected. Direct culture of all of the stool samples has been performed. In addition, all of the initial isolates (primary samples) received at the NIPH have been sequenced. Sequencing of the follow-up samples is ongoing and will, together with data analysis, be conducted throughout the autumn 2020 and the beginning of 2021.

JRP10-WP1-T4: Virulence of Salmonella strains originated from high and low-shedders (M7-M12)

In vitro virulence of *S. Typhimurium* and *S. Enteritidis* collected from super-shedders and low-shedders pigs and chickens have been evaluated by **Partner 18** (INRA) in pig and chicken epithelial and macrophage cell lines.

In vitro virulence of *S. Typhimurium* and *S. Enteritidis* collected from super-shedders and low-shedders pigs and chickens, respectively, was evaluated. Salmonella strains from two super-shedders and two low-shedders animals were used in each experiment in comparison with the strain which was inoculated.



Salmonella adhesion, invasion and intracellular multiplication to an intestinal pig epithelial cell line (IPEC-1) and a pig macrophage cell line (3D4) were measured to determine bacterial virulence. No differences were observed between Salmonella strains for any of the parameters evaluated.

Preliminary data evaluating the in vitro virulence of the different of *S. Enteritidis* collected from super-shedders and low-shedders chicken in LMH cells (an epithelial cell line originated from the chicken liver) do not show any differences between bacterial strains. The virulence on chicken macrophage cell line is ongoing.

In addition, the gene expression of pro-inflammatory and anti-inflammatory genes was evaluated in IPEC-1 cells and 3D4-cells. No differences were observed in the gene expression of CXCL8, IL8 or TGF β for any of the conditions evaluated.

In conclusion, our data do not show any alteration in the in vitro virulence of Salmonella strains from super-shedder and low-shedder origin. These results strongly suggested that the super-shedder and low-shedder phenotypes are not related to a modification of the virulence level of the bacterial strains. This conclusion should be confirmed by the ongoing experiments especially with chicken cell lines.

WP2. Prevention of the appearance of Super-shedder animals and asymptomatic carriage in humans and animals by modifying feed and/or microbiota (M1-M12)

JRP10-WP2-T1: Use of probiotics in chicken and pig (M7-M12)

Partner 8 (VRI) continued in the systematic culture of chicken gut anaerobes and laboratory collection currently consists of more than 450 isolates with known genomic sequences (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP101913&o=acc_s%3Aa). These strains are gradually used for oral inoculation of chicks on the day of hatching followed by verification of their presence 7 days later. The repeated observation and conclusion is that in this type of experimental design, bacterial species expressing outer membrane, i.e. Bacteroidetes, Proteobacteria and Veillonellaceae, can efficiently and persistently colonise chicken caecum. Rather unexpectedly, they never succeeded with the colonisation of newly hatched chicks with Gram positive bacteria from phylum Firmicutes and families Lachnospiraceae, Ruminococcaceae or Lactobacillaceae, despite the fact that these species are common microbiota members. Recently they have changed the protocol for culture of gut anaerobes and this seems to provide novel opportunities for culture of yet unculturable species. This modification consisted of culture under microaerophilic conditions and using Karmali agar. Several experiments have been performed to understand 1- why the inoculation of Lachnospiraceae, Ruminococcaceae or Lactobacillaceae which are otherwise common in gut microbiota on chickens 1 to 2 weeks old, were unable to colonise chicks after oral inoculation and 2- the origin of these bacteria in the caecum of chickens.

Other experiments were performed to determine whether the colonisation of chicken intestine with some strains modified on the long term the faecal and/or caecal microbiota composition. For this purpose defined mixtures consisting of bacterial species which can colonise chicken caecum after a single dose on day of hatch were gradually tested in real commercial farms. Altogether, over 130 000 chickens were treated with different mixtures consisting mainly of different Bacteroides, Prevotella and Megamonas species. Due to the financial scopes, these experiments were co-funded also by other projects of partner VRI. Central issue when upscaling from laboratory to field level was the administration of chicken gut anaerobes to flocks consisting of more than 10, 000 chickens. They tested oral administration via drinking water, resuspension of liquid cultures to pre-starter feed, jellyfying liquid probiotic cultures before their spread over the pre-starter feed or even feed fermentation by the probiotic strains.

Partner 22 (UoS) purified 20 porcine probiotic Lactobacillus strains. They have been genome sequenced and the data is currently being analysed. Four probiotic strains (2 chicken) and (2 pig) have been sent to Bulgaria for testing in chickens and pigs, respectively. Tissue culture studies are ongoing



to determine the safety and the inhibitory qualities (against Salmonella) of the porcine probiotics. Strain will also be shared with the AVANT H2020 project."

Partner 11 (NDRVMI) has analysed the effect in field conditions of several probiotics from **Partner 22**. they had formed 4 groups of day-old chicks of 18,500 in each group. In groups with probiotics 1 and 2, the birds were raised without antibiotics, as well as any other side effects. Growth and absorption of food were also excellent, even mortality was lower than in the control group (where antibiotics were still used). In the third experimental group, where probiotics 1 and 2 were collected and a prebiotic was added to stimulate probiotic microorganisms, they had trouble in the middle of the experiment with a sharp increase in mortality, finding that the causative agent was an aggressive pathogenic strain of E. coli and for 5 days they were treated with an antibiotic after which the mixture of probiotic 1 and 2 and prebiotic were delivered until the time of slaughter. This group was not a linear hybrid Ross like the other groups but a linear hybrid Gobb, which is much more susceptible and prone to disease. At the moment, the experiment is being repeated only with this compromised third group and of course the control, as the linear hybrid is Ross.

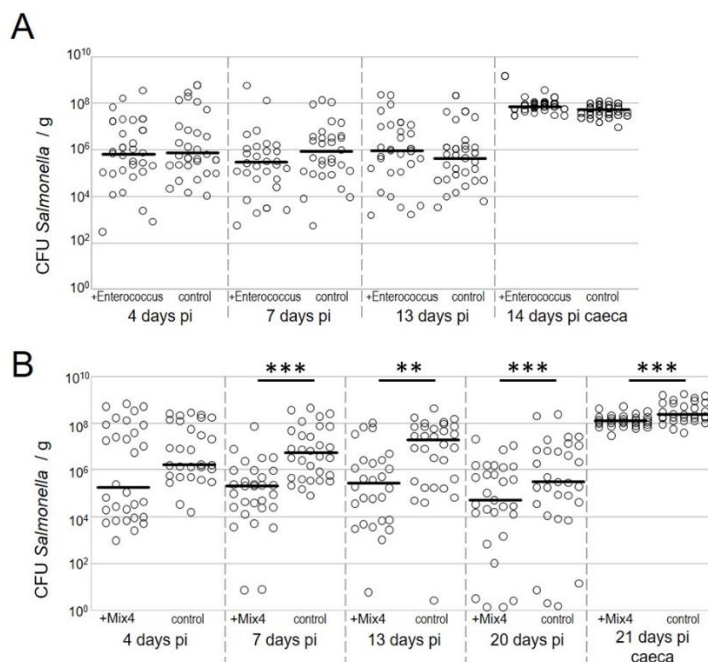
The experiment with the pigs will start on September 3. Three experimental groups of 40 piglets and of course a control group will be defined.

From the pig farm they expect to receive 10-12 pooled samples of feces from pigs after weaning, which in the meantime to test them for the presence of Salmonella. This farm has had Salmonella problems in the recent past. At least for now, they have an appointment with the owner of the pig farm. After the repetition of the experiment with the broilers, all samples will be sent to Partner 22 (UOS) for testing. This is likely to happen in late September.

In the laboratory, meanwhile, they repeatedly tested the obtained 4 strains of probiotics for growth in broth. **Partner 11** are also doing in-vitro tests of probiotics with various Salmonella serovars isolated from poultry and pig feces, using different concentrations of probiotic microorganisms to check their effect on Salmonella.

Previous works performed by **Partner 18** (INRA) demonstrates the role of gut microbiota in the susceptibility to S. Enteritidis infection and in the appearance of the low and super-shedder phenotypes. During the last period we showed that partial protection can be conferred by inoculation of four commensal bacteria prior to Salmonella infection (figure 4). This study paved the way for developing a protective mix of probiotics. An article describing this work has been accepted for publication in Microbial Biotechnol. The impact of the four commensal bacteria on the gut microbiota composition is under investigation by a 16S metabarcoding approach in collaboration with Partner 8 (VRI).

Figure 4: Level of S. Enteritidis after inoculation at one days of age of Enterococcus faecium (A) and the mix of four commensal bacteria (B).



JRP10-WP2-T2: Use of pre-biotics and nutraceutical already defined by the consortium partners in chicken and pig (M1-M12)

Partner 16 (VISAVET-UCM) has completed data analysis detailed in the last report, with both traditional culture and qPCR approaches. Both methods show a protective activity of the prebiotics even though few differences are observed between the traditional culture method and the qPCR. (Fig. 5).

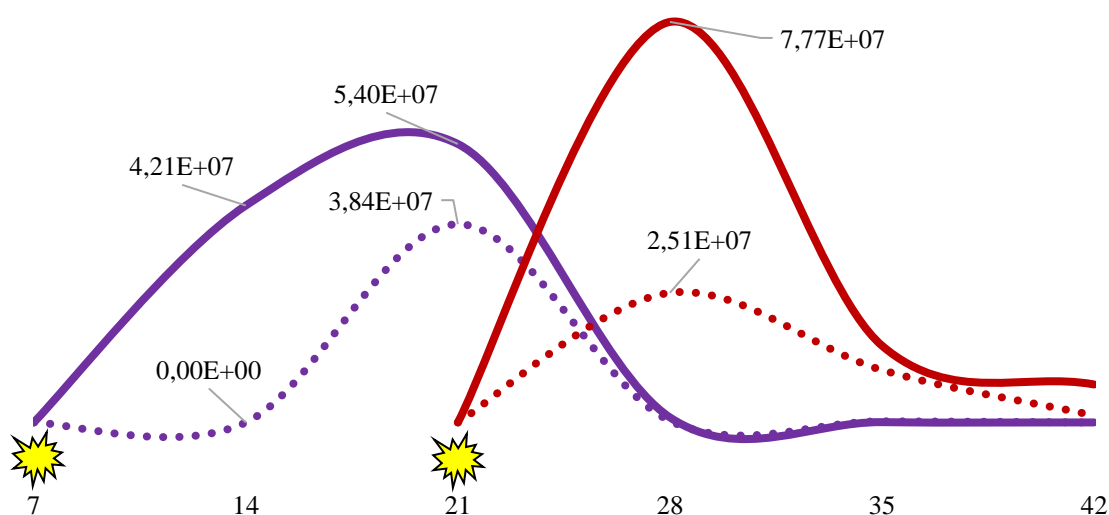


Fig. 5. Results of *Salmonella* spp. count in selective agar in broilers challenged with *Salmonella* Typhimurium at day 7 (purple lines) and at day 21 (red lines). Continuous lines represent control groups, whereas discontinuous lines represent treated groups, fed with fermented defatted 'alperujo'.

For these experiments, intestinal histopathology was performed at the different dates. Several differences have been observed between the different groups. These results are comforted by intestinal morphology, which was analysed at days 7, 14, 28, 35, and 42. Villi height and crypt size in the duodenum and the ceca were measured.



At this moment the metagenomics data is under statistical analysis, so the final results will be obtained soon.

On 22.06.2020 **Partner 11** (NDRVMI) started the experiment with the 4 groups of day-old chicks that received the tested probiotics and prebiotic. The experiment has been finished in August 2020. After the slaughter, materials and samples have been taken. NDRVMI and UoS are analysing them. During the experiment, the condition of the birds were taken into account, and photos were taken during sampling.

The experiment with pigs will start in September and will last until the end of October.

WP3. Modelling the transmission of zoonotic agents to improve intervention strategies on livestock farms (M1-M12)

JRP10-WP3-T1: Transmission modelling at within-host and between-host scales (M1-M12)

JRP10-WP3-T1-ST1: Within-host scale: modelling individual responses and shedding (M1-M12)

Completed, see second annual report 2019.

JRP10-WP3-T1-ST2: Between-host scale: modelling transmission, linked to within-host results (M1-M12)

The PhD student of partner 31 (CVI/DLO) has carried out modelling analyses of the outcomes of an experiment studying the indirect transmission of *Campylobacter* between broilers, and of other relevant historical and new data. In previous research consisting of a combination of experiments and mathematical modelling, a mathematical model of indirect transmission of bacteria between broilers was developed. This model assumes that bacteria are transferred from inoculated animals (source animals) to spatially separated susceptible animals (recipient animals) through random displacement of infectious material in the environment in combination with a loss of viability of the bacteria in time. Technically this model uses diffusion equations to describe the random displacement of material in the environment between the source and recipient animals. The experiment served to validate and refine the existing model, and to do so consisted of three different spatial setups that were each studied in two repeat animal rooms. The results showed no transmission at longer distances (above 130 cm), which is consistent with the existence of a threshold distance. A new experiment has been carried out, studying the indirect transmission of *Campylobacter* and *Salmonella* between broilers. Whereas the previous experiment used three different spatial setups, this experiment used one spatial setup but in this setup simultaneously studies the transmission from source animals to both animals in direct contact as well as to spatially separated animals. This enables us further validate (and possibly adjust) the mathematical model(s) describing indirect transmission by testing predictions in an experimental setting. The model can be used for designing and quantitatively assessing candidate bio-security based intervention strategies against indirect transmission of *Campylobacter* and *Salmonella*. The results of the experiment are currently being analyzed. This analysis is being delayed by COVID-19. This modelling subtask is planned to run until the end of the project (M42).

JRP10-WP3-T2: Interventions strategies: Identification and evaluation tools (M1-M12)

JRP10-WP3-T2-ST1: Systematic inventory of relevant intervention measures (M7-M12)

A draft inventory of relevant intervention measures against *Salmonella* in laying hens has been developed within the framework of a HACCP analysis and involved both literature study and elicitation of expert opinion. This draft still needs to be worked out to a systematic inventory. This was planned in M25-M30, but due to illness it is postponed to 2021, M35-M42.

JRP10-WP3-T2-ST2: Inclusion of potential interventions into the modelling (M10-M12)

At within-host scale, Partner 18 (INRAE-Jouy) completed the realization of a C++ software and the corresponding Matlab and R plugins allowing to analyse time series of microbial concentrations. We



have encountered problems for frequency time series analyses (see second annual report 2019) that are currently being fixed, and we plan to analyse time series from WP1 and include knowledge from probiotic strategies in WP2 to include probiotic based strategies in the model developed in WP3-T1-ST1. Due to COVID19 disturbances, this part of the subtask is postponed to the beginning of 2021.

JRP10-WP3-T2-ST3: Development of economic analysis tools (M1-M12)

This part is ongoing

WP4: Communication and Dissemination for Impact (M1-M12)

JRP10-WP4-T1: Dissemination of data within the project and management of data (M1-M12)

Completed, see second annual report 2019.

Participation to the OHEJP-ASM (virtual) conference (2020) with several posters and conferences.

As described in the report, Partners have exchanged for analysis numerous samples and thus the corresponding data from animal experiments. Partner 22 (UoS) and Partner 8 (VRI) have analysed gut microbiota composition of pigs and chickens respectively. Partner 16 (VISAVET-UCM) has performed histological analyses, Partner 18 (INRA) has performed virulence assays with strains recovered by other Partners. Partner 18 (INRA) and Partner 27 (ISS) have performed immune responses analyses. Partner 6 (NDRVMI) has performed field experiments with strains of Partner 22 (UoS). Data obtained by other Partners during experimental infections have been sent to Partners 18 (INRA-Jouy) and Partners 30, 31 (U. Wageningen). Consequently, all partners shared their results.

JRP10-WP4-T2: Dissemination of data outside the project and management of data (M9-M12)

Participation to several congresses. Due to the Covid19 crisis the high strategic meeting has been cancelled and the budget devoted to this activity has been reimburse to EJP (Partner 18 (INRA) and Partner 30 (NCOH)).



5.1.10.10.3 Progress of the research project: deliverables and milestones

5.1.10.10.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
10	D-JRP10-1.04	In vitro virulence levels of different <i>Salmonella</i> strains recovered from high and low-shedders in animals and humans (first round)	30		36	this deliverable will be merged with D-JRP10-1.05	8 report
10	D-JRP10-1.05	In vitro virulence levels of different <i>Salmonella</i> strains recovered from high and low-shedders in animals and humans (second round)	30		36	CO: until the publication. For strain of human origin, no available results yet. The microbiome analyses of the human stool samples are planned to start after the completion of the laboratory analyses of the stool samples, by June 2020.	8 report
10	D-JRP10-1.09	Definition of predictive microbiota markers associated to the high and low-shedders in chickens and in pigs.	32		36	Significant differences were noted in OTUs composition of high, intermediate and low-shedders in pigs and chickens. Further analysis are needed to understand the biological meaning of this result	1 ; 7 ; 8 report



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
10	D-JRP10-1.10	Definition of microbiota markers associated to the high and low-shedders in chickens and in pigs.	32		36	At least two microbiota markers have been identified in chickens. Small, but significant differences were noted in OTUs composition of high, intermediate and low-shedders in pigs. Further analysis are needed to understand the biological meaning of this result	1 ; 7 ; 8 report
10	D-JRP10-1.11	Recovery of all human samples	32		32	The sampling has been achieved. The sequencing is ongoing	10
10	D-JRP10-1.14	Identification, from in vitro studies, of immune parameters related to high and low-shedders	30	30		No differences have been detected. The report should be uploaded	7, 8 report
10	D-JRP10-2.01	In vitro effect of already characterized probiotics on Salmonella growth and cell invasion	30		36	In vitro growth inhibition studies are complete, but tissue culture studies have been delayed by COVID-19.	7, 8 report



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
10	D-JRP10-2.04	Characterization of protective commensal bacteria able to inhibit Salmonella colonization (two rounds)	30	30		An in vivo test performed in chicks demonstrated the protective activity of a mix of 4 commensal bacteria. The article has been uploaded	7, 8 report
10	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	30		36	The experiment has been completed. Data analysis is ongoing	7
10	D-JRP10-2.06	Impact of defined and undefined probiotics on Salmonella colonization in pig and chicken	26		36	Done for chicken. Studies with pigs are ongoing, but delayed by COVID-19.	7



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
10	D-JRP10-2.07	Impact of pre-biotics or feed on the immune parameters, the microbiome and resistome, gut physiology and welfare of pig and chicken.	30		36	Studies are ongoing, but delayed by COVID-19.	7
10	D-JRP10-2.08	Impact of pre-biotics or feed on Salmonella colonization in pig, chicken. Effect on the super-shedders and low-shedders.	30		36	Studies are ongoing, but delayed by COVID-19.	7
10	D-JRP10-3.03	Intervention measures inventory	30		39		6
10	D-JRP10-3.04	Economic analysis tools	28		39		9
10	D-JRP10-3.05	Definition of intervention measures to target super-shedders	28		39		6



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
10	D-JRP10-3.06	Evaluation scheme for cost effectiveness of the intervention strategies	28		39		9
10	D-JRP10-4.2	Data management policy and strategies	26		26	The deliverable will be uploaded	8, DMP

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity

5.1.10.10.3.2 Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
10	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)	30		36	The last cell line should be tested in the next months



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JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
10	M-JRP10-12	Comparison of immune response of high and low-shedders in chickens and pigs	26		36	The analyses are in progress
10	M-JRP10-21	First version of economic analysis tools completed	24		33	In progress
10	M-JRP10-22	Organization of consortium meetings (intermediate and closure)	24		36	The intermediate meeting has been done. The final meeting is planned.
10	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)	30		36	The last cell line should be tested in the next months.
10	M-JRP10-32	Final inventory of intervention measures completed	30		36	In progress



5.1.10.10.4 Ongoing collaborations

Probiotic strains will be shared with the AVANT H2020 project.

Member of ECDC and AFSA will be invited at the final meeting.

5.1.10.11 JRP11-FBZ4-MedVetKlebs

5.1.10.11.1 Summary of the work carried out in the JRP

The 1st semester of year 2020 has been deeply disturbed by the COVID crisis. However, a number of activities of the MedVetKlebs project did progress since January 2020.

Progress was made in setting up a combined qPCR detecting all *Klebsiella pneumoniae* complex members (the ZKIR target) with Kp1 (*K. pneumoniae* sensu stricto) phylogroup [task JRP-11-T2]. PCR protocol was largely optimized at partner INRA Dijon and is being tested at SSI. One issue that remains to be solved is the absence of detection of a minor phylogroup, Kp6.

Protocols for Kp isolation by culture from various food sources were disseminated/made publicly available [task JRP11-WP1-T3] through the protocols.io platform.

We have completed the genomic sequences of 132 *K. pneumoniae* strains from the chicken-salad multicentric study. Analyses and manuscript writing are in progress. 237 genomic sequences from soil and animal *K. pneumoniae* were also obtained and are being analysed [task JRP11-WP3-T1].

We have pursued our additional objective of setting-up a MALDI-TOF based identification web site for *Klebsiella*. A pilot web site is functional, and a publication is in preparation.

Deep sampling of selected sources [JRP11-WP2-T2]: we have decided to focus on three sources of potential One Health relevance and of scientific interest: veal gut carriage, seawater and onions. Sampling has been hampered by COVID19, but is starting currently. We have also started to analyse in depth, strains from a hospital setting, in order to describe the pattern of transmission of ESBL *K. pneumoniae* within a health care setting. In addition, we are exploring *K. pneumoniae* prevalence and diversity in human gut colonization using a targeted metagenomics approach.

Modelling work has started [JRP11-WP3-T2]. We are exploring models to simulate the diversification of a bacterial lineage that contaminates food, as a function of time and mutation rate of the bacteria. This approach will be used to define *K. pneumoniae* isolates that belong to a cluster, and could be generalized to all foodborne or environmental bacterial species; it will help to discriminate short-term transmission of pathogens to animals of humans.

We have obtained an extension of the project until end 2020, and revised the year 3 budget as suggested by the One Health EJP coordination.

Three publications were issued [JRP11-WP4-T3].

5.1.10.11.2 Progress of the project: description of activities

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)

The ZKIR qPCR for detection of *K. pneumoniae* in complex matrices such as soil or food have been set-up and was published in March 2020 (Barbier et al. Applied Env Microbiol). Besides, we had



disseminated the protocols ahead of publication (via protocols.io, see previous report) and we just heard from other consortia (KlebGAP project) that the method has been validated for their own purposes (quantification and detection of *K. pneumoniae* in gut samples).

Efforts are being pursued to set-up a combined qPCR detecting all *Klebsiella pneumoniae* complex members (the ZKIR target, which was published in March 2020 by the MedVetKlebs consortium) with phylogroup Kp1 (*K. pneumoniae* sensu stricto). A qPCR protocol was largely optimized at partner INRA Dijon and is being tested at SSI. This PCR assay has already been tested on a panel of environmental, food and sewage samples and worked very well.

One issue that remains to be solved is the absence of detection of a minor phylogroup, Kp6, due to a specific Kp6 DNA polymorphism where the probe hybridizes. To address this lack of detection, INRA Dijon developed a second probe, specific to Kp6. Trials are on-going to develop a combined PCR able to detect phylogroups 1 to 6 with Kp1.

JRP11-WP1-T3: Harmonization and alignment (M1-M24)

Protocols for Kp isolation by culture from various food sources were disseminated/made publicly available [task JRP11-WP1-T3] through the protocols.io platform.

WP2. Sampling

JRP11-WP2-T1: Broad sampling of potential reservoirs and sources of Kp (M1-M12)

Completed, see 12-months report 2019.

JRP11-WP2-T2: Deep sampling of selected sources (M13-M14)

After discussions among partners, and based on our broad sampling results, we have decided to focus on three sources of potential One Health relevance and of scientific interest: veal gut carriage, seawater and onions. Sampling has been hampered by COVID19, but is re-starting currently.

Regarding human sources, we have started to analyse in depth strains previously collected from humans in a hospital setting in order to understand better the pattern of transmission of ESBL *K. pneumoniae* in health care. We used data from the i-Bird study (Individual-Based Investigation of Resistance Dissemination, PI Didier Guillemot, IP; Collaboration with Dr Lulla Opatowski), a 4-month study that took place in 2009 in a rehabilitation hospital and followed up more than 600 patients and Health Care Workers. Weekly rectal swabs were performed in patients and human-human proximities were recorded from wireless captors. In total 604 ESBL-Enterobacteriaceae were isolated from 84 patients. Within the project, we have sequenced 61 *K. pneumoniae*. Interestingly, 50 out of the 61 *K. pneumoniae* isolates correspond to five clones belonging to the ST15, 20, 45, 348 and 630. We estimated that they account for 16 events of between-patients transfers. By sequencing multiple isolates per patient and comparing the within-host and between-host diversity, we will be able to infer the most likely transmission directionality, and compare it with confirmed epidemiological inference of transmission. We will also investigate cases of plasmid transmissions from *K. pneumoniae* to *E. coli*. With that objective, 48 additional isolates are currently Illumina sequenced to deepen our characterization of the within and inter-host evolution and to better characterize transmission. One representative isolate of each transmitted ST will be sequenced by using the long-read technology.

We are also exploring *K. pneumoniae* prevalence and diversity in human gut colonization using an innovative 'targeted metagenomics' approach. In short, mixed colonies and bacterial mass growing on the SCAI medium (WP1) from faecal material were harvested, DNA extracted, and Illumina sequenced en-masse. Sequence data analysis algorithms developed in the SpARK project (Ed Feil, PI) will be applied to the generated 'targeted metagenomics' data to quantify reads from *K. pneumoniae* or other *Klebsiella* species, and hopefully to define the diversity of clones within the samples. This project involves the coordinator center (IP) and NCOH/UMCU (Rob Willems) and is a collaboration with the SpARK project.



Finally, we have put emphasis on soil and other environmental sources for a dedicated 'One Health' study within a restricted geographic setting, Burgundy in France. The summary of this work is as follows: *Klebsiella pneumoniae* is of growing public health concern due to the emergence of multidrug-resistant and virulent isolates. Kp is able to occupy a broad range of environmental and clinical habitats, such as soils and vegetation, which could act as reservoirs of Kp to animals and humans. The high diversity of Kp population is well described, but the diversity and ecological adaptations of environmental Kp, as well as their relationships with clinical strains are poorly known. In this study, we collected 664 environmental samples in a single French administrative locality (Department of Côte d'Or, Burgundy, France) from July 2018 to July 2019. Most samples were collected monthly in a market gardener farm (n = 329) and in an organic cattle farming (n = 304) and consisted in soil/mud (n = 219), roots (n = 189), leaves (n = 106), water (n = 34), cow bedding and faeces (n = 50) and fertilizer/compost (n = 35). In addition, water and sludge were sampled in 31 wastewater treatment plants (WWTP) in the same department (n = 31). For comparison purposes, 47 clinical isolates collected in 2018 and 2019 by the Department of Bacteriology from the University Hospital of Dijon (the capital city of Côte d'Or), France, were included. Environmental samples screening with ZKIR qPCR assay followed by culture on SCAI media allowed the isolation of Kp strains in 24.7 % (164/664) of the environmental samples (soil/mud: 14.6 % of positive samples, roots: 20.1 %, leaves: 8.5 %, water: 44.1 %, cow bedding and faeces: 44.0 %, fertilizer/compost: 51.4 % and water/sludge from WWTP: 96.8 %). One to three Kp colonies were isolated from each sample, leading to an environmental samples collection of 237 isolates. Phylogenetic and genomic analysis are ongoing to evaluate the diversity of Kp in these environmental niches across one year, and to compare them with sewage and clinical isolates in terms of virulence factors, antimicrobial genes and strain taxonomy and genetic lineage.

WP3. Genomics and Modelling

JRP11-WP3-T1: Analyses of genomic sequences (M13-M24)

We have completed the genomic sequences of 132 *K. pneumoniae* strains from the chicken-salad multicentric study. Analyses and manuscript writing are in progress. 237 genomic sequences from soil and animal *K. pneumoniae* were also obtained and are being analysed.

JRP11-WP3-T2: Modelling and source attribution (M1-M24)

This task started in December 2019 with the recruitment of Audrey Duval, a post-doctoral fellow in mathematical modelling. In view of the presence and the huge diversity of clones found in all sources where we have isolated *K. pneumoniae* strains, the source attribution approach was deemed difficult to follow. While we are still exploring what best could be done, we have also adapted our objectives to address a related, albeit different, objective: we are exploring models to simulate the diversification of a bacterial lineage that contaminates food, as a function of time and mutation rate of the bacteria. This approach will be used to define *K. pneumoniae* isolates that belong to a cluster, and could be generalized to all foodborne or environmental bacterial species; it will help to discriminate short-term transmission of pathogens from food to animals of humans. The summary of this work, developed by our post-doc Audrey Duval, is as follows:

Whole genome sequencing (WGS) is now widely used to investigate foodborne outbreaks. Using genomic subtyping of *Klebsiella pneumoniae* isolated from food or environmental sources can inform on the relatedness of isolates with infection ones. Thresholds of genomic variation, typically recorded as single nucleotide polymorphism (SNP) 'cutoff' numbers, are used to define clusters. However, there should be no absolute SNP threshold, as variation within outbreaks is expected to depend on the strain, among which strong variations in mutation rates can occur, and outbreak duration. These parameters should be taken into account and included in dedicated models to define specific thresholds. Here, we propose a forward infinite-site model of bacterial evolution to simulate mutation within a population maintaining its size in a given source during a certain time. The model uses the mutation rate per site per year, the outbreak duration, the isolation dates (or sampled isolates) and the genome length as



input. A tool is being implemented to return the expected distribution of SNP (or cgMLST) distances. This distribution allows identifying a threshold of variation (e.g., 99th percentile) beyond which isolates can be ruled out as members of the outbreak according to their genotype. This threshold can thus be used to identify isolates that belong to a cluster. Furthermore, the framework enables, using MCMC sampling, the estimation of the date of contamination of the food source and/or the mutation rate, based on observed SNPs among genotyped isolates. This forward model could be generalized to all foodborne or environmental bacterial species and will help to discriminate short-term transmission of pathogens from food to animals of humans.

In addition to this modelling work, we decided to dedicate efforts to another additional objective. This work is described as follows:

The unsuitability of traditional clinical microbiology methods to distinguish species within the complex leads to high rates of misidentifications (most often as *K. pneumoniae*) that are masking the true clinical significance of each phylogroup and their potential epidemiological specificities. Extending our previous work showing that MALDI-TOF mass spectrometry technology can discriminate among *Klebsiella* species, we are developing a web-based tool named *Klebsiella* MALDI TypeR. Based on the MALDIquant TypeR algorithm, this new tool is a platform-independent and user-friendly application to upload raw data from MALDI-TOF mass spectrometer and identify an isolate at the species level. The tool will be based on the database of previously identified biomarkers (published by our team in two publications: Rodrigues et al., and Merla et al.) that are specific for species complexes and species/phylogroups or groups thereof. Our database also includes new biomarkers, which had not been described before. The *Klebsiella* MALDI TypeR tool will ease the analysis of *Klebsiella* spectra and thereby improve their identification.

WP4: Management, dissemination, exploitation

JRP11-WP4-T1: Implementation of the project management structure (M1-M24)

Nothing specific to report here.

JRP11-WP4-T2: Administrative, legal, financial and ethical support to the consortium (M1-M24)

We have asked, and obtained, an additional 6-months extension, resulting in a project end date on December 31st, 2020. The year 3 budget was revised as suggested by the coordination.

JRP11-WP4-T3: Exploitation of results and Intellectual Property rights management (M1-M24)

Three publications citing the MedVetKlebs project and One Health EJP funding were issued:

Barbier E, Rodrigues C, Depret G, et al. The ZKIR Assay, a Real-Time PCR Method for the Detection of *Klebsiella pneumoniae* and Closely Related Species in Environmental Samples. *Appl Environ Microbiol.* 2020;86(7):e02711-19. Published 2020 Mar 18. doi:10.1128/AEM.02711-19

Igor Loncaric, Adriana Cabal Rosel, Michael P. Szostak, Theresia Franziska Licka, Franz Allerberger, Werner Ruppitsch, Joachim Spengler. Broad-spectrum cephalosporin-resistant *Klebsiella* spp. isolated from diseased horses in Austria. *Animals* 2020;20(2):332. doi: 10.3390/ani10020332.

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



5.1.10.11.3 Progress of the research project: deliverables and milestones

5.1.10.11.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
11	D-JRP11-2.4	Genome sequence data	28		31	CONFIDENTIAL for MedVetKlebs consortium; Genome sequence data are available for internal analyses so far (see deliverable); a few were published in the Barbier et al paper and put on public repositories. In addition, we will have metagenomics data before month 36.	3
11	D-JRP11-3.1	Source distribution of clonal groups, plasmids and genes	30		36	CONFIDENTIAL for MedVetKlebs consortium; Although some data are available already (see deliverable), more data is still being analysed and will be added to the deliverable before the end of the project.	4



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
11	D-JRP11-3.2	Source attribution models by microbial subtyping and comparative exposure assessment	30		36	PUBLIC This objective will be rediscussed among involved partners given (i) the huge diversity found in our sampling surveys (see deliverable 3.1) and (ii) the presence of <i>K. pneumoniae</i> in all sources where we looked (deliverable 2.1, January 2020). Therefore, we will provide an alternative deliverable, before the end of the project. It will consist of a document (D-JRP11-3.2alt) containing a description of scenario for source attribution approaches as a function of pathogen ecological distribution and genotypic diversity.	4



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
11	D-JRP11-3.3	Computer program for dynamic models simulation	30		36	<p>PUBLIC Objectives corresponding to deliverables 3.3., 3.4 and 3.5 were abandoned given (i) the huge diversity found in our sampling surveys (see deliverable 3.1) and (ii) the presence of <i>K. pneumoniae</i> in all sources where we looked (deliverable 2.1, January 2020).</p> <p>Instead, we are proposing a novel deliverable, which will consist of a computer programme and attached publication, useful to model genomic diversification within food as a function of time and mutation rate (D-JRP11-3.345alt).</p>	4



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
11	D-JRP11-3.4	Estimates of the attribution proportion for different food, animal and environmental sources for the countries included.	30		36	<p>PUBLIC Objectives corresponding to deliverables 3.3., 3.4 and 3.5 were abandoned given (i) the huge diversity found in our sampling surveys (see deliverable 3.1) and (ii) the presence of <i>K. pneumoniae</i> in all sources where we looked (deliverable 2.1, January 2020).</p> <p>Instead, we are proposing a novel deliverable, which will consist of a computer programme and attached publication, useful to model genomic diversification within food as a function of time and mutation rate (D-JRP11-3.345alt).</p>	9



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
11	D-JRP11-3.5	Estimates of the relative contribution of different transmission routes for exposure to Kp in the different countries included.	30		36	PUBLIC Objectives corresponding to deliverables 3.3., 3.4 and 3.5 were abandoned given (i) the huge diversity found in our sampling surveys (see deliverable 3.1) and (ii) the presence of <i>K. pneumoniae</i> in all sources where we looked (deliverable 2.1, January 2020). Instead, we are proposing a novel deliverable , which will consist of a computer programme and attached publication, useful to model genomic diversification within food as a function of time and mutation rate (D-JRP11-3.345alt).	9
11	D-JRP11-4.1	Project Periodic Reports	24	24		PUBLIC (but for MedVetKlebs and OHEJP only)	10
11	D-JRP11-4.5	Final Report	24		36	PUBLIC (but for MedVetKlebs and OHEJP only) Delivery date rescheduled given the novel 6-months extension	10

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of



surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity

5.1.10.11.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
11	M-JRP11-6	Broad survey of Kp in multiple sources complete	28	Yes		
11	M-JRP11-7	Development of model frameworks for dynamic modelling and source attribution	29	No		Please see comment for Deliverable 3.2
11	M-JRP11-8	Initial prevalence, quantification and genomic data for model refining	28	Yes		
11	M-JRP11-9	1st batch of clonal groups, plasmids and genes defined, for refinement of models	24	Yes		
11	M-JRP11-10	Compilation and integration of the data produced in WP1 and WP2 to be used in the dynamic and source attribution models	31	Yes		Deliverables 2.4, 3.1 and 2.1
11	M-JRP11-11	Identification of a list of scenarios for control measures to be assessed through model simulations	30	No		Please see comment for Deliverables 3.3 to 3.5
11	M-JRP11-12	Consolidated prevalence, quantification and genomic data for modeling of Kp transmission	28	Yes		Deliverables 2.4, 3.1 and 2.1



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JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
11	M-JRP11-13	Application of dynamic and source attribution models to data collected from different countries	30	No		Please see comment for Deliverables 3.3 to 3.5
11	M-JRP11-14	Reporting of transmission and source attribution estimates	30	No		Please see comment for Deliverables 3.3 to 3.5



5.1.10.11.4 Ongoing collaborations

ECDC and EFSA we contacted along with multiple international scientists by the MedVetKlebs coordinator in the frame of the organisation of the KlebNET project meeting (April 2020), which was cancelled due to COVID19. We will reinitiate contacts before the end of the project (about protocols sharing and inclusion of Klebsiella in surveillance activities of these stakeholders).

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.

5.1.10.12 JRP12-AMRSH5-FARMED

5.1.10.12.1 Summary of the work carried out in the JRP

The first task of the consortium was to survey the different methods and experiences of institutes with both short and long read sequencing technologies, which highlighted the different matrices from a variety of human, animal, and environmental sources examined, the DNA extraction methods, and the bioinformatics tools used to detect bacterial species and/or AMR.

During kick-off and subsequent phone calls, the consortium decided on the sample matrices which were relevant for each institute and included simple (water and saliva) and complex (human/animal faeces, feed additives and boot swabs). We agreed that a defined microbial community (DMC) would be used to inoculate a simple (wastewater) or complex (animal faeces) matrix to test the effectiveness of the DNA extraction methods, used by each institute, to perform long-read sequencing.

In May 2020, FARMED were able to progress decisions needed so that WP1 could proceed and avoid further delay, following the COVID-19 related lockdowns. BfR and Sciensano (WP1 leaders), prepared a detailed plan for the defined microbial community (DMC) which will be used to assess the feasibility of long-read metagenome sequencing. The consortium agreed which six bacterial species (harbouring AMR), the concentration of inoculated bacteria, chemically inactivated, and the sample matrices (wastewater or animal faeces) will be used. Two bacterial isolates (DTU/ Sciensano) and wastewater (UCM) will be supplied to BfR for inclusion in this sample set. These inoculated matrices were sent to each institutes in July 2020.



To enable comparison of the DNA extraction methods only, WP2 will decide on the initial bioinformatics workflow to be used for the DMC analysis in WP1. The WP2 institutes held a phone meeting to discuss and decide on the bioinformatics analysis methods which should be used by each participant in WP1 to analyse the DMC samples. To ensure the bioinformatics methods were performed the same at each institute, a published dataset will be included. The bioinformatics analysis of the results from the DNA extraction methods used by each institute will be compared in October 2020 (planning for first face-to-face consortium meeting).

Just as laboratory work was being initiated, the COVID-19 pandemic was starting across Europe as well as worldwide, which resulted in lockdowns in many countries and reprioritisation of work conducted in the various institute laboratories. The majority of the FARMED project is based on wet laboratory work; therefore, the lockdowns or limited laboratories access has severely affected progress, losing approximately 3-4 months. Many institutes are not yet back to pre-COVID-19 activities and are involved in COVID-19 testing.

5.1.10.12.2 Progress of the project: description of activities

JRP16-WP1 - Assess feasibility of Long-read metagenome sequencing on exemplar matrices. Investigate the use of Hi-C metagenomics (M25-M44)

JRP16-WP1-T1 - Assess feasibility /perform long-read metagenomics MinION from 'defined' microbial community (M25-M40)

Planning initiated during the first teleconference 09/01/2020 with all partners, discussed which bacterial species to include in a defined microbial community (DMC) and which two matrices to concentrate on in the first experiments. During the teleconference of 20/05/2020 (originally planned 19/03/2020 as a face-to-face meeting), further planning prepared by Sciensano (4) and BfR (9), was discussed and a final agreement was decided with all partners. BfR conducted a literature search as well as screened and analysed isolates from the BfR strain collection to find feasible isolates for assembly of the defined microbial community (DMC). A DMC of four Gram-negative isolates and two with two Gram-positive with known complete sequences and different AMR profiles was designed and approved by the consortium, which are summarised in table below. BfR obtained the *S. epidermidis* from DTU in the beginning of June and will receive the *B. subtilis* from SCIENSANO shortly. In July, BfR assembled the six species to obtain two differently composed DMCs with a fixed log distribution of all strains (10^5) and a mixed concentration (different log distributions ranging from 10^3 to 10^7). Two matrices, water and animal faeces (both supplied by BfR), were spiked with six species in two different concentrations and sent to the project partners in WP1. BfR confirmed the faeces to be free of *Salmonella*, and ESBL and/ or Carbapenemase producing Enterobacteriaceae as well as *mcr-1* to *mcr-9* gene harbouring isolates.

Bacteria	Control	Fixed Concentration DMC	Mixed Concentration DMC
<i>Acinetobacter baumannii</i>	0	10^{-5}	10^{-3}
<i>Escherichia coli</i>	0	10^{-5}	10^{-3}
<i>Salmonella enterica</i>	0	10^{-5}	10^{-5}
<i>Staphylococcus epidermis</i>	0	10^{-5}	10^{-5}



Vibrio cholerae	0	10^{-5}	10^{-7}
Bacillus subtilis	0	10^{-5}	10^{-7}

Each partner will use DNA extraction protocols already used in their laboratories. DNA will be sequenced with Illumina short-read and ONT-MinION long-read sequencing technologies, aiming for 5Gbp per sample. The project partners will use the same long-read sequencing kits (Ligation sequencing kit and native barcoding Expansion) in order to obtain comparable sequencing results. All partners agreed to aim to have samples processed (DNA extraction/library prep), sequenced (short and long read) and analysed for species and AMR genes by 9th October 2020.

JRP16-WP1-T2 - Assess feasibility /perform long-read metagenomics MinION from 'simple' sample matrices (M27-M42)

Initial discussions on this task during the first teleconference 09/01/2020, but further planning was needed (which partner will do which matrix). This task will be delayed; however, discussion and decisions will be made during face-to-face meeting planned for October 2020.

JRP16-WP1-T3 - Assess feasibility/perform long-read metagenomics MinION from 'complex' sample matrices (M29-M44)

Initial discussions on this task during the first teleconference 09/01/2020, but further planning is needed (which partner will do which matrix). This task will be delayed; and will be discussed during the face-to-face meeting scheduled in October.

JRP16-WP1-T4 - Perform Hi-C metagenomics (M33-M42)

Although this task is yet to be initiated, BfR have made a start on preparing Hi-C libraries from two poultry faecal samples, from the EFFORT project and a 'defined' microbial community, containing four bacterial species (with complete genome sequences), consisting of Salmonella enterica, E. coli, A. baumannii and V. cholerae and harbouring different AMR plasmids. The resulting Hi-C libraries were sequenced with the Illumina short read technology. An approach for a Hi-C data analysis pipeline at BfR is currently in progress and will be tested on the defined microbial community Hi-C data.

WP2 - Bioinformatics tools to analyse the sequencing data and defining the characteristics within the sample (M28-M45)

JRP16-WP2-T1 - Development/adaptation of a pipeline that can predict species within sample/matrix (M28-M45)

KMA (k-mer alignment) pipeline is implemented and used for bacterial community assignments from long-read sequences. It is tested for Oxford Nanopore sequences. With minor adjustments to the bioinformatics codes, Oxford Nanopore sequences can be fed into the pipeline. To enable comparison of the DNA extractions used by institutes, using the DMC in T1.1, a single workflow for bioinformatics analysis is needed for identification of bacterial species and AMR gene content. WP2 will define the workflow to be used in the coming months and share this with the T1.1 participants by September 2020.

JRP16-WP2-T2 - Development/adaptation of a Resfinder-'like' pipeline for identification of AMR for long-read sequences (M30-M51)

ResFinder is currently being used and further optimised for AMR assignments after KMA mapping with long-read sequences. KMA and ResFinder are also compatible to short-read sequences, which facilitates a more consistent comparison of the same samples using both technologies: short- and long-



read. The pipelines will be made suitable to be carried out on 'simple' and mobile computers with limited access to internet so that they can be used on-site, for example on farm or in clinic.

WP3 - Implementation of on-site protocols for long-read metagenomic DNA sequencing (M25-M54)

JRP16-WP3-T1 - Literature search & Harmonisation of on-site DNA isolation (M25-M29)

WBVR (31) and APHA (21) designed and shared a questionnaire within the consortium to survey the DNA extraction, DNA short- (isolates and metagenomes) and long-read sequencing methods, as well as which matrices/samples were used by each institute. The bioinformatics analysis methods were also surveyed. All institutes contributed to this methods survey and this was collated by WBVR. This information along with literature searches on long read metagenomics and metagenomics analysis from animal faecal samples and food products will be undertaken. Based on these findings, any additional methods that are suitable for on-site DNA extraction will be tested and compared to the methods tested in WP1.

JRP16-WP3-T2 - Investigate the use of Voltrax (M30-M41)

This task is not yet initiated.

WP4 - Project management, coordination and training workshop (M25-M54)

JRP16-WP4-T1 - Annual physical project meetings (M30-M54)

The first physical day meeting was arranged for 19th March but had to be postponed due to the start of COVID-19 and travel restrictions across Europe. The consortium has provisionally planned to meet in October 2020; however, this will be subject to travel bans/restrictions being lifted.

JRP16-WP4-T2 - Teleconferences will be organised every 3 months, between partners (M25-M54)

The consortium has gathered on the phone on 9th January for a kick off meeting. Due to the COVID-19 situation, and not adding unnecessary stress on already closed lab, we had the next teleconference on 20th May when laboratories had a better idea of post COVID-19 activities. The consortium agreed a plan to start lab work and report findings in October 2020.



5.1.10.12.3 Progress of the research project: deliverables and milestones

5.1.10.12.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
12	D-JRP12-WP3.1	Review on current scientific literature and overview of commercially available methods for on-site DNA isolation.	M30		December 2020	Delay due to national lockdowns and reprioritisation/redeployment of staff to essential/statutory work, including involvement with COVID-19 testing.	8 (Summary of available methods, not publication)

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.12.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
12	M-JRP12-01	Review on current scientific literature and overview of commercially available methods for on-site DNA isolation presented to FARMED consortium.	M29	No	December 2020	Delay due to national lockdowns and reprioritisation/redeployment of staff to essential/statutory work, including involvement with COVID-19 testing.



5.1.10.12.4 Ongoing collaborations

Oral presentation of project “FARMED: Fast Antimicrobial Resistance and Mobile-Element Detection using metagenomics for animal and human on-site tests” by Manal AbuOun at the Cogwheel workshop with OHEJP and JPIAMR on 28th April (held online). As a result of attending this meeting we identified the K-Star project from jpiamr coordinated by Derek MacFadden, as they use very similar approaches to the analysis of sequencing data. We will contact this group once we have started developing the FARMED analysis workflows.

5.1.10.13 JRP13-AMRSH5-WORLDCOM

5.1.10.13.1 Summary of the work carried out in the JRP

NUI Galway has employed a post-doctoral researcher, Dr Owen Higgins, and a research assistant, Ms Alexandra Chueiri whose contract started on July 13th 2020, to the WorldCOM team. Progress has been made on WP1 Task 1, with NUI Galway contributing sequence information on AMR strains isolated locally to a WorldCOM AMR gene sequence database. Progress has also been made on WP2 Task 1 with completion of assay design using alignment analysis of available nucleotide sequence data from NUI Galway isolates, UoS and publically available sequence databases. Evaluation of these novel isothermal nucleic acid amplification assays for the detection of genes associated with beta-lactam resistance (CTX-M) found in *Enterobacteriaceae* has been completed. Progress has also been made on WP2 Task 2 with initial evaluation of sample preparation methods underway.

At the UoS, Dr Marwa Hassan had been recruited as a full-time research fellow and Dr Brian Gardner has joined the School of Veterinary Medicine to join the WorldCOM team. The team and sub-teams communicate and meet regularly to discuss and progress the project. At the UoS, the team is progressing with WP1 tasks by using the NCBI Pathogens database to analyse the trends in prevalence of antimicrobial resistance genes and their geographical distribution. All alleles of frequently identified resistant genes have then been extracted for a more in-depth sequence analysis. We are also progressing with WP2 where we are comparing extracted and downloaded sequences and sequence alleles to identify conserved regions for rapid diagnostics development. Conserved homologous regions of highly frequent resistance gene types are used to design targeted primer sets to target plasmid-mediated colistin resistance (*mcr-1* gene), KPC-mediated carbapenem resistance, OXA-48 and the most frequent alleles of the OXA-48-like variants.

UT has employed a post-doctoral researcher, Dr Aare Abroi to the WorldCOM team and progress is made with the *de novo* sequencing of AMR and comparative sensitive strains isolated locally from food, human and animal settings in Estonia.

FLI has recruited a post-doctoral researcher, Dr Belén González Santamarina, whose contract started on August 15, and a doctoral researcher, Ms Nicola Maria Pfeifer, whose contract will start on November 1. We are checking our isolate and sequence collections for the presence of the antibiotic resistance genes *bla*_{CTX-M-1/15}, *bla*_{OXA-48} and *mcr-1*.

UCM is actively participating in WP1, analysing sequence data to identify suitable targets for the development of the assays. For WP2, isolates from different settings are being sequenced using Illumina and/or Nanopore sequencing to detect known and emerging antimicrobial resistance genes and genetic platforms. The OH EJP Student Bosco R Matamoros is involved in these tasks, and a Short Term Mission from OH EJP has been approved to facilitate the visit and training on hybrid sequencing technology Nanopore-Illumina of Dr. Liam Burke to visit UCM in Summer 2021.

INSA have been delayed the recruitment of the fellow due to the COVID-19 crisis, but the process will start in October. The *de novo* sequencing of AMR and comparative strains isolated locally from human and veterinary settings is ongoing.



5.1.10.13.2 Progress of the project: description of activities

WP: 1 Generation of up to date sequence information for selected pathogens and antimicrobial resistance genes (M25-M36)

Task 1 (WP1-T1): Analysis of publically available sequences for antimicrobial resistance genes associated with *Salmonella*, *Campylobacter* and *E. coli* (M25-M28)

At the UoS, all *bla* antimicrobial resistance genes present in *Salmonella*, *E. coli*, *Klebsiella* and *Acinetobacter* genomes have been extracted from NCBI Pathogens database (<https://www.ncbi.nlm.nih.gov/pathogens/isolates#/search/>). For the initial phase of this work package, we have focused on ESBL-related AMR genes. As these genes are absent from *Campylobacter*, we have not included this bacterium in these analyses, and have used the important pathogens *Klebsiella* and *Acinetobacter*. Later work will include antibiotic resistances relevant to *Campylobacter*. All types and subtypes of Extended Spectrum β -Lactamases (ESBLs) and plasmid-mediated colistin resistance genes have been analysed for frequency among reported and extracted sequences. High frequency resistant genes subtypes have been highlighted for further sequence analysis to illustrate geographic distribution and geographic-specific single nucleotide polymorphisms (SNPs). The task is still ongoing.

Task 2 (WP1-T2): Targeted and whole genome de novo sequencing of phenotypically characterised isolates from various settings (M25-M30)

In the UK, no whole genome sequencing has been performed due to the COVID-19 lock down and it is currently postponed. However, the task is still ongoing.

In Estonia 120 *E. coli* strains have been *de novo* sequenced and the bioinformatic analysis of these genomes is ongoing. In addition, 88 *Campylobacter* sp strains have been prepared for *de novo* sequencing, delivered to the service provider in order to be sequenced in September 2020.

At UCM, enterobacteria highly resistant to aminoglycosides and beta-lactams from companion animals have been sequenced using Illumina and Nanopore sequencing, leading to the identification of new epidemic plasmids that will be studied within the Consortium in different countries. Further, 50 plazomicin resistant enterobacteria from a single location from animals, sewage, wastewater, humans and food have been isolated, sequenced also by Nanopore and Illumina and are currently being analysed bioinformatically.

At FLI, *E. coli* strains resistant to 3rd and 4th generation cephalosporins have been isolated from swine and are currently being characterised to allow the selection of strains for whole-genome-sequencing.

Task 3 (WP1-T3): Development of machine learning algorithms for the prediction of anti-microbial resistance. (University of Surrey, VISAVET Health Surveillance Centre UCM) (M30-M36)

The start date for this task has been partially delayed as Dr Gardner recently joined the UoS School of Veterinary Medicine. At UCM a new analytical tool has been developed, ARUflow, that elicits automated analysis of short and long-read sequences as a platform to complement in the next months with machine-learning technology.

Work-package 2 Assay Development (M31-M41)

Task 1 (WP2-T1): Development and performance evaluation of singleplex isothermal amplification assays for selected AMR gene targets (M31-M36)

NUI Galway has completed design of singleplex isothermal nucleic acid amplification assays for the detection of the selected AMR associated CTX-M gene targets. This initial assay design was achieved using existing genomic sequence data of *Escherichia coli* isolates from environmental samples collected and processed by NUI Galway. These *E. coli* isolates contained CTX-M Group 1 variants, type 1 and 15. Sequence alignment analysis was used to identify single nucleotide polymorphism (SNP)



differences between the type 1 and 15 variants. Loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) assays, six in total, were designed to detect and differentiate the type 1 and 15 variants. Standard LAMP assays, six in total, were also designed to detect both CTX-M Group 1 variants. Evaluation of these assays has successfully identified an optimal CTX-M-1 LEC-LAMP assay that detects CTX-M-1 targets while differentiating CTX-M-15 targets. A synthetic internal amplification control template and corresponding LEC-LAMP assay has also been designed and evaluated. Optimisation of these LEC-LAMP assays and development of a three target (CTX-M-1, CTX-M-15 and IAC) multiplex LEC-LAMP assay will be carried out in September.

At the UoS, *mcr*, *bla_{OXA}*, *bla_{CTX-M}*, *bla_{NDM}* and *bla_{KPC}* gene sequences have been extracted from genomes listed in the NCBI Pathogens database to represent all gene alleles. This showed the conserved regions across the different alleles of each *mcr*, *bla_{KPC}* and *bla_{OXA-48}* subtypes and the homology across the selected subtypes. The extracted sequences showed a species-specific trend across *mcr* gene subtypes with *mcr-1* being the most predominant in *E. coli*, *mcr-8* in *Klebsiella* and *mcr-9* in *Salmonella*. MSA of *bla_{KPC}* genes showed 99% homology among *bla_{KPC-2}* gene sequence alleles, while MSA of all *bla_{KPC}* genes and corresponding alleles showed 93% conserved homology. Thus, conserved regions were used to design LAMP primer sets for *mcr-1*, *mcr-8*, *mcr-9*, all *bla_{KPC}*, *bla_{OXA-48}* and some of the *bla_{OXA-48-like}* genes (*OXA-48*, *OXA-232*, *OXA-181* and *OXA-54* encoding genes) using both LAMP Designer 1.15 and Primer Explorer V5. The KPC LAMP assay was able to detect KPC positive strains with good sensitivity and specificity. Optimisation of the detection conditions is currently ongoing while collecting additional KPC positive isolates to facilitate further validation. The *mcr-1*, *bla_{OXA-48}* and *bla_{OXA-48-like}* variants LAMP primer sets are also currently being tested and optimised for further validations.

The task is still ongoing.

Task 2 (WP2-T2) : Evaluation and selection of sample preparation methods for use with laboratory and on-site tests (M31-M36)

NUI Galway has initiated work on evaluating sample preparation methods by collecting bovine faecal sample material and various nucleic acid extraction reagents for testing. Initial LEC-LAMP testing of synthetic templates spiked into and extracted from bovine faecal samples has been successful. Optimisation of these sample preparation methods and spiking experiments using CTX-M-1-producing *E. coli* strains will be carried out in September.

Task 3 (WP2-T3) : Development and performance evaluation of multiplex assays for pathogens and resistance genes (M33-M41)

This task has not started yet. It is due to start in M33.

Work-package 5 Project Management (M25-M54)

Task 1 (WP5-T1) - Project Management (M25-M54)

A WorldCOM consortium KOM was held on January 26th and 27th 2020 in Brussels, at which WP and Task priorities were agreed. Subsequent WorldCOM consortium meetings have taken place via Zoom meetings held monthly from March 2020 (March 24th; April 28th; May 20th; June 2nd). The consortium had planned a further consortium meeting to review progress and for future project planning in Prague, during the OHEJP ASM in May. However, the planned meeting was replaced with a Zoom meeting on June 2nd.

A draft Data Management Plan has been developed and was reviewed and approved by the WorldCOM consortium. The DMP is based on the Horizon 2020 FAIR DMP and was developed according to guidance provided by the OHEJP WP4 team in their D4.7 Guidelines for Data Management Plan implementation. The draft DMP is ready to submit. WorldCOM DMP leader Liam Burke attended online training on August 5th 2020 for the new OHEJP data management platform CDP. A data management guide for WorldCOM has been prepared for task leaders to help them comply with the DMP. The CDP



application will be updated with details of WorldCOM data throughout the project by Liam Burke, with information provided to him by task leaders on their datasets.



5.1.10.13.3 Progress of the research project: deliverables and milestones

5.1.10.13.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
13	D-JPR13-AMR2.1-WP1.1	Generation of a sequence database comprising publically available and newly generated sequences for <i>E. coli</i> , <i>Salmonella</i> and <i>Campylobacter</i> spp. and resistance genes CTX-M-15, NDM-5, KPC-2, OXA-48 and MCR-1.	M30 – June 30 th 2020		M31 - 31st July 2020	Confidential on relevant website section, and already shared with all consortium members during development.	3

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.13.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
13	M-JPR13-AMR2.1-01	Generation of sequence information for pathogens and resistance genes of interest.	M30 – June 30th 2020	No	M31 – July 31 st 2020	



5.1.10.13.4 Ongoing collaborations

NUI Galway participates in the MedVetKlebs OHEJP project, and is a member of the Med-Vet-Net association. Prof Morris is part of a JPIAMR network aimed at identifying robust, measurable surveillance indicators and methodologies for the monitoring of antimicrobial resistance (AMR) in the environment.

FLI participates in the JPIAMR consortia HECTOR and OASIS. HECTOR aims to identify determinants of host restriction of *Escherichia coli* strains and their potential association with AMR transmission and prevalence. OASIS aims to develop an AMR surveillance strategy, based on the Lot Quality Assurance Sampling approach, in a One Health context, and applicable in high-, middle-, and low-income countries.

UCM participates in the AVANT EU project, in ARDIG and FARMED, both from OH EJP, as well as in the MSCA CARTNET. Input from these projects will be relevant for WORLDCOM, and active participation between OHEJP projects will be encouraged for the benefit of OHEJP. Further, several TCs were organized between Dr. Burke and Prof. Gonzalez-Zorn to organize a visit to Madrid for training and organizing common research projects. The application for for Short Term Missions in OH EJP **has been approved**, and Dr. Burke will visit the lab at UCM in Summer 2021..

We are fully convinced that at the end of the project the tool obtained will be very useful, so it will be presented to EFSA and ECDC.

5.1.10.14 JRP14-AMR2.1-FULL-FORCE

5.1.10.14.1 Summary of the work carried out in the JRP

The goal of the Full Force project is to supply 17 EU partners with a technological toolbox and hands-on training in Single-Molecule Real-Time (SMRT) sequencing, and to apply this knowledge on six study cases. Using this state-of-the-art technology, public health and veterinary labs will have the capacity to perform full-length sequencing, and gain detailed insight in mobile genetic elements (MGEs) which carry antimicrobial resistance and virulence genes within and across species.

Unfortunately, this project set-up is hit hard by the Covid-19 pandemic. The basis of this project was supposed to be an on-site, three day workshop on SMRT sequencing, held at the State Serum Institute (SSI, DK), followed by a proficiency test to analyse each partner's capacity to perform SMRT sequencing. Due to restrictions imposed by all EU governments, we had to **postpone and reoriented this workshop to an online course held from September 7-9**. Moreover, all research activities were suspended for more than two months during lockdown, and many consortium members were reoriented towards Covid-19 surveillance. Likewise, the physical kick-off meeting, planned during ECCMID 2020 in Paris, was cancelled and replaced by a meeting in Brussels on October 8, unless the progress of the pandemic decides otherwise. It goes without saying that all this is causing significant delays in deliverables and milestones, as elaborated more in detail in the sections below. However, we are still confident that all goals of the Full Force project are still within reach.

- Applying intensive workload during the SMRT course, we still hope that our consortium partners with limited SMRT skills will be able to reach a sufficient technical level in plasmid sequencing. To maximize the output from this project, SSI scientists involved in WP1 are developing an easy-to-use software package (tentatively named Full Force Plasmid Assembler – FuFoPA), which will automatically perform hybrid assemblies through the build-in UniCycler program from a combination of short and long sequence reads. This package will be presented and applied during the online course.
- Although WP2 (five cases studies implementing long-read sequencing on existing datasets) has



suffered some delays, substantial progress has been made. Focus will be hypothesis generation based on short-read sequence data for the five defined studies cases (T2.1-2.5). Short-read Illumina data will allow to do a comparison of total plasmid content and relatedness of isolates and based on those results choose a subset of isolates for MinION runs.

- WP5 has not been impacted by the pandemic, as no lab work is required. The design of a transmission spread model of pAMR in the simulation framework SimInf has been initiated by collaboration between consortium partners.

5.1.10.14.2 Progress of the project: description of activities

WP 0: PROJECT MANAGEMENT (M25-M54)

This overarching WP ensures proper coordination at both the overall project and the individual WPs, as well as timely reporting of results and budgets according to the formal EU requirements.

Task 0.1: MEETINGS AND TELCALLS (M25-M54)

The two major consortium meetings, planned during ECCMID (Paris) and the SMRT sequencing workshop (SSI), were **postponed** due to Covid-19 measures. These were replaced by one-to-one teleconferences held by the PI with each individual partners, and by specific calls organized by task and WP leaders. To date (June 10, 2020), the workshop is planned for September 7-9, and the kick-off/progress meeting is set to take place October 8 in Brussels.

Task 0.2: REPORTING (M25-M54)

Three public deliverables (D-JRP19-WP1.D1/2/4) were uploaded to Zenodo. The nine month rapport was submitted in due time.

Task 0.3: CENTRAL DATA REPOSITORY (M25-M36)

FULL_FORCE will use a centralized data repository to upload sequence- and metadata which are generated during WP1 and WP2. Originally, we planned to use the AMR data hub of the European COMPARE Consortium and the European Nucleotide Archive (ENA). However, we were not successful in reaching an agreement with ENA, who is still negotiating single-subcontractor model for hubs created during COMPARE. Therefore, we have decided to use a commercial cloud tool (OwnCloud) for temporal data storage during Full Force.

Task 0.4: DATA MANAGEMENT PLAN (M25-M54)

Given the guideline update, was published in July 2020, we will provide a first DMP by September 2020, in collaboration by the EJP WP4 responsible, Géraldine Boseret.

WP 1: SMRT IMPLEMENTATION (M25-M36)

In the pre-pandemic planning of Full Force, we scheduled three-day workshop on practical implementation of long-read sequencing in Copenhagen. The main goals was to get less advanced users of SMRT sequencing up to speed, and to use the technological know-how in WP2-4. However, as explained below, we were forced to postpone this workshop and suffer from delays in deliverables and milestones.

Task 1.1 METHODOLOGY FOR MGE SEQUENCING (M25-M27)

In two rounds of teleconferences (January and March 2020) led by RIVM, task participants shared experiences in SMRT sequencing. In short, SSI results are between N50 of 15-20k, RIVM results N50 of 35k. Regarding DNA extraction methodologies, there was a choice between faster (semi-)automated protocols using magnetic beads (as used by Sciensano, APHA and SSI), and more elaborate protocols based on DNA precipitation as used by RIVM. As N50 of the RIVM protocol is clearly higher, it was



decided to go for the longer procedure to produce highest-quality data. Both protocols can be compared during the proficiency testing of Task 1.3.

A final protocol for SMRT sequencing was elaborated by RIVM, based on the rapid library generation protocol from Nanopore. Input was generated by bilateral conversations with John Rossen, PI of the JPIAMR SOLIDNESS project. They agreed on sharing strains and results from their project EQA on SMRT sequencing.

Task 1.2 SMRT SEQUENCING WORKSHOP (M28-M30)

The basis of this project was supposed to be an on-site, three day workshop on SMRT sequencing, held at the State Serum Institute (SSI, DK) in Q2 of 2020, followed by a proficiency test to analyse each partner's capacity to perform SMRT sequencing. Due to restrictions imposed by all EU governments, we had to **postpone and reorient this workshop to an online course to be held on September 7-9, 2020.**

Task 1.3 PROFICIENCY TEST FOR MGE SEQUENCING (M31-M36)

Each institution's proficiency in SMRT sequencing will be assessed afterwards using a proficiency test, organised and coordinated by SSI. Given the delay in the workshop, this EQA will be organised in M37-42.

WP 2: GENOME STUDIES (M25-M54)

In WP2, the acquired SMRT toolbox will be applied in the (re-)sequencing of AMR strains from various research and surveillance projects in WP2 including EU projects such as EFFORT, COMPARE, ENGAGE and ARDIG, as well as national and EU surveillance activities for which short read sequences are available. Given the postponed SMRT workshop, focus in WP2 in the first months of Full Force was lead on **hypothesis generation based on short-read sequence data** for the five defined studies cases (T2.1-2.5). Short-read Illumina data will allow to do a comparison of total plasmid content and relatedness of isolates and based on those results choose a subset of isolates for MinION runs.

Task 2.1 MGE evolution in longitudinal sample sets (ARDIG, ABRES) (M25-M54)

During a teleconference coordinated by Muna Anjum (APHA, UK), task participants decided to focus on plasmid evolution in longitudinal datasets from livestock and human samples. It was agreed to focus on plasmid evolution within the IncI1 plasmids encoding bla_{CTX-M-1}. All partners will extract sequence data from their databases to enable selection of strains for long-read sequencing.

Task 2.2 MGE evolution in cross-sectional data sets (EFFORT, ENGAGE & National Surveillance) (M28-M54)

Jens-Andre Hammerl (Bfr, GER) coordinates the group studying cross-sectional datasets, but he was absent due to illness. At the time of writing of this report, a first teleconference with task participants was being planned.

Task 2.3 Klebsiella pneumoniae: the canary in the coalmine (M28-M54)

Alma Brolund (PHAS) organised teleconferences with task participants, in which it was decided to focus on K. pneumoniae isolates with reduced susceptibility to carbapenems. Participants from Norway, Denmark, Sweden, The Netherlands and Portugal agreed that isolates with proposed high variation in genetic context were seen as most interesting to study. A separate work group will be initiated where Klebsiella isolates from the animal (and environmental?) sector can be further discussed. The task leader send around a metadata sheet serving as basis for strain inclusion. This groups estimates that short-read sequencing will be completed by M36.

Task 2.4 ESBL-producing Enterobacteriaceae in horses – A separated epidemiology of plasmids? (M28-M54)



SVA (Stefan Borjesson) coordinated a teleconference with task participants, in which it was decided to focus on *E. coli* isolates from horses encoding *bla*_{CTX-M-1} and *bla*_{SHV-12} genes. This group estimates that sequencing will be completed by M34. It is also worth noting that task leader (Stefan Borjesson) will be replaced by Joost Hendrickx (RIVM, NL) due to changes in job positions.

Task 2.5 *Salmonella* Infantis and *S. Kentucky* across reservoirs: role of MGEs (M28-M54)

ISS (Laura Villa) coordinated a teleconference among task participants, in which it was decided to focus on the pESI virulence plasmid of *Salmonella* infantis from animal and human origin. All task participants completed a metadata sheet, and samples were selected for short-read sequencing which should be completed by M34.

Task 2.6 Evaluation of publicly available and in-house tools for MGE typing (M30-M54)

This task has not yet been initiated at the time of writing.

WP 3: CULTURE-INDEPENDENT TYPING AND METAGENOMICS (M30-M54)

This work package has not yet been initiated at the time of writing.

Task 3.1 MGE ANALYSES IN METAGENOMIC DATASETS (M30-M54)

This task has not yet been initiated at the time of writing.

Task 3.2 CULTURE INDEPENDENT METHODS FOR PLASMID IDENTIFICATION (M30-M54)

This task has not yet been initiated at the time of writing.

WP 4: FUNCTIONAL CHARACTERIZATION OF AMR MOBILE GENETIC ELEMENTS (MGE)-CARRYING AMR GENES AND BACTERIAL HOST ASSOCIATIONS (M25-M54)

The overall goals of this WP are to (i) gain knowledge on molecular mechanisms of spread and persistence of main MGEs carrying critically/highly important antimicrobial resistances, (ii) identify key molecular interactions between AMR-MGEs and bacterial host important for dissemination and maintenance.

Task 4.1 SELECTION of MGEs and HOST STRAINS FOR DETAILED CHARACTERIZATION (M25-M42)

Given the delay caused by postponing both the kickoff meeting as well as the workshop on SMRT sequencing, it was decided that the selection of MGEs will be performed within tasks 2.1-2.5. Therefore, a current focus lies on pESI of *S. Infantis*, pKpQIL of KPC-producing *Klebsiella pneumoniae*, IncX3-SHV and IncHI1-CTX-M plasmids from horses, IncI1-AmpC/ESBL plasmids of *E. coli*. To allow smooth exchange of reference strains and/or donor-acceptor strains for conjugation experiments, a Material Transfer Agreement is circulating for approval by all WP4 partners.

Task 4.2 FUNCTIONAL CHARACTERIZATION of MGEs (M31-M54)

This task has not yet been initiated at the time of writing.

WP 5: MODELLING (M25-M54)

The objectives of WP5 are to address: i) gaps in quantitative knowledge on the spread of pAMR which will be essential to direct future focused research, ii) insight in the uncertainty around the effect of measures reducing pAMR prevalence in the food production chains, and iii) identification of key elements in the production chains to mitigate the risk of human exposure.

Task 5.1 MODEL DESIGN for AMR TRANSMISSION (M25-M42)

The design of a transmission spread model of pAMR in the simulation framework SimInf has been initiated. SVA (Stefan Widgren) has coordinated two teleconferences with task participants. The first teleconference was a startup meeting and the second teleconference was a meeting to discuss horizontal vs. vertical AMR transmission.



Task 5.2 EXPOSURE ASSESSMENT of HORIZONTAL and VERTICAL TRANSMITTED AMR (M25-M54)

This task has not yet been initiated at the time of writing.



5.1.10.14.3 Progress of the research project: deliverables and milestones

5.1.10.14.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
14	D-JRP14-WP0.D1	Start-up meeting report	M27	NA	M34	PUBLIC; Meeting originally planned at ECCMID 2020. Replaced by progress meeting in Brussels in October.	10
14	D-JRP14-WP0.D3	Recorded webinar tutorial ENA AMR data hub	M27		M33	CONFIDENTIAL; ENA was unable to collaborate and give access to the AMR data hub. We have therefore decided to opt for another, commercial platform to internally share sequence data during WP2-4. A webinar will be recorded at the SMRT workshop in September. At the end of the project and/or upon publication, sequences will be made publicly available at ENA.	3
14	D-JRP14-WP1.D1	Teleconference to assess required protocols and infrastructure	M25	M26		Public; 10.5281/zenodo.3733393	10



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
14	D-JRP14-WP1.D2	Teleconference to discuss proposed protocols and infrastructure (follow-up)	M26	M26		Public; 10.5281/zenodo.3759335	10
14	D-JRP14-WP1.D3	Completion of final protocol for SMRT sequencing	M27		M32	CONFIDENTIAL UNTIL PUBLICATION; The protocol will be finalized and published before the SMRT workshop in September 2020.	2
14	D-JRP14-WP1.D4	Invitation to workshop delivered to all participating institutions	M28	M27		Public; 10.5281/zenodo.3693741	10
14	D-JRP14-WP1.D5	Completion of workshop organization plan including selection of course material	M29		M32	The protocol will be finalized and published before the SMRT workshop in September 2020.	10
14	D-JRP14-WP1.D6	Selection of proficiency test data	M32		M36	CONFIDENTIAL UNTIL PUBLICATION; Proficiency test will be organised as follow-up of the postponed online course. Therefore, this delivery dates shifts backwards.	10



* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity

5.1.10.14.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
14	M-JRP14-M1	Creation of specific data hubs in ENA AMR hub	M27	Yes		Password-protected data hubs for each individual task were created at OwnCloud
14	M-JRP14-M5	Publication of first version of data management plan	M30	No		Due for September 2020
14	M-JRP14-M6	3-days workshop on SMRT sequencing event	M30	No		Due for September 2020
14	M-JRP14-M7	Shipment of proficiency test data and strains	M32	No		Due for January 2021
14	M-JRP14-M3	Selection of MGEs and host strains to be studied T4.2	M28	No		Due for January 2021
14	M-JRP14-M8	Sharing of protocols, recipient- and host-strains, molecular tools	M33	No		Due for January 2021



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JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
14	M-JRP14-M2	A teleconference or physical meeting on horizontal vs. vertical AMR transmission	M27	Yes		
14	M-JRP14-M4	A teleconference or physical meeting on input/output relationship between SimInf and sQMRA	M29	No		Due for October 2020
14	M-JRP14-M9	An implementation of a SimInf model designed for pAMR transmission to be studied in T5.1	M33	No	M36	



5.1.10.14.4 Ongoing collaboration

The SOLIDNESS network (JPIAMR, 2019-2020) which grouped experts in sequencing, plasmid biology and bioinformatics, and aims to streamline procedures for MGE sequencing. The PI of SOLIDNESS will give a lecture during the SMRT workshop.

Cross-sectional and longitudinal bacterial samples of ENGAGE, EFFORT (Horizon 2020, 2013-2018) and ARDIG (OHEJP, JRP2, 2018-2020) projects will be selected for long-read sequencing during WP2.

The KENTUCKY PhD project (OHEJP, 2020-2022) will use fully sequenced *S. Kentucky* strains (T2.4) to focus on the cell biology behind MGE transfer.

Potential collaborations with ECDC and EFSA might be envisioned, for sustainable implementation of long-read sequencing technology in surveillance of AMR in Europe.

5.1.10.15 JRP15-AMR2.1-FED-AMR

5.1.10.15.1 Summary of the work carried out in the JRP

In the FED-AMR project, extracellular DNA (exDNA) is presented as an important environmental reservoir for antimicrobial resistance genes (ARGs). Furthermore, bacterial transformation contributes to the horizontal gene transfer (HGT) of antimicrobial resistance genes (ARGs). However, empirical data on the impact of bacterial transformation in the environment are lacking.

Since the beginning of the project on 1st January 2020, and to fulfil its aims, we are conducting a longitudinal study over a one-year crop-growing period by monitoring and comparing 11 different matrices ("Compartments") from agricultural research areas (or alternatively, from production units) located in four European regions. Indeed, the FED-AMR project aims are 1) to analyse microbial biodiversity and ARGs along food/feed chain, 2) to evaluate the relevance of free exDNA in the HGT of ARGs over ecosystem boundaries 3) to identify points for intervention to reduce the spread of AMR via exDNA 4) to compare geographical differences and trends in AMR and antimicrobials in the natural environment 5) to put a focus on multidrug and emerging resistances.

Overall, we have been able to coordinate the sampling (WP1) and generate all sampling protocols (WP2) for the 11 compartments, the general culture protocol (WP2) and the *C. difficile* culture protocol (WP3), as well as the three protocols for analysis of antibiotics, elements and herbicides in environmental samples (WP4). Also, we finalized the description of the catchment areas and collectors within the four European regions (WP2), the guidelines for sample distribution, transportation and conservation (WP2), the sampling timeline for each collector (WP2) and the unified sample references (WP2). In addition, protocols including extracellular DNA extraction, Whole Genome Sequencing (WGS) of bacterial isolates and shotgun metagenomics are finished as well. The only protocol that is under discussion to be able to complete D-JRP15-FED-AMR-WP2.1 is the qPCR protocol, since the planned budget for metagenomics can be tight, and therefore we are considering to divert funds to metagenomics and obtain quantitative data rather than using qPCR.

All FED-AMR partners have been affected by COVID-19 as many have been directly involved in the work and laboratory activities, which have been shut down to handle only essential work. Additionally, in many cases, sample collection, laboratory work and analysis of the samples were delayed, as well as the recruitment of post-doctoral fellows, PhD students or technicians. However, very recently, 10-FLI and 13-SSI have recruited a PhD student each (Ines Dost and Semeh Bejaoui, respectively), 25-NUIG and 36-NSA a MSc student each (Charitini Nikolaidou and Rita Castro, respectively). Likewise, a PhD student (Krõõt Arbo) and two technicians (Jelena Kiprovskaia and Viia Kõiv) have been recruited by 14-UTARTU. Two Postdocs (Marwa Hassan and Brian Gardner, respectively) are already integrated and learnt about the FED-AMR project at 23-UoS.



Finally, many other projects and tasks from OHEJP projects have been delayed and the new plans for their execution will take place differently as initially foreseen, which may affect the timeline of the FED-AMR work (laboratory, data analysis and results dissemination).

Also, as described in WP1 and WP2 (see corresponding WPs below), the huge amount of experimental and sampling protocols needed exceeded the expectations. However, for these and all WPs, we are committed to make good use of the time lost in order to compensate the delay in milestones and deliverables. Nevertheless, all partners hope that the period of the project can be extended after June 2022, as this will help in weighting decisions, in more detailed analysis of results and in a greater dissemination, so that the project can have an improved impact on decision makers.

5.1.10.15.2 Progress of the project: description of activities

All WPs have started, but with delays in one or several tasks. Additional information on the progress can be found below for each task and subtask in the different WPs. As the recruitment of the last fellows has not happened until recently we will only be able to adjust all budget changes in December for the FED-AMR Annual Report, due to changes in several tasks of the project.

WP1: Project Management and Communication (M25-M54)

AGES is responsible for the project management and for all communication within FED-AMR (WP1). The leader of the WP1 is now Werner Ruppitsch and his deputy leader is Adriana Cabal Rosel.

JRP15-WP1-T1: Scientific Management (M25-M54)

Manuela Caniça (36-INSA) is now the responsible person for the scientific management of the project, acting as leader of task 1 within this WP (**WP1-T1**). She is supported by her deputy leader Adriana Cabal Rosel (2-AGES). This task is **ongoing** and it will comprise the whole project duration (M25 to M54). Up to now, this task together with task **WP1-T2** has involved the creation of a Scientific Supervisory Board (SSB) composed by experts within the consortium and the nomination of the local administrative representatives. The outcome of both tasks can be seen as part of the deliverable D-JRP15-FED-AMR - WP1.1 (before named as D-JRP17-FED-AMR -WP1.1).

A risk management strategy is being implemented and it is intended to be regularly updated by the Administrative Manager (Karin Rainer, 2-AGES) and the Scientific Manager (Manuela Caniça, 36-INSA), to ensure that adverse situations are properly handled along the course of the project.

JRP15-WP1-T1.1: Coordination of sampling, laboratory experiments and building a database (M25-M33)

Manuela Caniça (36-INSA) is now the responsible person for this **ongoing** sub-task, and her sub task deputy leader Adriana Cabal Rosel (2-AGES) supports her. Initially, this subtask was planned to start in M25 and to finish in M28. The task started on the expected month (M25), but it will end now in M33. This is partially due to SARS-CoV-2 crisis that prevented many partners to hire staff, work in the laboratory or coordinating sampling campaigns.

The deliverable associated to this sub-task (now named as D-JRP15-FED-AMR-WP1.2) was delayed as well because it is strongly associated with WP2 and its corresponding deliverable D-JRP15-FED-AMR-WP2.1. Both deliverables are expected by M33.

Contributing partners collaborated to generate new guidelines and harmonized protocols for sampling and experimental analysis. The high number of newly designed protocols exceeded the initial expectations and contributed to this delay, even when using some available from the EFFORT project, as well as from COMPARE and from reference institutions (e.g. DTU in Denmark), as foreseen. Those protocols included sampling protocols for 11 compartments, protocols for the molecular techniques, or culture protocols, among others.



In this sub-task (WP1-T1.1), the leader and the deputy leader, coordinated the sampling and the experimental protocols. Within the sampling, project partners were asked about the type of samples they could collect. As stated in the proposal, 11 different compartments were proposed for each European Region. However, not all partners could collect samples from each of the compartments. Most of the experimental protocols have been finished, but those related to genomics are finishing in M33. For additional information, see WP2.

JRP15-WP1-T1.2: Webinar forum and Skype meetings for instant scientific interactions (M30-M50)

Task WP1-T1.2 is **ongoing** and two webinars were already held. The first webinar on the topic “Environmental reservoirs of antimicrobial resistance genes” took part of the deliverable related to this task (D-JRP15-FED-AMR-WP1.4), which took place in M31. Professor Elisabeth Wellington was proposed as the first speaker. The second webinar was celebrated in M33 and it collected information about the first metagenomics and gene enrichment tests carried out with samples from the FED-AMR project. For the third webinar, which will take place in M33, the WP leader proposed Markus Wögerbauer as speaker and moderator.

Regarding the online meetings, the vast majority of project partners have attended through a different online platform other than Skype at the beginning of each month, since the kick off meeting. Minutes of the meetings were registered and shared with the project partners for approval. A definitive version incorporating all suggestions received was distributed and published in the AGES site (<https://fed-amr.ages.at>). In addition, weekly calls were arranged between the deputy leader of WP1 and the leader of WP1-T1.1 to discuss the evolution of the different WPs and tasks.

JRP15-WP1-T1.3: Project Meetings (M25-M52)

The Kick off meeting took place in Vienna at the end of the M25. The next one is planned in Lisbon in M41. This task is **ongoing**.

JRP15-WP1-T2: Administrative Management (M25-M54)

The administrative management (AM) is supported by the infrastructure of the AGES academy and the secretariat of the AGES knowledge transfer department. The coordination of joint activities in the frame of the FED-AMR project is being coordinated by AGES. Additionally, each partner had appointed an Administrative Representative who is and will be in direct contact with the AGES Administrative Manager (AM) whenever necessary. A risk management strategy for the project is being put in place by the Administrative Manager (AM) and the Scientific Manager (SM), in consultation with the Scientific Supervisory Board (SSB) to ensure that adverse situations are properly handled along the course of the project, which will be highlighted in the Data Management Plan. This task is **ongoing**.

JRP15-WP1-T3: Data and Protocol Management (M25-M52)

The Data and Protocol Management Plan has been delayed and therefore, its deliverable is expected by M34. DMP leader attended online training on August 5th 2020 for the new OHEJP data management platform CDP, provided by the OHEJP WP4 team. The CDP application will be adapted and updated with details of FED-AMR data throughout the project, with information provided to the leader and deputy leader by task leaders on their datasets. This task is **ongoing**.

WP2: Field experiments: Determination of the naturally occurring ARG background load and microbial biodiversity in the tested environmental compartments (M25-M50)

WP2 takes place over the first, second and third year of the project (Y3, Y4 and Y5) after the end of the project have been delayed to M50. Thus, Tasks WP2-T1. and WP2-T2. take place in the first year (Y3). Tasks WP2-T3, and WP2-T4. take place over the first and second year of the project (Y3 and Y4). Tasks WP2-T5. and WP2-T6. take place over the second year (Y4). Task WP2-T7 was delayed up to the third year, so now is planned to take place over the three years of the project (Y3, Y4 and Y5).



Overall, in this WP the prevalence, quantity and movement of AMR via free exDNA will be monitored along different compartments of the food/feed chain within the HOAL catchment: “human/animal gut -> manure -> soil -> crop -> drainage -> surface water -> groundwater -> human/animal”. All matrices (pig faeces, manure, agricultural soil, crop plants, drainage, surface and ground-water) will be analysed for the presence of clinically relevant ARGs encoded on free exDNA taking into special account antimicrobial treatments of the pig herds. Cultivable, resistant soil and gut bacteria will be characterized with standard microbiological methods. The results will be compared with data obtained from similar testing locations and environmental compartments from different regions. The establishment of the bacterial biodiversity in the tested compartments will be carried out, as well as the identification of the most prevalent naturally transformable species in agricultural soils and the monitoring of their fate in different environmental compartments.

JRP15-WP2-T1: Assemble list of sampling compartments and points. Determination of test areas representative for the European regions (North, West, East, South) (M25-M30)

The consortium members contributed to compile the final list of sampling compartments and points, having been carried out according to the collectors from East (Czech Republic, Poland), West (Austria, Ireland and Great Britain), North (Estonia and Norway), and South (Portugal). The final list of compartments according to partners was achieved and is already available to the participants via the AGES site (<https://fed-amr.ages.at>), as well as the sample timeline by collectors, the sample distribution, transport and conservation of sampling by compartment. A unique identifier by compartment and time point was given to all samples planned to be collected in the frame of the FED-AMR project, ensuring a correct traceability of all samples from their collection until their processing and analysis at the laboratory. The description of the HOALs and main catchment areas within FED-AMR was elaborated. Participants of WP2 (2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 36-INSa) provided input and advice according to their expertise and involvement in the sampling. The sampling list provided in this task supports harmonization of testing procedures and enhances comparability of the results obtained from those regions of Europe. As the leader and deputy leader of this task changed being actually 2-AGES and 36-INSa, they provided preparatory and final work, and the remaining participants took part, namely during the teleconferences made monthly by the project leader with all from the consortium. End month was delayed to 30. This task is **finished**.

JRP15-WP2-T2: Establish common protocol for sampling and data analyses to facilitate comparability of the results between European test areas (North, West, East, South) and local sampling locations (M25-M33)

Common protocols for sample collection were made available by the leader and the new deputy leader; it is finished later than planned (end month M33) due to the number of compartments and procedures and in view of the participants 2-AGES, 7-SZU, 14-UT, 23-UoS, 25-NUIG, 33-NVI, 36-INSa being more directly involved. At present, all compartments already have the respective protocols of sampling, which were uploaded on AGES site (<https://fed-amr.ages.at>) for FED-AMR, available for all partners. The protocols of culture are also available and provide a harmonizing framework in the microbiology laboratory, for the isolation, detection and identification of specific bacterial isolates from different samples collected, such as faeces (from pigs, wild animals and farmers), manure, soil, water, crops and feed. This procedure applies to bacterial isolates that may have human, veterinary, zoonotic or environmental bacteria, in such samples, focusing on six species (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* spp, *Staphylococcus aureus*, *Enterococcus faecium* and *E. faecalis*). Since joining the project in April, one 23-UoS Postdoc has been engaged in helping revising those protocols in which UoS is involved under WP2, e.g. pig feces, manure and culture, and in writing the susceptibility testing protocol, which is an example of cooperation of the partners in this task. The protocol for DNA extractions (eDNA and Total DNA) was finished for samples of all compartments (pig and wild animals feces, and feces from farmers, manure, soil, crops, river water, groundwater, wastewater and feed). The protocols for genomics are finished by the respective task and deputy task leader (see task WP2-



T3; WP2-T3.2 and WP2-T4). The only protocol that is under discussion to be able to complete D-JRP15-FED-AMR-WP2.1. is the qPCR protocol, since the planned budget for metagenomics can be tight, we are considering to divert funds to metagenomics and obtain quantitative data rather than using qPCR.

These common protocols support harmonization of testing procedures and enhance comparability of the results obtained from different regions of Europe; those protocols already concluded are available via the AGES site (<https://fed-amr.ages.at/home/>) for FED-AMR and under the members area in the OHEJP website (<https://onehealthjep.eu>). 36-INSa and 2-AGES provided preparatory work; the remaining participants provided input and advice according to their expertise and take part in voting and the final decision. This task is finishing in M33.

JRP15-WP2-T3: Assess microbial and ARG diversity with NGS in the selected test environments (metagenomics). Compare microbial and ARG diversity between ecosystems and over ecosystem boundaries. Characterization of cultivable environmental bacteria on complete nutrient and minimal media (M27-M48)

JRP15-WP2-T3.1 Shot gun sequencing and bioinformatic analyses of AMR genes and MGEs

We don't have enough work performed in this subtask according to the protocols that are missing (see task WP2-T2). Even that, the harmonized protocols already decided in **WP2-T1** and **WP2-T2** are using different samples interconnected with several environmental compartments during over a crop growing period of one year, so results will be achieved during this period and end by M48. This task is **ongoing**.

JRP15-WP2-T3.2: Gene enrichment with gene capture probes (M31-M42)

This subtask is being evaluated regarding the pros and cons to do it in terms of achieving better results in metagenomics, as samples with complex communities and various sources of DNA (e.g. soil) need to be well thought out to take decision. For this a pilot and QC studies are being processed between AGES and an enterprise to help on the decision according to the results obtained. The start of this task was delayed to M31.

JRP15-WP2-T4: Quantify clinically relevant ARGs in the tested compartments (qPCR; qPCR arrays) (M34-M42)

ARG qPCRs protocol was planned to be performed. However, since the planned budget for metagenomics may be too tight, we are considering diverting funds to metagenomics and obtain quantitative data from it instead of by using qPCR. A member of the Scientific Supervisory Board (SSB) is contributing with 2-AGES for this decision. If performed, the start of this task will be delayed to M34.

JRP15-WP2-T7: Isolate and assess quantity, diversity and stability of free extracellular ARG encoding DNA in the tested environments. Sequence comparisons (M34-M50)

The start and end of this task were delayed to M34 and M50 (in year 5), respectively.

WP3: Elucidating the role of *Clostridium difficile* as an ARG transfer platform over ecosystems boundaries and its linkage between human and non-human (zoonotic) reservoirs (M25-M50)

There is increasing evidence that *C. difficile* may have a foodborne or zoonotic aetiology, challenging the One Health paradigm. *C. difficile* has also been suggested as a reservoir/receptor of resistance genes that might be transferred to other species in the host gut as well as in the environment. WP3 aims therefore to investigate the epidemiology of zoonotic *C. difficile*, the genetic overlap between human and non-human *C. difficile* lineages and the role of *C. difficile* as an ARG transfer platform over ecosystems boundaries.

JRP15-WP3-T1 - Epidemiological survey of zoonotic ribotypes across participant countries. (M25-M40)

In M25 a first survey was carried out among the six WP3 participating partners, regarding available *C. difficile* strains, sources of these strains, year of isolation and available phenotypic and AMR data.



All involved partners have at the moment *C. difficile* strains that can be already integrated into the project, despite they will need to be complemented with isolates from other sources. Therefore, the possibility to collect new isolates from different sources was also evaluated, and the majority of the partners gave a positive response. Therefore the existing collection will be enriched in order to meet a One Health frame. Due to COVID-19 pandemic, the collection of new isolates is forecasted to start in M34. We are currently identifying in human *C. difficile* strains, the most likely zoonotic ribotypes. WP3 team has also prepared a harmonized protocol for *C. difficile* isolation and characterization from fecal, soil and water samples, to be applied in WP3, T3.4. This task is **ongoing**.

JRP15-WP3-T2 - WGS and AMR characterization of human and non-human *C. difficile* isolates (M34-M46)

In normal conditions, this task should start in M31, but due to COVID-19 pandemics, its start will be delayed to M34.

WP4: Determination of the selection pressures in the tested compartments of human, animal and environmental ecosystems (M25-M50)

Due to the COVID-19 crisis, the start of tasks WP4-T2 to WP4-T7 was delayed. The main reason was the impossibility of shipping samples to the laboratory performing the analysis (34-PIWET), due to national COVID-19 restrictions. Meanwhile, 128 samples from HOAL Austria were shipped on 22th June and 1st September 2020 to 34-PIWET (antibiotics), UBA Vienna (herbicides) and 23-UoS (elements), after the creation of a detailed list of shipping companies. All other samples will be shipped from other HOALs between September and October 2020. If everything goes according to the plan, analyses of the first set of collected samples of soil (WP4-T5), manure (WP4-T3) and water (WP4-T2) will be done by the end of September.

JRP15-WP4-T1 Selection of essential antimicrobials to be quantified in the tested compartments (published antibiotic consumption data, farmers' questionnaire, personal experience, expert interviews (veterinarians) (M25-M30)

This task is **finished**. Task WP4-T1, was planned for M25 to M26. The task was delayed, but it has been finished in M30 and the corresponding deliverable (D-JRP15-FED-AMR-WP4.1) was uploaded into the members area of the OHEJP website; this deliverable contains three protocols as annexes on the quantification of antibiotics, elements and herbicides in the different compartments.

Corresponding to the ARGs (tet(M), tet(W), tet(Z), sul1, sul2, sul3, erm-like genes, PMQR-encoding genes) to be investigated in the environment (faeces, manure, agricultural soil, drainage, surface and ground-water; see WP2), the four antimicrobial classes to be tested in these compartments were selected: tetracyclines, macrolides, sulphonamides and fluoroquinolones (task JRP15-WP4-T1). From these antibiotic groups the most important (according to published antibiotic consumption data and EFSA report on antibiotic residues in live animals and food) were included in the analytical method by liquid chromatography-tandem mass spectrometry (LC/MSMS) performed by 34-PIWET (WP4-T2 to WP4-T5).

Herbicides were chosen in the same Task, among those that are often used in agriculture, such as glufosinate and glyphosate, as well as its degradation product aminomethylphosphonic acid (AMPA), 2,4-Dichlorophenoxyacetic acid (2,4-D). Quantification of these substances (Task WP4-T6) will be performed by an AGES associated sister company (UBA Vienna).

Heavy metals and trace elements were also already chosen among those that are triggering co-selection and that have been used in co-selection studies: Cd, Cr, Cu, Ni, Hg, Co, Pb, Zn. The samples will be analysed by inductively coupled plasma mass spectrometer (ICP/MS) carried out by 23-UoS (Task WP4-T7).

JRP15-WP4-T2 Quantification of four antimicrobial classes (tetracyclines, macrolides, sulphonamides and fluoroquinolones) in aqueous matrices (water) (M31-M50)



Samples from some HOALs were taken and collected: 5 water samples from different compartments including wastewater (inlet and outlet), river water, ground water and drainage water.

Due to the COVID-19 crisis, the start of tasks WP4-T2 to WP4-T7 has been delayed. The main reason was the impossibility of sending samples to the laboratory performing the analysis, due to national COVID-19 restrictions. Meanwhile, 10 samples from HOAL Austria were shipped on 22th June and 1st September 2020. If everything goes according to plan, analyses of these first set of collected samples will be done by the end of September. The expected starting date for this task was M27, but it has been delayed to M31 (M-JRP15-FED-AMR-31 to -36, except -32).

JRP15-WP4-T3 Quantification of four antimicrobial classes (tetracyclines, macrolides, sulphonamides and fluoroquinolones) in manure (M31-M50)

The expected starting date for this task was M27. The start date for analyses is now M31 (see task WP4-T2). However, samples from some HOALs were already collected. 4 samples from HOAL Austria were shipped on 22th June and 1st September 2020. Other HOALs will ship samples in September 2020.

JRP15-WP4-T4 Quantification of four antimicrobial classes (tetracyclines, macrolides, sulphonamides and fluoroquinolones) in faeces (M35-M50)

The expected starting date for this task was M30. The new starting date is delayed to M35.

JRP15-WP4-T5 Quantification of four antimicrobial classes (tetracyclines, macrolides, sulphonamides and fluoroquinolones) in soil (M31-M50)

The expected starting date for this task was M27. The start date for analyses is delayed to M31 (see task WP4-T2). Samples from some HOALs have been already collected. 28 soil samples from HOAL Austria were shipped on 22th June and 1st September 2020. Other HOALs will ship samples from September 2020 onwards.

JRP15-WP4-T6 Quantification of herbicides in agricultural soil (M31-M50)

The expected starting date for this task was M27. The start date for analyses is delayed to M31 (see task WP4-T2).

Samples from some HOALs were collected. 41 samples (28 soil, 3 manure, 10 water) from HOAL Austria were shipped on 22th June and 1st September 2020. Other HOALs will ship samples from September 2020 onwards.

JRP15-WP4-T7 Measurement of the concentration of trace elements in environmental samples gathered across participants countries (M31-M50)

Since the start of the project, the work has been done in order to design a sampling strategy suitable for the analysis of trace elements in variety of samples. These include soils, crops and animal feed, water, manure and sewage sludge. The researchers have worked very closely with the leaders of WP2 in order to harmonize the sampling strategy and make sure that the sampling procedures and processing were compatible with all types of analyses across the consortium. The team has also completed the protocol for sample preparation for solid and liquid samples, as well as the procedures for instrumental analysis and the sourcing of the certified reference material for validation. Arrival of the first set of samples to the ICP-MS Facility at Surrey was planned for March 2020, however this was postponed due to the lock-down and restrictions to research activities at the University of Surrey, starting on 23rd March. The labs have started to reopen gradually since June, and after assessing the risk posed by COVID-19 epidemics, the 1st set of samples is expected to be completed by the end of September 2020.

Samples from HOALs have been already taken, namely at the Austrian HOAL, where 21 soil, 5 water, 2 manure and 2 feed samples were collected. 45 samples (28 soil, 3 manure, 10 water, 3 feed, 1 crop)



from HOAL Austria were shipped on 22th June and 1st September 2020. Other HOALs will ship samples from September 2020 onwards.

The expected starting date for this task was M27. The start date for analyses was delayed to M31.

WP5: Identification of environmental conditions modulating transformation frequencies in soil microcosms and an in vitro porcine gut model (poGutMo) (laboratory studies) (M32-M52)

The start of this work package has been delayed for two reasons. First, it took longer than anticipated for 23-UoS to recruit the PDRA to work on this work package. Second, the laboratories at 23-UoS have been closed due to national COVID-19 restrictions since March. Marwa Hassan was recruited successfully to the project at the end of April 2020. In the second week of June, the first members of staff have begun a phased return to the laboratories. M. Hassan was able to access the laboratories early in July. From mid-June, she has focussed on preparing the protocols and ordering the consumables to start work on WP5 in M32. This represents a seven-month delay to the anticipated start of WP5. As such, WP5-T1 will now be extended into year 4, as will WP5-T1-ST3 and WP5-T1-ST4. We anticipate getting the project back on track by M45.

Acinetobacter was proposed as a model organism for transformation experiments; however, our preliminary experiments proved the inability of this pathogen to grow anaerobically. After consultation with the team and FEM-AMR consortium, we are going to perform the transformation experiments using *E. coli* as a model organism in the anaerobic gut model. Currently, the selection of strains to use for the transformation and conjugation experiments has started. *E. coli* J53 (a derivative of K-12) and 912 (isolated from pigs) will be kindly provided by AGES and Ana Herrero-Fresno (University of Copenhagen), respectively, to be used for the transformation experiments. For the conjugation experiments, *C. difficile* strains 630 and CD37 will be kindly provided by Prof. Peter Mullany, University College London, in the near future. A further investigation is still ongoing to identify other potential isolates to use in the pig gut model. We are currently finalising the protocols and involved risk assessments for WP5, including procedures for analysis of trace elements and heavy metals in samples from the pig gut model.

JRP15-WP5-T1 Establish baseline levels of HGT in the model organism (*E. coli*) arising from transformation in the poGutMo (M32-M45).

Preliminary experiments proved the inability of *Acinetobacter* to grow anaerobically; thus, *E. coli* was chosen as a model organism for the transformation experiments in the anaerobic gut model.

Start date delayed to M32. New end month: 45.

JRP15-WP5-T1-ST1 Ability of *E. coli* strains to acquire AMR to serve as a donor DNA (M34-M36).

Start date delayed to M34. New end month: 36.

JRP15-WP5-T1-ST2 Determine the optimal growth parameters for cultivating *E. coli* strains within the gut model (M36-M39).

Start date delayed to M36. New month: 39 (Year 4).

JRP15-WP5-T1-ST3 Rates of transformation calculated by taking samples from the gut model and plating on TSC agar plates supplemented with the appropriate antibiotics (M41-M45).

Start date delayed to M41. New end month: 45 (Year 4).

JRP15-WP5-T1-ST4 DNA transfer rates via bacterial conjugation will be calculated using the endpoint method (M34-M45).

Start date delayed to M34. New end month: 45 (Year 4).

WP 6: Probabilistic and mechanistic models of the links between antimicrobial usage in animals, AMR in the environment, and the risks for public health (M32-M54)



The start of this work package has been delayed as it took longer than anticipated for 23-UoS to recruit the PDRA to work on this work package. A Postdoc (Brian Gardner) was recruited successfully to the project at the beginning of May. Thus, the project and milestones were delayed by two months.

JRP15-WP6-T1 Build a probabilistic mathematical model of the emergence of AMR in target bacteria and the relative contribution of transformation and conjugation to ARG acquisition (M32-M54).

Gardner has started to define the protocol for a systematic search of the literature on environment and AMR. The output of the search will also provide the data to be used as input for the machine learning approach. An initial preliminary search returned about 3000 papers, and we are now finalizing the protocol and inviting other members of the team to start the review. As Barnaghi has recently resigned from the University, Mirek Bober has agreed to help with WP6-T1, mentoring Brian Gardner and advising the group.

JRP15-WP6-T1-ST1 Data Integration, Annotation and Association Analysis (M32-M37)

Start date delayed to M32.

JRP15-WP6-T2 - Develop mechanistic models to address key questions regarding the spatio-temporal changes observed in microbiological communities (M32-M54)

Since May, Gardner has been fully engaged in reviewing the relevant literature. He has been in contact with team in UoS (Chambers, La Ragione, Horton and other members of their group) to explore different sources of data that can be used as input/validation for the modelling approach and to refine the research questions. He is also exploring different modelling approaches (e.g. agent-based model) relevant to the task.

JRP15-WP6-T2.1 - Modelling microbial communities I (M34-M41)

Start date delayed to M34.



5.1.10.15.3 Progress of the research project: deliverables and milestones

5.1.10.15.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
15	D-JRP15-FED-AMR - WP1.1	Scientific Supervisory Board (SSB) installed. Local administrative representatives nominated (T1, T2)	M25	M25		Confidential (contains e-mail addresses of the members of the consortium)	10
15	D-JRP15-FED-AMR - WP1.2	Unified sampling and experimental protocols (T1.1.)	M27		M33	Public	2
15	D-JRP15-FED-AMR - WP1.3	Data and protocol management plan (T3)	M27		M34	Public	8
15	D-JRP15-FED-AMR - WP1.4	Webinars (T1.2.)	M30	M31		Public	5



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
15	D-JRP15-FED-AMR-WP2.1	List of sampling compartments, points and European test areas and harmonized protocols in alignment with EFFORT project protocols available in data repository (T2.1, T2.2)	M26		M33	Public	2
15	D-JRP15-FED-AMR-WP4.1	Standardize protocols for sampling and testing of environmental samples	M26	M30		Public	2
15	D-JRP15-FED-AMR-WP5.1	<i>E. coli</i> strains demonstrated to be suitable for transformation	M26		M36	Confidential until published	10
15	D-JRP15-FED-AMR-WP5.2	Optimal growth parameters for cultivating <i>E. coli</i> within the porcine gut model and the time after inoculation at which its concentration is maximal determined	M27		M39	Confidential until published	10



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
15	D-JRP15-FED-AMR-WP5.3	Optimal growth parameters for cultivating the clostridial strains within the gut model determined	M28		M37	Confidential until published	10
15	D-JRP15-FED-AMR-WP5.4	Results from pilot experiments using PCR amplicons as ARG donors	M30		M43	Confidential until published	10
15	D-JRP15-FED-AMR-WP5.5	Conjugation-mediated HGT between the clostridial donor and recipient strains within the gut model determined	M31		-M45	Confidential until published	10
15	D-JRP15-FED-AMR-WP5.6	Results from using chromosomal DNA derived from <i>E. coli</i> as ARG donor	M33		M45	Confidential until published	10



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
15	D-JRP15-FED-AMR-WP6.1	Main code for the mathematical modelling made available in public repository (e.g. GitHub) with associated documentation (which can be used as "Material and Method" section of the forthcoming publications).	M30		M37	Public	3

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.15.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
15	M-JRP15-FED-AMR-01	Kick off meeting	M26	Yes		
15	M-JRP15-FED-AMR-02	Database repository active	M27	Yes		
15	M-JRP15-FED-AMR-03	Webinar forums started	M30	Yes		The consortium initiated the scientific exchange via online teleconferences and formally installed regular webinars by M31.



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M25-M36



JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
15	M-JRP15-FED-AMR-07	List of sampling compartments, points and European test areas available. Harmonized protocols for sample collection + transportation, DNA extraction, qPCR, metagenomics, shotgun sequencing, gene capture and bioinformatics and statistical analysis of sequence data available. Alignment with EFFORT project protocols (T2.1, T2.2)	M26	No	M33	The list of sampling compartments, points and European test areas have been defined. Harmonized protocols for sample collection and transportation and DNA extraction protocols are already available for all project members. Protocols for WGS, metagenomics, gene capture and bioinformatics are still under development. When possible, sampling protocols were aligned with e.g. EFFORT project.
15	M-JRP15-FED-AMR-30	Starting the selection of essential antimicrobials to be quantified in the tested compartments	M25	Yes		
15	M-JRP15-FED-AMR-31	Starting the analysis of antimicrobials in aqueous matrices	M27	No	M31	



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Third Year - 2020
M25-M36



JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
15	M-JRP15-FED-AMR-32	Starting the analysis of antimicrobials in manure	M31		M31	
15	M-JRP15-FED-AMR-33	Starting the analysis of antimicrobials in faeces	M29	No	M31	
15	M-JRP15-FED-AMR-34	Starting the analysis of antimicrobials in soil	M31			
15	M-JRP15-FED-AMR-35	Starting the quantification of herbicides in agricultural soil	M31			
15	M-JRP15-FED-AMR-36	Starting the measurement of the concentration of trace elements in environmental samples	M27		M31	15
15	M-JRP15-FED-AMR-37	Bacterial strains supplied to UoS	M25		M34	15



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Third Year - 2020
M25-M36



JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
15	M-JRP15-FED-AMR-38	Porcine gut model set up using faecal samples obtained through WP2, samples stored for trace element analysis (WP4) – experiments can start	M26		M40	15
15	M-JRP15-FED-AMR-39	Samples from gut model experiments stored for trace element analysis (WP4)	M30		M41	
15	M-JRP15-FED-AMR-40	Samples from gut model experiments stored for trace element analysis (WP4)	M31		M42	



5.1.10.15.4 Ongoing collaborations

There are complementarities between the FED-AMR protocols and those available from EFFORT and COMPARE projects, and some from the DTU National Food Institute, the International Organization for Animal Health (OIE), and also needed information from European Food Safety Authority (EFSA). Several partners of FED-AMR participate in other JRP and JIP projects from OHEJP (e.g. AGES participates in the MedVetKlebs, INSA participates in Matrix, etc).

The synergies between these projects could be established at a later stage, once the project partners within FED-AMR share their first results. The same will apply to possible synergies with EFSA, ECDC, the SSB and POC members.

5.1.10.16 JRP16-ET2.2-TELE-Vir

5.1.10.16.1 Summary of the work carried out in the JRP

The worldwide COVID-19 pandemic has had a great impact on the TELE-Vir project. There has been national lockdowns, laboratories have been closed or allocated for COVID-19 diagnostics. There has been a worldwide shortage of basic laboratory reagents and equipment, which have influenced the ability to perform basic laboratory experiments. At this time point (June 2020) the COVID-19 crisis is less severe and countries are opening up which means that the TELE-Vir partners can begin to increase their activities.

At the TELE-Vir kick-off meeting held at IZSAM, Italy (20th-22nd of January 2020), it was decided to use Coronaviruses and Influenza A virus as model viruses for the proof-of-principle studies. Many of the TELE-Vir partners have been involved in COVID-19 diagnostics and due to the shortage of reagents for NA extraction, the partners have been forced to develop alternative methods for NA extraction which is in line with the development of a field based protocol for MinION sequencing using a minimum of laboratory equipment (the poi tool box). In addition, surveillance programs for the novel coronavirus (SARS-CoV-2) are based on sequencing of the virus, which has resulted in an upgraded version of the INSAFLU software. The new version is better to accommodate identification and genome-based analyses and to display metadata on phylogenetic trees, thus facilitating integration of relevant epidemiological and/or clinical data and pathogen genomic data (https://insaflu.readthedocs.io/en/latest/change_log.html). Further developments are ongoing.

Overall the COVID-19 pandemic has had a positive impact on the TELE-Vir project and many of the experiences and problems encountered during the crisis can be used or translated to the development of the TELE-Vir poi-tool box.

5.1.10.16.2 Progress of the project: description of activities

WP1: Coordination and data management (M25-M54)

WP1-T1: Coordination and project management (M25-M54)

Project management and coordination of the project is proceeding according to the plan. This is ongoing.

WP1-T2: Data management (M25-M54)

The OHEJP project management team has postponed the deadline of first DMP delivery. We await their decision on a specific intuitive use-friendly tool that can help us create our DMP. This is ongoing.

WP1-T3: Kick-off-meeting at IZSAM, Italy (M25-M26)



The kick off meeting of the TELE-Vir project was held on January 21st – 22nd 2020 at the International Centre for Veterinary Training and Information "F. Gramenzi" (CIFIV) of Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (Via G. Caporale, Teramo, Italy). All partners were present. This task is completed.

WP2: Development of a Bioinformatics tool-kit for POI data analysis (M25-M54)

WP2-T1: Survey and collection of databases for genotype-phenotype associations (M25-M36)

At the kick off meeting it was agreed that the first stage was selection of important phenotypic characteristics for the chosen model virus (influenza and coronaviruses), followed by assessment of the amount and quality of data available. A literature review has been performed to identify coronavirus phenotypes of relevance to tropism, emergence, and clinical disease, and any data available for their prediction based on genotype. A summary has been prepared for circulation to partner institutes and reference laboratories for elicitation of expert opinion. A similar exercise is still to be performed for influenza A virus. These reviews will form the basis for a manuscript, to be submitted for publication in a scientific journal. Work on this task is ongoing.

WP2-T2: Development of bioinformatics modules for third-generation sequencing analysis and pathogen identification (M25-M39)

In the context of pathogen detection and field genome sequencing, there are multiple advantages in either using online bioinformatics tools or running the platforms locally. As such, a Docker version of the online INSaFLU platform has been built and distributed publicly (<https://github.com/INSaFLU/docker>) in order to facilitate the local installation process. INSaFLU users, TELEVIR partners and other stakeholders (e.g., ECDC) were notified of this novel feature. INSaFLU has been successfully installed and run locally 'offline' on partner computer servers including at UoS.

As a response to COVID-19 pandemic, both the locally installed INSaFLU version and original website (<https://insaflu.insa.pt/>) were adapted to better accommodate the identification and genome-based analyses of the novel coronavirus (SARS-CoV-2), as follows:

a new module for rapid assignment of Human Betacoronavirus (BetaCoV), including the novel coronavirus (SARS-CoV-2), has been developed and implemented, and the rationale behind the classification and outputs was documented (https://insaflu.readthedocs.io/en/latest/data_analysis.html#influenza-type-and-sub-type-identification-and-human-betacoronavirus-classification-as-of-march-2020; check more details in the list of current INSaFLU genetic markers used for Influenza type and sub-type identification and Human Betacoronavirus classification);

the publicly available SARS-CoV-2 reference genome sequence (NCBI accession number MN908947) was inserted as default in the INSaFLU reference database;

multitasking configurations were changed, considerably speeding up the analyses, and the maximum upload file size was made more flexible; and,

a new tab "Settings" was created making software parameterization more flexible and tailored to SARS-CoV-2 NGS analyses.

Work on this task is ongoing.

WP2-T3: Development of bioinformatics modules for sequence curation and phenotypic association (M25-M42)

This task is highly dependent on the collected databases for genotype-phenotype associations, so the design of the bioinformatics approach cannot be fully drawn at this stage. Meanwhile, we will investigate the feasibility of inferring biochemical and immunological properties using existing models



and machine learning approaches being tested at UoS. Also, novel software parameterization features at the INSaFLU sequence curation module have been developed and will be implemented soon. Work on this task is ongoing.

WP2-T4: Development of bioinformatics modules for genomic and metadata integration towards enhanced surveillance (M25-M54)

Following the kick off meeting a strategic approach has been agreed to achieve this task. First, Nextstrain (<https://nextstrain.org>) tools will be implemented for temporal and phylogeographical analysis. Then, we implement novel functionalities focused on fitting the needs of labs working in different sectors (vet, PH, etc) and that can be handled by users from multidisciplinary fields. In this context, INSaFLU was also upgraded to easily display metadata on phylogenetic trees (through user-defined node colouring and metadata blocks), thus facilitating integration of relevant epidemiological and/or clinical data and pathogen genomic data (https://insaflu.readthedocs.io/en/latest/change_log.html). Still, further developments are ongoing, dependent on tasks 2 and 3.

WP2-T5: Development of a user- and surveillance-oriented web-based interface (M25-M54)

An application for an STM is in preparation for UoS and INSA to align bioinformatic approaches and share knowledge of the existing INSaFLU bioinformatics pipeline, in the context of WP2-T2 and WP2-T3 and to facilitate the eventual design of the user interface. Work on this task is ongoing.

WP3: Development of a protocol for POI MinION sequencing (M25-M48)

WP3-T1: Development and validation of a S.O.P for sample handling & pre-treatment (M25-M48)

A detailed research plan for testing of the virucidal activity of MPLB buffer against different animal viruses under various temperature-time conditions was developed. The experiments will meet the requirements of EN 14675: 2015 standard document. For propagation of selected animal viruses (myxoma virus, MYXV; canine adenovirus type-2, CAV-2) representing different virus families, the following cell lines were used: rabbit kidney (RK13), madin-darby canine kidney (MDCK) and A-72. Viruses were grown in cell cultures to the titres of $10^{5.84}$ TCID₅₀ /ml for MYXV and $10^{7.47}$ TCID₅₀ / ml for CAV-2. Additionally, to increase MYXV titre in cell culture suspension a virus concentration step was performed. The virus stock suspensions were prepared for subsequent inactivation studies. This study is ongoing.

Development of a protocol to verify the inactivation of Bovine Beta-CoV (Bov-CoV) using different inactivating buffers: MPLB and eNAT™-buffer. The last one is a Guanidine-thiocyanate based medium that stabilizes the RNA and DNA of Viruses. Bov-CoV strain because it belongs to the same genus of SARS-CoV-2 and can be a valuable model for validating virus inactivation by using several inactivating buffers. The protocol is based on the ultracentrifugation of the virus-inactivating buffer compound in order to remove the toxic effect of the inactivating buffer on cell cultures. Two different virus titers and two different contact times were considered. The reduction in the virus titers expressed in TCID₅₀ was then calculated. This study is ongoing.

Development of virus inactivation protocols without the use of lysis buffers. Heat inactivation of SARS-CoV-2 virus has been tested by heating nasopharyngeal swabs positive for SARS-CoV-2 for 5min at 98°C. The heated swabs were filtrated and used for infectivity studies on Vero E6 cells. Preliminary results show that simple heating for 5min. inactivates SARS-CoV-2. This study is ongoing.

WP3-T2: Development and validation of a S.O.P for sample NA purification (M25-M48)

Selected nasopharyngeal swabs have been processed with different lysis/inactivation buffers trying to circumvent the NA extraction. The first trials resulted in a lesser viral RNA detection by real-time PCR when compared to the established diagnostic procedure, resulting in the ultimate loss of the weak



positive samples. Preliminary assays using samples collected on FTA cards have also led to lower detection of viral RNA. This study is ongoing.

Heating of nasopharyngeal swabs has also been tested in order to circumvent NA extraction (Fomsgaard and Rosenstjerne, Euro Surveill. 2020 Apr;25(14):2000398. doi: 10.2807/1560-7917). Other alternative methods such as the use of detergents in the RT-PCR mastermix is currently being validated. Whether or not these alternative methods are compatible with library preparation for MinION sequencing will be investigated. This study is ongoing.

WP3-T3: Development and validation of a S.O.P for NGS library preparation & MinION sequencing (DNA & RNA) (M25-M48)

A SOP for MinION sequencing of Avian influenza virus (AIV) has been tested. Four primer PCR protocol and the PCR Barcoding Kit (SQK-PBK004) from Oxford Nanopore Technologies (ONT, Oxford, UK) was used to sequence and typing of avian influenza virus. Preliminary amplification of RNA was conducted prior to sequencing on MinION utilizing SuperScript IV One-Step RT-PCR System with Platinum Taq (Invitrogen/ThermoFisher Scientific, Waltham, MA) and universal Influenza A primers designed for the conservative ends of all AIV segments (Zhou et al., 2009, J Virol 83:10309-10313). Spot on Flow Cell, R9 version (FLO MIN 106D; ONT) and basecaller Guppy (v3.29; ONT) was used for the real-time basecalling to produce sequencing data and monitor the run. Sequencing data from two samples were analyzed using CLC Genomics Workbench (Qiagen-CLCBio). Study is ongoing.

A SOP for targeted sequencing of SARS-CoV-2 on MinION has been tested and validated and is used for comparison to a metagenomics approach on the same clinical sample material from humans and mink. The SOP is based on the ARTIC nCoV-2019 amplicon sequencing protocol (<https://artic.network>).

For the metagenomics approach, a sequence independent isothermal amplification step will be included before library preparation and two methods are currently being tested. Sequencing independent single primer amplification (SISPA) and Recombinase polymerase amplification (RPA). Both have the potential to amplify sample NA before sequencing and thereby increase the sensitivity of viral detection. This study is ongoing.



5.1.10.16.3 Progress of the research project: deliverables and milestones

5.1.10.16.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category * (1 to 10)
16	D-JRP#-WP1.1	Kick-off meeting in Italy (IZSAM)	M25	31 st of January 2020		Public https://zenodo.org/record/3734134#.Xwbj0Sgz a70	10
16	D-JRP#-WP1.2	1st version of the DMP	M30		M36 postponed by OHEJP management team	Public	5

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity.

5.1.10.16.3.2 Milestones

No milestones before M36



5.1.10.16.4 Ongoing collaborations

National project RTA2015-00002-C02-01-E. Analysis of new **West Nile virus encephalitis outbreaks** in Spain and its geographical expansion. Field samples obtained in this national project will be used in the WP4 of TELE-VIR project.

Activities as **EU Reference Laboratory for African Swine fever** will allow to compile some field samples to be used in the WP4 of TELE-VIR project.

MEDILABSECURE (CoE Project 037 Ct N° IFS/2013/330 961) (www.medilabsecure.com): INIA coordinates the animal virology network of the «One-Health» based MedilabSecure project, incorporating (in addition to animal virology) human virology, entomology, public health & veterinary services and early warning modelling networks from 22 non-EU countries in the Mediterranean, Sahel and Black Sea regions, aiming at improving diagnostic capacities and integrated surveillance for better response against emerging zoonotic diseases. TELE-VIR can assist in these efforts providing an easy portable tool for a rapid identification and response against viral threats not only for EU but also to our neighbouring non-EU countries.

A collaboration between SSI, Aalborg University and the Danish hospitals have successfully been established during the COVID-19 crisis in order to setup a **national COVID 19 Surveillance system**. The consortium has whole genome sequenced more than 2000 SARS-CoV-2 positive samples using the Nanopore platform and the ARTIC network protocol. The Danish Ministry of education and science (UFM) and Grundfos Foundation have funded the consortium (1.5 mio. €).

As the project progresses contact to ECDC, EFSA and the Swedish Food Agency as EU-RL for foodborne viruses (<https://www.livsmedelsverket.se/en/production-control-and-trade/eurl-foodborne-viruses?AspxAutoDetectCookieSupport=1>) will be made in order to inform them of our platform.

5.1.10.17 JRP17-ET2.2-IDEMBRU

5.1.10.17.1 Summary of the work carried out in the JRP

Project has started as planned in January 2020. After initial kick off meeting on December 17th 2019, several meetings had been done on organizing the project work packages (WP), hiring personnel and starting the works on literature data analysis and samples collections. Unfortunately, in March everything had to be stopped due to COVID-19 sanitary crisis. Due to a still ongoing crisis, it is hard to restart scientific activities. Sample collection had to be postponed due to strict movement restrictions. One post-doctoral fellow started working on the project in January, while another one started in May due to restricted rules for movement from the USA to EU. Other employments had to be postponed for later period. The work on the project interactive data and Large file Storage platforms as well as Data management are finished. Second draft of Material Transfer Agreement (MTA) has been created and sent to partner institutions for verification. DMPs were created for first two WPs. However, with new EJP guidelines and DMP toolkit, the consortium decided not to publish it and wait to finish necessary trainings in order to use the platform. Existing sample collection data has been defined and draft or analysis strategy has been prepared in order to detect atypical *Brucella* spp. in different biotopes. Harmonized SOPs for sample treatment are drafted and will be tested in several consortium laboratories within following months. Due to all delays and state of emergency still active in several partner countries, the consortium had to postpone all deliverable and milestone deadlines and annual meeting for six months (estimated delay).



5.1.10.17.2 Progress of the project: description of activities

WP 1-Recording the situation of brucellosis in emergent wild and environmental reservoirs (M25-M44)

Task 1 started on January 2020. Due to all delays, this task has to be hindered until December 2020. Task 2 had to be delayed from May to November 2020, and will be finished until February 2022.

Common database for all samples collected and treated before and/or during the project had been created. Each partner will include their sample collection of atypical *Brucella* spp. originating from animal species and environment as well as samples collected during the project. This database will include all results generated during the project from WP-1 to WP-5.

Multiple animal and environmental samples will be represented.

Further animal and environmental sampling had to be postponed due to currently ongoing sanitary crisis. Therefore, consortium decided to use the existing collection of various previous projects performed by other teams in partner institutions, to generate initial data on which sampling strategy can be based.

WP2 - Recording the situation of brucellosis in humans (M25-M44)

Due to all delays, task 1 had to be delayed from January to July 2020, and will be finished until December 2020. Task 2 had to be delayed from May to November 2020, and will be finished until February 2022.

The question was raised about what kind of network the project should produce: Just a *Brucella* mailing list or interactive network.

Future actions:

Create a survey for the experts in Bruce list to ask how would they diagnose emerging *Brucella* and/or differentiate classical from atypic species cases.

Identify the expert point person in ECDC.

It was decided to develop diagnostic kit for emerging *Brucella* in humans.

WP-2 members will test potential antigens on humans, negative for classical *Brucella* species, first.

Inclusion criteria for other human samples will be: feverish patients and/or potentially exposed to atypical *Brucella* sources/reservoirs.

WP3 - Genomic characterisation of *Brucella* detected from samples and selected isolates (M25-M48)

Due to all delays, Task 1 had to be delayed to November 2020, and will be finished until April 2022. Task 2 will start in July 2020 and will be finished by June 2021, while Task 3 will start in July and finish until April 2022.

Due to the high costs of metagenomics, it was decided to apply it only to the existing strain collection.

WP-3 members will perform standardization of DNA extraction protocols, which will be tested in several consortium laboratories by performing it in parallel with "partner's reference method".

PCR detection will be designed/optimized in order to detect *Brucella* spp. from animal, human and environmental samples.

WP4 - Phenotypic characterisation of *Brucella* detected from samples and selected isolates (M25-M54)

Due to all delays, Task 1 had to be delayed to July 2020, and will be finished until June 2022. Task 3 will start with a delay in July this year and will finish in December 2022.



Atypical *Brucella* strains from existing collections of the project partners and strains newly isolated during the project period will be typed using classical phenotyping methods

The aim is to characterize novel emerging *Brucella* species which will be isolated within the project from the project partners using the Micronaut system. Due to the delay of the whole project the task is still ongoing.

Drafted RNA extraction protocol will be established to ensure isolation of high-quality RNA for RNA-seq approach. We are currently improving and refining the protocol of choice.

We are still working on the evaluation of cDNA library preparation and sequencing protocols

WP5 - Zoonotic potential and virulence (M35-M54)

Due to all delays, this task had to be delayed from November 2020 to May 2021, and will be finished until December the same year.

WP7 - Coordination, management and communication (M25-M54)

The tasks started on January 2020. Due to all delays, this task has to be hindered until December 2022. Task 2 started on time, and due to all delays, it will be finished by December 2022. Tasks 3 and 4 had to be delayed until July 2020, and will be finished until December 2022.

Initial kick-off conference call was done on December 17 2019, for organization of the project (M-JRP19-M2).

Conference calls with all WP leaders and deputies regarding the project organization for WP-1, WP-2, WP-3, WP-4 and DMPs were done on March 5, on April 02 and June 09 2020 (M-JRP19-M4).

Individual meetings with WP-2 and WP-3 members were done on June 17 in order to define WP status, delays and strategies.

Kick-off meeting had to be postponed to second part of 2020.

Master data table is finished and an Interactive platform (SharePoint) made by ANSES will be used in this project.

DMPs for the first two WPs were done. However, after communication with EJP DMP coordinators we discovered that new platform and guidelines would soon be available.

Large storage file (DNA and RNA seq, and analyses, mapping data...) platform is available to the consortium since the end of June 2020.

WP7-T3: Risk management (M25-M54)

Due to the ongoing situation, on the conference call held on June 09th, consortium reported the delays, which were finally estimated to be 6 months.

WP7-T4: Synthesis and dissemination of recommendations coming from the project outputs (M25-M54)

WP-1 T-1 and WP-2 T-1 will produce the base for the reporting system of emerging atypical *Brucella* spp.



5.1.10.17.3 Progress of the research project: deliverables and milestones

5.1.10.17.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
17	D-JRP17-WP1.Del1	Creation of a common database to share information about emerging <i>Brucella</i> and reservoirs among the consortium partners	30		July 2020	Public	3
17	D-JRP17-WP2.Del1	Set-up of a human brucellosis network	30		December 2020	Public	5
17	D-JRP17-WP7.Del1	First draft of data management plan	30		December 2020	Public	8
17	D-JRP17-WP7.Del2	Creation of a data sharing common platform	32		February 2021	Confidential	3

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.17.3.2 Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
17	M-JRP17-M6	Information on worldwide emerging <i>Brucellae</i> (shared database)	30	No	December 2020	Delayed due to Coronavirus crisis (labs overloaded by COVID diagnostic; lockdown conditions; movements restrictions; delayed hiring of collaborators)
17	M-JRP17-M7	Definition of sampling and testing protocols (molecular, bacteriology and serology)	30	No	December 2020	Delayed due to Coronavirus crisis (labs overloaded by COVID diagnostic; lockdown conditions; movements restrictions; delayed hiring of collaborators)
17	M-JRP17-M1	Mapping of existing human <i>Brucella</i> species (shared database)	28	No	October 2020	Delayed due to Coronavirus crisis (labs overloaded by COVID diagnostic; lockdown conditions; movements restrictions; delayed hiring of collaborators)
17	M-JRP17-M5	Creation of an epidemiological questionnaire	29	No	September 2020	Delayed due to Coronavirus crisis (labs overloaded by COVID diagnostic; lockdown conditions; movements restrictions; delayed hiring of collaborators)
17	M-JRP17-M8	Definition of sampling and testing protocols (serology, bacteriology and molecular biology)	30	No	December 2020	Delayed due to Coronavirus crisis (labs overloaded by COVID diagnostic; lockdown conditions; movements restrictions; delayed hiring of collaborators)



JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
17	M-JRP17-M9	Drafted RNASeq protocols to identify regulatory differences between classical species and emerging <i>Brucella</i> strains	30	No	December 2020	Delayed due to Coronavirus crisis (labs overloaded by COVID diagnostic; lockdown conditions; movements restrictions; delayed hiring of collaborators)
17	M-JRP17-M2	Conference call of the steering committee regrouping WP leaders + deputy leaders on data management	28	Yes		An initial kick-off conference call was organised in December 2019, regrouping all WP's deputy and leaders.
17	M-JRP17-M3	Definition of type of data generated for each WP and structure of the data sharing platform	28	Yes		Template of database provided to IDEMBRU partners; Data sharing Platform organised by ANSES
17	M-JRP17-M4	Conference call of the steering committee regrouping WP leaders and deputy leaders	28	Yes		Conference calls with all WP leaders and deputies regarding the project organization for WP-1, WP-2, WP-3, WP-4 and DMPs were done on March 5, on April 02 and June 09 2020 (M-JRP19-M4).
17	M-JRP17-M10	Definition of the programme of the annual workshop	30	No	December 2020	Delayed due to Coronavirus crisis (labs overloaded by COVID diagnostic; lockdown conditions; movements restrictions; delayed hiring of collaborators)



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M25-M36



JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
17	M-JRP17-M11	Organisation of accommodation and logistic aspects	32	No	February 2021	Delayed due to Coronavirus crisis (labs overloaded by COVID diagnostic; lockdown conditions; movements restrictions; delayed hiring of collaborators)



5.1.10.17.4 Ongoing collaborations

The ongoing EURL (**European Reference Laboratory for Brucellosis**) work programme is mainly dedicated to classical *Brucella* species and is restricted to the domestic species, especially ruminants (in relationship with European regulation, including surveillance and control plans). Public results issued from the IDEMBRU project will be disseminated within this lab network in order to improve understanding of new atypical and emerging classical species in reservoirs without any current monitoring.

For the sampling steps, **national stakeholders and policy makers** are currently contacted as the project starts in order to organise the sampling and to establish the list of existing collections. For the human side, INSA is in contact with a national network of hospitals, which will contribute for dissemination of public results. The “BRUCE list” (list of contacts provided by the international society for Brucellosis) will be contacted within the WP2 task in order to interact with a large *Brucella*-focused network.

ECDC and EFSA will be contacted within WP-2 and WP-6 to establish an interactive network and look for experiences in setting up the toolkit. Collaboration with other regional, national and OH EJP projects will be established in order to share experiences and achieve objectives.

5.1.10.18 JRP18-ET1.1-MEmE

5.1.10.18.1 Summary of the work carried out in the JRP

MEME is aiming to fill the research gaps highlighted by international agencies for the detection and control of cystic and alveolar echinococcosis. MEME is focusing on standardization, harmonization and validation of existing parasitological and molecular methods, and the development and comparative assessment of innovative molecular tools and biomarkers to detect *Echinococcus multilocularis* (Em) and *Echinococcus granulosus s.l.* (Eg) in the food chain. Production of epidemiological data on the presence of Em/Eg eggs in the food chain is focusing on vegetables for human consumption as well as canine faeces in selected endemic countries. MEME will provide a comprehensive set of integrative activities to harmonize procedures, improve detection of *E. multilocularis* and *Echinococcus granulosus s.l.*

Core activities during the first year of the project:

- Production of Standard Operating Procedures (MEME WP1-T1) for the sampling of matrices.
- Start of sampling of different matrices from naturally or experimentally infected definitive and intermediate hosts (WP1-T2, T3).
- Start validating the established parasitological (Segmental Sedimentation and Counting Technique, SSCT) and novel molecular diagnostic procedures (m-PCRs and MC-RT-PCR assay) to detect *E. multilocularis* and *E. granulosus s.l.* in different matrices along the food chain (WP2-T1, T2, T3).
- Start developing and validating new tools such as new molecular markers for *Echinococcus* species from rapid diagnostics to source attribution; new multiplex qPCR for detection and discrimination of Em/Eg and Eg genotypes; sequencing using Region-Specific Extraction (RSE) and NGS for the detection of Em/Eg in complex samples; biomarker discovery in exosomes from sheep plasma for diagnosis of CE infection (WP3-T1, T2, T3, T4).
- Start producing of data relevant for epidemiological assessments on: contamination of vegetables for human consumption by eggs of Em/Eg; prevalence of Em/Eg in dog faeces; identification of potential risk factors for human CE/AE infection through questionnaires; molecular epidemiology studies on Eg genotype diversity (WP3-T6, T7, T8, T9).



- Disseminating project results at different levels (general public, populations at risk, biologists, veterinarians, clinicians, health authorities, policy makers and media) (WP4-T2).

5.1.10.18.2 Progress of the project: description of activities

WP: 1 SAMPLING STRATEGY (M25-M48)

The aim of this WP is to collect in the field and in the research facilities and to produce in the laboratory, the biological material needed to implement the activities of MEME. In the reporting period the collection of the samples in the field was started. Most of participants begin to organize sampling in the field from intermediate and definitive hosts. Part of planned material were collected and preserved for further purposes (red fox intestines and faeces, worms, cysts, dog faeces). Detailed SOPs for sampling of matrices are in preparation. In experimental model, *E. multilocularis* strain was maintained in different groups of mice infected at different period in order to have protoscoleces available around every two months. These protoscoleces will be used soon to infect two red foxes in order to obtain faecal samples infected with worms. Simultaneously, an infection of red fox will be done using *E. granulosus* s.s. cysts from slaughterhouses in order to obtain eggs to infect sheep (WP3-T4).

JRP-WP1-T1. SOPs for sampling in matrices (M25-M28)

The following Standard Operating Procedures (SOPs) were prepared:

Sampling of faecal material in dog faeces collected from the environment (WP1T2, Responsible ANSES) and their processing for molecular analysis.

Sampling of *E. granulosus* cysts from naturally infected sheep and pigs at abattoirs (WP1-T2, Responsible PIWET) and experimentally infected sheep (WP1T3) and their processing for molecular analyses.

Sampling of faeces and small/large intestines from experimentally infected foxes (WP1-T3, Responsible ANSES) and their processing for parasitological and molecular analyses.

Sampling of blood from experimentally infected sheep (and sheep at abattoirs) (WP1-T3, Responsible ANSES) and its processing to obtain plasma suitable for proteomic analysis of exosomes.

Sampling of vegetables for human consumption (WP3-T6, Responsible ANSES) and their processing for molecular analysis.

JRP-WP1-T2. Matrices collection in the field from intermediate and definitive hosts (M27-M36)

The aim of this task is to collect different matrices from naturally infected definitive and intermediate hosts for their use in other WPs. Most of participants started to organize sampling from red foxes, dogs, and intermediate hosts. Two hundred and ninety six intestines of red foxes from endemic areas were collected; some of them were examined with SCT/SSCT. From positive intestines *E. multilocularis* worms were isolated. Additionally, faeces from distal part of large intestine were collected. Sixty three red fox intestines from areas supposed to be free of *E. multilocularis* were collected. Other parasites (*Mesocestoides* spp., 3 samples; *Taenia* spp., 2 samples) were isolated from small intestines of red foxes - for specificity controls. Ninety samples of pigs' livers with suggestive lesions of tapeworm larvae were collected and were prepared for identification. Additionally, few *E. multilocularis* and *Taenia hydatigena* sp. lesions isolated from *Arvicola terrestris* and *E. granulosus* cysts isolated from sheep were collected. Organization of collection of faecal samples from dogs for epidemiological study was started. Participants sent information and collection kits for owners and veterinary cabinet in rural areas of highly endemic regions. Additionally, hundreds dog faecal samples from environment were collected, all negative for *E. multilocularis* (examined with qPCR) as a potential matrix for validation.

JRP-WP1-T3. Matrices collection from experimental animal models (M27-M48)



ST1. All the authorizations for animal testing have been obtained at ANSES for the infection of mice and foxes with *Echinococcus*. They are in compliance with the ethical authorizations for animal experimentation (Decree n° 2013-118 of February 1st 2013) and the ethical authorizations for the experimental infection of carnivores by EM (from mice to foxes) (16-073 N° APAFiS: 20160913348095). Unfortunately, production, of matrices (faeces, worm and eggs) on Fox has been delayed because of the COVID-19 pandemic in absence of workers on the animal facilities in Atton and because impossibility to modify cages to facilitates faeces collection in biosafety. The infection will be programmed ASAP. Only mice metacestodes production have been maintained during this period. A first production of metacestodes material, corresponding to 200 µl of protoscoleces, has been produced and 200 references DNA/protein samples are available. Several infected groups of mice are available (i.e. fertile lesions with protoscoleces) around every two months starting since July 2020. The return to a "normal" post COVID 19 situation, have permit to perform an experimental infection of two foxes. The drawers adapted to the fox cages have been produce and put in place to permit the collect of fox faeces in safety conditions. In the beginning of July two foxes have been infected with 35,000 protoscoleces each from infected mice. The faeces have been collected daily to control the infection. Collected faeces have been decontaminated at -80°C for safety manipulations. These faecal samples have been analysed by qPCR and flotation. We have obtain reference positive faeces matrices (with or without eggs) for our partners for future development of diagnostic protocol. A study about the detection of *E. multilocularis* DNA by copro-qPCR during prepatent and patent infection is in progress in order to obtain a better understanding of Em DNA detection in fox faeces. An infection of red fox will be programmed using *E. granulosus* s.s. cysts from slaughterhouses in order to obtain *E. granulosus* eggs to infect sheep in Portugal.

ST2. Sheep were selected and kept in animal facilities in Portugal according to animal welfare procedures. Concerning ethics approval for the experimental infection of sheep, this was prepared and submitted to the local ethics committee in Portugal. Because of the COVID-19 pandemic, we are still waiting the approval from the local ethics committee of the veterinary authority.

WP: 2 VALIDATION of PARASITOLOGICAL and MOLECULAR ASSAYS (M29-M44)

The harmonization and validation of selected parasitological and molecular procedures have been impacted by COVID-19 pandemic, some task are delayed, time has been used to collect samples. Validation will include estimates of the analytical and diagnostic performance characteristics of tests.

JRP-WP2-T1. Segmental Sedimentation and Counting Technique, SSCT (M29-M36)

The SSCT method was chosen due to its high sensitivity (98.3%) compared to reference SCT and above all a significant reduction of the analysis time due to the analysis of only two out of five segments of the whole intestines (Umhang et al., 2011). According to the lifecycle of *E. multilocularis*, surveillance of foxes is considered the best approach. According to the SOP (WP1-T1), each segment of fox intestines collected in the different countries will be independently analysed for the observation and counting of the number of worms. The data obtained will be used for an international validation of the method using the best combination of two segments resulting in the higher sensitivity. Previous results reported from 117 infected fox intestines from France in the context of the original description of the method will be added. According to the protocol, the analyses of the first ten segments of intestines has already started in France and Poland and will be extended to other participants during the year. Due to COVID-19 pandemic the real start of the analyses was delayed, which will probably need to be delayed until 2021.

JRP-WP2-T2. Comparison of multiplex PCRs (M29-M44)

This task focuses to the validation of two molecular assays widely used for the detection of *Echinococcus* in definitive and intermediate hosts, targeting mitochondrial and nuclear markers:



Boubaker et al. (PLoS Negl Trop Dis 2013), a single-tube multiplex PCR allowing discrimination of *Echinococcus* at the level of species/genotypes ;

Trachsel et al. (Parasitology 2007), a single-tube multiplex PCR targeting the definitive host for the identification of eggs belonging to *E. granulosus*, *E. multilocularis* and *Taenia* genus.

The assay 1 was tested resulting in conclusion that it is not possible to validate this method because of the unreliability of the molecular markers used in the multiplex PCR. Next step will be the validation of the assay 2.

JRP-WP2-T3. Magnetic Capture - Real Time PCR assay (M29-M44)

The validation of the Magnetic Capture-PCR will be performed according to stage 3 and 4 of the OIE chapter on validation of diagnostic methods, since basic diagnostic characteristics of the test have already been published. Stage 3 validation means testing the assay in different laboratory settings, using equally aliquoted panels of more than 20 samples sent out to each laboratory. For this to be possible, the method needs to be set up and verified before this panel is sent out and tested. So far, we have identified the laboratories interested in participating, we have more or less finished SOPs for the methods and a SOP for the validation is in progress. Next steps are to get samples from ANSES and prepare reference material for setting up and verifying the method in the participating labs, and also arrange workshops for the persons in need. Due to COVID-19 measures limiting personnel access to the laboratories and travel restrictions due to quarantine requirements these workshops have been postponed. We are exploring alternative methods to carry out the training and are in dialogue with the project partners to find a viable alternative for early 2021, such as digital training platforms. Once the method is set up and verified in the labs, the stage 3 validation panel can be sent out. This is planned to take place late summer 2021. Not until the data is analyzed, can stage 3 validation be considered finished.

WP: 3 DEVELOPMENT/VALIDATION of NEW TOOLS and PRODUCTION of DATA RELEVANT for EPIDEMIOLOGICAL ASSESSMENTS (M27-M52)

WP3 aims at generating new innovative tools for rapid detection, differential diagnosis, and tracking of infection, both at large and small-scale settings.

Most tasks of work package 3 are delayed due to the COVID-19 lockdown as many laboratories were closed. It was therefore not possible to work on several tasks of the work package. In course of the task WP3-T1, samples from various countries worldwide were collected and of these, the mitochondrial genomes of 39 isolates from Turkey and Armenia were sequenced. Further samples will be collected and analysed by mitogenome sequencing. Four TaqMan® probe-based qPCRs were developed in task WP3-T2. At this stage, they can be used as a single-step genotyping technique for the diagnosis of *E. granulosus* genotypes in four epidemiologically relevant subgroups, i.e. *E. granulosus* s.s. (G1 to G3), *E. equinus* (G4), *E. ortleppi* (G5) and the *E. canadensis* complex (G6 to G8 and G10). In task WP3-T4, the study protocol was submitted for approval to the ethics committee in March 2020. Due to the difficulties caused by COVID-19 pandemic, the ethics committee in Portugal (responsible INIAV) has not yet responded to the application.

JRP-WP3-T1. New molecular markers for Em and Eg s.l.: from rapid diagnostics to source attribution (M27-M48)

Following the use of the two published microsatellites EgSca6 and EgSca11 for *E. granulosus* s.s. (M'rad et al., 2020), the presence of two copies in the genome of EgSca11 complicates its interpretation and prevent its use for phylogenetic analyses. There is a need to substitute this microsatellite by one or several other classical microsatellites. A new screening of the *E. granulosus* genome has resulted in the identification of 15 new microsatellites targets, which are currently evaluated for their polymorphism, reproducibility, limit of detection, quality of the electrophoretic profiles and their specificity. The selected profiles will be associated to EgSca6 in order to obtain a panel with a high discriminatory



power allowing highly discriminant identification which is necessary for accurate source attribution and for large phylogenetic studies. *E. granulosus* s.s. DNA samples from North Africa (Morocco, Tunisia) and Europe (France, Moldova) are already available and will be completed by others for testing polymorphism of the microsatellites.

JRP-WP3-T2. New multiplex TaqMan qPCR for detection and genotyping of Eg s.l. and Em (M27-M48)

At this stage, Four TaqMan® probe-based qPCRs can be used as a single-step genotyping technique for the diagnosis of *E. granulosus* genotypes in four epidemiologically relevant subgroups, i.e. *E. granulosus sensu stricto* (G1 to G3), *E. equinus* (G4), *E. ortleppi* (G5) and the *E. canadensis* complex (G6 to G8 and G10). The technique also allows differentiating *E. granulosus* samples from other *Echinococcus* or *Taenia* species in samples derived from cystic or faecal material. The qPCRs show high efficiency (ranging between 99% and 106%), analytical specificity (100%) and sensitivity (ranging between 0.6 and 1.4 copies/μl) when used with DNA obtained from cysts or from cloned PCR products. Therefore, they are suitable for a PCR-based diagnosis of cystic echinococcosis in intermediate hosts, including humans as aberrant intermediate hosts. These qPCRs will now be combined to develop a TaqMan probe-based multiplex Real-Time qPCR as a tool for a simultaneous, rapid diagnosis and typing of *E. granulosus sensu lato* and *E. multilocularis* infections. Moreover, further experiments are initiated to develop a TaqMan® probe-based qPCR assays that differentiate between the individual members of the *E. canadensis* complex (G6, G7, G8 and G10).

JRP-WP3-T3. Detection of Em/Eg in complex samples: sequencing using Regions Specific Extraction (RSE) and NGS (M27-M48)

Initial RSE with NGS have been performed by FLI in Q1-2 2020. Data analysis still has to be performed. Analysis of Em DNA positive control material (surveillance extraction method) with RSE and Nanopore using Flongle was planned for summer 2020 at NVI. However, restrictions to laboratory access as a result of preparedness measures for the COVID-19 pandemic, has delayed this work. Restrictions are still in place but we hope to be able to schedule this work for late autumn 2020.

JRP-WP3-T4. Proteomic study on biomarker discovery in exosomes from animal plasma (M27-M52)

The collection of blood samples for the inception of this activity will start one-year after the infection of sheep with *E. granulosus* egg in Portugal. The protocol for the experimental infection was prepared and submitted to the local ethics committee in Portugal (INIAV). Because of the COVID-19 pandemic, we are still waiting the approval from the ethics committee in Portugal.

JRP-WP3-T6. Contamination of vegetables for human consumption by Em/Eg (M29-M48)

The use of a robust and reliable method able to detect few *Echinococcus* spp. eggs from vegetables is a prerequisite to estimate the contamination of vegetables for human consumption. The sensitive technique based on sequential sieving coupled with molecular detection recently developed by the team of Prof. Peter Deplazes at the Zürich Institute of Parasitology (Guggisberg et al., 2020) was chosen to investigate lettuce. This method using 300g of lettuce leaves sample was transferred to ANSES. After sieving and DNA extraction, the detection of Em DNA was realized using qPCR (Knapp et al., 2016) which proved to detect one egg (Ct: 30). Using different spiked numbers of Em eggs obtained from faeces after experimental infection of foxes (WP1-T3-ST1), the limit of detection in 95% of the time (LoD₉₅) was estimated at three Em eggs (23/24). When spiked with two eggs a positive detection was obtained for 87.5% (21/24) of the lettuce leaves samples and the one for one Em egg still needs to be estimated. Additionally, the efficiency of washing lettuce leaves under running water and of spinning with salad spinner and on paper towel are also currently under evaluation in order to provide recommendations. The collection of 160 lettuces from private kitchen gardens (2 lettuces each) and directly from producers (4 lettuces each) in high endemic area for Em in north-east France. These analyses detected DNA of Em in two pools from producers and DNA from *Hydatigera* sp. in six pools from both private kitchen garden and producers. Hundreds of lettuce from others partners are planned



to be collected and analysed by ANSES. For each lettuce the solution obtained at two additional steps (40µm filter and filtrate from 20µm) will be also collected in order to later estimate contamination by other parasites (mainly *Toxocara* spp. and *Toxoplasma gondii*) using dedicated molecular tools.

JRP-WP3-T7. Prevalence of Em/Eg in dog (faecal samples) from selected geographical areas (M29-M48)

The design of the study was discussed among participants of the task. The target group are dogs originating from highly endemic areas (i.e. with high prevalence of *E. multilocularis* in foxes), or from regions with relatively high prevalence of *E. granulosus* in sheep. In most cases, they are rural dogs with better access to infected intermediate hosts. Specific conditions in individual countries will be taken into consideration (i.e. sled dogs from Svalbard in Norway). Samples must be collected individually (i.e. to be identifiable with individual dog described in questionnaire). Moreover, organization of collection of faecal samples from dogs was started. Number of samples has been statistically estimated. The questionnaire (to complete by owners/vets) with questions concerning data which will be used in epidemiological analysis was elaborated. Following data are included in questionnaire: data about dog (age, breed, sex), place of living, activity sites and types of activity, deworming (date, frequency, drug), feeding, eating rodents and others. Some participants sent information and/or collection kits to owners and veterinary cabinet in rural areas of highly endemic regions. Additionally, the collection of around 200 hundreds of faeces from hunting dogs in France (cooperation with hunting federation) has been launched. Till now, overall, 65 samples from dogs were collected in Poland and Estonia in preliminary sampling and preserved for further molecular testing. Next step is to discuss and choose the best molecular method (PCR) for identification of *Echinococcus* spp. in faecal samples.

JRP-WP3-T8. Potential human risk factors by means of questionnaires (M29-M48)

Semi-structured questionnaires on potential risk factors for human infection with CE and AE will be designed and submitted for ethical approval to the relevant committees of the participating hospitals. Upon written informed consent, questionnaires will be administered to volunteers with CE/AE and matched (by age, sex and country of origin) uninfected controls accessing clinical centres participating to the study. This task has few months delay because of COVID-19 pandemic. Task WP3-T8 will be achieved in the due time.

WP: 4 TRAINING, DISSEMINATION and PROFICIENCY TESTING SCHEMES (M27-M54)

This WP focuses on establishing the most effective methods for disseminating project results at different levels and training scientists from institutions participating to MEME in the parasitological and molecular identification of *Echinococcus* spp.

JRP-WP4-T1. Trainings (M27-M48)

This task has few months delay because of COVID-19 pandemic. Task WP4-T1 will be achieved in the due time.



5.1.10.18.3 Progress of the research project: deliverables and milestones

5.1.10.18.3.1 Deliverables

TELEVIR							
JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category * (1 to 10)
18	D-JRP18-WP1.1	SOPs for sampling in matrices shared with the Consortium	M28		M33 (docs ready; September online)	Public	2
18	D-JRP18-WP5.2	Periodic technical and financial reports to EJP/Commission	M36	M33 (9mth report)	/	Public	10
18	D-JRP18-WP5.5	Kick-off annual meeting in Malzéville (France) by ANSES	M27	10/04/20	/	Public	10

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.18.3.2 Milestones

TELEVIR						
JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
18	M-JRP18-03	Ethics approval for the use of animal model	M28	yes for ANSES; no for INIAV	Depending on ethics committee feedback at INIAV	<u>COVID-19 pandemic</u> delayed the ethic approval for the sheep model at INIAV, Portugal. This is the most relevant delay with domino effects on proteomic study (WP3-T4)
18	M-JRP18-04	SOP for validation of SSCT	M30	yes	M33	
18	M-JRP18-05	SOP for validation of multiplex PCRs	M30	no	M36	Task delayed but achievable
18	M-JRP18-06	SOP for validation of mc-RT-PCR assay	M30	no	M38	SOP preparation is depending on the requirements of the people participating to the training on RT-PCR assay which was delayed by <u>COVID19</u> event



TELEVIR						
JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
18	M-JRP18-07	Protocols for new molecular methods, NGS included	M30	yes for WP3-T2; draft protocol for the others	M35	Task delayed but achievable before the end of this year
18	M-JRP18-08	Protocol for proteomic analysis in animals plasma	M30	no	Depending on ethics committee at INIAV	Because of <u>COVID19 delay</u> : serious concerns on feasibility of this task in due time because of the huge amount of time needed for the growth of parasites after animal infection (>1 year)
18	M-JRP18-09	Protocol for contamination of vegetables by Em/Eg	M30	yes	M33	
18	M-JRP18-10	Protocol for prevalence of Em/Eg in dog	M30	yes	M33	
18	M-JRP18-11	Questionnaire scheme for potential human risk factors	M30	no	M36	Task delayed but achievable before the end of this year



TELEVIR						
JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
18	M-JRP18-12	Interim evaluation of collection of samples in the field	M32	yes	M32	Lockdown delayed the start of sampling but this deadline was achieved in due time.
18	M-JRP18-01	Tasks and responsibilities allocation	M25	yes	/	Kick off meeting in France used for the allocation of the tasks and responsibilities
18	M-JRP18-02	Organization of Kick-off annual meeting by ANSES	M26	yes	/	Nancy, 5-6 February 2020



5.1.10.18.4 Ongoing collaborations

Ongoing and planned collaborations with the following entities:

- the other parasitology-JRPs: PARADISE and TOXOSOURCES;
- the European Reference Laboratory for Parasites (EURLP);
- the WHO Collaborating Centre WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis (in humans and animals);
- PERITAS project (molecular ePidemiological studiEs on pathways of tRansmission and long lasTing cAPacity building to prevent cyStic echinococcosis infection). 2018-2021. Funded by the EC under EULAC-Health.

5.1.10.19 JRP19-ET1.1-PARADISE

5.1.10.19.1 Summary of the work carried out in the JRP

The Kick off meeting (KOM) held at ISS (Rome, 10-11 February 2020) allowed a detailed presentation of the planned activities, followed by ample discussion among the Consortium members. The most important progresses for the research-orientated activities are as follows:

-We reached a consensus on the criteria for the selection, preservation and shipment of the samples. Despite the restrictions linked to the COVID-19 emergency, many partners were able to collect and ship samples and the first genome sequencing effort has taken place, resulting in the generation of 24 novel *Giardia duodenalis* and 29 novel *Cryptosporidium parvum* genomes. We performed an inventory of the available genome data and bioinformatics tools, and the tests of the pipelines are rapidly advancing. The in silico metagenomics approach demonstrated specific parasite sequences in metagenomes from various matrices and the amplicon-based sequencing approach was more robustly tested. Reference material (cysts) for spiking experiments has been produced.

-In consultation with the associated partner CRU, markers that can be typed using fragment analysis or by Sanger sequencing were compared, and it was concluded that both should be considered while identifying an informative panel. An inventory of the parasite samples (genomic DNA, faeces, and other relevant matrices) available at each partner Institute was completed and judged well suited for the planned tests.

-The animal immunization experiments were performed as planned and the steps towards the identification of nanobodies are in progress. For the aptamers, various rounds of SELEX were done for both *Cryptosporidium* and *Giardia* at ANSES and ISS. Two capture systems were designed for *Cryptosporidium* (18S, gp60) and one for *Giardia* (beta-giardin); optimization of the methods is in progress.

In conclusion, although delay in some activities has occurred, considerable progresses has been made and the project is proceeding as planned.

5.1.10.19.2 Progress of the project: description of activities

WP JRP-PARADISE_WP1 Coordination and impact (M25-M54)

JRP-PARADISE-WP1-T1 T-0.1 Management, coordination and communication (M25-M54)

The KOM of the project was organized at ISS in Rome on February 10-11, 2020 (Deliverable). The members of the Steering committee have been nominated, while the Advisory Board needs to be completed. Communication has mostly taken place among WP and Task leaders, but scheduled meetings are planned.



WP: JRP-PARADISE_WP2 NGS-based genomics and metagenomics (M25-M52)

JRP-PARADISE-WP2-T1 NGS-based genome study of selected isolates of *C. parvum* and *G. duodenalis* (M25-M36)

The planned sample collection has been partially achieved, due to restrictions imposed by the COVID-19 epidemics. Nevertheless, partners Institutes (SVA, SSI, NVI, PIWET, OKI, and RKI) and associated partners (BIOR) collected a relevant number of *Cryptosporidium* (~ 60) and *Giardia* (~ 30) samples. SVA, RKI and ISS prepared high quality genomic DNA, and ANSES performed the genome sequencing of the first *Giardia* (24) and *Cryptosporidium* (29) isolates. Furthermore, ANSES, SVA, RKI and ISS are testing the analytical pipelines for data processing. A cloud storage space to share NGS data has been set up by SVA.

JRP-PARADISE-WP2-T2 In silico analyses of metagenomes for detection of foodborne parasites (protozoa and helminths) (M25-M52)

Promising results (RIVM) were obtained by mining calf intestinal metagenome datasets, which resulted in the detection of many *Cryptosporidium*-specific reads. When a metagenome from irrigation water sampled at the Sao Paulo zoo was investigated, a complex array of protozoa was detected, demonstrating the suitability of the approach for detection of multiple parasites. Planned experiments include the creation of metagenomics datasets spiked in silico with known number of reads from given parasites, in order to explore the detection limit. A more systematic search is planned to identify public metagenomes to be mined for the presence of FBPs. The reference database is close to be completed.

JRP-PARADISE-WP2-T3 Experimental amplicon-based and shotgun metagenomics for detection of foodborne parasites (M25-M52)

Further testing of the amplicon-based detection platform (SSI), mostly on calf samples, has been done. This confirmed a good performance in the detection of a wide range of parasites, but also highlighted specific issues with the detection of *Giardia*. Tests of different samples with a different primer pair are ongoing to address this issue. Experiments with spiked matrices are planned, and will take advantage from the availability of highly purified oocysts and cysts generated at SVA and ISS.

JRP-PARADISE_WP3 Design, implementation and validation of multi-locus typing schemes (M25-M54)

JRP-PARADISE-WP3-T1 In silico selection of informative loci from comparative genomics data. (M25-M42)

This activity is strongly dependent on the outcome of WP2-T1 (new genome information), which, although delayed, results in the generation of >50 new genomes. A first in silico selection will be made based on all publicly available genomes, on the additional, unpublished genomes from the project's partners, and the newly generated genomes. A common repository to upload and share raw sequence data has been established by SVA.

JRP-PARADISE-WP3-T2 Development of MLST schemes for *C. parvum* and *G. duodenalis* (M31-M50)

All partners Institutes have been asked to confirm availability of samples in view of the test experiments; the feedback has been very positive. SVA has discussed with CRU (associate partner) about markers for fragment typing, and CRU has agreed to share a list of the most promising markers they have identified in previous projects.

JRP-PARADISE_WP4 Parasite enrichment strategies (M25-M54)

JRP-PARADISE-WP4-T1 Development of pre-DNA extraction enrichment strategies (M25-M54)

Nanobodies: Two new world camelids were immunized either with extracts from *Giardia duodenalis* cysts or *Cryptosporidium parvum* oocysts, respectively. ELISA and IFA tests showed that both animals



reacted to the respective antigens. The cDNA libraries of variable domains of heavy-chain only antibodies have been prepared for both *Giardia* and *Cryptosporidium*.

Aptamers: ANSES has completed ten rounds of SELEX for five aptamer pools for both *C. parvum* (IOWA strain) and *G. duodenalis* (assemblage B). ISS has successfully produced in vitro *G. duodenalis* cysts (assemblage A) and completed six round of SELEX for three pools for both *G. duodenalis* assemblages A and B. Melting curve qPCR assay has been set up independently and used to evaluate aptamer pool diversity decrease. A decrease in pool complexity was evident starting from round six (maximum at round 10). The melting peak increase was evident from cycles 5-6 and more so at cycle 10. To ensure the necessary range of diversity, ANSES established a dedicated panel (from 1N to 20N on the 80 bp sequences aptamers). ISS is setting IF condition with the aptamers.

JRP-PARADISE-PARADISE-WP4-T2 Development of post-DNA extraction enrichment strategies (M25-M54)

SVA has designed a capture system for *Cryptosporidium* (18S ribosomal DNA) and one for *Giardia* (beta-giardin gene). A test panel of FACS-purified sorted oocysts has been produced for optimization and sensitivity testing. A consistent detection of 10 *C. parvum* oocysts has been achieved. The beta-giardin capture system is a more recent acquisition, and more work remains to be done. RIVM has designed capture probes for *Cryptosporidium* (gp60 gene), and testing of spiked faecal samples resulted in promising results. In fact, gp-60 capture probes detected the spiked samples and allowed to concentrate *Cryptosporidium* DNA.



5.1.10.19.3 Progress of the research project: deliverables and milestones

5.1.10.19.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
19	D-JRP19-PARADISE-WP1.1	Report of the kick off meeting	M26	April 2020		Public	10
19	D-JRP19-PARADISE-WP2.1	Protocol for 18S rDNA-based amplicon sequencing for detection of relevant FBPs	M30		M36	Public	2
19	D-JRP19-PARADISE-WP3.1	Report on the <i>in silico</i> selection of highly polymorphic sequences in <i>C. parvum</i> and <i>G. duodenalis</i> genomes	M30		M36	Public	10

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.19.3.2 Milestones

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
19	M-JRP19-PARADISE-1	Kick-off Meeting (WP1)	M26	Yes		
19	M-JRP19-PARADISE-2	Study visit (ISS-ANSES) for optimizing aptamer selection strategy	M28	No	Not planned	Study visit impossible due to the COVID-19 epidemics
19	M-JRP19-PARADISE-3	Key isolates of <i>C. parvum</i> collected	M30	No	M33	Delay in the collection/shipment of samples due to the COVID-19 epidemics
19	M-JRP19-PARADISE-4	Key isolates of <i>G. duodenalis</i> collected	M30	No	M33	Delay in the collection/shipment of samples due to the COVID-19 epidemics
19	M-JRP19-PARADISE-5	Referenced database of foodborne parasite genomes established	M30	No	M36	Delay due to limited access to working places
19	M-JRP19-PARADISE-6	Pipeline for metagenome data analysis for FBPs optimized	M30	No	M36	Delay due to limited access to working places



JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
19	M-JRP19-PARADISE-7	First set of candidate markers for MLST development available	M30	No	M36	Activity impacted by delay in WP2-T1
19	M-JRP19-PARADISE-8	Animal immunization and cDNA library of nanobody sequences for <i>C. parvum</i> and for <i>G. duodenalis</i> completed	M30	No	M33	Animal immunization and cDNA library construction completed



5.1.10.19.4 Ongoing collaborations

- Complementarities with the OHEJP research project “Toxosources”. Both projects use genomics as a tool to derive new typing schemes, and try to determine the role of specific food matrices (ready-to-eat salads).
- Complementarities with the OHEJP integrative project “Harmony-CAP”, in which *Cryptosporidium* has been selected as a model organism for evaluation of the current and best practices and the development of harmonised protocols.
- Link with the objectives of the **European Union Reference Laboratory for Parasites** in terms of new typing schemes for FBP that, in perspective, may become official, validated methods.
- Complementarities with the **EFSA-funded research project “IMPACT”** for the definition of optimized protocols for the detection and typing of FBP on selected food matrices.

5.1.10.20 JRP20-FBZSH3-DISCoVeR

5.1.10.20.1 Summary of the work carried out in the JRP

In these first 9 months of the project, we have achieved the following:

1. Identified members of the Project Management Team (PMT)
2. Held one face-to-face kick-off/annual meeting at DTU on February 10-11, 2020.
3. Held two web meetings for all project partners.
4. Held three project management team web meeting to discuss plans and progress.
5. Developed data inventories of focus hazards to map existing and available data.
6. Mapped knowledge gaps for source attribution through the completion of a rapid systematic review (D-JRP FBZ-1-WP1.1)
7. Determined for each focus hazard (*Salmonella*, *Campylobacter*, VTEC/STEC and antimicrobial resistant (AMR) bacteria with a focus on ESBL/AmpC *E. coli*) which attribution approaches that will be applied, further developed and compared.
8. Held four hazard-specific web meetings to discuss and decide on improvement of datasets to work with throughout the project.
9. Selected for each focus hazard relevant datasets and proposed how these can be improved by additional sampling of particularly non-animal food reservoirs and sequencing of existing isolates.

5.1.10.20.2 Progress of the project: description of activities

WP 1. Project coordination and administration (M25-M54)

Task: JRP FBZ-1-WP1-T1 Project management (M25-M54) - ongoing

Since the start of the project, we have had one face-to-face kick-off meeting hosted by DTU on February 10-12, 2020, and 2 web meetings for all partners and 3 web meetings for the project management team (PMT) consisting of all work package leaders, their deputies and task leaders.

A share-site hosted by DTU has been set up, where project partners can share documents and data, including minutes of meetings and the developed data inventories.

Task: JRP FBZ-1-WP1-T2 Mapping of existing knowledge gaps and recommendations on how to fill them (M25-M28) - completed



For the mapping of existing knowledge gaps a systematic literature search by means of a rapid review was performed. Based on predefined search queries publications relevant for source attribution of the bacterial species *Salmonella*, *Campylobacter*, VTEC/STEC and for antimicrobial resistant (AMR) bacteria (*Salmonella*, *Campylobacter*, *E. coli*) were identified in the two databases Scopus and Web of Science. Each identified publication was tagged according to categories like the organisms and sources they considered or the methods they employed. Using the data of the tagged publications knowledge maps were created which showed how much publications were found for each method-source combination for each of the above-mentioned bacterial species and AMR. The methodology and results of this mapping together with suggestions how to fill the identified knowledge gaps were put together in a report and submitted as draft for deliverable D-JRP FBZ-1-WP1.1 to the project coordination.

Task: JRP FBZ-1-WP1-T3 Data Management Plan (DMP) (M25-M30) - ongoing

The development of the DMP is in progress. Considering the structure of the new DMP tool, we plan to upload information about our selected datasets, whenever we have a 'finalised' dataset ready for data analysis. Currently, we have just finalised selecting appropriate dataset and decided on how to improve these.

WP 2. Data – Coordination of the collection of genomic data, other microbiological data and epidemiological data (M25-M48)

During this period of the project, several meetings took place with WP2 partners to organize data collection.

Task: JRP FBZ-1-WP2-T1 Mapping of existing data and establishing a joint data-sharing platform (M25-M36) - ongoing

A data inventory form in Excel was adapted to each pathogen subgroup. The purpose of the inventory was to get a quick overview of strains and sequences available in order to pinpoint areas with limited data availability to i) direct further sampling, and ii) identify for which pathogens and models we can expect to have sufficient data for source attribution. The data inventories have been filled-in by partners and hazard-specific web meetings have been organized to present the data available and agree on further sampling of mainly non-animal food reservoirs and/or isolate characterization (mainly sequencing) and transmission to the other WPs for source attribution analysis. Ideally, when these proposed further sampling and sequencing activities are done, we will have a selection of appropriate datasets for source attribution for the focus hazard and attribution approaches (see WP4 description for the approaches selected for each organism).

We are currently exploring, if the Virtual Research Environment (VRE) hosted by D4science would be useful for DiSCoVeR for sharing data, models, and scripts. Until a suitable solution is found, we will continue using the DiSCoVeR share-site at DTU.

Task: JRP FBZ-1-WP2-T2 Data collection for *Salmonella* (M25-M48) - ongoing

Overall, nine participants from eight countries (Belgium, Czech Republic, Denmark, France, Netherlands, Poland, Portugal and Spain) delivered data about *Salmonella* serovars in their collections. Seven institutes showed data from animals, food and environment, four concerning humans.

Overall, the most numerous *Salmonella* serovars are *S. Enteritidis* (>15100 isolates/> 800 sequences), *Typhimurium* (>9600 isolates/> 170 sequences), monophasic variant of *S. Typhimurium* (>6500 isolates/> 300 sequences) and *S. Infantis* (>2290 isolates/ 40 sequences). Those serovars occur in all main sources. SE and ST were found as a top 1 and 2 in humans and animals. In food *S. Infantis* and *S. Typhimurium* are dominant. The environment is represented by the least number of isolates (>300 isolates/ 0 sequences). Some countries declared ongoing sampling or being ready to sequence selected isolates if needed.



The data inventory was discussed during a meeting and proposals for improving specific datasets were made. For WGS-based source attribution, it was decided to focus on the following serovars: Typhimurium, monophasic Typhimurium, Enteritidis, Infantis, Derby, Agona, Newport and Kentucky. However, we will also attempt to build a multi-country model based on phenotypic information, as this will increase the amount of available data.

Task: JRP FBZ-1-WP2-T3 Data collection for *Campylobacter* (M25-M42) - ongoing

The *Campylobacter* specific questionnaire was filled-in by 10 institutes from 7 countries. A total of >5000 whole genome sequenced strains were reported to be available for the project. This includes genomes of human clinical strains, food, animal and environmental strains. After discussion with the WP3 task leaders on the data needs for modelling, it will be decided if more isolates should be sequenced and/or if sampling of some of the under-represented sources should be done.

The data inventory was discussed during a meeting and proposals for improving specific datasets were made. Fairly good data sets are available already and the modellers expect to be able to use the data, despite the incompleteness when it comes to full coverage of countries, sources, years. New sampling should primarily focus on environmental samples and other sources that are not well covered already. Focus is on *C. jejuni* and *C. coli*. Sequencing should focus on WGS of isolates from recent years, 2016-2020.

Depending on the budget at each institute, the institute should select a number of isolates for WGS giving priority to categories with low representation in general (e.g. environmental isolates) as well as categories that specifically are low/missing in the country (e.g. human isolates, different foods, ...).

Task: JRP FBZ-1-WP2-T4 Data collection for Verocytotoxin-producing *E. coli* (VTEC) (M25-M48) - ongoing

The STEC specific questionnaire was filled in by 13 partners from 11 countries. Five partners from five countries shared information regarding human STEC isolates including more than 2400 isolates whole genome sequenced of different serotypes. Information regarding STEC isolates from animal, food and environmental were shared by 11 partners from 10 countries. More than 2800 STEC isolates from animal, food and environmental sources was described, not all isolates were whole genome sequenced. The number of isolates from sources other than food-producing animals was sparse. The main serogroups in the combined dataset are *E. coli* O157 and O26. For now no data has been shared, only information on what is available.

The need for further sampling of other sources than food-producing animals has been highlighted and sampling has been planned. However, due to Covid-19 the sampling has been postponed for many partners.

As data from different sources are sparse a preliminary comparison between human data reported to ECDC (Tessy) and our dataset have been performed to see if it's possible to merge data from several countries with similar distribution of human cases to make the source attribution at regional levels.

Task: JRP FBZ-1-WP2-T5 Data collection for AMR (M25-M48) - ongoing

Overall, seven countries (Czech Republic, Denmark, Ireland, Netherlands, Poland, Portugal and Spain) have in their strain collections clinical/indicator *E. coli* (including ESBL-*E. coli*) for task 4. Number of available isolates/sequences ranges from less than 100 (Ireland) to 500-700 (Czech Republic, Poland) to > 1.000 (remaining countries). Most of the isolates however come from animals/food products, so that only Denmark and the Netherlands report a large number (>100) of isolates of human origin.

The data inventory was discussed during a meeting and proposals for improving specific datasets were made. It was agreed that it was necessary to focus on years with overlapping data from multiple reservoirs, and thus the period 2013-2020 was considered the best period to concentrate on for further analyses/sequencing. It was proposed to collect additional information for countries with



available strains/sequences (possibly at the strain level), including: 1) Availability of phenotypic AMR information, and 2) Specifics on the genotypic information available (for non-sequenced strains): gene families/specific genes.

WP3. Methods - Critical assessment/improvement of existing and development of new source attribution models. (M25-M48)

Two web meeting has been held and monthly meetings are scheduled for after the summer break.

It is planned to use the systematic literature review from BfR (D-JRP FBZ-1-WP1.1) to identify existing models/methods and pinpoint any methodological gaps.

Task: JRP FBZ-1-WP3-T1 Assessing and developing source attribution methods based on microbial subtyping (M25-M48) - ongoing

A Danish dataset of *Campylobacter* sequences collected from humans, animals incl. pets, foods and environments from 2015-2017 is currently being processed through different bioinformatics pipelines to obtain cgMLST, wgMLST, SNPs and Kmer data. These different types of output data will be explored in different source attribution models using machine learning to identify any host-associated genetic (groups of) markers.

Task: JRP FBZ-1-WP3-T2 Assessing and developing source attribution methods based on phylogenetic data (M25-M48) - ongoing

A workgroup has been assembled and the research objectives has been defined: Using phylogeny of surveillance data to apply weights in source attribution models to move from reservoir attribution towards source attribution that is directly actionable by public health and food authorities, by better reflecting exposure evidence. Focus will be on *Campylobacter* and *Salmonella*.

Task: JRP FBZ-1-WP3-T3 Evaluation of microbial subtyping source attribution by infectious disease modelling (M25-M48) - ongoing

This task sets out to develop a method for measuring the quality of source attribution based on subtyping. The initial idea was to simulate infections from different sources using an infectious disease modelling approach, but we are now looking to an approach based on simulating bacterial population using the software: Bacmita <https://doi.org/10.1093/bioinformatics/bty093>

Task: JRP FBZ-1-WP3-T4 Assessing and developing approaches for source attribution of antimicrobial resistance based on metagenomics (M31-M48) - ongoing

Nothing to report.

Task: JRP FBZ-1-WP3-T5 Assessing and developing source attribution approaches based on case-control study results (M30-M36) - ongoing

A Bayesian evidence synthesis model that is able to combine the percentage attribution estimates from different studies has been developed. The next step is to extend the model to also combining population attributable fractions and odds ratios, which are the typical association measure used on case-control studies.

Task: JRP FBZ-1-WP3-T6 Assessing and developing source attribution approaches based on data from reported outbreak investigations (M28-M36) - ongoing

It has been decided to use outbreak data reported by EFSA or this task.

Task: JRP FBZ-1-WP3-T7 Assessing and developing source attribution approaches based on Risk-assessment (M30-M38) - ongoing

Activity not started yet, but the plan is to make a systematic review of comparative assessment models.



WP4. Results – Quantifying the contribution of various sources of foodborne zoonoses and AMR (M30-M49)

In WP4, data collected in WP2 and the methods assessed/developed in WP3 will be used to quantify the contributions of the main sources of the three focus pathogens and AMR. Results will be presented per pathogen, attribution method, type of data and, when/if applicable, geographical region/country. Particular attention will be given to environmental and non-livestock (pets and wildlife) sources besides the ‘traditional’ livestock/food sources. The results of applied methods for each pathogen will be compared in the light of data availability and robustness, underlying uncertainties, the point in the food production chain where source attribution takes place, and the usefulness of different methods to answer different One Health questions. Before performing any attribution, it has become evident in the past months that it is necessary to compare the typing data between countries using, e.g. PCA and/or similarity metrics like PSI. In this way, geographical regions could be identified as the “epidemiological units” for the attribution analyses. Moreover, such an analysis would already be very informative in itself as it would provide information on the distribution of relevant subtypes among the DISCOVER partner countries and it would also offer the opportunity to identify potential sources of surrogate data for the attribution analysis. Regarding the exposure assessment, data for all DISCOVER partner countries are incomplete or non-existent. A comparative exposure assessment, to be “comparative” needs to include different sources or to be performed in different regions/countries, which would also be in line with the spirit of OHEJP projects. It was therefore decided that a reasonable approach would be to perform an exposure assessment for a specific source for which we expect to have few typing data, i.e. pets like dogs and cats, for each of the 3 target pathogens and AMR in different countries that have the necessary data, and to compare the exposure estimates for this same source between the countries, as well as to check whether possible differences are also reflected in the attributions based on the other methods.

Task: JRP FBZ-1-WP4-T1 Salmonella source attribution and comparison of results from different approaches (M30-M40) - ongoing

In the past months, the specific activities of this task have been critically re-assessed and structured as follows: 1) attributions based on microbial subtyping; 2) analysis of outbreak data; 3) meta-analysis case control data; and 4) exposure assessment. People/partners contributing to each activity have also been identified. As an activity of interest for all tasks within WP4 and in collaboration with WP2, we worked together to define a minimum set of (meta)data to be collected to perform source attribution in a meaningful way. A document has been prepared and shared with the consortium. This document will be integrated in the upcoming milestones M-JRP FBZ-1-07 (Format for results presentation (standard structure) for all pathogens and AMR) and M-JRP FBZ-1-08 (Protocol for presentation of results for Salmonella, Campy, VTEC and AMR).

Task: JRP FBZ-1-WP4-T2 Campylobacter source attribution and comparison of results from different approaches (M30-M42) - ongoing

Alike the previous task, also for this one on Campylobacter, 3 activities have been defined: 1) attributions based on microbial subtyping; 2) meta-analysis case control data; 3) exposure assessment. This is because the scarcity of documented campylobacteriosis outbreaks would make an analysis of outbreak data not very useful. The document with the minimum set of metadata mentioned in the previous task also include specific indication for Campylobacter data.

Task: JRP FBZ-1-WP4-T3 VTEC source attribution and comparison of results from different approaches (M30-M49) - ongoing

Also for this task on VTEC, activities have been more clearly defined as: 1) attributions based on microbial subtyping; and 2) exposure assessment. This is because there are already recent works on the analysis of outbreak data and meta-analysis of case-control studies available performed by Sara



Pires (deputy leader of WP4), among others. The document with the minimum set of metadata mentioned in the previous task also include specific indication for VTEC data.

Task: JRP FBZ-1-WP4-T4 AMR source attribution results presented regionally and by region/country, for each applied method and integrated (M30-M49) - ongoing

Nothing to report.

WP5 - Conclusions, recommendations and policy translation. (M33-M54)

Nothing to report

Task: JRP FBZ-1-WP5-T2 Translating source attribution estimates into options for control policies (M33-M50) – not started yet

Nothing to report



5.1.10.20.3 Progress of the research project: deliverables and milestones

5.1.10.20.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
20	D-JRP20 FBZ-1- WP1.1	Mapping of knowledge gaps and recommendations for new data generation and method development	28	August 20 th 2020		Confidential until it has been published as a scientific article.	10
20	D-JRP20 FBZ-1- WP1.2	Data Management Plan			36	We will upload information about selected and complete datasets to the DMP database as they get ready to use. Some of them will be public, others confidential.	8 (3?)

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.20.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
20	M-JRP20 FBZ-1-01	Identification of Project Management Team	25	Yes		
20	M-JRP20 FBZ-1-02	First annual project meeting	27	Yes		
20	M-JRP20 FBZ-1-03	Completion of mapping of knowledge gaps and recommendations for new data generation and method development	28	Yes		
20	M-JRP20 FBZ-1-04	Identification of types of samples to investigate and for which species to include in the sampling	29	Yes	32	
20	M-JRP20 FBZ-1-05	Framework for evaluation of Microbial subtyping methods	33			
20	M-JRP20 FBZ-1-06	Methods for source attribution based on outbreak data evaluated	33			



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JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
20	M-JRP20 FBZ-1-07	Format for results presentation (standard structure) for all pathogens and AMR	33			



5.1.10.20.4 Ongoing collaborations

As part of WP5, we will organize a stakeholder webmeeting in the end of 2020 inviting EFSA, ECDC and the relevant EURLs. In addition, one of our partners are also part of OHEJP WP5, and she will inform about DiSCoVeR's activities in relevant fora (Stakeholder committee, Scientific Steering Board (SSB) and Program Owner Committee (POC)), and potentially asking DiSCoVeR to give a presentation at a later stage.

5.1.10.21 JRP21-FBZ3.1-BIOPIGEE

5.1.10.21.1 Summary of the work carried out in the JRP

The BIOPIGEE project is successfully running since January 1st 2020.

In WP1, a communication infrastructure was set up among participating institutes (email-lists, repeating teleconferences, website for data exchange). The kick-off-meeting was held at BfR in Berlin and brought all actors together. A poster and a BIOPIGEE leaflet were generated. Plans are being developed how to cope with the consequences of the CoVID-19 outbreak for the BIOPIGEE project. The project lead will change. Chris Kollas left the project at the end of June 2020. A new project leader is being searched (BfR), meanwhile Elke Burow (deputy) is to be contacted for the 9M report.

In WP2 T2.1 the development of the biosecurity protocol for pig farms has been completed (consisting of around 55 biosecurity questions for indoor/outdoor situation). Evidence from literature, existing protocols and expert opinions were brought together to build the questionnaire. The questionnaire was transferred into an electronic survey tool, translated into the languages needed and installed and tested as a mobile survey app on devices of interviewers in the participating countries. In T2.2, the sampling protocol, the laboratory testing protocol and the farm recruitment protocols have been designed and discussed with the team. Farm recruitment has started and the first farm visits have taken place. Most countries plan to start visiting farms in autumn and these visits will continue through 2020 and 2021. T2.3 has started to list existing biosecurity protocols for slaughterhouses from partner countries. In T2.4, the three longitudinal studies have been designed and coordinated to ensure any synergies between the studies are utilised and to assist with making the results comparable.

In WP3, discussions are in progress to choose the panel of *Salmonella* isolates and to compare the methods for disinfectant effectivity testing, and to define the methods that will be used to screen the isolates for biofilm forming abilities. Furthermore, work is continued on the further development of an HEV infectivity assay.

WP4 successfully developed a questionnaire covering around 24 questions about farm performance and provided this input to WP2. Information needed from WP5 (T5.2 systematic review/meta-analysis) for the modelling in WP4 is being specified.

WP5 T5.1 generated an online table starting the catalogue on effective biosecurity measures with regard to HEV and *Salmonella* prevalence. Data from WP2-4, especially from a brief review in T2.1, were integrated into the catalogue and all partners continuously fill that catalogue with their knowledge. A systematic review has started (T5.2). The precise search question and terms as well as inclusion and exclusion criteria have been discussed between participants in a web conference and are getting further developed with the help of an online document. A first expert panel was built and rated biosecurity measures. This panel is currently being extended with more and different types of stakeholders (T5.4).

WP6 initiated the collection of best biosecurity illustrations (pictures) which will be gathered during farm visits (WP2 T2.2) and which is described in the sampling protocol of T2.2. Due to the CoVID-19 outbreak, there has been a delay in visiting farms for some partners with subsequent delays in



collection of illustrations. A BIOPIGEE flyer/leaflet as brief information about the project has been produced (WP6 T6.4). Activities around the workshop series have been put on hold due to CoVID-19 outbreak and its consequences. We are preparing for online and/or national/regional workshops instead.

5.1.10.21.2 Progress of the project: description of activities

WP: 1 Project coordination and integration of results (M25-M54)

The project coordination, link between Tasks and WPs, and integration of results is ongoing until the end of the project. Teleconferences have been organised, conducted and followed up (minutes). Email lists for all WPs and Tasks have been created. Links to all relevant online tables and documents were listed in the BIOPIGEE private groupsite. Survey material including data protection forms, tutorials for the technical use of the survey and an invitation letter to farmers were prepared and, together with the survey app, made available to T2.2 participants via a download link. Incoming survey data from T2.2 get regularly and continuously checked. Thanks to a little tool, the income gets automatically listed in an online table. Thereby interviewers in T2.2 can follow the data income. For a double check, interviewers can in turn indicate to have sent survey data in the online table. The export of the survey data from the survey company's server has been tested and editing of spreadsheets is currently being prepared for quarterly data reports to participants/interviewers of T2.2. Overarching problems have been solved on a higher level (e.g. invitation of expert opinions). Pressing questions in subtasks have been solved/forwarded.

JRP21-WP1-T1 Project management and meeting organisation (M25-M54)

The BIOPIGEE Kick-off meeting was organised and successfully conducted at BfR in Berlin/Germany on 29th-30th of January 2020. 41 BIOPIGEE participants took part, all participating organisations were represented. Participants stated a good forthcoming of the project and a good organisation of the event. Minutes of the meeting were written, re-worked with all participants and finally published internally.

The BIOPIGEE OHEJP website was filled with content, participants were invited to join and organised as all having allowance to moderate in the group. All general and important documents are uploaded to the page and status updates were written. Participants were encouraged to use that website for exchange within the project.

Production of a BIOPIGEE flyer in WP6/ OHEJP Communication Team was supported with text modules and images.

JRP21-WP1-T2 Development of data management plan (M25-M30)

The elaboration of the data management plan was started. All WP-leader were invited to fill the "old" xls-sheet regarding their data. Later, we were informed that the former tool DMPonline will not be supported by OHEJP any further and a search for a better tool is under way. The deadline for the first version of the DMP was delayed by the OHEJP-PMT. Hence, work on the DMP was put on hold. Introduction to the new tool for the DMP took place on September 9th 2020. We have started to fill the DMP in CDP and are waiting for further instructions from OHEJP-PMT.

JRP21-WP2 Biosecurity effectiveness studies (M25-M50)

JRP21-WP2-T1 Development of biosecurity protocol (M25-M28)

The development of the biosecurity protocol has been completed. The biosecurity measures were reworded as questions (55 biosecurity questions for indoor, 56 for outdoor situation), and questions



(10) on general farm characteristics were added. Evidence from literature review and expert opinion was used to inform the content of the questionnaire. Existing protocols were assessed but it was decided to design a new protocol specific to the needs of the project. The protocol was transferred into an electronic survey tool and translated into the necessary languages of the countries participating in T2.2. An app for this survey was produced, set up on devices of interviewers in each country participating in T2.2 and the function of the app and data transfer to a central server have been tested, with support of WP1. This system enables standardised data collection, and facilitates data income and evaluation.

JRP21-WP2-T2 Application of the biosecurity protocol (M27-M42)

This task is ongoing.

Farm recruitment has started and first farm visits (about 15) took place. Incoming survey data are being reported to the interviewers quarterly to check and prepare for later data evaluation. The task is delayed due to different restrictions in EU member states concerning epidemiological situation related to worldwide pandemic of coronavirus disease COVID-19. NL decided not to collect fecal samples that they had planned in this task for Salmonella testing but instead will attempt to use results from routine meat juice sample surveillance to identify high and low risk farms. There have been difficulties in all countries to visit farms and sample as expected. The current situation in the countries is collected by WP1 and WP2 and updated in monthly catch-up conferences between partners. Currently, most countries expect to start farm visits in autumn 2020. The partners will hurry to fulfil the planned design yet. However, reduction in the number of farm visits, sample size and contributing partner countries could occur unless the deadlines for the project can be extended.

The sampling, laboratory testing and recruitment protocols have been designed. Details of the HEV-testing methods are still in discussion between partner labs and participants of T2.2 and T2.5 in order to harmonise methods as much as possible and to reach comparability of results that can also supplement the HEVnet database. For this, experts of HEV testing from all partner institutes have formed a group and conferences on method harmonisation are taking place. A harmonised test protocol is being developed.

JRP21-WP2-T3 Slaughterhouse biosecurity practices (M37-42)

This Task was planned to start at the beginning of next year (M37). We pulled it forward and have already started to collect existing national and local assessment protocols on biosecurity measures in slaughterhouses. A first meeting will take place in September 2020.

JRP21-WP2-T4 Field studies (M25-M48)

The study plans for the three proposed studies have been in design and the teams have been in contact to assess synergies and harmonisation of techniques to improve comparison of potential findings. The protocol has been uploaded to the BIOPIGEE page and is thereby accessible to the whole consortium. The task is ongoing.

WP3: Impact of disinfection on persistence of pathogens in biofilm (M25-M54)

JRP21-WP3-T1: Comparison of methods for testing the effect of disinfectants (M25-M40)

Subtask is ongoing. Discussions and planning on methods and reference strains to be used by all participating laboratories are ongoing.

JRP21-WP3-T2: Effect of disinfectants on biofilm-associated wild type Salmonella (M25-M50)



Subtask is ongoing. The subtask is dependent on the outcome of JRP21WP3-T1. Discussions and planning are ongoing among the participants in JRP21WP3 participants. Method establishment has started.

JRP21-WP3-T2-ST1: Selection of wild type salmonella isolates (M25-M36)

Selection of wild type Salmonella isolates (M25-M36). Discussions are in progress to choose the panel of Salmonella isolates, and finalise the method that we will use to screen the isolates for biofilm forming abilities. The sub-task is going ahead as planned and will be completed by the deadline.

JRP21-WP3-T3: Study of HEV stability in relation to disinfection approaches (M25-M50) and

JRP21-WP3-T3-ST1 Implementation of HEV infectivity assay for testing biofilms (M25-M36)

Over the first 9 months of the BIOPIGEE project work has been done on the further development of a HEV infectivity assay. Primary hepatocytes were isolated from liver tissue of (HEV free) piglets using a collagenase treatment. The obtained primary hepatocytes were aliquoted and stored at -180 Celsius until use. For the actual assay the hepatocytes were seeded onto plates and inoculated with different concentrations of HEV. First HEV replication plots were established.

As a second option for testing of HEV infectivity, precision-cut liver slices (PCLS) were used. PCLS cuts will be directly transferred to an ice-cold organ preservation solution and inoculated with different concentrations of HEV and tested daily using qRT-PCR for increasing levels of HEV RNA. Single replication rounds could be observed in some cases but the method needs to be optimized.

WP4 Modelling of the cost and effectiveness of biosecurity measures (M25-M50)

Task 4.1 is finished, and T4.2 and T4.3 have just started.

Task 4.1: Development of questionnaire on biosecurity costs (M25-M26)

A questionnaire with respect to health and performance data of pigs located in farms was developed which covers 24 questions. The questionnaire was delivered to the partners of WP2.1, who prepare a protocol on biosecurity practice and who will collect these economic data during their empirical survey at the farm level. Questions about the costs of biosecurity were not included. The reason is that the costs of the selected biosecurity measures will be difficult to answer for the farmers. Thus, the costs of the biosecurity measures will be estimated using usual country prices such as disinfection costs per litter and via monetary values from the scientific literature.

Task 4.2: Stochastic simulations on the effectiveness of biosecurity measures (M33-M49)

Task 4.2. has started in September 2020. A first online meeting was performed to coordinate and harmonize the different task of 4.2 and 4.3 between the partners. In this meeting we have discussed the adaption of currently available transmission models such as QMRA for Salmonella and SimInF model for HEV and/or Salmonella of the consortium partners in order to analyse the impact of biosecurity measures on prevalence reduction of the zoonotic pathogens. During the meeting the data requirements (e.g. type of transmission data, meta-data on the animal population, and necessity data inputs of the other WPs 2, 3 and 5 about the effectiveness of biosecurity measures on the reduction of prevalence values etc. was discussed during the meeting) for the transmission model was discussed as well as the In- and Outputs of the models.

Sub-Task 4.2.1: Data collection for the transmission models (M33-M36)



Task 4.2.1 has started in September 2020 and in the first meeting the data requirements for the transmission model was discussed between the partners as well as the outputs of the single models considering different type and aggregation of input data from the other WPs.

WP: 5 Benchmark of biosecurity practice (M27-M52)

JRP21-WP5-T1 Data integration from WP2-4 in catalogue of biosecurity measures (M27-M50)

With support of WP1, an online table ("BIOPIGEE: Biosecurity measures Salmonella and HEV") was set up starting the WP5 catalogue on effective biosecurity measures with regard to HEV and Salmonella - prevalence. Data from WP2-4, especially from a brief review in T2.1, were integrated into the catalogue.

JRP21-WP5-T2 Literature review/meta-analysis (M27-M50)

All BIOPIGEE participants were invited to supplement the BIOPIGEE catalogue of biosecurity measures. Further references have been added in the online table.

A systematic review has been started. The search question, terms, period, and in-/exclusion criteria have been discussed and are currently getting précised in an online document.

In the review, we wish to concentrate on biosecurity measures included in the questionnaire in T2.1 and for which no references on proving evidence of effectiveness has been identified in T2.1. Therefore, knowledge gaps in information from T2.1 is being analysed.

JRP21-WP1-T4 Expert panel to add estimations on effectiveness/ weights (M33-M51)

This task will build on and expand the expert panel set up and surveyed in T2.1 (scientists) to additionally incorporate knowledge of other stakeholders like practitioners, advisors, etc.. We are currently précising the list of types of experts to recruit in an online table.

JRP21-WP6 Dissemination (M25-M54)

JRP21-WP6-T1: Assembly and development of biosecurity information (M25-M54)

This task is dependent on data provision from the other WPs and results have not yet been obtained.

Collection of best biosecurity illustrations (pictures) during farm visits of WP2 T2.2 has been initialised and is described in the Sampling protocol of T2.2. Due to the CoVID-19 outbreak, there has been a delay in the initiation of farm visits for some partners with subsequent delays in collection of illustrations. This task is ongoing.

Sub-Task JRP21-WP6-T1-ST1 Identification of appropriate websites or other online channels (M25-M54)

An excel file and an online table are in use to list appropriate channels for dissemination per country. About 21 web sites in 6 countries have already been identified for dissemination. This task is ongoing.

Sub-Task JRP21-WP6-T1-ST2b Provision of slaughterhouse protocol to slaughter industry/related associations (M31-M52)

This task has not started yet and is postponed to start in M43 when effective biosecurity measures for slaughterhouses have been analysed in T2.3.

Task JRP21-WP6-T3: Organisation of a workshop-series (M25-M54)



WP5 (expert panel) und WP6 (workshops) are working on description of stakeholder groups to consider, include and address in each of the two work packages.

Due to the current CoVID-19 situation, the workshops have been postponed. At the moment, it is not possible to make any concrete plans for the workshops based on physical meeting.

Sub-Task JRP21-WP6-T3-ST1 Identification of relevant stakeholders (M25-M52)

This task is ongoing. An excel file and an online table are in use to list relevant stakeholders.

WP5 and WP6 are working on detailed description of stakeholder groups to consider, include and address.

Sub-Task JRP21-WP6-T3-ST2 Identification of relevant conferences (M25-M26)

This task had to be postponed due to the current CoVID-19 situation but will be resumed as soon as possible.

Sub-Task JRP21-WP6-T3-ST3 Organisation of Workshop 1 (M25-M30)

This task had to be postponed due to the current CoVID-19 situation but will be resumed as soon as possible. It's being discussed on whether the workshops can be held in a web meeting. If possible, we prefer national or regional workshops.

Additional Sub-Task JRP21- WP6-T4 Production of BIOPIGEE flyer (M29-30)

WP6 (supported by WP1 and the OHEJP Communication Team) produced a BIOPIGEE Flyer as brief information material about the project and which is planned to hand out to farmers (T2.2), external collaboration partners, at conferences, workshops (T6.3) and when recruiting experts for the panel (T5.4).



5.1.10.21.3 Progress of the research project: deliverables and milestones

5.1.10.21.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
21	D-JRP21-WP2.1	Biosecurity protocol (addressing Salmonella and HEV) designed for data collection in the field	28	Will be uploaded and made public when results are published Uploaded on private space: 24.08.2020	It was finished in M28, then translated and transferred to a technical tool, uploaded as pdf in M32	Confidential until publication.	1
21	D-JRP21-WP1.2	First draft of data management plan finished	30 – postponed by OHEJP-PMT		Assume 34	Introduced to new DMP tool on 9 th Sep. 2020, waiting for further instructions from OHEJP-PMT	8



JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
21	D-JRP21-WP6.3	Workshop 1 completed	30	48	48	WP-leader decision: the 1 st workshop is postponed due to problems of physical contact provoked by the CoVID-19 outbreak	8
21	D-JRP21-WP6.4	BIOPIGEE Flyer	(new)	Uploaded on private space: 24.08.2020	30	Public, https://zenodo.org/record/4009015	8 (for recruiting farmers, expert panel, dissemination)

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.21.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
21	M-JRP21-01	Kick-off meeting successfully organized	26	Yes		
21	M-JRP21-02	Questionnaire on biosecurity costs	26	Yes		Farm performance questions included. Biosecurity cost question excluded; data will be gathered from other sources.
21	M-JRP21-03	Relevant conferences for workshops to be held are identified	26	No	Not possible under the current circumstances	The CoVID-19 situation has put a stop to conferences including workshops for now. It is planned to disseminate findings at local/national workshops at the end of 2021/ beginning of 2022 instead
21	M-JRP21-04	Biosecurity protocol designed for Salmonella and HEV	28	Yes		
21	M-JRP21-05	Relevant stakeholders identified	28	Yes	32	Was postponed due to the current CoVID-19 situation; List of stakeholder groups is being developed in an online table (link in BIOPIGEE private webgroup); Task is ongoing



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M25-M36



JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
21	M-JRP21-06	Appropriate websites or other online channels for dissemination identified	30	Yes	32	Was postponed due to the current CoVID-19 situation; List of web sites is being filled in an online table (link in BIOPIGEE private webgroup); Task is ongoing
21	M-JRP21-07	Workshop 1 completed	30	No	Not possible under the current circumstances	The CoVID-19 situation has put a stop to conferences for now
21	M-JRP21-08	Design of field study protocols	32	Yes		



5.1.10.21.4 Ongoing collaborations

Collaboration is planned with Ghent University (developed BioCheck®). We invited Jeroen Dewulf to evaluate our farm survey and to participate in the slaughterhouse study. Unfortunately, he was not funded. Hence, he indicated that his input may be limited.

A cooperation with HEVnet is ongoing. Agnetha Hofhius was our contact until August 2020, she also participated in our Kick-off-Meeting in January 2020. As she is now working on Covid-19 fulltime, our new contact persons are Annelies Kroneman and Claudia Swart-Coipan. Annelies Kroneman participates in our group (covering HEV-test-experts from participating institutes) which is working on the harmonisation of the HEV test protocol to ensure that information from samples collected and tested on HEV in BIOPIGEE (T2.2) can be included in the European harmonised database HEVnet. We are working on expanding the HEVnet data base with animal/farm/biosecurity related variables.

Frank Boelaert (EFSA) has been invited to support the expert panel (T5.4) informing our benchmarking system. He was also asked for information and contacts related to current projects and new information on biosecurity measures related to Salmonella/HEV prevalence.

We plan to inform DG HEALTH, DG AGRI and EFSA, if our findings suggest recommendations to improve existing surveillance programmes.

National collaborations with e.g. animal health services/vet services are currently supporting in accessing farms for sample collection (these services are less restricted in Covid-19 situation) and are planned to contribute in our expert panel (contacts are currently listed).

Collaborations with universities in several partner countries are initiated. This gives the opportunity to find support of students to carry out systematic literature reviews on BIOPIGEE relevant questions. For instance, a diploma thesis is currently being prepared in WP 4, dealing with the topic "Financial impact of biosecurity and vaccination measures to minimize the use of antimicrobials in pig farms". Additionally, the cooperation with universities also makes it possible to obtain data and information from on-going national projects to fill any data gaps in our WPs. For instance cooperation with Austrian swine clinics enables to recruit farms to participate in questionnaires and to obtain information about existing data sources.

Through these contacts, we may also increase dissemination of findings.

5.1.10.22 JRP22-FBZ4.1-TOXOSOURCES

5.1.10.22.1 Summary of the work carried out in the JRP

TOXOSOURCES is a 2.5-year Joint Research Project of the One Health EJP that focuses on *Toxoplasma gondii* at the interface between humans, animals, food, and the environment.

The protozoan parasite *Toxoplasma gondii* is a highly prioritized but so far not systematically controlled zoonotic foodborne pathogen in Europe and globally. The infection can be acquired by ingesting oocysts (in food, water, or the environment contaminated with feces of infected, shedding felids; environmental pathway) or tissue cysts (in meat of infected animals; meatborne pathway). The relative contribution to infection and disease of the different transmissible stages and transmission pathways remain unknown, partly due to lack of appropriate methods.

TOXOSOURCES Consortium comprises 20 One Health EJP partners and several external partners. The TOXOSOURCES research question: **What are the relative contributions of the different sources of *Toxoplasma gondii* infection?** is addressed using several multidisciplinary approaches and novel and improved methods, to yield robust estimates that can inform risk managers and policy makers.



TOXOSOURCES started efficiently and adapted well to the challenges related to the COVID-19 situation. The Kick-off Meeting was held in Copenhagen, Denmark, in February 2020. The collection of input data and building of a quantitative microbiological risk assessment model were started. An extensive literature review was performed, and complemented by a survey of expert opinions, current practices and experiences, to select the most suitable molecular method for *T. gondii* oocyst detection in fresh produce. Data were collected for designing a sampling strategy for a multicentre survey of *T. gondii* oocysts in fresh produce. Bioinformatic selection of promising protein candidates for a novel serology method was finalized, and recombinant expression of selected proteins was started. Availability of suitable sera for assessing the proteins of interest was confirmed, and decisions were made regarding technical details of the method to be developed. The retrieval of key *T. gondii* isolates and DNAs from across Europe for Whole Genome Sequencing was successful. Using the sequences, polymorphic marker regions are being identified for the establishment of a new typing method to detect within-genotype variation.

Challenged by the COVID-19-pandemic, the TOXOSOURCES Consortium has shown impressive resoucefulness and adaptability, and the careful risks-and-dependencies planning proved useful. All Milestones, Deliverables and reports have been reached and submitted by their planned deadlines, and no delays are expected in reaching the future measurable outcomes by their planned deadlines.

The main outcomes of TOXOSOURCES will be quantitative estimates of the contribution of the main sources and transmission routes of *T. gondii* infection based on improved source attribution models covering both meatborne and environmental exposure, new data filling the knowledge gap regarding the role of increasingly popular but unstudied ready-to-eat fresh produce, a novel serological method specifically detecting infections caused by oocysts, and a novel typing method enabling detection of introduction of atypical *T. gondii* strains by import and tracing the infection sources in outbreaks. The results of TOXOSOURCES will contribute to developing efficient interventions at national, regional, European and global levels.

5.1.10.22.2 Progress of the project: description of activities

The work in TOXOSOURCES is organized into five work packages (WPs) with tasks (T) and some subtasks (sT). The 2.5-year project spans three Annual Periods (Y3-Y5). All the tasks that take place in Y3 continue to Y4; no task is planned to be completed in Y3.



Kick-off Meeting of TOXOSOURCES, February 2020, at Statens Serum Institut, Copenhagen, Denmark.
JRP22-WP1 Coordination and impact (M25-M54)



The project started efficiently, the first milestone 'Kick-off Meeting held by WP1' was reached, first version of 9M report, the first Deliverable 'Data Management Plan', and the final version of 9M report were all submitted on time. The impact of the COVID-19 situation was followed up closely.

JRP22-WP1-T1 Management, coordination and communication (M25-M54)

The Kick-off Meeting was held February 3–4, 2020, at SSI, Copenhagen, Denmark, with a possibility to participate remotely. The established key structures for management of the project include monthly online meeting with TOXOSOURCES WP-Leaders and Co-Leaders, Consortium emails, use of the online group for sharing and storage of relevant documents, and WP-level online meetings. Dissemination of the outcomes started with several presentations to relevant audiences and first article manuscripts. The first version of Data Management Plan was drafted, and the PL participated in the work of the DMP committee. The collaboration with the Interest Group started by establishing key contacts.

JRP22-WP2 Multicentre quantitative microbiological risk assessment for *T. gondii* infections (M25-M54)

The outlined work of all TOXOSOURCES-WP2 tasks – collection of input data and building of the QMRA model – started well.

JRP22-WP2-T1 QMRA modelling for human *T. gondii* infections (M25-M54)

The Consortium members involved in QMRA modelling met at RIVM, The Netherlands, to discuss plans. The development of the structure for the QMRA model for environmental transmission of *T. gondii* was finished, and expansion of both the meatborne and environmental QMRA models to include multiple countries was started.

JRP22-WP2-T2 Review of prevalence of *T. gondii* infection in animals (M25-M54)

A list of European countries and a list of key animal species raised or hunted for human consumption in Europe were collated. These were used to develop a search strategy for data on prevalence of *T. gondii* in animals. The screening of retrieved records was finished, and data extraction has started.

JRP22-WP2-T3 Quantitative exposure survey (M25-M48)

A draft questionnaire was developed taking into account experiences from the Dutch National Food Consumption Survey as well as risk factor information from a prospective case-control study in the Netherlands. Adaptation of the questions to the different countries was started.

JRP22-WP2-T4 Overview of processing parameters for relevant meat products (M25-M45)

Information on food consumption in the different countries was collected from the EFSA FoodEx2 database. The information is used to identify country-specific relevant products.

JRP22-WP2-T5 Review of prevalence and risk factors for human *T. gondii* infection (M25-M54)

Results from the literature review on *T. gondii* source attribution (including risk factor analyses) for COST-action Euro-FBP were presented at OHEJPASM2020, emphasizing the continuation of the work in TOXOSOURCES. The synergy with WP2-T2 will be useful for the new searches.

JRP22-WP3 Multicentre survey to fill the key existing gap: role of fresh produce (i.e. Ready-to-Eat salads) (M25-M54)

The collection of data on molecular detection methods for *T. gondii* oocysts and on *T. gondii* oocyst prevalence in fresh produce and environment was completed, and collection of information on fresh produce production, trading and consumption progresses. The first deliverable 'Report on available analytical procedures for detection of *T. gondii* in fresh produce and list of promising analytical procedures' was submitted on time.



JRP22-WP3-T1 Selection, evaluation and implementation of detection procedure for *T. gondii* oocysts in fresh produce (M25-M39)

An extensive literature review and multi-attribute assessment of the different steps (oocysts recovery, DNA extraction and DNA amplification), complemented by a survey on expert opinions, current practices and experiences on molecular detection of *T. gondii* (DNA), was performed to select the most suitable molecular method for *T. gondii* oocyst detection in fresh produce. The first Deliverable was submitted on time and provided a good starting point for developing a standard operating procedure (SOP) for the multicentre survey of *T. gondii* oocysts in fresh produce. The comparative experimental work was completed and pre-SOP is in preparation. The location of the technical workshops was changed from Rome (IT) to Brno (CZ), and preparations were made for possible need to change the workshops to be virtual.

JRP22-WP3-T2 Design of a risk-based sampling strategy (M26-M48)

An extensive review of peer-reviewed literature on prevalence of *T. gondii* oocysts in fresh produce was performed, as well as a review of literature on *T. gondii* prevalence in environment (soil and water) and bivalves. These were complemented by an online questionnaire to consortium partners to collate grey literature on the topic.

The design of a risk-based sampling strategy for the multicentre survey of *T. gondii* oocysts in fresh produce was started by preparing an online questionnaire, in collaboration with the TOXOSOURCES interest group, to gather relevant information on trade and consumption of ready-to-eat salads from local and international industry.

JRP22-WP4 Serology method based on novel antigens to discriminate *T. gondii* infections acquired from oocysts (M25-M54)

The bioinformatic selection of promising protein candidates was finalized and the first milestone 'Bioinformatic selection of oocyst/sporozoite-specific antigens completed in WP4' was reached as planned. The recombinant expression of the first set of proteins of interest was started following the timeline, and the availability of suitable sera for assessing them was secured.

JRP22-WP4-T1 Identification and production of *T. gondii* stage-specific antigens for source attribution (M25-M48)

The predicted proteome of the *T. gondii* oocyst/sporozoite was analysed bioinformatically to identify the best stage-specific and antigenically relevant protein candidates. A list of 96 proteins with source-attributing potential was defined; the milestone was reached on time. Main selection criteria were exclusive expression in oocysts, evidence for secretion, and a high score in linear epitope prediction. The first set of 10 proteins of interest was expressed and purified (ongoing).

JRP22-WP4-T2 Development of a novel stage-specific antigen-based ELISA to diagnose oocyst- and bradyzoite-driven *T. gondii* infections (M29-M48)

Availability of suitable sera for assessing the proteins of interest from WP4-T1 was determined.

JRP22-WP4-T2-sT1 Standardization of a POI-based ELISA to diagnose oocyst- and/or bradyzoite driven *T. gondii* infections using reference pig sera

Availability of suitable panel of sera from pigs experimentally infected with either oocysts or tissue cysts was confirmed. The sera were characterized using commercially available and routinely used serological tests and the selection of the best secondary antibody for the novel stage-specific antigen-based ELISA was started.

JRP22-WP4-T2-sT2 Validation of a novel stage-specific antigen based ELISA to diagnose oocysts-and/or bradyzoite-driven *T. gondii* infections using reference sera from several relevant host species including humans



Availability of suitable sera from sheep experimentally infected with oocysts was confirmed. The sera were characterized using commercially available and routinely used serological tests and the best secondary antibody for the novel stage-specific antigen-based ELISA was selected.

JRP22-WP5 Novel *T. gondii* typing method to detect within-genotype variation (M25-M54)

The retrieval of key *T. gondii* isolates or DNAs was successful and the aims set for the first months were achieved. The work progressed following the timeline: the first milestone 'Key isolates summarized in WP5' was reached 2 months ahead of its deadline and one subtask could start earlier than anticipated.

JRP22-WP5-T1 Retrieval of relevant *T. gondii* isolates or NGS-quality DNAs for NGS and NGS-MST (M25-M44)

T. gondii isolates, WGS-quality DNAs or WGS-data on isolates were collected from across Europe. Isolates were expanded in-vitro, and DNA was extracted for WGS. The focus was on *T. gondii* Type II; Type III, atypical or recombinant isolates were included as well. This retrieval of key *T. gondii* isolates or DNAs was successful and the aims were reached. The Milestone was reached 2 months before its deadline, and the total number of isolates already retrieved for the work is markedly higher, $n > 60$, than the original target. All DNAs in the panel are also characterised based on polymorphism of fewer markers using existing standard techniques (PCR-RFLP and microsatellite (MS) typing). Isolates of northern and eastern European regions are slightly underrepresented on the list, but further efforts were successful to gather more isolates from these regions. Moreover, for particular regions, we aim to include additional isolates to investigate the resolution of typing to trace differences of local isolates.

JRP22-WP5-T2 Novel, standardized high-throughput direct NGS-MLST *T. gondii* genotyping method (M25-M48)

Whole genomic sequences are being generated. Based on these sequences a novel, standardized, high-throughput, targeted NGS-MLST genotyping method is established to separate closely related *T. gondii* strains.

JRP22-WP5-T2-sT1 Whole Genome Sequencing (WGS) of key *T. gondii* isolates and WGS-quality DNAs (M25-M36)

Due to the COVID-19 situation, sending samples was challenging, and we decided to apply a batch-approach to sequencing. The first batch, $n = 22$ isolates, were WGS-sequenced de-novo and together with already existing data, sequences from $n = 41$ isolates were available by M30. The already existing sequences were from ongoing EURLP-FLI and UCM-CVI (Craig-Venter-Institute) collaborations, and included also sequences of a few Type III and recombinant isolates already available at FLI and UCM. A second batch, $n = 21$ isolates, were WGS-sequenced by M32.

JRP22-WP5-T2-sT2 Establishment, validation and refinement of a novel, standardized high-throughput targeted NGS-MLST *T. gondii* genotyping method (M31-M44)

Based on the sequences from WP5-T2-sT1, highly polymorphic marker regions (partially focusing on introns and/or particular gene regions of e.g. virulence associated genes) are identified and selected for the establishment of a new typing method. This subtask was started a bit ahead of time.



Screenshot from an online meeting of TOXOSOURCES-WP-Leaders and WP-Deputy-Leaders



5.1.10.22.3 Progress of the research project: deliverables and milestones

5.1.10.22.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
22	D-JRP22-WP3.1	Report on available analytical procedures for detection of <i>T. gondii</i> in fresh produce and list of promising analytical procedures	M28	M28		Public. 10.5281/zenodo.3778719	9
22	D-JRP22-WP1.1	Data Management Plan	M30	M30		Public. 10.5281/zenodo.3924450	8

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.22.3.2 Milestones

JRP Co de	Milestone number	Milestone name	Delivery date from AWP 2020	Achie ved (Yes/ No)	If not achieved: Forecast achieveme nt date	Comments
22	M-JRP22-01	Kick-off Meeting held by WP1	M26	Yes		Kick-off Meeting was held on 3.-4.2.2020 at SSI, Copenhagen, Denmark. Milestone reached M26 (on time).
22	M-JRP22-02	Bioinformatic selection of oocyst/sporozoite-specific antigens completed in WP4	M28	Yes		20.4.2020 TC of TOXOSOURCES-WP-leaders: Selection is done. Milestone reached M28 (on time).
22	M-JRP22-03	Key isolates summarized in WP5	M30	Yes		15.4.2020: TC of TOXOSOURCES-WP5: Summary list is compiled. Milestone reached M28 (2 months ahead of time).



5.1.10.22.4 Ongoing collaborations

TOXOSOURCES builds largely on previous work and is actively looking for collaborations and synergies.

- An example of building on previous work, which was emphasized in the oral presentation at OHEJPASM2020, is the building on the work performed within **COST-Action Euro-FBP**. A literature review of source attribution for *T. gondii* infections in Europe was performed within the COST-Action Euro-FBP; TOXOSOURCES will address the identified data gaps. The literature review also covered risk factor analysis; within TOXOSOURCES this will be updated and extended with prevalence studies.
- Another example of building on previous work is that the TOXOSOURCES-WP3-T1 survey questionnaire was developed based on outlines of the **project IMPACT** (Standardising molecular detection methods to improve risk assessment capacity for foodborne protozoan Parasites, using *Cryptosporidium* in ready-to-eat salad as a model organism”; Partnering Grant Project Grant Agreement Number GP/EFSA/ENCO/2018/03 – GA03).
- TOXOSOURCES established a collaboration with **SafeConsume project** (<http://safeconsume.eu/>), and representatives of TOXOSOURCES were invited to participate in SafeConsume Multi-Actor Workshop, which was unfortunately cancelled due to the COVID-19 situation. Both projects are interested in the relevance of *Toxoplasma gondii* contamination in fresh produce, with different focus: SafeConsume focuses on consumer behaviour on safe handling of fresh produce at home, whereas TOXOSOURCES WP3 focuses on fresh produce from harvest to packaging.
- Collaboration with **International network for environmental Toxoplasma studies (INETs)** is another important established collaboration. INETS is a global network that organizes e.g. workshops.
- **One Health EJP PhD project ToxSauQMRA** (PhD candidate Filip Damek) is closely linked to TOXOSOURCES.
- There are discussions about collaborations with **other One Health EJP projects**, including COHESIVE, ORION, MEME, and PARADISE. Synergies and complementary approaches have been identified.
- To enable and encourage collaborations, the QMRA models will be made available via a repository (<https://foodrisklabs.bfr.bund.de/rakip/>)
- European Reference Laboratory of Parasites and network of National Reference Laboratories are well represented in the consortium.
- TOXOSOURCES collaborates with and builds on the results of several national and regional projects.

5.1.10.23 JRP23-FBZSH5-ADONIS

5.1.10.23.1 Summary of the work carried out in the JRP

Although challenging due to the COVID19 situation the project is running so far without major/critical problems and delays. Detailed work plans have been made following the kick-off meeting in January and are monitored regarding their progress with project management video calls. During this first period the project especially focussed on preparation for data analysis and data gathering. For WP2 this includes the gathering of *Salmonella* National Control Programs (NCP) audit reports, the preparation of a survey to collect information on the main characteristics of NCP for *Salmonella* in laying hens at the country level, and the design of a study protocol for primary production on-field



investigations (WP2). For WP3 this includes the selection of countries for evaluation of human surveillance systems and detailed epidemiological trend analysis. For WP4 this includes the preparation of an inventory of available sequence data and a pilot GWAS experiment (finding DNA markers for specific phenotypes). For WP5 this included the identification of determinants possibly associated with the reversal of the decreasing trend in *Salmonella* incidence and the possible interventions options.

5.1.10.23.2 Progress of the project: description of activities

JRP23-WP1 Project management (M25-M54)

JRP23-WP1-T1 Coordination

Ongoing.

Although challenging due to the COVID19 situation the project is running so far without major problems. The project has its kick-off meeting at 15 and 16 January 2020 which was very successful. The meeting produced detailed planning for each WP (see D-JRP23-WP1.1). Regular TCs within the project management team and within WPs are conducted.

JRP23-WP1-T2 Aligning and communication

Ongoing.

JRP23-WP1-T2-ST1 Reporting

Draft 9M report is being made (this document)

JRP23-WP1-T2-ST2 Alignment and communication. Ongoing.

Efforts are undertaken to align the EJP projects of ADONIS, DISCOVER and BEONE in the area of *Salmonella*. The RIVM appointed a PhD that will work primarily on ADONIS but will also do work regarding source attribution of *Salmonella* (DISCOVER) and genomics-epi integration (BeONE). In this way we maximize the cross-fertilization of the different projects.

JRP23-WP2 *Salmonella* controls at the primary production level (M25-M54)

This WP covers comparative analysis and management measures between MS in the EU based on current NCP and on questionnaire to collect data on farms. On-field investigations are also expected in two MS (France and the UK) to collect data which will contribute to the comparative analysis performed in this WP and to the analysis of SE increase within the project.

JRP23-WP2-T2.1 Comparative analysis of *Salmonella* controls and management measures at MSs level (M25-M48). Ongoing.

Analysis of *Salmonella* National Control Programs (NCP) audit reports is ongoing. 232 recommendations were identified in 38 publicly available reports from 24 countries. Most (143) recommendations concern not full compliance on implemented programs with EU regulations on *Salmonella* control. The others refer mostly to legislation on official controls and food hygiene. The ongoing work focuses on identification of specific issues raised in recommendations.

A questionnaire has been developed to document control and management measures for *Salmonella* in laying hens at the country level in several participating MSs. The questionnaire is presently circulating in MSs among the veterinary agencies. The survey contains questions directed at the population and scope of the program as well as some specific questions on the type of controls performed by the Food Business Operators and Competent Authorities.

The first preliminary review of historic data from controls performed by competent authorities and food business operators has been delayed due to the COVID-19 crisis but is expected to be performed in the upcoming months.



JRP23-WP2-T2 Comparative analysis of *Salmonella* controls and management measures at farm level (M25-M48). Ongoing.

JRP23-WP2-T2-ST1 Field studies during outbreaks and sensitivity testing

The study protocol of on-field investigations was designed by APHA and Anses. In France, this protocol was presented and agreed by the competent authority. Recruitment of flocks presenting *Salmonella* cases will start by September 2020 with the help of local authorities.

JRP23-WP2-T2-ST2 On farm surveys

Currently, intensive preparatory work is underway regarding the developing of the questionnaire and guidelines for completing it. The questionnaire will be a kind of survey that will be distributed / carried out on poultry farms in which the presence of *Salmonella* infection was found as well as on farms where such infection has never occurred. The questionnaire contains detailed questions about the herds on which the survey will be conducted / implemented, on the welfare of herds, breeding conditions, their feeding, detailed therapy if were carried out, if preventive vaccinations were carried out from the moment the chickens were introduced to the farm until the chickens were sent to the slaughterhouse.

After completing the questionnaire and its guidelines, it will be sent to WP2 partners to disseminate prepared surveys in other EU countries.

JRP23-WP3 Surveillance, epidemiology and source attribution (M25-M48)

Determinants of the recent increase in *S. Enteritidis* incidence could be related to changes in the performance of the human surveillance systems/diagnostic standards in place, as well as changes in the epidemiological and exposure patterns of *S. Enteritidis* in the population at large.

JRP23-WP3-T1 Evaluation of surveillance systems in humans (M26-M48). Ongoing.

National surveillance systems for *S. Enteritidis* of three countries will be evaluated (three scenarios); one country where a decrease in the number of human salmonellosis cases has been observed, one country where the number of human salmonellosis has remained stable, and one country where the number of human salmonellosis cases increased.

Trend analyses were performed (Tau parameter) of the increasing/decreasing incidence of *S. Enteritidis* of multiple countries in the period 2013-2018 based on ECDC data (<https://www.ecdc.europa.eu/en/surveillance-atlas-infectious-diseases>). Countries with low *S. Enteritidis* numbers or without *S. Enteritidis* data were excluded. Countries should have experienced a decreasing trend observed in the 2009-2012 period to meet the research question of ADONIS. The non-parametric Mann-Kendall Test was used to detect monotonic trends. Kendall's Tau permits a comparison of the strength. To select countries with a decreasing/increasing trend in 2013-2018 period, they were ranked according to the Tau value (strength of the trend). Stable trends in the 2013-2018 period were selected based on a Tau value close to 0. The Pettitt's test was used to identify a point at which the values in the data change over time (breakpoint). A document was prepared, showing the trends of each of the countries, as well as the breakpoints. This was further discussed during a teleconference meeting with all partners of WP3. Breakpoint analyses showed that the breakpoint (i.e. change in trend) for most countries was in 2013. Therefore, it was decided to compare the time period 2007-2012 and 2013-2019. Concerning the selection of countries, it was decided to focus on Spain and UK as countries with an increasing trend (WP2 also focusses on these countries), Netherlands and Belgium as countries with a stable trend, and Norway as country with a decreasing trend. Next steps are to approach the relevant persons in Spain, UK, Netherlands, Belgium, and Norway to assess the feasibility of obtaining the data required to complete WP3.

Key elements for description and key attributes and indicators for evaluation were defined using an adapted version of the 2014 ECDC evaluation framework of public health surveillance systems,



focusing only on those surveillance attributes that could potentially affect the number of captured human *S. Enteritidis* cases. A detailed list with these key elements for description and key attributes and indicators for evaluation was composed. Next step is to describe and evaluate the surveillance systems of the selected countries within the selected time periods once the surveillance data from these countries has been obtained. Eventually it will be assessed whether identified changes in the surveillance systems over time have affected the number of captured human salmonellosis cases over time.

JRP23-WP3-T2 Assessment of changes in the epidemiology of human *S. Enteritidis* cases and other relevant serovars (M29-M48). Ongoing.

This task just started. At RIVM a PhD student is appointed that will start working on data extraction and analysis for The Netherlands. Changes over time in *Salmonella* serotypes will be assessed in relation to epidemiological parameters such as gender, age, travel-relatedness, and severity of symptoms. This methodology will subsequently be applied to the other selected countries.

JRP23-WP3-T3 Assessment of human exposure to *S. Enteritidis* (M32-M48)

To be started.

JRP23-WP4 *Salmonella* Genomics (M25-M54)

JRP23-WP4-T1 Collection overview (M25-M30). Ongoing.

All partners are involved in WP4, especially in the collection of sequences. During the Kick-off meeting, we agreed on the creation of an inventory to get an overview of the available strains and sequences at each partner institution. The inventories were collated and presented at a teleconference where we then discussed the available strains and decided what to sequence. At present we are awaiting the final lists of strains to be sequenced from all partners and are also awaiting the sequences to be finalized, both are influenced by the COVID-19 pandemic.

D-WP4.1 : Sequence Inventory Report (July 2020): Ongoing.

The lists of strains to be sequenced and already available sequences have been provided by most partners. Report will be written once all partners have provided this information.

M-7: Sequence collection to be shared amongst partners (July 2020): Ongoing.

Certain partners have experienced delays in the sequencing due to COVID-19 reorganisation in their institutions. A data sharing site (data hub) has been ordered at European Nucleotide Archive (ENA), however they are also experiencing delays.

M-8: Phylogenetic trees describing the sequence collection (August 2020): Not yet started.

Once all sequences will have been collected, the analysis will start. Expected one month after all sequences are finalized.

JRP23-WP4-T2 Population structure and comparative genomics (M25-M48). Ongoing.

This task is awaiting the sequence inventory in order to really begin. Small pilot studies on limited datasets have been initiated on Mobile Genetic Element (MGEs) detection and analysis.

JRP23-WP4-T3 Phylodynamics and Phylogeography (M31-M51). Ongoing.

This task is just beginning and is also awaiting the sequenced genomes to be collected and shared at ENA.

JRP23-WP4-T4 Mutant creation and testing including GWAS studies (M31-M54). Ongoing.

A pilot GWAS study was performed on a limited dataset of 120 genomes. The study detected 6 plasmid related genes slightly associated to with the *Enteritidis* increase since 2014. More analysis is needed to confirm this pilot study.



WP5: JRP23-WP5 MCDA model to support priority setting (M28-M52)

As it is possible that no single factor is able to explain the observed changes in *S. Enteritidis* incidence in humans and poultry, but that the reasons for these changes is multifactorial and interconnected in nature, WP5 will conduct a MCDA to determine: 1) what are the main determinants that may explain the reversal of the decreasing trend in the incidence of human *S. Enteritidis* infections in the ADONIS partner countries; 2) which interventions are expected to have the largest impact on stopping the reversal of the decreasing trend in incidence of human *S. Enteritidis* infections in the ADONIS partner countries.

Task: JRP23 FBZ-4-WP5-T1 Framework building (M25-M48). Ongoing.

The MCDA framework has been defined. The determinants possibly associated with the reversal of the decreasing trend and the possible interventions (i.e. options) are being identified based on the (hierarchical) structure of the transmission chain and all possible interrelation between primary production, exposure and pathogen characteristics: a literature review helps with this process. The MCDA framework is being based on the Analytic Hierarchy Process (AHP) approach, which also requires independent evaluation criteria and sub-criteria for both the determinants and interventions: these have also been defined. A list of experts to conduct the MCDA has also been compiled, including 2 to 5 experts per ADONIS partner country that cover all three domains of the project (i.e. poultry primary production, *Salmonella* genomics, salmonellosis epidemiology) and a balanced representation of the medical and veterinary fields. The next step will be to organize a first round of elicitations to define the weights for the criteria.



5.1.10.23.3 Progress of the research project: deliverables and milestones

5.1.10.23.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
23	D-JRP23-WP1.1	Detailed work plan developed (Kick-off meeting minutes)	28	April 20, 2020	-	Public Detailed project management plans with goals and actions (in x-matrix format)	10
23	D-JRP23-WP2.1	Report on the evaluation of the questionnaires	30		43	Public Following the preparation of the detailed work plan we noticed that a deadline in M30 is a mistake made in the proposal. It should be M43.	10
23	D-JRP23-WP4.1	Sequence inventory report	31		33	Public The COVID-19 pandemic has had an impact on laboratory performance.	10

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



5.1.10.23.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
23	M-JRP23-1	Kick-off meeting organised	26	Y (15/16 january 2020)		
23	M-JRP23-2	Communication partners mapped	28	No	M32	Delayed due to COVID
23	M-JRP23-3	Inventory of documents available for the comparative analyses	30	Yes		
23	M-JRP23-4	Farm survey planed and questionnaires developed	30	Yes		The questionnaires are developed; they are circulating among partners to be addressed.
23	M-JRP23-5	Selection of three countries of which their national S. Enteritidis surveillance systems will be evaluated	27	Yes	M28	Trend analyses were performed of the increasing/decreasing incidence of S. Enteritidis of multiple countries in the period 2013-2018 based on ECDC data



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JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
23	M-JRP23-6	Obtained national surveillance data on <i>S. Enteritidis</i> and other relevant serovars	30	Yes		For NL; the methodology and data needs (incl gaps) will be used for the other selected countries
23	M-JRP23-7	Sequence collection to be shared amongst partners	31	No	M35	The COVID-19 pandemic has had an impact on laboratory performance.



5.1.10.23.4 Ongoing collaborations

Within the EJP consortium The ADONIS project strongly liaises with the EJP projects DISCOVER regarding source attribution and BeONE regarding the genomics and bioinformatics. We have strengthened this at RIVM with a PhD that is involved in all three projects.

Outside the EJP consortium the ADONIS project maintains strong interactions with ECDC and EFSA. The project leader especially has intensive connections within the ECDC Food- and Waterborne Disease network and acts as a member of the FWD steering committee. In that role updates will be given to the ECDC with respect to ADONIS and feedback can be absorbed. The project also plans videomeetings with EFSA and ECDC, for which WP3 of the EJP will be contacted.

5.1.10.24 JRP24-FBZSH9-BeONE

5.1.10.24.1 Summary of the work carried out in the JRP

The main research and development takes place in WorkPackages (WPs) 1-4. WPs 3 and 4 are largely progressing as planned. WP 1 has experienced some delays, mainly due to the Covid-19 situation. Especially critical is the delay in dataset collection, since this blocks dependent WP 1 tasks as well as WP2-T2 and subtasks, as well as the testing of the outputs of WPs 3 and 4 (WP5-T2, M-BeONE.5.3).

The kick-off meeting (KOM) and subsequent teleconferences (TCs) successfully aligned the partners with regard to a common goal, and provided insights into the different realities of institutes from the animal health, food safety and public health sectors.

The goal to develop a decentralized system for collaborative outbreak surveillance and investigation has crystallized into an architecture for the platform and a model for data exchange (M-BeONE.4.2). This has been implemented in a prototype for testing and continued development. The prototype features automated basic strain characterization (WP4-T2), and user driven searching and subsetting of sequences, and display of certain data categories like geospatial data and phylogeny (WP4-T1, M-BeONE.4.3). The prototype submits compute tasks to the Norwegian High Performance Computing (HPC) infrastructure metacenter.no.

We have defined a meta-data schema (WP3-T2+3) for samples leading to a data model implemented in the database and dashboard prototype.

A literature review of factors impacting outbreak detection has been undertaken and the results are being implemented in a model for farm-to-fork tracing of bacterial pathogens. (WP2-T1)

5.1.10.24.2 Progress of the project: description of activities

WP1: Typing comparability and nomenclature (M25-M48)

The goal of WP1 is to provide a solid foundation for the remaining WPs to build on. WP1 has faced some difficulties both with hiring additional staff at INSA (due to changed hiring rules in Portugal as discussed with the OHEJP-PMT), and especially during the ongoing Covid-19 crisis. Following a previously agreed contingency plan, if the recruitment is not possible until month 9, some sub-tasks (specially Tasks 2) may require a higher involvement of other partners. Also, the WP leader (Vitor Borges) has been almost entirely occupied by the public health effort during the crisis. Impacts will be stated under the specific tasks. During the KOM and subsequent Coordination team monthly meetings, the WP1 workflow was amply discussed, leading to an agreement on the approaches to be applied for each task (see details below). The discussion benefited from data collected before and during the KOM through both surveys and parallel sessions.

Task: BeONE-WP1-T1 Establishment of state of the art (M25-M30)



Status: ongoing

At the KOM, it was discussed the need of this task, considering the huge amount of available literature on this subject and/or the outputs of other ongoing EJP projects (namely ORION). This prioritization was done both to avoid duplication of work and to decrease the workload at INSA due to expected hiring difficulties. It was agreed that BeONE would not focus on delivering an exhaustive state of the art of methods and existing public platforms for WGS-based typing. Instead, BeONE will build-on the ORION project report by adding not-covered topics related to the BeONE project, namely a discussion around the advantages and disadvantages of centralized versus decentralized approaches. It was also decided to assemble documentation on the confluence site, about the state of the art of different currently available surveillance platforms to regard to 1) software, 2) Data Management and 3) Analysis Pipelines. The deliverable D-BeONE.1.1 was delayed by the COVID-19 response.

Task: BeONE-WP1-T2 Dataset selection and curation (M25-M32)

Status: Ongoing

Sub-Task: BeONE-WP1-T2-ST1 Dataset selection and collection

After the KOM, a survey was conducted within the BeONE project partners to define their WGS and metadata contribution. However, presumably due to the Covid-19 crisis not many participants responded. Following a discussion on the needs of each WP among Coordination team members, guidelines for WGS data and metadata collection were built. For metadata collection and harmonization, the strategy was developed within “WP3 Task 2 - Metadata acquisition and standardisation” activities (see below). This task and the accompanying milestones are currently pending by decisions on what metadata is needed on the samples. Since the dataset is to be used for testing, and the needed data is quite sensitive, a fully anonymised dataset is required. Because of the anonymization it is not possible to request additional data once the data have been collected, without recreating the dataset anew. Apart from these constraints, it is expected that at M33 / M36, a curated template along with guidelines for data anonymization is already sent to each partner and all data is filled out and uploaded to the HPC platform (metacenter.no, see below). As agreed, this strategy allows for the efficient collection of data required for WP1, WP2 and WP3 to be performed in a single instance. In summary, this task should have finished in M30, but is still ongoing due to delays caused by the COVID-19 pandemic.

Sub-Task: BeONE-WP1-T2-ST2 Quality check and assurance, and genome assembly

During the KOM, this sub-task was redefined and inserted within WP1-T3 activities (see next section).

Task: BeONE-WP1-T3 Clustering congruence and thresholds (M25-M48)

Status: Ongoing

Sub-Task: BeONE-WP1-T3-ST1 Selection of WGS-based typing methods to be evaluated

Status: Completed

Due to the high heterogeneity of methods and parameters used for WGS-based typing, at the kick off meeting, it was decided that the full independent pipelines used by each BeONE partner would be run on the collected dataset. Thus, once the dataset is fully collected and stored, partners that volunteered to test their own pipeline will install it and run it up to the clustering steps (which will be handled by INSA in WP1-T3-ST2). As such, this will bypass the evaluation of the impact of variations in any given parameter within each step of a pipeline while providing a more grounded and realistic approach to the comparison of different WGS-typing methodologies currently being used for foodborne pathogen surveillance. This task was completed before M30 as planned.

Sub-Task: BeONE-WP1-T3-ST2 Assessing clustering congruence between different methods at different hierarchical levels



This task is not planned to start until M33 as it is dependent on the completion of the dataset collection that is currently ongoing. Still, it was decided that the different hierarchical levels of clustering would be defined for each method/pipeline used by the partners in task WP1-T3-ST1 (by determining cluster stability threshold ranges and cluster thresholds in association with known outbreak data), which then would be used to correlate cluster congruence.

Sub-Task: BeONE-WP1-T3-ST3 Correlating clustering congruence with existing nomenclature schemes
Not planned to start until M39.

WP2: Joining molecular and epidemiological methods (M25-M48)

WP2 will make the link between the genomics and epidemiology by building knowledge and algorithms on outbreak detection. The first task of the package will summarize the existing knowledge on epidemiology of the targeted pathogens, while the second will implement elements of that knowledge in an algorithm for detection of outbreak episodes. The already incurred or expected to incur delays, and the reasons for it are detailed for each task.

Task BeONE-WP2-T1: Conceptualization of epidemiological and biological factors impacting on fine resolution clustering and outbreak detection (M25-M34)

Status: ongoing

Start delayed due to circumstances surrounding the COVID-19 outbreak. Therefore, the expected delivery date for D-BeONE.2.1 is two months later than planned (M36). The delivery date for the milestone M-BeONE.2.1 will accordingly be postponed two months later than initially planned (M34).

We have initiated a literature review on the factors potentially impacting on the detection of an outbreak with any of the four targeted microorganisms. Differences in ecology and biology of the various pathogens will dictate the population dynamics and environmental persistence of bacterial clones, but also on the range of food products likely to be contaminated by any of the selected pathogens, and subsequently on the transmission pathways to humans. These aspects will be documented and compared between the targeted pathogens. From this study we expect to be able to build a conceptual model to serve as a soft guideline for the selection of the variables to be used in the outbreak/cluster detection algorithm that will be developed in BeONE-WP2-T2. We have started the build-up of the conceptual model in a generalized form of the idea of “farm-to-fork” tracing of bacterial pathogens. We aim to quantify some of the most relevant variables composing each compartment, and assess their influence on dependent compartments/variables and, ultimately, on the clustering of the targeted microorganisms and its interpretation for the purpose of outbreak detection. For example, the Gram-positive property of *Listeria monocytogenes* makes it more resilient to environmental factors, and therefore, likely to survive longer in the food production facilities; this in turn, can be translated to human infections that do not cluster in time, although they might cluster genetically; the use of the time variable in an outbreak detection algorithm for *Listeria monocytogenes* might, therefore, have a lower predictive value than for Gram-negative bacteria, such as *Campylobacter*.

In a first draft, simplified version, the conceptual model has the form depicted in Figure 1

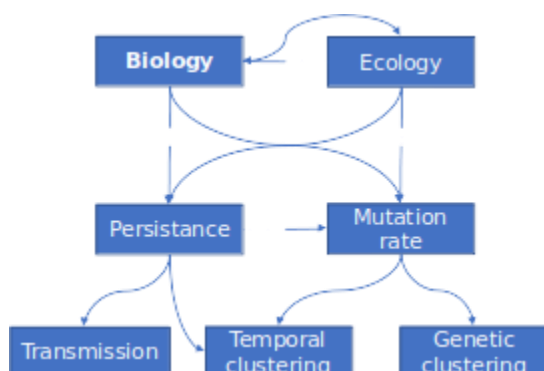


Figure 1 Conceptual model for factors potentially impacting on outbreak detection

Task BeONE-WP2-T2: Integrating genomics with epidemiology (M33-M46)

Not planned to start until M33. Although the development of pathogen-specific algorithms is expected to be delayed, as it would be partly based on the outcome of tasks BeONE-WP1-T2 and BeONE-WP2-T1, we expect to be able to make a head start with a proof of principle, generalizable algorithm, on a smaller dataset of *Salmonella* Enteritidis; this will be updated at a later stage.

Sub-Task: BeONE-WP2-T2-ST1 Comparison of the epidemiologic clusters with the phylogenetic tree of the *Campylobacter jejuni* isolates

Dataset has been identified and an initial meeting for the BeONE-WP2-T2-ST1 work group has been held.

Sub-Task: BeONE-WP2-T2-ST2 Comparison of the epidemiologic clusters with the phylogenetic tree of the Shiga toxin producing *Escherichia coli* (STEC) isolates

Dataset has been identified and an initial meeting for the BeONE-WP2-T2-ST2 work group has been held.

Sub-Task: BeONE-WP2-T2-ST3 Comparison of the epidemiologic clusters with the phylogenetic tree of the *Salmonella enterica* isolates

Dataset has been identified and an initial meeting for the BeONE-WP2-T2-ST3 work group has been held.

Sub-Task: BeONE-WP2-T2-ST4 Comparison of the epidemiologic clusters with the phylogenetic tree of the *Listeria monocytogenes* isolates

Dataset has been identified and an initial meeting for the BeONE-WP2-T2-ST4 work group has been held.

WP3: Storage, management, and sharing for meta- and molecular data (M1-M30)

In WP3 we have made substantial progress regarding the conceptualization and implementation of the (meta)data management within the BeONE framework. For all tasks in this work package we aimed to harmonize our developments with existing approaches and projects via communication with scientists within BeONE but also from other projects. We were able to decide on a database system to build on and design compatible data structures which capture the complexity of intersectional surveillance data while keeping a strict focus on usability. The concrete proceedings are described within the belonging tasks and subtasks.

Task: BeOne-WP3-T1: Evaluate national level data sharing (M25-M34)

Status: Ongoing



As discussed at the KOM and described in Task BeONE-WP1-T1 Establishment of state of the art, we decided to base the output of this task on existing literature and bilateral communication. The key hurdles described by project partners at the KOM focused on both technical restrictions and data privacy concerns.

A preliminary status of the art will be taken by the questionnaire conducted in COHESIVE: ["Questionnaire on available databases and Information Systems for WGS DATA MANAGEMENT"](#).

The COHESIVE deliverable D-4.1.2 and D-4.1.3 provides the status of the feasibility studies for sharing, integrating and harmonizing WGS data and related epi/metadata between human and veterinary organizations at member state level. The involved member states are Italy, The Netherlands and Norway.

Task: BeOne-WP3-T2: Metadata acquisition and standardisation (M25-M48)

Status: Ongoing

As part of BeONE-WP1-ST1 (Dataset selection and collection), a survey was conducted by WP1 among BeONE partners to estimate their data contribution and to inquire minimal-to-optimal sample attributes that each participating institute is allowed to share publicly (s. Sub-Task: BeONE-WP1-T2-ST1 Dataset selection and collection). These covered historical, geographical and source attributes acquired during sample collection as well as provisional analysis results. Strikingly, varying institutional privacy restrictions impose a heterogeneous depth of metadata information.

Sub-Task: BeONE-WP3-T2-ST1 Metadata acquisition

Status: Ongoing

To relieve the constraints of a least common denominator approach at the level of information, we opted for gathering metadata information encoded by hierarchical controlled vocabularies (CVs) to an individual granularity each submitter is able to publish.

The European Food Safety Authority (EFSA) provides controlled vocabulary in their Data Collection Framework (DCF) catalogues (doi.org/10.5281/zenodo.3243215) for many aspects relevant to surveillance and with excellent coverage of European market products and culture. The geographic catalogues for European (NUTS) and world regions (GAUL) as well as the consumer-exposed matrix catalogues (FOODEX2 - subsections food, feed, non-food matrices) were integrated into the BeONE sample submission table for drop-down-menu query in Microsoft Excel. Multiple attributes per sample may be stated by concatenating catalogue codes. This simple yet powerful approach is computer-readable, complies with strict institutional IT security policies that prohibit executable code in shared documents, e.g. VBA macros, and is approved by WP1-4 collaborators.

Sub-Task: BeONE-WP3-T2-ST2 Standardisation using ontologies

Status: Ongoing

As described in Sub-Task: BeONE-WP3-T2-ST1 Metadata acquisition, we have selected controlled vocabularies to enable the implementation of ontology systems when after seeing the metadata that will be provided by data submitters in BeONE-WP1-T2-ST1 Dataset selection and collection. We are in close contact to an ontology expert group (s. Task: BeONE-WP6-T2 Communication) to enable compatibility with existing ontology systems. Beyond the planned use of ontologies for acquired metadata, we currently assess the possibility to assign ontologies to technical metadata such as results from tools, used references, tool versions etc. to enable easier development of APIs for different tools and analysis pipelines.

Task: BeOne-WP3-T3: Database design and implementation (M25-M54)

Status: Ongoing



Database design was looked at to incorporate a diverse set of data. Structure will be put in but an expectation that regions can put additional fields for their own needs is taken into consideration. Another key part of the design is that it needs to fit our planned data sharing model where each member controls their own data and what others can see. This follows the decentralized approach so that data is not stored in a master database. The database needs to be designed to handle WGS and epi data and should function as the source of information for the dashboard.

The data structure for the database has been determined to be based on Bifrost (<https://github.com/ssi-dk/bifrost>), a platform developed and in use internally at SSI. The bifrost platform uses a flexible mongoDB which will enable a decentralized sharing model and storage of varied data and data types.

Sub-Task: BeONE-WP3-T3-ST1 Determine and implement data structure for database

Status: Ongoing (due by M33)

Database schemas have been established for WGS data though some details need to be finalized. Database design regarding epidata has also begun but still needs to be established.

Sub-Task: BeONE-WP3-T3-ST2 Implement API for import of data

Status: Complete (by M33)

As we have chosen to implement the Bifrost system from SSI we have access to their processed data and can port data from their existing pipelines.

Sub-Task: BeONE-WP3-T3-ST3 Data porting from an existing pipeline

Status: ongoing - not due to start until M35.

The SSI-bifrost pipeline was set up at BfR to analyze its native mongoDB data structure. The results of the NGS quality control pipeline AQUAMIS, developed and used by BfR, were restructured to match the mongoDB collections "runs", "samples" and "sample-components". This requires a mapping of AQUAMIS result values to the appropriate bifrost stores or to extend the documents with AQUAMIS-specific attributes and is ongoing. Ontologies will guide this result mapping procedure in a later phase.

Sub-Task: BeONE-WP3-T3-ST4 Data porting from further pipelines

Status: Not starting until M41.

The data structures and schemas are being designed with compatibility to a number of different pipelines in mind. The data porting frameworks are not yet in development.

Sub-Task: BeONE-WP3-T3-ST5 Expansion of the API for referencing/exporting of entries in other reference databases

Status: Not starting until M41.

Sub-Task: BeONE-WP3-T3-ST6 Expansion of the API for queries from the dashboard WP4

Status: Not starting until M47.

WP 4: Development of a user-oriented interface for analysis and sharing of epi and molecular data (M25-M54)

A public Github repository has been set up to collect and manage work on the Dashboard and it is available here: <https://github.com/ssi-dk/BeONE>.

Task: BeONE-WP4-T1 Dashboard (M25-M42)

Status: Ongoing



A prototype for the dashboard has been created by using mainly Plotly's (<https://plotly.com/>) Dash platform for Python-based web applications for data analysis and visualization. The prototype has been built based on a Dash web application currently being developed at SSI with the name of Bifrost Dashboard (the repository can be found here <https://github.com/ssi-dk/bifrost>) as well as on publicly available Dash web applications that can be found here <https://dash-gallery.plotly.host/Portal/>.

In addition to the Dash main framework, additional tools used to build the web application come as JavaScript based web components created through the React JavaScript library (<https://reactjs.org/>). Dash basic core components are JavaScript and the additional React components expand the available and core set with new, richer and customized web page objects.

Sub-Task: BeONE-WP4-T1-ST1 Core display components

The Dashboard contains the following main display areas and visualization components:

A sidebar. The sidebar enables the user to browse through different available databases, to define a range of dates to collect documents from, to choose specific organisms as targets for collecting documents and perform other similar choices.

A tabs bar. A set of tabs open different visualization areas with different tools and plots available to the user. Specifically, four different bars have been defined at this stage: 1) an Isolates view, 2) a survey view, 3) an Analysis view and 4) a Reports view.

Page buttons. These buttons direct the user to different pages within a specific tab, which contain different features and tools. In particular, two types of pages have been defined at this stage: 1) a table-view that allows data browsing in table format, and 2) a graph view to explore data as histogram, scatter or other type of plot. The latter contains, for example, geolocalization and phylogenetic tree plotting support.

Sub-Task: BeONE-WP4-T1-ST2 Component integration

Components in the Dash-based Dashboard are integrated dynamically via the use of call backs. The input/output implementation of call backs enable the flow of data and information to/from individual components. In addition, Dash web applications allow the storage of data in the web page, which is then available for further processing.

Components created with the React JavaScript library are managed at the library/package level with either npm or yarn package manager tools for JavaScript programming and its Node.js runtime environment. Dash provides a boilerplate framework (a set of tools collected in a github repository <https://github.com/plotly/dash-component-boilerplate>) for the creation of custom components from templates.

A React component for the phylogenetic tree visualization tool PhyloCanvas (<http://phylocanvas.org/>) has been integrated in the BeONE dashboard.

Task: BeONE-WP4-T2 Back end analysis implementation (M25-M36)

Status: Ongoing

Back end analysis draws significantly from the Bifrost platform being developed at SSI (see above). This framework employs Snakemake workflow management pipelines, the non-relational database MongoDB (<https://www.mongodb.com/>) and a Python-based set of tools to handle data flow from bioinformatic tools to database storage.

Task: BeONE-WP4-T4 Data sharing front end (M29-M54)

Status: Ongoing

Significant investigation has been carried out with regard to defining the best implementation of user-user data sharing of sensitive and non-sensitive data. This has been described extensively elsewhere.



Sub-Task: BeONE-WP4-T4-ST1 Web-based input system

Status: Ongoing

The dashboard has support to select specific databases and type of data to have access to and share among users. The dashboard will have an entry point and database drop function for these data types:

Sequence data in .fasta format

Read data in .fastq format

Allelic profiles in tabular format

Tree graphs in Newick format

The implementation is ongoing.

Sub-Task: BeONE-WP4-T4-ST2 Import/Export front end for reference databases

We have at this stage defined the import/export of core genome MLST reference schemas to be used for cgMLST typing.

Sub-Task: BeONE-WP4-T4-ST3 BeONE data exchange system

Status: Ongoing

We are currently investigating a database-centric implementation of data exchange based on the idea of a two public-private databases system. The front end dashboard will contain functionalities that enable a user to select sets of data and make them available to collaborators, for example by copying them to a public database.

WP5: Dissemination, Testing, Evaluation and Sustainability (M25-M54)

Task: BeONE-WP5-T1 Dissemination (M25-M54)

Status: Ongoing

The base code is kept and shared in the form of Github repositories, publicly available. These repositories have support for thorough documentation, which is used to disseminate the scope, functionalities and technical aspects, such as installation, of the BeONE platform.

Task: BeONE-WP5-T2 Continuous Testing and Feedback (M25-M54)

The BeONE platform, including the dashboard web application and computational layer, is installed and made accessible to all project partners on a secure server hosted at the norwegian Metacenter infrastructure (<https://www.metacenter.no/>). The fully functioning prototype released as beta (under development) version to users and testers within BeONE will enable the testing of the different functionalities.

We have put in place a reporting system on our project documentation system for gathering feedback from users, which will then be translated in a prioritized list of features and improvements to be implemented within each release cycle. The testing will be realized through collaborative efforts of participants from all work packages and all the user categories. The testing of the dashboard will comprise functional, non-functional, integration, and regression testing.

The strategy and planning for the initial and continuous testing of the dashboard will be compiled in an Initial Test Plan. Due to circumstances surrounding the COVID-19 outbreak, the start of this task has been postponed. While progress is ongoing, we expect to have a three months delay on milestone M5.3. (M31).

Task: BeONE-WP5-T4 Sustainability (M25-M54)

Status: ongoing.



As described in the project proposal, sustainability is at the forefront of this project's priorities. The issues of sustainability are identified and documented in close collaboration with each WP leader. The most pertinent sustainability material will be compiled in a yearly sustainability document, alongside with recommendations for future work.

WP6: Management (M25-M54)

Task: BeONE-WP6-T1 Management (M25-M54)

Status: Ongoing

The coordination team has been set up. Project management tool Jira has been set up for task management. Further details are covered below in *BeONE-WP6-T2*.

Task: BeONE-WP6-T2 Communication (M25-M54)

Status: ongoing

This task comprises Internal and external communication

Internal communication:

Monthly meetings have been held by the coordination team except in April.

An active slack group has been made for rapid informal communication.

A digital oceans server has been set up to host various web content.

A confluence site has been set up on the digital oceans server as a more structured repository for documentation and decisions.

Frequent web meetings have been held in the WP3+4 group.

The project has applied for and been granted HPC resources at the norwegian national infrastructure for computational science (Metacenter) to set up the computational back end for a test implementation of the BeONE system.

External communication:

Communication has been established with EFSA contact Mirko Rossi, especially with the following focus:

aligning and avoiding duplication of work on the EFSA cgMLST pipelines

aligning metadata terms and ontology use

Contact has been established with IRIDA contact Aaron Petkau, this was an informal talk focusing on:

Broad decision process on development of Irida and how they compare to Bifrost

Possibility of sharing data between the systems

Differences between the systems that came from both technology used and requirements of stakeholders

Communication with COHESIVE on data structure

Initiation of an ontology expert group, including Emma Griffiths (BCCDC), Damian Dooley (BCCDC), Fernanda Dorea (SVA, ORION), Mirko Rossi (EFSA)

Communication with Mario Ramirez (INNUENDO) on their software on microbial surveillance.

Sub-Task: BeONE-WP6-T2-ST1 Kickoff meeting

Status: Completed

Kick-off meeting was held at RIVM.



Task: BeONE-WP6-T3 Data Management (M25-M54)

Status: Ongoing

Data management plan has been created using the new DMP tool.



5.1.10.24.3 Progress of the research project: deliverables and milestones

5.1.10.24.3.1 Deliverables

JRP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
24	D-BeONE.1.1	Report on the state-of-art	30		36	Public. Scope changed to avoid duplication of work with ORION, and mitigate delays due to COVID-19. Delivery date changed to wait for ORION delivery fall 2020.	10
24	D-BeONE.6.1	Initial data management plan	30	33		Public	8
24	D-BeONE.1.2	Finalized BeONE dataset	32	33		Public	3
24	D-BeONE.2.1	Draft manuscript on the conceptual model	34		36	Public	8
24	D-BeONE.4.1	Back-end analysis pipeline	36			Public	4
24	D-BeONE.4.2	Web input system	36			Public	4



* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity

5.1.10.24.3.2 *Milestones*

JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
24	M-BeONE.4.1	Initial requirement list from workshop at kick-off meeting	26	Yes		
24	M-BeONE.3.1	Agreement on minimal and optimal set of metadata	28	Yes		
24	M-BeONE.4.2	Implementation plan	28	Yes		
24	M-BeONE.5.3	Initial test plan	28	Yes	31	Delayed but achieved by M31
24	M-BeONE.1.1	State-of-the-art completed	30	Yes		
24	M-BeONE.1.2	Collected dataset	30	Yes		



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JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
24	M-BeONE.1.4	Agreement on the methods/solutions to evaluate	30	Yes		
24	M-BeONE.1.3	Curated WGS dataset completed	32	Yes		
24	M-BeONE.2.1	The critical factors for outbreak detection have been identified	32	No	34	
24	M-BeONE.4.3	Prototype dashboard	32	Yes		
24	M-BeONE.3.2	Finalized evaluation of data sharing experiences	34			
24	M-BeONE.3.3	Implementation of data structure	34			
24	M-BeONE.4.4	Plan for data sharing system	34			
24	M-BeONE.3.4	Finished implementation of controlled data entry	36			



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JRP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
24	M-BeONE.3.5	Plan for ontology implementation	36			
24	M-BeONE.3.6	Implementation of API for import of data	36			
24	M-BeONE.4.5	Basic system for display component integration	36			
24	M-BeONE.5.2	Yearly sustainability document	36			



5.1.10.24.4 Ongoing collaborations

Collaboration on-going with **OHEJP projects** ORION and COHESIVE, and to a smaller extent with CARE, MATRIX and OH-HARMONY-CAP.

Collaboration on-going with **Danish FVST**, on cross sector sequence analysis platform for investigation of foodborne outbreaks.

Collaboration with **EFSA** as described in *BeONE-WP6-T2*.

Collaboration with **ontology expert group**, including Emma Griffiths (BCCDC), Damian Dooley (BCCDC), Fernanda Dorea (SVA, ORION), Mirko Rosso (EFSA)

5.1.11 Additional WP3 activities

On 28 May a Project Leaders Forum TC was organised for both first and second call project collaborators. The aim was to briefly remind the objectives of the One Health EJP and to give guidance on the reporting procedures. Also additional information on integrative activities (JIP and others), the Data Management Plan, the dissemination procedure and scientific publication policy were given.

The progress of the second round projects is, as for the first round projects, through the 9M-report (see paragraph 'Detailed follow up of JRP').

With a total of 28 research and integrative projects ongoing from January 2020, it became clear that many of them touched similar or related activities, be it techniques (New Generation or Whole Genome Sequencing, machine learning or artificial intelligence, ontologies; bioinformatics scripts and pipelines etc.), protocols (harmonization of protocols, databases & data sharing, setting up collections of strains, bio-banks, etc.) or other (activities that have particular interest for ECDC or EFSA, GDPR, the implementation process (e.g. reach out to local, national or international stakeholders), environmental issues; etc.). For this reason, WP3 Team developed an online survey that aimed at identifying these common activities among all projects and, in addition, the available expertise among the One Health EJP partners. By sharing this information among peers, Project Leaders were encouraged to unite forces and to do certain general or transversal tasks together, or to build on knowledge that is already available. Such an approach creates synergy that should lead to a more efficient workflow and a more significant outcome. A report on the online survey regarding the identification of common activities in the One Health EJP projects (JRP & JIP), and expertise to be shared among projects is in preparation.

5.1.12 Task 3.4: Organisation of annual scientific meetings (ASM) where results from JRP are presented.

The ASM2020 was announced at the end of ASM2019 in Dublin. Although both Czech partners (Statni Zdravotni Ustav, SZU) and Vyzkumny Ustav Veterinarniho Lekarstvi, VRI) were mobilized at the end of May 2019 and a local professional organizer was identified, complicated local administrative procedures had a considerable impact on the organisation of the conference. In addition, the various sanitary measures following the COVID-19 crisis in Europe forced the organizers to turn the conference into an online event. WP3 and the Communication Team at the University of Surrey, together with a private company Purple Patch, designed and performed the happening. The entire programme, including opening and closing remarks, keynote lectures, oral and poster presentations was successfully transformed in a highly attended scientific event.

The abstract book of the ASM2020 is available as Deliverable D3.12.



5.2 Deliverables and Milestones

5.2.1 Deliverables

Del. Ref.	Deliverable title	Submission
D3.11	2nd periodic report on JRP	M27
D3.12	Abstract book for 2nd Annual Scientific Meeting (ASM)	M29
D3.13	Report n°1 on evaluation of finalised JRP	M36
D3.14	Report on the recently started projects, 2nd call	M32

5.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS34	Kick-off meeting (2nd call projects)	M26	Organized in Berlin, 13 November 2019
MS35	Final reports sent to external evaluators (#n°1)	M33	New deadline: Sep 2020. Final Report MAD-VIR sent to external evaluators in July 2020
MS36	Feedback questionnaire sent out to Project Leaders n°2	M27	Questionnaire sent out on 25 February 2020
MS37	Second Annual Scientific Meeting	M29	Organised as webinar on 27 to 29 May 2020
MS38	Preparation of final evaluation report (to contact external evaluators for assessing Final Reports of projects ending M36)	M33	Will be done towards November M35 or December M36 2020
MS39	Interim and final reports preparation (to contact PL1 and PL2 to submit Final Report or 12M report) n°2	M36	Planned before December 2020



6 WP4 - Joint integrative projects

6.1 Work carried out to date

6.1.1 Task 4.1: Development of procedures and guidelines for submission and selection of JIP proposals, and for reporting and evaluation

Together with WP3, guidelines for evaluation of the final reports is being prepared (deliverable D4.19 Guidelines for evaluation of final Joint Integrative Project reports). The guidelines build on the corresponding deliverable D3.9 for JRP reports, which was prepared by WP3 with input from WP4 in Y2. Since both JIP projects from the 1st call have been extended with 6 months, the final reports will not be submitted before Y4. The need for a revision and update of D4.12 Guidelines for reporting on JIPs (M26), will be investigated later during Y3. External evaluators will not be recruited until the beginning of Y4.

6.1.2 Task 4.2: Supervision of JIP

There is a continuous follow-up of all JIPs. Every third month there is a common digital meeting between the WP4 leaders and the JIP leaders. At these, the PLs are updated on upcoming procedures like reporting and there is also a sharing of experiences between the PLs, where especially the second call projects can learn from the first call projects. Between the meetings there are informal contacts continuously between the PLs and the WP4 support team. The start-up meetings for the new JIPs were all visited by the WP4 leader, who also informed about OHEJP common procedures. Because of the COVID-19 situation all PLs have been asked to make plans for how possible effects on the projects will be handled. ORION and COHESIVE have asked for an extension and both have been granted an extra six months. During the ASM 2020 all PLs also participated in a common PL Forum with updates from the OHEJP project managing team and the PLs got an opportunity to reflect and discuss on different aspects.

In M27, WP4 submitted the 2nd periodic report about the ongoing JIPs, ORION and COHESIVE (D4.17).

A series of actions are taken as a follow-up:

- The projects are requested to make sure that the specifications of the final deliverables (title, number) match the specifications stated in the project description. If there are any modifications, they should be carefully described to facilitate the traceability to the original title and number of the deliverable.
- The projects are reminded to upload the deliverables to the OHEJP website as soon as they are finalized. The same regards the uploading on Zenodo. WP4 will provide some guidance for the JIPs on how to do this.
- The projects are instructed to follow the Scientific Publication Policy and the Scientific Dissemination Procedure, published in the end of year 2. These documents aim to formalise and harmonise the publication and dissemination processes.

6.1.3 Detailed follow-up of JIPs



6.1.3.1 JIP01-R1-IA1.1-ORION

6.1.3.1.1 Summary of the work carried out in the JIP

The ORION project progressed successfully from the “Improvements and new resources” (2nd) phase into the “Evaluation” phase as described in the PERT chart of the project proposal. This third phase includes the implementation of WP-specific / country-specific One Health (OH) pilots as well as the continuous improvement of resources developed during the second phase - based on the end-user feedback collected in the pilots.

In WP1 the evaluation and application of the ORION “OH Surveillance Codex” (OHS Codex) was performed in the national and the supranational pilot with EFSA & ECDC. As a result, a first major revision of the OHS Codex could be released and this latest version was presented during ASM2020. WP1 further developed new resources that help to maintain and exploit the OHEJP Glossary, e.g. a new “text processing” web service that allows to automatically find matches between a user-defined document and the OHEJP Glossary. Also, the OH-CRAC was extensively validated, improved and applied in several pilot applications. In addition, a new web-based tool could be created that will promote its easy adoption by end-users (<https://aflex.vrac.iastate.edu/checklist/?t=OH-CRAC>). In WP2-Epi the main resources were invested into the preparation and implementation of national pilots. Some sub-tasks were affected by delays due to staff fluctuations and delays in recruitment processes due to COVID-19. WP2-NGS continued to enrich the content of the OH NGS Handbook and successfully implemented during the national pilot the technical analysis platform IRIDA (<https://www.irida.ca/>) at the Norwegian Research and Education Cloud (<https://www.nrec.no/>). During this process it was discovered that some of the promised IRIDA pipelines were non-functioning, so additional resources had to be allocated towards creating new functional pipelines in collaboration with the IRIDA project. WP2-Integration organized an ORION pilot integration workshop in January 2020 to synchronize the work done in the country specific pilots under the overarching OHS Codex framework. A generic template for evaluating impact and outcomes of each pilot study was established as well as a strategy for how lessons learned from the individual pilots will be captured and communicated as part of the OHS Codex framework. Work in WP3 focused on establishing the foundations for practical application of new interoperability tools during the national pilot. This process already resulted in an improved collaboration among the national OH sectors and a better understanding of each other’s surveillance activities and results. Specifically, new instructions and guidelines were produced that will be adopted in future national zoonosis reports as well as paving the way to the application of data interoperability tools developed by WP3.

The project continued with holding trimonthly web meetings for the whole ORION consortium (including stakeholders and interested EJP members) and monthly calls for the WP leaders & deputy leaders. The project contributed to the EJP DMP steering board, initiated new collaborations and information exchange with other EJP projects (e.g. MATRIX) or initiatives (e.g. RAKIP, BeONE). Members of the ORION project presented research results at the ASM2020, and several scientific publications are currently under preparation. ORION continued to use innovative web technologies for webinars.

6.1.3.1.2 Progress of the project: description of activities

WP1: “OH Surveillance Codex”

JIP1-WP1-T3: One Health pilot (M7-M39)

JIP1-WP1-T3-ST2: Planning and performing work necessary to prepare the execution of the OH pilot (M13-M18)



- The WP1 OH Pilot is specifically testing and improving two new resources that are available within the OHS Codex: the OHEJP Glossary (formerly ORION Glossary) and the One Health Consensus Report Annotation Checklist (OH-CRAC). These two resources have been further extended in the third year of the ORION project.
- The ORION WP1 and WP3 planned and started in M25 an additional pilot called “EJP ORION WP1 & WP3 supra-national pilot with EFSA & ECDC”. This pilot evaluates the applicability of the four solutions developed by WP1 (OHS Codex, OHEJP Glossary, OH-CRAC) and WP3 (Health Surveillance Ontology) to improve the process of generating information valuable in a One-Health context with EFSA and ECDC. In this context, apart from the updates and developments on the aforementioned resources, a new web service was developed to facilitate the semi-automatic mapping of concepts from the EFSA’s catalogues into the new Health Surveillance Ontology (HSO) developed in WP3. This web service helps to create an easy to maintain link between the constantly evolving EFSA catalogues and HSO that can be exploited by future semantic search technologies and services that build on linked open data (LOD).

JIP1-WP1-T3-ST3: Execution of the OH pilot - applying the “OH Surveillance Codex” guide (M19-M39)

- For the WP1 OH Pilot, the EJP project “Antibiotic Resistance Dynamics” ([ARDIG](#)) and “German One Health Initiative” ([GOHI](#)) were chosen as use cases to validate the WP1 pilot study (for details see annual report 2018, or deliverable D-JIP1-1.1) in AMR and AMU domains. During this third year, meetings with representatives of these projects in BfR have been organized to present the different resources and evaluate its practical applicability. In these meetings, we have mainly focused on improving the coverage and quality of AMR and AMU related terms within OHEJP Glossary and validating the text processing tool and the OH-CRAC checklist with surveillance data reports provided by ARDIG and GOHI.
- The EJP ORION WP1 & WP3 supra-national pilot with EFSA & ECDC incorporated a new research scientist at the beginning of this year. An on-site visit was organised in February with EFSA to define the specific objectives and working plan. Since then, we have hosted monthly calls and work together applying the ORION solutions with specific examples on Salmonella, Campylobacter and AMR surveillance to improve the usability of our tools.

JIP1-WP1-T4: Evaluation and Recommendations (M31-M42)

- The first draft of “OH Surveillance Codex” was finalized by month 24 and the details are described in Deliverable “D-JIP1-1.2 Draft on OH Surveillance Codex”. The OHS Codex and the solutions described in there were envisioned as a “living” framework and therefore, it undergoes regular reviews and updates as the project and the developed resources evolve. As a result of these reviews, the OHS framework has been recently updated and this new version has been implemented as a new open source community resource that is available via: <https://oh-surveillance-codex.readthedocs.io/en/latest/index.html>. In the future versions, the OHS Codex will also include “lessons learned” summaries from pilot studies performed in the context of ORION.
- The OHEJP Glossary (publicly available in <https://onehealthejp.eu/jip-orion/>) is the result of the collaborative effort of three EJP projects namely ORION, NOVA and COHESIVE, with support from OH experts of EJP stakeholders. Based on feedback received during application of the resource in several pilots we have focused on the development and maintenance of the OHEJP glossary, which implies the continuous extension, curation and validation of its content. This process is supported by the implementation of new infrastructure, which provides the necessary technical and operational resources to curate and semi-automate the content management of the glossary in a collaborative environment. A new web-based “text processing” service has been implemented to provide added-value to the OHEJP Glossary (<https://foodrisklabs.bfr.bund.de/ohejp-glossary/>). End users can now automatically search



within any user-provided text document for terms that are contained in the OHEJP Glossary. Currently only users with credentials (username: ORION, password: OneHealth) can access this service for testing purposes. Once all the feature requests from the different pilot applications and certain IT security constraints will be solved the tool will be accessible without login request.

- The OH-CRAC supports the harmonized reporting of surveillance meta-information in future sector-specific or OHS data reports. The OH-CRAC gives recommendations on what meta-information should be provided and how to structure it within future surveillance data reports. During this year, we performed a joint analysis of existing cross-sectoral guidelines and frameworks that were mapped to OH-CRAC schema to support end-users in the checklist completion and we also improved the provided sub-headings, definitions and added examples of linked metadata. This resource has been recently made available as web-based interactive annotating tool (<https://aflex.vrac.iastate.edu/checklist/?t=OH-CRAC>) for functional meta-information extraction in reports/documents uploaded as pdf files.

WP2: Epi

JIP1-WP2-T2: Improving OH Knowledge Base – Epi (M13-M24)

The framework for the OH Knowledge Base has been developed (web applications) and an application server was set up at FLI.

JIP1-WP2-T2-ST1 - Data collection and integration

- The final data collection has not been started yet as we had to consolidate all internal input, and input from ECDC and EFSA. The data input tables have been consolidated. The data collection was discussed with EJP MATRIX. As they are very interested in analysing the data and proceeding with the data collection, a webinar was carried out in M31. The data collection is delayed because of missing personnel resources (the recruitment process was delayed by COVID 19).

JIP1-WP2-T2-ST2 - Data analysis and validation

- A data analysis model (Rasch model) was developed and tested with data from preliminary data collection. The Rasch model is used to quantify properties that cannot be ascertained directly but only indirectly, for example by means of questionnaires. The Rasch model is a probabilistic model with which latent variables are inferred. We can use the Rasch model the more successful the more complete the questionnaire response will be. The model is developed, data analysis is delayed as the input data collection is not finished yet.

JIP1-WP2-T2-ST3 - Knowledge integration and Decision support

- In the OH context we are dealing with a transdisciplinary process, and knowledge integration aims at the integration of two types of knowledge: the theoretical and abstract knowledge of the sciences and the concrete and empirical knowledge of practice. The integration of science and practice ensures that both political decisions are based on scientific knowledge and insights and that questions of science are directed to decision-relevant aspects of practice. Especially with regard to the fundamental uncertainties that run through the entire context of the OH approach, integration in the sense of mutual learning is relevant. The knowledge integration is realized in the knowledge databases, because besides the pure listing of theoretical knowledge also the context is given in which this knowledge was generated. Further links to literature and application examples are provided for this purpose. The first version of the database can be accessed at https://joerngethmann.shinyapps.io/EJPORion_AHS. The final version will be published on a server at FLI. Currently, we are testing the server; the final version will be published soon. More data will be added (see JIP1-WP2-T2-ST1).



JIP1-WP2-T3: Epi - OH pilot studies (M7-M39)

JIP1-WP2-T3-ST1: One Health Pilot 1: *Toxoplasma gondii* (carried out by FLI and BfR, Germany) (M7-M39)

- In this pilot study, in a first step will analysed the currently available data on *T. gondii* surveillance from reports of the different sectors. In a next step, we carried out a literature review on seroprevalence data and risk factors for the infection with *T. gondii* in the relevant livestock species. The study on the risk factors was published in January 2019 (Stelzer et al. 2019).
- Additionally, an analysis of the data on *T. gondii*-seroprevalence in participants of the study on the "Status of Health in Pomerania" (SHiP) and comparison with the national cohort (Wilking et al, 2014) will be conducted, as well as a literature analysis on seroprevalence and source attribution of *T. gondii* in humans.
- The One Health Pilot 1 is delayed because of missing personnel resources (the recruitment process was delayed by COVID 19).
- At BfR, a study on surveillance systems on *T. gondii* in feed and food will be carried out. With this pilot study, we will be able to test the inventory and show its practical applications. Additionally, we will be able to make a gap analysis and show opportunities and challenges for stakeholders.

JIP1-WP2-T3-ST2: One Health Pilot 2: Salmonella (M7-M39)

- The plan for the pilot study has been developed and agreed. A document detailing experiences regarding whole genome sequence data sharing processes and lessons learnt from experiences during 2019 is in development, which will be used to develop recommendations for the final project outputs. A data sharing protocol and draft Memorandum of Understanding is in development.

JIP1-WP2-T3-ST3 One Health Pilot 3: Hepatitis E (carried out by WBVR and RIVM, the Netherlands) (M7-M39)

- Data about hepatitis E surveillance done in different institutes belonging to different sectors are gathered and put in a country map. The country map consists of surveillance components or monitoring projects per sector and institute and data sharing among these sectors for a disease or pathogen. To make the country map broadly useable for others, an instruction of how to complete the map will be made. Following the results on our country map, the goal is to set up and enhance inter-sectoral (all identified institutes in the country map) collaboration for hepatitis E. This has come to a standstill, due to the corona-crisis.

JIP1-WP2-T3-ST4 One Health Pilot 3: AMR (carried out by Sciensano, Belgium) (M7-M39)

- The plan for the pilot study was developed. The plan includes the objectives, the expected outcome as well as information on collaboration and reporting of the results. The aim of this pilot project is to enlighten the network of collaboration between the authorities and the scientists in term of surveillance of antibioresistance in Belgium, in analysing the participation of scientists in national meetings where topics related to OH AMR surveillance or monitoring are discussed (focus: operations and communication).
- Tasks: After each meeting where assigned Sciensano scientists participate in and topics related to OH AMR surveillance are discussed, specific information is collected in an Excel/Word file (eg: Title / reason of meeting; Main points discussed; Outcome ; Number of participants per sector; Affiliation of participants; Comments).
- Expected outcomes: Description in a report of the main terms used by the different disciplines and sectors, in a view to put forward terminology misunderstanding. Describe the usefulness of ORION tools (especially glossary) to facilitate these meetings and activities.



- Pillar/ principle of OHS Codex that will be addressed: Collaboration principle: mutual understanding via Glossary (WP1) and sharing experience of collaborative strategic planning within Idea Catalogue (WP2 int).
- Advance of the project: the scientists involved in the pilot were informed begin of March 2020, and ready to collaborate, but the CoV-SARS-2 outbreak put the whole project on hold, as all AMR-related-meetings were cancelled. Moreover, the Competent Authority (due to the Cov-SARS-2 situation) has delayed the implementation of a National Action Plan on AMR. The beginning of the pilot project is then postponed to September 2020 and will be performed during two months (September and October 2020).

JIP1-WP2-T3-ST5 One Health Pilot 5: Application of Rasch model on data collected in questionnaires (see above **JIP1-WP2-T2-ST1** - Data collection and integration)

- Based on the questionnaires describing the properties of surveillance projects a set of quality scores are planned to be calculated by the use of a Rasch model. By this application of a method developed and validated in **JIP1-WP2-T2-ST2** - Data analysis and validation on data collected in **JIP1-WP2-T2-ST1** - Data collection and integration we optimize the information gain and further examine the practical applicability of the method collection. With the results we provide a base for a ranking of surveillance systems and for the discussion of the properties of recent surveillance projects under the framework of one-health. Project status: Model is coded and validated. Results will be calculated immediately after feedback of questionnaires
- The model is developed, data analysis is delayed as the input data collection is not finished yet.

Task T-2Epi.4: Evaluation and Recommendations Epi (M31-M42)

1 Survey and/or interviews with internal / external experts:

- The preparation for an update survey with a new version of the tables for inventory of surveillance systems were performed
- 2** OH knowledge base-epi:
- The established knowledge base is a living document that is continuously updated by the WP members.

WP2: NGS

JIP1-WP2-T5: Improving OH Knowledge Base – NGS (M13-M24)

- With regards to the NGS handbook, the focus for the reporting period has been on building the framework for the book, and on content creation. For this purpose we have run several writing workshops during the spring of 2020. The technical platform that is being used for the handbook is Github, with display of the handbook via Readthedocs. The contents that will go into the handbook were more closely described in the M21 report. Our pathogen of choice for the pilot has been *L. monocytogenes*, and due to that we have had a particular focus on methods for typing and characterizing that pathogen for the handbook. In connection with this we are also developing an R package for conducting systematic reviews for pathogen related bioinformatics analyses.

JIP1-WP2-T6: NGS OH pilot studies (M7-M39)

- For the OSLO pilot project, the main focus has been on getting the technical analysis platform up and running. We have chosen to use IRIDA as our platform (<https://www.irida.ca/>). We are using the Norwegian Research and Education Cloud (<https://www.nrec.no/>) as our infrastructure provider, and have established a test bed with them. We have so far installed IRIDA there, and we have an instance up and running. We have during this process discovered that some of the pipelines that we were wanting to use inside of IRIDA are non-functioning. We are thus working on creating new ones in collaboration with the IRIDA project.



- For the applied part of the pilot, we have so far focused on the pathogen *L. monocytogenes*. We are currently in the process of gathering information on analysis tools and pipelines with the goal of creating a viable analytical process within the IRIDA platform. We are planning a tabletop exercise for surveillance and outbreak detection in the fall of 2020. For this purpose we are gathering a set of isolates that we can use as a test set within various possible scenarios.

JIP1-WP2-T7: Evaluation and Recommendations (M31-M42)

- Feedback gathered from the work done in the pilot studies is used to improve the OH Knowledgebase - NGS. This optimization process will continue to the end of the project due to the interactions with the pilots.

WP2: Integration

JIP1-WP2-T8: Improving OH Knowledge Base – Integration

- The WP hosted a workshop in January 2020 to integrate the work done in the country specific pilots into the overarching work of ORION, by discussing what tools were developed and trialled in the pilots and how they fitted within the CODEX framework. The workshop was held in Copenhagen and co-hosted by SVA and DTU.
- Each pilot was presented by a flash-presentation after which everyone else had the opportunity to provide input, suggestions and identify synergies with other work. A generic template for evaluating impact and outcomes of each pilot study was also discussed and as was the capture and reporting of lessons learned. Furthermore, the OHS CODEX was discussed from the perspective of how the pilot studies feed into the pillars. A strategy for how lessons learned would be captured and communicated as part of the OHS Codex framework was created.

JIP1-WP2-T9: Integration OH pilot studies (M7-M42)

- The three pilot projects in WP2 integration have been progressing, albeit slowly in 2020. The COVID-19 pandemic has not affected the studies directly, but have caused a drain on both epidemiology and laboratory expertise in public health. Even though we have attempted to make up for this on the food and veterinary side, the pilots do need public health inputs as the collaboration is the nature of the projects.

Pilot: Enhancing the One Health-ness of interpretation of AMR and AMU surveillance data for zoonoses.

- In 2019, this pilot generated One Health focussed way of reporting AMR in *Campylobacter* by integrating data integrating the five relevant data streams of AMU for treatment in humans and animals and AMR in animals and food and people. [DANMAP link](#)
- In 2020, the work focussed on a similar objective for *Salmonella* and the project group met early in the year to plan the flow in the chapter and the sections. The objective was to emphasise the One Health focus, whilst still ensuring that information in other serotypes relevant for national control plans was maintained. The work on the chapters is currently ongoing and data is in the process of integration and mutual data interpretation is taking place. Even though the ambition for the *Salmonella* chapter may have been reduced due to COVID-19, the chapter for DANMAP 2019 will still contain an improved One Health *Salmonella* chapter. DANMAP 2019 will be published late September and after that the templates will be written up as resources for the ORION CODEX. The templates will provide a best practice guideline of how to integrate interpretation and communication of AMR and AMU surveillance data to facilitate and support One Health policies and decisions.



Pilot: Explore the additional value of integration of multiple *Campylobacter* surveillance data streams from animal health and food safety

- This objective is integrating surveillance data and register-data from 3-4 surveillance components across animal health and food safety for *Campylobacter* in Denmark. The aim is to enhance the surveillance value by looking at the whole farm-to-fork-to-human exposure chain by carrying out integrated analyses and cross-sector interpretation. Data from 2013-2018 was received from industry and government, which were combined with surveillance data sets available at DTU. The data was cleaned and harmonized using R-programme files and outliers and missing values were identified and discussed with data owners. The data and metadata were described and the analyst acquired an in-depth understanding of the surveillance components and the data. Towards the end of the 2019, the data was further collated with the national CHR database to allow for geo-referencing and identification of production type of the flocks at the time of sampling.
- Integration between the surveillance data and the national register data at flock-level over time was not beneficial. Due to data quality, too many missing values were created and the attempt was abandoned and instead the data was integrated at farm/premise level. Encouragement for better and more precise registration in the surveillance programme was fed back to data providers. Better data quality will increase the sustainability of the data by expanding its uses. Analyses plans were drawn and the data is in process of being analysed across data-streams and sectors to add value to the traditional sector specific surveillance.

The expected outcome is to improve knowledge of the *Campylobacter* epidemiology and demonstrate added value from surveillance data by integrating data from different sectors. The aim is to improve scientific advice to policy on *Campylobacter* control and risk-based surveillance at national level. If implemented, a reduction in human campylobacteriosis could be expected.

Pilot: A coherent description of a surveillance system from farm-to-patient was developed and is being prepared for publication.

- The work continues to be developed and modified to encompass animal, food and human surveillance data. Processes along the surveillance chain were described including target populations, sampling methods, laboratory methods, data analyses, actors and stakeholders. Descriptive results of the different components will be presented alongside the detailed data and metadata descriptions. This will create a mutual understanding of the whole surveillance programme across sectors to identify potential gaps or One Health opportunities.

WP3 OH Surveillance Harmonisation Infrastructure

JIP1-WP3-T3: One health pilot (M7-M39)

- Every year since 2009 a national report on the outcome of surveillance activities of infectious diseases in animals and humans is produced in Sweden. The report is produced with contributions from the animal health-, public health- and food safety sector. The Swedish National Veterinary Institute (SVA) coordinates the production of the report. The purpose of the pilot is to strengthen the OH-focus of the report and to create and implement a work-process supporting the collaboration between sectors that will persist after the project ends. Moreover, the agencies will through the process gain a better understanding of each other's activities, data sources and results.
- For this OH pilot, we decided initially to focus on three important chapters of foodborne zoonotic agents; *Salmonella*, *Campylobacter* and VTEC/STEC. The work with the pilot started during 2018. Workshops were organized individually for each chapter. Joint decisions were made on main issues to lift in the report, from the OH perspective. The process resulted in an improved collaboration



among the sectors involved in zoonoses surveillance and a better understanding of each other's surveillance activities and results.

- The new process that was introduced in the pre-pilot (during 2019) has been tested and further developed during the real pilot, finished in month 30, as planned. For the execution part of the pilot (M19-M30) new instructions were produced to clarify the role of the main responsible author of each chapter. It was also decided that all zoonotic chapters/diseases that are produced by authors from more than one sector should follow new guidelines to make all the zoonotic chapters more alike. To improve the OH work in all zoonotic chapters it was mandatory for the main authors to contact the other authors for a meeting to discuss the results of the previous year's surveillance and what to highlight in the report. Lessons learned in all harmonisation aspects – collaboration among sectors, data sharing, data publishing, and gathering « One Health highlights » - will be compiled in the following task, and made available through the data interoperability tools developed in WP3, and the OH Codex published by WP1.

JIP1-WP3-T4: Evaluation and Recommendations (M31-M42)

- This task has been postponed to *after* the conclusion of the pilot, therefore starting on month 31, and extended to the end of the project, now also extended to month 42.

WP4 : Coordination, Communication, Training and Sustainability

JIP1-WP4-T1: Internal project coordination (M1-M42)

- The project coordination continued to use the shared project management resources on Google, the ORION Virtual Research Environment (VRE) and promoted the adoption of the EJP Website and EJP ORION groups (an internal and a public group).
- The coordination holds trimonthly web meetings for the whole ORION consortium (including EFSA, ECDC, EJP WPs and interested other EJP project leads) and a monthly call for the WP leaders & deputy leaders.
- For all web-meetings the ORION coordination created meeting minutes that were shared via email and the ORION VRE.

JIP1-WP4-T2: External project integration (synchronized with EJP WP5) (M1-M42)

- The project coordination contributed to relevant overarching EJP activities and continued to extend collaboration and information exchange specifically with the new EJP projects.
- EFSA / ECDC were actively informed on project results via the “EJP ORION WP1 & WP3 supra-national pilot with EFSA & ECDC”
- ORION successfully applied to host the 2020 CPD Module. This event will take place in February 2021.

JIP1-WP4-T3: Sustainability roadmap (M7-M42)

- No update

JIP1-WP4-T4: Training and Dissemination (M1-M42)

JIP1-WP4-T4-ST1: Internal training (sharing knowledge on currently available national solutions) (M1-M12)

- See reports from WP1 to WP3

JIP1-WP4-T4-ST2: Knowledge integration (web portal, Wiki, curricula, tutorials, videos, sample data) (M7-M42)



- Continuous updates performed on ORION's main online platform "OHS Codex" as well as on individual web resources that each partner created for their specific solution, e.g. for OHEJP Glossary linked resources the website <https://foodrisklabs.bfr.bund.de/ohejp-glossary/> was created and updated regularly

JIP1-WP4-T4-ST3: Training and support for other EJP projects & partners (M7-M42)

- ORION co-organized the NGS online workshop in April 2020
- Participation in ASM2020 with 2 presentations & 2-3 posters
- ORION will present own results during a dedicated EJP Cogwheel workshop on 6th October 2020 to EJP members
- A number of extended webinars on specific tools (e.g. EJP Glossary text processing tool, the LOD tool, OH-CRAC etc.) are in preparation and will be performed in fall 2020
- ORION will present the status from the WP1-WP3 supra-national pilot with EFSA-ECDC during EFSA's 38th meeting of the Scientific Network for Zoonoses Monitoring Data on 19-20 October 2020
- ORION results were accepted as posters / oral presentation during several international conferences: ICBO 2020 Integrated Food Ontology Workshop (IFOW) (16th September 2020), WOHC 2020 (30th Oct - 03rd Nov. 2020) and the 4th ICAHS 2020 (11th -13th November 2020)
- Selected ORION project results will trained during the 2020 EJP CPD module (workshop planned from 15th- 19th February 2021)

Participation in ASM2020 with 2 presentations & 2-3 posters



6.1.3.1.3 Progress of the integrative project: deliverables and milestones

6.1.3.1.3.1 Deliverables

JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
ORION	D-1.3	Revised OH Surveillance Codex, including lessons learned from the OH pilots	M36		M42	Revised OH Surveillance Codex : Expected to be delivered on time Lessons learned: Delayed until M42	4, 5
ORION	D-2.7	Revised OH Knowledge Base - Epi, including lessons learned from the OH pilots	M36		M42	Revised OH Knowledge Base – Epi : Expected to be delivered on time Lessons learned: Delayed until M42	5
ORION	D-2.8	Revised OH Knowledge Base -NGS, including lessons learned from the OH pilots	M36		M42	Revised OH Knowledge Base – NGS : Expected to be delivered on time Lessons learned: Delayed until M42	1,2,3,4
ORION	D-2.9	Revised OH Knowledge Base - Integration, including lessons learned from the OH pilots	M36		M42	Revised OH Knowledge Base – Integration : Expected to be delivered on time Lessons learned: Delayed until M42	



JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
ORION	D-3.3	Revised OH Harmonisation Infrastructure Hub, including lessons learned from the OH pilots	M36		M42	Revised OH Harmonisation Infrastructure Hub : Expected to be delivered on time Lessons learned: Delayed until M42	
ORION	D-4.4	Revised Sustainability Roadmap	M36		M42	A final revision will be created in M42	9
ORION	D-4.5	OHS Knowledge Hub populated with resources from WP1, WP2 and WP3	M36		M42	A final revision will be created in M42	4,5
ORION	D-4.6	Two additional training workshops and two webinars	M36		M42	Delayed due to CoV-19 situation	9

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



6.1.3.1.3.2 *Milestones*

JIP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
ORION	M-IA1.ORION.3	ORION evaluation workshop	M33	No	M39	Some ORION pilots had delays in implementation.

6.1.3.1.4 *Follow-up of the recommendations and comments by the Ethics Advisors*

Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
The applicants must confirm the compliance with GDPR.	<p>In WP1, no data from individuals will be collected or used. We see no risk of infringing the GDPR.</p> <p>In WP2Epi, no data from individuals will be collected or used. We see no risk of infringing the GDPR.</p> <p>In WP2-NGS, we might utilize some human related pseudonymized metadata for sequences that we might seek to analyze in collaboration with other EJP projects, however, if so</p>	As regard to the proposed interviews with volunteering key personnel (WP2int 2.3), an informed consent procedure should have been established and proposed to the research participants. It must mention the rights to participants as	Informed consent was established with reference to GDPR by the Data Protection Officer and according to the existing rules. In the end, contact persons emails were omitted from the report, only publishing the organisational contact point	As a reminder of one important part of GDPR, the beneficiaries must provide the contact addresses of the Data Protector Officer of the institution in charge of processing the data obtained.	<p>BfR: dsb@bfr.bund.de</p> <p>SVA: jerker.plobeck@sva.se</p> <p>DTU: Joell@food.dtu.dk for data from ORION</p> <p>The overarching DPO of DTU is: anesa@dtu.dk</p>



Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
	<p>these will be stored on an e-infrastructure approved for use for human sensitive data set up in Norway.</p> <p>For WP2int 2.3 - Requirement analysis for an "OH Knowledge Base – Integration", professional email addresses of individuals were volunteered in the screening questionnaires. Interviews with volunteering key personnel of some initiatives was recorded and the original sound files will be kept in a restricted folder complying with ORION data management plans, DTU data management plans, DTUs Policy of the Retention of Primary Materials and Data, GDPR, until deletion on the last day of the ORION project.</p>	<p>described in GDPR, among which the contact address of the Data Protector Officer of the institution in charge of processing the data obtained through the interviews.</p> <p>It is not clear that the above documents were used for the questionnaire work</p>	<p>emails already available on the web.</p>		<p>FLI: Martina.Rychly@fli.de</p> <p>NVI:</p> <p>Siv.Gunhild.Boe-Tondevold@vetinst.no</p>



Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
	In WP3, no data from individual cases or laboratory tests will be used, only data already aggregated at the surveillance level, and already made public by the owner institution. We see no risk of infringing the GDPR.				

6.1.3.1.5 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP2 integration	M36	M40	D-2.9	M36	M42	Shortage of staff and closed laboratories		The budget will be extended and some unused funding carried over into 2021 due to the approved project extension – not necessarily due to COVID-19



Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP2 integration	M36	M40	D-2.9	M36	M42	Shortage of staff and closed laboratories		The budget will be extended and some unused funding carried over into 2021 due to the approved project extension – not necessarily due to COVID-19
JIP1-WP2-T2	M36	M42	D2.7	M36	M42	Personnel left, COVID-19		x
JIP1-WP2-T3	M30	M42	D2.7	M36	M42	Personnel left, COVID-19		x
JIP1-WP2-T5 and T6	M24	M42	D-2.8	M36	M42	Personnel participating in the WP were reallocated due to the COVID-19 work that various institutions had to engage in		Existing budget will be pushed to 2021



Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JIP1-WP4-T4	M36	M42	D-4.6	M36	M42	Physical training workshops could not be organized due to travel restrictions		Existing budget will be pushed to 2021

Comments:

JIP1-WP2-T2 delayed due to a combination of personnel left and delay in recruitment process due to COVID-19.

JIP1-WP2-T2-ST1 delayed due to a combination of personnel left and delay in recruitment process due to COVID-19.

JIP1-WP2-T2-ST3 delayed due to corona-crisis. The scientists involved in one of the WP2-Epi pilots were informed begin of March 2020, and ready to collaborate, but the CoV-SARS-2 outbreak put the whole project on hold, as all AMR-related-meetings were cancelled. Moreover, the Competent Authority (due to the Cov-SARS-2 situation) has delayed the implementation of a National Action Plan on AMR. The beginning of the pilot project was then postponed to September 2020 and will be performed during two months (September and October 2020)

Other general COVID-19 related effects on ORION work:

- There were unforeseeable delays in performing certain planned research work in Year 2 of the project, specifically in areas where sector-overarching synchronized actions were required. Due to COVID-19 crisis partners could not catch up on these delays until the end of M33.
- Due to the COVID-19 crisis, several project partners were not able to present their research outcomes at international conferences, as these were postponed or cancelled, e.g. IAFP Europe was cancelled completely.



6.1.3.1.6 Publications

N/A

6.1.3.1.7 Additional output

- M. Filter, T. Buschhardt, T. Günther, E. Lopez de Abechuco, E. M. Sundermann, J. Ellis-Iversen, J. Gethmann, K. Lagesen, V. De Waele, G. Boseret, F. Dórea. The OH Surveillance (OHS) Codex - a high level framework supporting mutual understanding and information exchange between One Health sectors. OHEJP ASM 2020, 27th-29th May (Oral presentation).
- E. Lopez de Abechuco (BfR): Introduction to ORION project. Cogwheel workshop, 28th April 2020
- E. Lopez de Abechuco, F. Dorea, T. Buschhardt, T. Günther, E. Sundermann, N. Scaccia, A. Foddai, M. Dispas, M. Umaer, M. Holmberg, J. Gethmann, M. Filter. One Health Consensus Report Annotation Checklist (OH-CRAC): a generic checklist to support harmonization of surveillance data reports. OHEJP ASM 2020, 27th-29th May (Oral presentation).
- N. Scaccia, T. Guenther, T. Buschhardt, L. Valentin, M. Filter. Text mining technology as added-value infrastructure to the One Health EJP Glossary. OHEJP ASM 2020, 27th-29th May (Poster).
- Gethmann, J. et al Poster Presentations during the ASM Scientific conference 2020: The ORION project – OH knowledge base “surveillance systems” OHEJP ASM 2020, 27th-29th May (Poster).

6.1.3.1.8 Data Management Plan

1. Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.

=> No, so far not.

2. A draft final DMP should be submitted for validation at the latest three months prior to the end of the project. Have you initiated the work with the final DMP?

=> Will be done in M39.

6.1.3.1.9 On-going and planned collaborations with national or European projects or networks

- WP1 – collaborated with MATRIX, NOVA, COHESIVE project on joint improving the WP1 tools like the OHEJP Glossary, OHS Codex, CRAC.
- WP2Epi - collaborated with representatives of EFSA and ECDC on the details and sustainability questions of the inventories and with COHESIVE and RaDAR project.
- WP2integration - pilot projects are intertwined with other projects such as the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) and The Danish Action Plan on Campylobacter in Food and Environment.
- WP2-NGS – collaborated with COHESIVE (ontologies & metadata databases), LISTADAPT (building bioinformatics pipelines for Listeria) and the IRIDA project (improving their platform and bug-fixing)
- WP3 – established a collaboration with the BeONE project concerning data harmonisation and interoperability in OH; also regular meetings with the IRIDA consortium in Canada, and their team developing the GenEpiO ontology.
- WP4 – continued to maintain the contact with the Federal Food Safety and Veterinary Office



(Switzerland) and the SISOT team at WHO, FAO and OIE (in collaboration with the MATRIX project)

6.1.3.2 JIP02-R1-IA1.2-COHESIVE

6.1.3.2.1 Summary of the work carried out in the JIP

For Task 2.1 the main goal is to develop guidelines for *national* One Health structures or other ways to strengthen human-veterinary collaborations, with the aim to improve signalling, risk assessment and response (risk analysis) to zoonoses. A lot of information has already been gathered, which is currently turned into the guideline. The guideline will be web-based and eventually coupled to the MedVetNet association website in order to be able to maintain the guideline also after OHEJP ends.

For Task 2.2 the goal was to develop an online decision support tool to help the user decide on the most appropriate method to use when tasked with conducting a risk assessment for a specific situation. The tool will direct the user to one of fourteen resources, which include guidance documents, tools and models. The tool is nearing completion and it is expected to go live soon, a web address will be circulated to EJP members as soon as it is publicly available. The deliverable associated with this task was delivered in Dec 2019, however it has been significantly improved since. The tool is designed so that it can be updated as new risk assessment resources emerge.

In Task 2.3 several pilots are planned in which countries will go through the first steps of the guidelines. Belgium, Portugal, Norway and Italy will take part. In three countries a core group (change agents) has been made and a stakeholder analysis is performed. However, due to COVID-19 the systems mapping workshops have all been delayed.

Task 3.1: The task focuses on identifying factors that contribute to well-functioning systems or ways to share signals of zoonotic events within and between countries. Participating countries have finalized most of their planned interviews, but COVID-19 has affected some of the interviews. The participating countries have finalized the preliminary analysis of the national data, but COVID-19 has also affected this work. A planned workshop for joint analysis of data needed to be cancelled and is planned for autumn 2020. Instead, a joint analysis of the data will be performed by sharing documents and having digital meetings. A poster presentation and an oral presentation were given at the annual OHEJP meeting in Prague.

For Task 3.2 a follow-up of the horizon scanning pilot Exercise regarding One Health that took place in Nov 2019 has been conducted and the results were presented in a poster at the One Health EJP Annual Scientific Meeting in May 2020.

For Task 3.3 all UK-government publications relating to Hantavirus and Q-fever have been compiled and their data-sources tracked. A general European-level Q-fever surveillance map has been made in a similar way, using input from consortium members and available online publications. Systematic mapping of Q-fever detection systems within the UK continues, seeking guidance from members of the UK Q-fever surveillance network. We aim to document UK surveillance protocols to assess their strengths and weaknesses.

For Task 3.4, pilot One-Health Early Warning System, the aim is to build on the findings of the previous tasks (i.e. tasks 2.1, 3.1, 3.2) and host an online meeting to share low threshold potential signals for pathogens with One-Health impacts across multiple countries within the consortium. The aim is for countries to briefly present what zoonoses reports they produce and prompt informal discussions about country trends and opinions. Through this we can build relationships between countries and start a forum that can continue post-project and is seen as being of value for those involved to continue to support financially.



For Task 3.5, Development of tool for systematic cost-benefit analysis (CBA), the aim was to build on previous experience in the area of CBA to produce a tool and/or method that is applicable to zoonotic pathogens. As CBA can vary due to within country specific conditions that affect both costs and benefits, having a common method attempts to make the CBA comparable across countries. A literature review of CBA methods is underway, with the aim of producing a report focussing on the similarities, differences, strengths and weaknesses of different models and methods. This will incorporate input from other OHEJP members in order to gather CBA experience from across the OHEJP consortium. The systematic review will culminate in a framework for optimizing CBA studies which would help to ensure that future studies are harmonized and directly comparable. However, this task was predominantly scheduled for the final year of COHESIVE and COVID-19 has severely limited the resource available for this task. It is likely that the original scope of this task will need to be reassessed.

In WP4.1, the foreseen feasibility studies on the three Member States (The Netherlands, Italy, Norway) are ongoing. Data coming from the organizations of the Member States have been inserted in the corresponding prototypes information systems (cis-holland, cis-italy, cis-norway) which are online and accessible for the involved organizations.

The sub-task “Linking of the national databases with the COHESIVE prototype information system and the epidemiological analysis tools” is started. A meeting with BFR and IZSAM is planned for the integration among the three information systems developed of the WP4.

In WP4.2, the development of the tracing web portal - FoodChain-Lab Web - advanced further. A revised login procedure and data security documents are currently being developed and the production mode is set up. Also, a JSON-based data exchange format allows for analysing delivery data from a data collection mask developed in a national project in FCL Web.

In WP4.3, the development of risk modelling framework – quantitative riskanalysis - we have completed our prototype a web application in Shiny R for quantitative risk models. In the next months we will be supported by an external partner in the work of further developing our web application.

6.1.3.2.2 Progress of the project: description of activities

WP1: Coordination, communication and sustainability

JIP2-WP1-T1: Coordination (M1-M36)

The steering group is meeting online every 5 weeks. In these meetings all tasks are discussed shortly, including (possible) problems. Also general tasks are discussed and decided, such as annual meetings and reports. There have been several contacts with EFSA, mainly concerning WP4, but also regular video conferences are organized between the contact persons of EFSA and the coordinator of COHESIVE. Unfortunately, ECDC no longer has a contact person available for COHESIVE. ORION and NOVA are other OHEJP projects to which COHESIVE closely relates. ORION, NOVA and COHESIVE are working together at a glossary including terms used within the different projects. A publication is in preparation.

JIP2-WP1-T2: Communication/dissemination (M1-M36)

Since in 2019 two general (annual) meetings were organized, the annual meeting that was planned in March 2020, was postponed. Due to many planned conferences (i.e. World One Health Conference, ASM OHEJP, IAFP European Symposium on Food Safety Food) in Spring it was postponed to Autumn. Due to COVID-19 it is now questionable whether it will be possible to actually have a physical meeting. We have decided to arrange an online annual meeting. Since, it is still important for the progression to meet, we had hoped to have WP-meetings physically for WP2/WP3 combined and a separate one for WP4 followed by an online annual meeting with the whole consortium. However, it has become clear that also the smaller meetings cannot take place physically. We will organize online alternatives,



although we have to realize that the online meetings might have lower efficiency and quality of the discussions and therefore might affect the output of the WPs 2, 3 and 4. As a spin-off, we had organized a workshop on systems thinking as a satellite workshop before the ASM in Prague. However, this could not go through. Also the workshop during the IAFP European Symposium on Food Safety conference was cancelled. And, we were involved in the organization of an outbreak preparedness workshop, which was cancelled only a few days in advance due to the large COVID-19 outbreak. This was a real pity, because it was about one of the key issues COHESIVE wants to address (be prepared in a One Health fashion for emerging zoonoses) and more than ever relevant. Dissemination activities that did take place, related to the other work packages, are indicated below.

WP2. Integrated risk-analysis at the national level

JIP2-WP2-T1: Development of guidelines for national One Health structures (M1-M26)

The main goal of this task is the development of guidelines in order to support countries to set up/strengthen the collaboration between the human-vet-food domains with respect to signaling, risk assessment, response and control of zoonoses. During the general meeting in Rome in November 2019 it was decided to disseminate the guidelines as a website. This would facilitate easy searching of dedicated information, based on the needs of the user. An outline of the website has been made. We have finalised the contract with the web designer. Already a lot of information has been gathered during the workshops in 2018 and 2019 and we have started filling the website. In the workshops also information has been gathered on barriers that can be faced when trying to organize risk analysis of zoonoses in a One Health fashion. Important barriers such as obtaining political will and building trust are deepened by performing dedicated interviews. The interviews are currently analysed and the results will be translated into tips and tricks in the guidelines to support countries with these kind of barriers. After the OHEJP ends, the guideline-website will be transferred to the MedVetNet Association (MVNA) and linked to their website, to secure long term sustainability.

There is regular contact with the FAO with respect to the [Tripartite Zoonoses Guide](#) (TZG) of OIE, WHO and FAO, specifically in relation to the SISOT work group, and now also contacts are made with the WHO in relation to the governance part of the TZG.

It became clear in the first 2 years, that physical workshops are the best way to co-create the guidelines. Because the results from a workshop are input for the next and good preparation is key to a successful meeting, the workshops cannot be too close together. Unfortunately, due to COVID-19 no workshop has taken place this year yet. We will organize an online workshop in September and we were hoping to have a physical workshop in November. Now, it turns out that is not possible as well and we will try to organize another online workshop. Since, due to COVID-19, we will not be able to have three physical workshops this year, this task will be delayed. Although we will be able to deliver a website with guidelines, the quality most likely will be affected by the COVID-19 crisis.

JIP2-WP2-T2: Development of structured decision making (M1-M24)

The deliverable for this task was delivered during 2019, this produced a prototype tool of a decision tree framework to help the user decide on the most appropriate method to use when tasked with conducting a risk assessment for a specific situation. The tool has been significantly improved since the delivery in 2019. The tool is designed so that it can be updated as new risk assessment resources emerge. The tool will direct the user to one of fourteen resources, which include guidance documents, tools and models. The tool, now run in a javascript-enabled browser, has supporting text outlining purpose and caveats, and has adopted EJP colours. It references the grant agreement in a footer, and also provides links to the OHEJP homepage and EJP homepage. No further dissemination activities have been done, however a short communication to accompany the tool has been drafted. The tool is nearing completion and we are currently seeking web-space to host the tool, aiming to do so through collaboration within the consortium.



JIP2-WP2-T3: Knowledge transfer and dissemination (M25-M35)

Different dissemination activities of WP2 have taken place during the OHEJP ASM; an oral presentation and a poster both related to the development of the guideline in WP2.1. The preparation for the first pilot in Belgium started in 2019. A systems mapping workshop should have taken place on March 27, but was cancelled a few days in advance. After trying to postpone to June, the new aim was to have it in October. However, due to changing COVID-19 situation in Belgium it turned out not to be possible after all and it will be further delayed. Also, the other pilots were strongly hampered by the COVID-19 crisis. However, Norway managed to make a draft systems map and performed a stakeholder analysis. The actual pilot has been postponed to January. Italy has done the first part of the stakeholder analysis, the systems mapping workshop will take place in 2021. When a physical WP2/3 meeting can take place in Portugal, we will try to have a systems mapping workshop in the same week. If not, then the workshop has to be further postponed. Depending on the COVID-19 crisis, we have to realize that one or more pilots will be delayed so far that they will not be done before the end of COHESIVE.

WP3: Towards an EU zoonoses structure

JIP2-WP3-T1: Explore current ways for exchanging signals between countries and cross disciplines – pathway analysis (M1-M24)

One of the overall purposes of the task is to find good examples of ways to exchange signals cross disciplines or cross borders and to learn from each other. During a workshop in Uppsala in 2019 the focus of the task was set to identify different factors that contribute to well-functioning systems or ways to share signals as well as factors that prevent sharing of signals, both within and between countries, focusing on the persons in the systems rather than the technical or organizational solutions. In depth interviews were considered to capture more in-depth information than a written questionnaire.

Six countries have been involved and finalized the interviews, although the COVID 19 situation has affected some interviews. The first round of national data analysis has been completed although COVID-19 has delayed the analysis. A planned joint workshop was cancelled and is planned for the autumn 2020. However, alternative ways to progress with the joint thematic analysis without meeting in person, have been scheduled. In general, the participating countries have experienced that they through the interviews have received new information which is relevant when understanding signal sharing. One preliminary theme that has emerged in several countries, is trust and the importance of knowing someone in person. Two presentations were given at the OHEJP annual conference in Prague, one oral presenting the results from the Italian part of the study, and one poster describing the whole project. The work drafting the manuscript has continued. Further, another project, close to Task 3.1, was presented for the DG Santé in January 2020 while they performed an audit in Sweden on “Early detection and notification systems in place for emerging animal diseases”. The representatives from DG Santé were very interested in the study and in the preliminary findings presented.

JIP2-WP3-T2: Horizon Scanning Pilot Exercise (M25-M36)

The pilot horizon scanning exercise that took place in November 2019 was based on an assembly of information sources and an assembly of analysis teams with assigned topics. Within task 3.2 a horizon scanning team composed experts within public health, animal health, food safety has been established. To explore the potential application of such a technique, the pilot exercise first identified more than 30 potential One Health issues. From these a summary of six topics were identified to have a key impact in the next coming years. The conducted pilot horizon scanning exercise showed to be useful but needs to be further developed for foresight applications related to One Health in Europe. The One Health pilot horizon scanning exercise took place (Nov 2019) prior to the covid-19 pandemic. Even though the foresight was focused on One Health the exercise identified many issues that have been of relevance during the last months. The task leader participated in a horizon scanning exercise



organised by the WHO, using similar methodology as in task 3.2. A scientific report on the horizon scanning exercise is in progress.

JIP2-WP3-T3: Retrospective systems analysis of detection of outbreaks (M6-M30)

Data gathered from the November 2019 workshop has been used to map One-Health surveillance structures in consortium countries. Cross-comparing these has identified the 'common core' of components that are present in all those countries. Due to COVID-19 work on this component paused in mid-March but has since resumed. Collaboration with participants in refining, adding detail and finalising the One Health structures in each country is still required, and may be significantly delayed by COVID-19. In tandem, a map of Q-fever surveillance protocols in the UK veterinary sector has been made to identify local strengths and weaknesses. We hope to expand this across public health and food safety in the UK.

JIP2-WP3-T4: Pilot One-Health Early Warning System (M25-M36)

The core feature of this pilot was originally envisaged to be a face to face meeting with one-health professionals across the COHESIVE project. Due to COVID-19 it is unlikely that such a meeting can take place. We have amended the plans to convert this to an online format and are currently focused on identifying One Health representatives in institutes involved in COHESIVE. Aims, objectives and terms of reference will be drafted for the meetings and development of the documents can be carried out online.

There will be additional challenges to developing such a group only online as trust plays a significant part in open sharing of information and ideas, this may slow the effective development of such a group and will require innovative approaches.

JIP2-WP3-T5: Development of a tool for systematic cost-benefit analysis (CBA) (M25-36)

Due to COVID-19 work on this task has not progressed as much as originally planned as key staff members have been redirected to COVID-19 related activities. An email was circulated to the whole COHESIVE project seeking active contributions to the task. A number of positive responses were received. An abstract for a poster was accepted to the EJP ASM also looking to recruit more members to the project however the revised online format of the meeting made this less effective than hoped. Current work is focused on performing a systematic review of cost-benefit analysis studies within the foodborne zoonosis remit. This will incorporate input from other OHEJP members in order to gather CBA experience from across the OHEJP consortium. The systematic review will culminate in a framework for optimizing CBA studies. Work on this objective has been delayed during 2019 due to secondment of staff on to COVID-19 related tasks.

WP4: Data platform to facilitate risk-analysis and outbreak control

JIP2-WP4-T1: Molecular typing data and metadata – database creation

The feasibility studies on the three Member States (The Netherlands, Italy, Norway) are ongoing. Three prototypes information systems (cis-holland, cis-italy, cis-norway) are online and have been filled with information provided by the involved member states. RIVM and WBVR of The Netherlands have provided details of their coding systems. They are currently under the harmonization phase. ISS and NRL are conducting a sharing exercise of WGS data and metadata of *Listeria* and STEC.

JIP2-WP4-T1-ST5: Filling of DBs (M15-M26)

- In the new T4.1 description, the sub-task title is "Linking of the national databases with the COHESIVE prototype information system and the epidemiological analysis tools".
- During the General Meeting on November 2019 in Rome, it was decided to start the activity of integration among the three information systems of the WP4. A telco was planned for the beginning of 2020 followed by face-to-face meeting.



- The activity has been affected by COVID-19 crisis.

JIP2-WP4-T1-ST6: Design and implementation of pipelines (M21-M32)

- In the new T4.1 description, the sub-task title is *“Study of available pipelines”*.
- We are connected with the EJP ORION project, which has a WP specifically dedicated to this activity.
- The activity might be affected by delay of ORION deliverables due to COVID-19 crisis.

JIP2-WP4-T2: Development of a platform-independent tracing framework

JIP2-WP4-T2-ST2

In subtask 2, several modules for data collection, cleaning, visualisation and reporting – in part developed in the framework of other projects - will be unified in one platform:

- A data collection module
- An interactive analysis module
- A WGS-data integration module
- A reporting module
- A synchronization module with the desktop version of FoodChain-Lab (FCL)

The overall status and progress of the whole FCL project can be inspected at <https://foodrisklabs.bfr.bund.de/foodchain-lab>.

The specific status and new software versions of the FCL tracing web portal are deployed automatically to a test server where new features of the tool can be seen live. Currently, the production system of the tracing portal is set up and will be accessible at <https://fcl-portal.bfr.berlin> in the second half of 2020. Also, a revised login procedure including obtaining the user's consent to gather personal data as well as data security documents are being developed now.

In addition, a JSON-based data exchange format allows for visualising and analysing delivery data obtained from a data collection mask developed in a national project in FCL Web.

JIP2-WP4-T2-ST3

In several presentations on FoodChain-Lab the audience highlighted the importance for integration of WGS data into tracing network visualisations. In addition to the work performed in 2018 and 2019, an interface to the COHESIVE Information System, the whole genome sequencing database mentioned in JIP-WP4-T1 is planned and work will be intensified in the upcoming weeks. This subtask will not be finished as planned until M32. Hence, we plan to elongate this subtask.

JIP2-WP4-T3: Development of a platform-independent risk modeling framework

JIP2-WP4-T3-ST1: Requirement analysis (M1-M9)

Typical components have been identified that support quantitative microbiological risk assessment, advanced simulation techniques, documentation and extended usability. Selection of minimal models for testing and development is completed.

The prioritization of building blocks for implementation in web application of rrisk is finished.

JIP2-WP4-T3-ST2 Implementation (M10-M30)

Various minimal models from the literature and from project partners were tested and defined. Risk questions and scenarios as well as quantitative risk models were provided by partners in COHESIVE and other EJP projects. As part of the implementation of standards, we were also provided with data sets and use-cases in cooperation with the FLI. Currently, also the search of models and data suitable as case study (ideally with input from project partners) is ongoing. Due to the corona pandemic we



have a delay of 3 months. Furthermore, in cooperation with project partners we have prioritized different building blocks. For the web application of risk we developed various mocks to define the workflow and the individual steps of the user interface in R shiny.

In the first step we have successfully implemented the one-dimensional Monte-Carlo simulation in the web application. The Prototype of “quantitative Riskanalysis” was developed and is finished. With “quantitative Riskanalysis” different risk models can now be implemented and calculated. The implementation of further statistical methods for quantitative risk assessment is ongoing (this subtask will be finished in Q4 2020).

In order to create a stable application which can be further developed in the future, the programming task was handed over to a software development company. Due to the financial volume of 100.000€ the contract had to be publicly tendered.

Due to the Corona pandemic this tender process has been delayed by 3 months.

JIP2-WP4-T4: Dissemination

Although dissemination duties of WP4 achievements start only from M25, a lot of dissemination work for FCL and the tracing portal that was set up in COHESIVE was done in the framework of other projects in the years 2018 and 2019 (workshops and presentations on FCL, meetings with stakeholders). In the framework of COHESIVE annual meetings, presentations and workshops for WP4 tools were conducted for the COHESIVE community.

WP4 activities were presented at the 2020 OHEJP Annual Scientific Meeting in an online format (presentation on COHESIVE Information System, poster on FoodChain-Lab Web). In 2020 a joint interactive workshop on WP4 tools and how they interact is envisioned as a satellite event for the COHESIVE annual meeting in autumn which is open for the COHESIVE community and other interested users. Furthermore, an online presentation of the WP4 tools is planned for 2020 as well.



6.1.3.2.3 Progress of the research project: deliverables and milestones

6.1.3.2.3.1 Deliverables

JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category * (1 to 11)
COHESIVE	D-JIP2-1.5	Annual meeting	26		35	Since we have had a second meeting with the whole consortium in November 2019, this annual meeting was already postponed to Autumn 2020. Due to COVID-19 we cancelled the physical meeting and we will have an online annual meeting	11
COHESIVE	D- JIP2-1.6	Annual report	28	14-01-2020			
COHESIVE	D- JIP2-1.7	Closing symposium	36	Not due	42	An extension of 6M months has been granted. Therefore, this closing symposium will be postponed to month 42	11
COHESIVE	D- JIP2-1.8	Annual report	36	Not due			



JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category * (1 to 11)
COHESIVE	D- JIP2-2.5	Development of common procedures and tools	26		38	Very dependent on physical meetings. Appeared to difficult to arrange frequently and has also been hampered by the COVID-19 crisis	11
COHESIVE	D- JIP2-2.6	Knowledge transfer and dissemination	34	Not due		Very much dependent on physical workshops. This has been extremely hampered by the COVID-19 crisis	11
COHESIVE	D- JIP2-3.3	Pathway analysis of exchanging signals	10		36	Interviews have been performed in six countries. National thematic analysis has been performed and a joint thematic analysis is ongoing. Due to COVID this deliverable is further delayed. Work with manuscript for scientific publication ongoing.	11
COHESIVE	D- JIP2-3.5	System analysis of detection of outbreaks	24		36	This deliverable had already been extended. Draft of UK systems map completed Dec-2019. Work currently paused due to COVID-19 and hoped to restart June 2020	11



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JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category * (1 to 11)
COHESIVE	D- JIP2-3.6	Knowledge transfer and dissemination	34	Not due	40	Delayed as transfer and dissemination will wait until completion of all deliverables.	11
COHESIVE	D- JIP2-3.7	Drivers of change in One Health– a horizon scanning exercise (new deliverable)	30		36	New deliverable, not listed in the proposal. A horizon scanning exercise was done at the general meeting in November 2019. Material compiled within WP3.2 (including the exercise) will be compiled.	11
COHESIVE	D- JIP2-3.8	Tool for systematic cost-benefit analysis	36	Not due	40	New deliverable, not listed in the proposal. Started on collating the results of literature review between AGES and APHA, however work is paused due to COVID-19	11
COHESIVE	D- JIP2-4.1.3	Databases containing the country data	26	30-03-2020		The three prototypes information systems (cis-holland, cis-italy, cis-norway) have been filled with information provided by the involved member states. Details are on the deliverable uploaded to the EJP platform end of March 2020.	4



JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category * (1 to 11)
COHESIVE	D- JIP2-4.1.4	Description of the pipelines implemented to feed the analysis tools with the data stored in the three databases of this project	32		36	<p>Partial information is gathered from involved member states.</p> <p>Pipelines specification will be taken from an ORION deliverable specifically dedicated to this activity. Pipelines integration will start just after ORION deliverable publication.</p> <p>Information was expected to be shared and harmonized among the others softwares from WP4. Covid crisis delayed this point.</p>	4
COHESIVE	D- JIP2-4.2.2	FoodChain-Lab with integrated network analysis module as a web-service browser-ready, open source and implemented in the BfR cloud	32	31.08.2020		FCL Web is already accessible at https://fcl-portal.bfr.berlin .	4, 11



JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category * (1 to 11)
COHESIVE	D- JIP2-4.2.3	Integration and analysis of epi-data from 4.1 → WGS-Epi module and selected features from IA-1, WP2, WP3	32		38	This deliverable will be delayed. Delay due to travel restrictions and shortage of staff due to COVID-19 associated issues	4, 11
COHESIVE	D- JIP2-4.3.2	Populating a model project model repository Harmonised graphical user interface prototypes Technical documentation and user guidance	30		36	A public tender for further development of our prototype for quantitative risk analysis was conducted to have some parts of the web application programmed by an external company Due to the corona pandemic and a very long process of the tendering, we have suffered considerable delays.	4, 11



JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category * (1 to 11)
COHESIVE	D- JIP2-4.3.3	Final testing design elaborated Report section about testing results (internal standards) Report section about testing results using external comparative models Final release (open access)	36	Not due	40	Deliverable will be delayed by 4 months	4, 11
COHESIVE	D- JIP2-4.4.1	Each subtask conducts workshops, provides documentation and tutorials	36				9

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities



and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity; 11: Co-creation of common procedures, tools and information to support One Health risk-analysis of zoonoses (national and international)

All COHESIVE products are public unless otherwise stated

6.1.3.2.3.2 *Milestones*

JIP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
COHESIVE	M-AI2.COHESIVE.1.3	Annual report	28	Yes		
COHESIVE	M-AI2.COHESIVE.1.4	Closing symposium	36	Not due	42	An extension of 6M months has been granted. Therefore, this end symposium will be postponed to month 42
COHESIVE	M-AI2.COHESIVE.1.5	Annual report	36	Not due		
COHESIVE	M-AI2.COHESIVE.2.2	Development of common procedures and tools	26	No	38	Very dependent on physical meetings. Appeared difficult to arrange frequently and has also been hampered by the COVID-19 crisis
COHESIVE	M-AI2.COHESIVE.2.3	Knowledge transfer and dissemination	34	Not due	40	Very much dependent on physical workshops. This has been extremely hampered by the COVID-19 crisis



JIP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
COHESIVE	M-AI2.COHESIVE.3.1	Development of common procedures and tools for horizon scanning	26	No	36	Due to the COVID-19 crisis much of the correspondence needs to take place via emails and other electronic tools.
COHESIVE	M-AI2.COHESIVE.3.2	Pathway and system analysis of signal exchange and outbreak detection	24	No	33	Task 3.1 is currently on-going and the results will be compiled in 2020, the plan was to finalise the work in spring 2020 but due to COVID-19 the work is delayed. The preliminary draft of Task 3.3 which was done at UK level needs to be adjusted to other countries. The joint work of tasks 3.1 and 3.3 started in 2020.
COHESIVE	M-AI2.COHESIVE.3.3	Local dissemination	34	Not due	40	Delayed due to the cancellation of many conferences due to take place over the summer 2020. Alternative approaches will be required for dissemination activities and methods.
COHESIVE	M-A2.COHESIVE.4.7	Databases containing the country data	26	Yes		The three prototypes information systems (cis-holland, cis-italy, cis-norway) have been filled with information provided by the involved member states. Details are on the deliverable uploaded to the EJP platform end of March 2020.



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JIP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
COHESIVE	M-A2. COHESIVE.4.8	Prototype for risk modeling framework ready	30	Yes		Prototype “quantitative riskanalysis” for risk modelling frame is available on github.
COHESIVE	M-A2.COHESIVE.4.9	Availability of the pipelines from the databases to the analysis tools	32		36	<p>Partial information is gathered from involved member states.</p> <p>Pipelines specification will be taken from an ORION deliverable specifically dedicated to this activity. Pipelines integration will start just after ORION deliverable publication.</p> <p>Information was expected to be shared and harmonized among the others softwares from WP4. Covid crisis delayed this point.</p>
COHESIVE	M-A2. COHESIVE.4.10	Analysis of epi-data into tracing framework enclosed	34	Not due	40	<p>Some epi-data (case information, sample data) already integrated into FCL Web.</p> <p>However, for this milestone i.e. the WGS data to be integrated and analysed in FCL Web, will be delayed due to travel restrictions and shortage of staff due to COVID-19 associated issues</p>



JIP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
COHESIVE	M-A2. COHESIVE.4.11	Final release of risk modeling framework	34	Not due	40	Delayed due to Covid-19 crisis and a very long tender process

6.1.3.2.4 Follow-up of the recommendations and comments by the Ethics Advisors

Requirements of Ethical Reviewers, January 2018	Project's measures and actions taken at the end of 2018	Comments of Ethics Advisors, January 2019	Comments Project Leaders, end of 2019	Comments of Ethics Advisors, January 2020	Comments Project Leaders, mid-2020
None	None	None	None	None	



6.1.3.2.5 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP1.1	M36	M42	D-JIP2-1.5	M26	M35	The delay was actually due to successfully organizing 2 general meetings in 2019. The new proposed deadline is M35. However, it is questionable whether that is realistic, due to travel restrictions and availability of large enough rooms at institute (restrictions of number of people per room)	0€	100.000€
WP1.1	M36	M42	D-JIP2-1.7	M36	M42	An extension of 6M has been granted, therefore the closing symposium has been postponed to M42.	0€	150.000€
WP2.1	M26	M38	D-JIP2-2.5	M26	M38	Very dependent on physical meetings. Extra delay due to travel restrictions related to the COVID-19 crisis and shortage of staff due to reallocation to COVID-19 work	10.000€	150.000€



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Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP2.3	M35	M42	D-JIP2-2.6	M34	M42	Very much dependent on physical workshops. Delayed due to travel restrictions and shortage of staff due to reallocation to COVID-19 work	0€	150.000€
WP3.1	M24	M36	D-JIP2-3.3	M10	M36	Extra delay due to travel restrictions and shortage of staff due to reallocation to COVID-19 work. Alternative ways, e.g. electronic meetings, are foreseen.	0€	50.000€
WP3.2	M24	M36	D-JIP2-3.7	M30	M36	Delayed mainly due shortage of staff due to reallocation to COVID-19 work	0€	50.000€
WP3.3	M30	M36	D-JIP-3.5	M24	M36	Extra delay due shortage of staff due to reallocation to COVID-19 work	0€	25.000€
WP3.4	M36	M40	D-JIP2-3.6	M34	M40	Delayed due to travel restrictions and shortage of staff due to reallocation to COVID-19 work. Alternative ways, e.g. electronic meetings, are foreseen	0€	150.000€
WP3.5	M36	M40	D-JIP2-3.8	M36	M40	Delayed due shortage of staff due to reallocation to COVID-19 work	0€	60.000€



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Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP4.1.5	M26	M36	D- JIP2-4.1.3	M36	M36	A meeting at BFR for coordinating activities is delayed due to travel restrictions	0€	25.000€
WP4.1.6	M32	M36	D- JIP2-4.1.4	M36	M36	Delay due to travel restrictions and shortage of staff due to reallocation to COVID-19 work. Additionally, delay on deliverable from EJP ORION impact this task	0€	25.000€
WP4.2.3	M36	M38	D-JIP2-4.2.3	M32	M38	Delayed due to travel restrictions and shortage of staff due to COVID-19 associated issues	0€	20.000€
WP4.2.3	M36	M40	M-A2. COHESIVE. 4.10	M34	M40	Delayed due to travel restrictions and shortage of staff due to COVID-19 associated issues	0€	20.000€
WP4.3.2	M30	M36	D-JIP2-4.3.2	M30	M36	Delays due COVID 19 lockdown impairment of regular work	0€	40.000€
WP4.3.2	M34	M40	M-A2. COHESIVE. 4.11	M34	M40	Delays due COVID 19 lockdown impairment of regular work	0€	90.000€



Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP4.3.3	M36	M40	D- JIP2-4.3.3	M36	M40	Delays due COVID 19 lockdown impairment of regular work	0€	40.000€

Comments:

It is very complex to get a good impression of the delayed budgets due to Covid and preferably we would not give any estimates. Even though it feels uncomfortable, we did give a number. However, these numbers are not more than an indication that could be off in both directions and should not be used for any financial calculations or actions.



6.1.3.2.6 Publications

No peer-reviewed publications

6.1.3.2.7 Additional output

WP2 Presentations at the OHEJP annual meeting in Prague 27-29 May, 2020

6.1.3.2.7.1 *Oral presentation:*

COHESIVE: Development of implementation guidelines to support countries with early warning, response and control of (emerging) zoonoses in a One Health fashion

Frank Koenen, Sandra Cavaco Gonçalves, Solveig Jore, Charlotte Cook, Elina Lahti, Karin Nyberg, Malin Jonsson, Frits Vlaanderen, Ines Mogami, Mathilde Uiterwijk, Rosangela Tozzoli, Kitty Maassen on behalf of the COHESIVE WP2 consortium

6.1.3.2.7.2 *Poster presentation:*

COHESIVE: Understanding the needs for European implementation guidelines for a One Health Risk Analysis System for zoonoses

Ines Mogami-Asselin, Caroline Ribeiro, Malin Jonsson, Frank Koenen, Ewa Pacholewicz, Bart Regeer, Hendrik-Jan Roest, Simon Rüegg, Mathilde Uiterwijk, Frits Vlaanderen, Cecilia Wolff, Kitty Maassen

WP3 Presentations at the OHEJP annual meeting in Prague

Oral presentation:

Factors affecting signals sharing of zoonotic events: a qualitative exploratory study in Italy

Michele Luca D'Errico, Maria Nöremark, Chiara Cattaneo, Rosangela Tozzoli, Gaia Scavia

Poster presentation:

To share or not to share - the exchange of signals of zoonotic events within and between countries in Europe

Maria Nöremark, Sandra Cavaco Gonçalves, Charlotte Cook, Michele Luca D'Errico, Rob Dewar, Gry Marysol Grøneng, Malin E Jonsson, Trude Marie Lyngstad, Ines Mogami Asselin, Karin Nyberg, Ewa Pacholewicz, Gaia Scavia, Barbara Schimmer, Cecilia Wolff, Elina Lahti

Poster presentation:

Horizon scanning pilot exercise regarding One Health

Rickard Knutsson, Elina Lahti, Renata Karpiskova, Ivana Kolackova, Ludovico Pasquale Sepe, Rosangela Tozzoli.

WP4 Presentations at the OHEJP annual meeting in Prague

Oral presentation:

cgDIST: a new methodology to inferring phylogeny



Adriano Di Pasquale, Iolanda Mangone, Antonio Rinaldi

Poster presentation:

The COHESIVE Information System: a cross EJP projects example

Adriano Di Pasquale, Fernanda Dorea, Karin Lagesen, Francesca Cito, Alessio Di Lorenzo, Paolo Calistri, Kitty Maassen

Poster presentation:

The FoodChain-Lab Web application – an integrative tracing tool to analyse complex global food supply chains in foodborne crises

Marion Gottschald, Birgit Lewicki, Alexander Falenski, Marco Rüger, Isaak Gerber, Dominic Tölle, Annemarie Käsbohrer, Armin A. Weiser

Abstracts can be accessed from the proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats.
<https://onehealthejp.eu/wp-content/uploads/2018/12/D3.12-OHEJP-ASM-2020-Abstract-book.pdf>

6.1.3.2.8 Data Management Plan

Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.

No

- A draft final DMP should be submitted for validation at the latest three months prior to the end of the project. Have you initiated the work with the final DMP?

N.a.

6.1.3.2.9 On-going and planned collaborations with national or European projects or networks

Co-operation with other EJP projects, such as **NOVA** and **ORION**. Together, an online glossary in under development. Within WP4.1.6 there is a complementary collaboration with ORION. And for WP4.2, there are links to the EJP NOVA project in which a module for analysis of sales data should be integrated into FCL. Within task 3.1 there are links to a task within NOVA on early detection and notification of transmissible animal diseases.

In WP2.1 there are regular meetings with **FAO** and **WHO** in relation to their TZG and our guideline to help one another with tools and experiences. Also to align the initiatives and prevent overlap.

In WP4.2 there is a close collaboration with **EFSA** with focus on tracing solutions in the framework of an EFSA-BfR Framework Partnership Agreement. Also, FCL was part of joint ECDC-EFSA crisis trainings. On the national level, FCL is involved in a project with the German federal state North-Rhine Westphalia on developing a data entry mask for supply chain data. Several modules of the tracing portal were developed in the framework of these projects and the aim of COHESIVE is to unify all of them under one umbrella – the FCL tracing portal.



6.1.3.3 JIP03-R2-IA2.3-CARE

6.1.3.3.1 Summary of the work carried out in the JIP

In the reporting period, the EJP CARE has progressed as planned and according to agreed work plan meeting the deliverables and milestones in time. The coordination runs smoothly with both scheduled virtual meetings between the two lead organizations as well as also among the WP leads. Additional calls have been conducted within the WPs to ensure compliance to the work plan. The progress is monitored in details and comprehensive way with bi-monthly reporting by the WP lead into a monitoring framework. The two lead organizations have participated in all activities organized by the OHEJP WP4 promoting and disseminating information about the scope of CARE through posters.

A mapping review has been conducted identifying available and existing External Quality Assurance (EQA) programmes offered to the National Reference Laboratories for zoonotic bacterial agents including antimicrobial resistance (AMR) from both the public health and veterinary side embracing the One Health concept. The mapping review will serve as a guidance in setting up new EQA scheme expanding to whole genome sequencing (WGS) which for many laboratories in EU are still a novel technology and approach. The scope is also to assess if EQAs could be facilitated in a jointly way using the same reference material across the EQA providers for assessing the quality of testing zoonotic bacterial agents including antimicrobial resistance. The mapping review is still in a draft version and expected to be delivered in July according to the proposal.

During the kick-off meeting, the pilot EQA on isolation, detection and characterization of pathogens were extensively elaborated in terms of approach and material deliverable (matrix). This discussion is still ongoing since deemed more complicated as anticipated but not anticipated to be delayed due to the later scheduled deliverable date. The project lead anticipates to have a schedule and plan for this EQA prior to the fall of 2020. The second EQA included the proposal progress as scheduled with the first output being available. Prior to selecting reference material for the genomic EQA assessing WGS quality control (QC) metrics and prediction of characteristics such as serotypes and AMR determinants, a target list was created of emerging clones at risk identifying relevant epidemiological traits such as MLSTs, virulence genes and AMR determinants to be tested in the EQA. The WP4, conducting risk assessment provided the basis of the list, which was further endorsed by the EURLs in question. The next step is to identify relevant candidates for the EQA. Similarly, to the EQA on isolation, detection and characterization of pathogens, a simulated exercise has been elaborated to assess the laboratories ability to conduct a reliable outbreak investigation based on a genomic cluster analysis to mimic a real scenario. This activity is in its early planning phase as scheduled later in the project period.

Progress has also been observed identifying the priority pathogens for which reference material should be collected. A list of nine pathogens; all known to be responsible for foodborne human infections, were agreed upon among the WP2 partners. The target list will serve as a basis for which gaps will be identified in available reference material to cover all known serotypes, genotypes and generally pathogenic variants of the selected pathogens which are relevant for human health, also including AMR mechanisms and associated metadata within the nine pathogens completing a repository/ database of reference material. A questionnaire has been drafted to inquire CARE partners about available reference material. Subsequently, the survey will be expanded to outside of CARE to complete the repository. The latter activity is about to be initiated after the summer of 2020 break.

During the kick-off meeting WP2 and WP3 discussed how to enable the use of and visibility of the reference material repository/ database with a mutual agreement on the structure of the repository/ database. This was further elaborated through a questionnaire survey conducted assessing also the Microbial Resources research Infrastructure (MIRRI) database structure. MIRRI is part of the European Research Infrastructure which aims to facilitate provision of high quality microorganisms through an European reference database centralizing strains and their metadata associated. MIRRI-IS will deploy



an integrated, high-quality, manually annotated, non-redundant micro-biological resource database which provides all relevant information data and associated contextual data. The final agreement and layout of the structure is about to be formulated.

The planned risk assessment activities have gained speed and momentum identifying initially whom to be surveyed including the target audience from the EFSA network for microbial risk assessment. The target group will be approached with a developed questionnaire to query criteria to assess the data quality and accessibility as well as what type of risk assessment data and associated data to record. A draft survey has been created for further enhancement and distribution.

In summary, the CARE project progress as planned meeting milestones and deliverables in time.

Please consult the attached progress monitoring framework schemes.

6.1.3.3.2 Progress of the project: description of activities

WPO

WP0-T1 Over-all project coordination

The coordination runs smoothly with bi-weekly meetings between the two lead organizations as well as also among the WP leads. In addition, one meeting every quarter with all partners. The dissemination plan and deliverables have been submitted and the DMP in preparation.

WP1 Develop of cross-sectional PT's (proficiency testing)

WP1-T1 Mapping of existing and proposals for new PT schemes

The purpose is T1 is to map the currently available PT schemes and to identify existing and propose new PT schemes that can be used in a cross sectorial context. The outcome of the work will be a short report (D.1.1). The T1 work was initiated at the CARE kick-off meeting in Copenhagen. There has been developed a draft paper, that includes a review of the currently available relevant PT schemes that potentially can be relevant form a one health perspective. The draft is summarizing the usefulness of the currently available PT's and gives a roadmap for the work in W1-T2. The final version of the document is still pending.

WP1-T2 Pilot trials and documentation of outcome (start month 31)

WP1-T2-ST1 Pilot PT on isolation/detection and characterization of pathogens

The task was discussed briefly during the kick-off meeting.

WP1-T2-ST2 Pilot PT on typing/characterization including WGS

The task was discussed briefly during the kick-off meeting. Some work has already been undertaken in order to identify the target organisms that should be included in the study.

WP1-T2-ST3 Pilot PT on outbreak surveillance based on WGS data

The task was discussed briefly during the kick-off meeting and there have been developed a short document describing a set up for the exercise.

WP1-T3 Development of guidance document with suggestions for design of future cross-sectorial PT schemes (start month 49)

This part of the WP has not started yet.

WP2 Creation of EUROpanelOH, a reference database of strains and genomes for effective quality control analysis in food safety and public health protection across sectors



WP2-T1 Inventory of current use and existence of reference materials across CARE / OHEJP partner institutes, for selected, prioritized pathogens and antimicrobial resistances

The time period has developed a minimum set of descriptive standard information to be associated with individual biological resources among CARE partners. A focus was made on bacteria listed as prioritized pathogens during the kick-off meeting. These attributes include qualitative characteristics and physical location of the biological resources as well as contact information of the supplier. Feedback on the attributes selected by the task leaders has been asked by using a questionnaire. Some additional attributes have been suggested and included in the final excel template of the inventory of resources. The template was sent to CARE partners on April 27th. The duly filled templates are expected before June 30th.

WP2-T2 Gap analysis with respect to accessibility, quality and usefulness of existing and potential new reference materials from a One-Health perspective

Nothing to report

WP2-T3 Production of additional RM to fill in gaps and/or improve characterization if needed

Nothing to report

WP2-T3-ST1 WGS characterization

Nothing to report

WP2-T3-ST2 MALDI-TOF characterization

Nothing to report

WP2-T3-ST3 Completing metadata

WP3 Access and sustainability of well-defined microbial reference materials (RM)

WP3-T1 Development of an information system for making RM more widely accessible and visible

The period was used to define the structure (Tables and fields) of standard information to be used to build the RM catalog. The resulting database structure was shared with other WPs for comments. It is planned to finalize it before sharing it with all the partners for the month of July.

WP3-T2 Ensure the long-term sustainability of RM collections

Nothing related to task 2 was performed during the period.

WP3-T3 Ensuring the long-term accessibility of the RM collection and its existence

Nothing related to task 3 was performed during the period.

WP4

WP4-T1

The initial first nine months of the project were dedicated to define the list of targeted institutes to who the survey will be addressed. Criteria to assess the data quality and accessibility were also defined. A draft survey has been designed and tested. The survey has been addressed to the institutes to collect the available risk assessment and associated metadata throughout EU.

WP4-T2 Metadata web platform

No specific tasks have been carried out related to task 2 during that period.



WP4-T3

No specific tasks have been carried out related to task 3 during that period.



6.1.3.3.3 Progress of the research project: deliverables and milestones

6.1.3.3.3.1 Deliverables

JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
CARE	D.0.1	Consortium agreement	M25	June 2020		confidential	9
CARE	D.0.2	Internal progress reporting templates	M26	June 2020		confidential	9
CARE	D.0.3	Dissemination plan	M28	June 2020		public	9
CARE	D.0.4	Minutes of the kick off meeting - 2020	M26	June 2020		public	9
CARE	D.1.1	WP1-T1: Report - Mapping of existing and proposals for new PT schemes	M30		Not due		
CARE	D.2.1	Shared database gathering all information	M34		Not due		
CARE	D.2.2	Sub-database for antimicrobial resistances	M35		Not due		



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JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
CARE	D.3.1.1	Database Structure for RM catalog	M32		Not due		9
CARE	D 3.1.2	Synthesis Documents after questionnaire processing 1/ partners expectations 2/ list of existing softwares 3/ technical choice	M35		Not due		9
CARE	D.4.1.1	A survey on (meta-)data relevant for risk assessment	M31	July 2020		co	9
CARE	D.4.1.2	The establishment of connection of this CARE activity with the databases and initiatives already in place	M35	November 2020		co	9

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



6.1.3.3.2 *Milestones*

JIP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
CARE	M.0.1	Kick off meeting - 2020	M25	Yes		This activity was conducted with participation of all partners as well as WP4
CARE	M.1.1	Kick off meeting for WP1	M25	Yes		This activity was conducted during the Project kick off.
CARE	M.1.2	Review of task 1 and description of relevant pilot PTs	M33	Yes		A draft review has been compiled and await comments from the WP1 partners.
CARE	M.2.1	Identification and localization of available RM among CARE and OH-EJP partners	M30	was due	Sept, 2020	The process is on-going by compilation of the inventories of strains (> 3000) for the prioritized pathogens.
CARE	M.3.1.1	Define list of fields for the RM database	M31	Yes		
CARE	M3.1.2	Building questionnaire on expectations of CARE partners for the RM online catalog	M31	Yes	Dec,2020	It was considered that a scenario approach submitted to partners would be more effective



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JIP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
CARE	M.4.1.1	Define the list of targeted institutes to who the survey will be addressed	M27	Yes		Based on MRA EFSA network
CARE	M.4.1.2	Definition of criteria to assess the data quality and accessibility	M28	Yes	M34	The criteria for assessing data availability were inserted in the survey. The list of criteria (data quality) might evolve in the next few months.
CARE	M.4.1.3	Definition of types of risk assessment and associated metadata	M28	Yes		QMRA types were defined according to codex definitions. The most commonly stated objectives (associated to risk questions) have been identified.
CARE	M.4.1.4	Test the draft questionnaire of a small panel of risk assessors	M30	Yes		<u>The draft has been tested by RIVM and ANSES during months 31 and 32.</u>



6.1.3.3.4 Follow-up of the recommendations and comments by the Ethics Advisors

Requirements of ethical reviewers in 2020	What measures and actions do you propose?
Satisfactory ethics self-assessment: Well addressed. Comprehensive completed but Nagoya section needs some further clarification.	Please see below.
(1) Biological samples: However, from the statement on Nagoya compliance, is the CARE team stating that all the biological resources that will or potential will be used in this project that may have been subject to Nagoya have been collected before October 2014 so are not subject to Nagoya regulations. The statement was not completely clear in the ethics checklist.	No, biological resources used in this project will be owned and originate from the partners. Should other biological resources other than that be used, a MTA or NDA be signed to ensure that CARE comply to the Nagoya protocol.
(2) Health and Safety: none	
(3) Other: Considering the implications of this work and what the CARE team hopes to achieve, are there any potential IPR or data access issues raised by this work? In WP4 who will have access to the “unique web platform”, the text implies only the OHEJP partners? Will all results, datasets and the reference collections “facilitated” through CARE be fully on Open Access? The team implies this in WP3 but can the team reconfirm please, thank you.	<p>There are discussions at DTU if the web platform will be withdrawn from the project.</p> <p>The datasets and the reference collections “facilitated” through CARE will be fully Open Access</p>

6.1.3.3.5 5. Impact of COVID-19 crisis on the project

N/A



6.1.3.3.6 Publications

None produced within the reporting period.

6.1.3.3.7 Data Management Plan

1. Have you uploaded a first version of the project's DMP to the DMP group on the OHEJP website?

No, as CARE has not yet received the template and no data has been generated.

2. Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.

Yes, CARE has not yet received the template and informed not being due by the WP4.

6.1.3.3.8 On-going and planned collaborations with national or European projects or networks

Collaboration with the EURL AR and potentially other EURLs about the WP1/T2/ST2 will be established to make most out of the generated reference materials for other PTs. Some RM will be included the task already developed and used by some of the other EURLs.

6.1.3.4 JIP04-R2-IA2.2-OH-HARMONY CAP

6.1.3.4.1 Summary of the work carried out in the JIP

Specifically, this summary will include: The composition of the OH-Harmony-Cap project group, an updated work plan, an overview of the various outcomes and agreements going forward, such as what was decided on the kick-off meeting (KOM).

On January 21st, the day before the KOM meeting in APHA, Addlestone UK, the WP leaders and WP deputy leaders met. The purpose of the meeting was to: firstly, form the OH-Harmony-Cap project group and formalize a common understanding of the roles and responsibilities, output specifics and expectation. Secondly, to give a project overview, discuss and prepare for the workshop next two days. The OH-Harmony-Cap project group consists of Project leader Nadia Boisen, Deputy Project leader Flemming Scheutz and WP leaders and deputy leaders, Mélanie Gay, Mónica Oleastro, Rosangela Tozzoli, Morabito Stefano, Patrizia Rossi, Tryntsje Cuperus, Joke van der Giessen, and Declan Bolton. Twenty-eight participants attended the KOM on the January 22nd and 23rd as well as ECDC contact person, Mike Catchpole. Specific outcomes and decisions included:

The WP2 survey should consist of one pilot survey, running M25-M36 followed by an adjusted survey, which will become the OHLabCap

Ten parasites using EURO-FBP criteria were chosen. Can be adjusted after the pilot survey Y3

Questions for WP2, incorporating capability, capacity, and interoperability was formulated

Questions for WP3 directed at sampling and testing was formulated

WP4 collection strategy was decided

The next OH-Harmony-Cap meeting will be held June 17th-18th in Uppsala, Sweden.

Originally, the development of the OHLabCap was divided into two pilot surveys targeting the National reference Laboratories (NRLs) and the primary diagnostic services. During the KOM it was determined, due to time constraints, to combine the two pilot surveys into one pilot survey running Y3 following an adjusted survey, Y4. Fortunately, this early change to the project will most likely help avoid further



delays to WP2. It was conveyed to all the participants, via email, on April 1st that a tentative way forward, and as a direct cause of the Covid19 crisis that it would be necessary to make changes to the work plan and time line which would affect and delay the deliverables and milestones in Y3.

The changes included: 1. WP3 questionnaire will be split into four specific questionnaires, based on model organisms, to ensure a better quality response. In addition, to design the questionnaire as quantitative as possible which will “simplicity” the output and analysis. 2. For WP4, a letter to the ECDC Food and Waterborne Diseases and Zoonoses Network (FWD-Network) and to the EURLs for E. coli, AMR and Parasites would be drafted in order to expand the collection of protocols. Of note, the collection of WP 4 protocols has commenced. 3. Related to WP2 and WP3, and to ensure timely response we agreed that the pilot survey and questionnaires should be sent to the EFSA and ECDC contact list. 4. Given the circumstances they will most likely not be sent out before October 2020 (we have, now, changed this to September 2020) and 5. The second OH-Harmony-Cap meeting, in Uppsala, will be postponed to September 2020. However, this might not be feasible.

It needs to be noted that after our KOM we were making good progress but that was quickly hampered by the COVID-19 pandemic. Currently, our primary concern is getting timely response and data to WP2, WP3, and WP4. The output of WP2 and WP4 feeds directly into WP5; here, we will 1. setup a workshop for communicating and discussing the outcome of the OHLabCap survey conducted in the framework of WP2 and 2. Conduct practical workshops and e-learning activities dedicated to the application of the harmonised protocols developed in WP4

Lastly, the OH-Harmony-Cap project changed project leader from Flemming Scheutz to Nadia Boisen on April 7th 2020.

6.1.3.4.2 Progress of the project: description of activities

WP1 Project coordination

WP start month: M25-M54

WP Leader: 13-SSI

WP participants: 1-ANSES, 9-BfR, 13-SSI, 30-RIVM, 26-Teagasc, 41-SVA

JIP4-WP1-T1: Internal coordination

Progress: As part of the internal engagement it has been important to effectively communicate the progress and status of the project.

- At the KOM we communicated a common understanding of the roles and responsibilities, output specifics and expectation.
- Formed the OH-Harmony-Cap project group
- Several emails to all participants with project overview, status, and decisions.
- Frequent conference calls with WP2, WP3, and WP4
- Three conference calls with OH-Harmony-Cap project group
- Conference call with the OH-Harmony-Cap consortium on July 14th

JIP4-WP1-T2: External coordination, outreach and engagement

Progress: As part of the external engagement and to effectively find synergies with other project, disseminate our project, progress and results, OH-Harmony-Cap has participated and presented

- Presented at the CARE, KOM on Feb 12-13th of February 2020.
- Presented at the Fifth cogwheel workshop between OHEJP and JPIAMR April 28th 2020.



- Presented at OHEJPASM2020 May 29th 2020.
- Stayed connected with external stakeholders; ECDC and EFSA by invited them to KOM and comment on Surveys and questionnaires.
- Established contact with Mike Catchpole (ECDC) and Frank Boelaert (EFSA).
- Reached out to Rachel Chalmers Head, Cryptosporidium Reference Unit, Public Health Wales for input and collection on Cryptosporidium protocols.
- Participation in other OHEJP teleconferences on February 14th, May 20th, and May 28th.

JIP4-WP1-T3: Data management

Progress: I participated in the Data Management Platform (CDP) – Training on July 29th and intend to start using the platform as soon possible most likely in line with the WP2 and WP3 survey process.

JIP4-WP1-T4: Sustainability

Progress: At this stage, sustainability activities are mainly entailed to external outreach and detailed in JIP4-WP1-T2

WP2: Development of the OHLabCap

WP start month: M25-M54

WP Leader: 13-SSI

Deputy WP Leader: 30-RIVM

WP2 participants: All OH-Harmony-Cap participants

JIP4-WP2-T1: Development and testing of a pilot survey (NRL)

Task start month: M25-M30

Task Leader: 13-SSI

Deputy Task Leader: 30-RIVM

Task Participants: All OH-Harmony-Cap participants

Progress: As mentioned in the summary above the development of a pilot survey, targeting NRLs, was expanded to include the primary diagnostic services (primary sector). This pilot survey, which will later become the OHLabCap, has been drafted covering; [1] capability, [2] capacity, and [3] interoperability. The pilot survey covers six priority bacteria and ten priority parasites have been chosen, as model organisms, together with the antimicrobial resistance (AMR) testing of *Salmonella* and *Campylobacter*.

They include:

- Shiga Toxin-producing *E. coli* (STEC), *Salmonella*, *Campylobacter*, *Shigella*, *Yersinia*, and *Listeria*.
- *Echinococcus multilocularis*, *Toxoplasma gondii*, *Trichinella spiralis*, *Echinococcus granulosus*, *Cryptosporidium* spp., *Trichinella* spp. other than *T. spiralis*, *Giardia lamblia*, *Anisakidae*, *Toxicara* spp. *Taenia solium*

The next stages include; [1] adapt the survey for the EU online survey tool; [2] distribute the pilot survey via the OH-Harmony-Cap consortium and via EFSA to the European Food and Waterborne Diseases and Zoonoses Network (FWD-Net) (humans) and the national contact points of the National Reference Laboratories (NRLs) of 28 MS, Iceland, Norway and Switzerland (food, feed and animal testing) for completion; [3] revise the pilot survey as required.



The current plan is to have stage 1 completed by the end of June (M30), stage 2 commenced in September. Note, the survey has been sent to EFSA (contact person, Frank Boelaert) and ECDC (contact person, Mike Catchpole) for comments

JIP4-WP2-T1-S1 (M25-M27): Compilation of indicators

Progress: The compilation of indicators (questions included in the pilot survey), across three dimensions from the NRLs and primary diagnostic laboratory services, have been completed. The scoring options used is similar to the EULabCAP survey and the pilot survey includes 63 questions covering National reference Laboratories and Primary diagnostic services.

JIP4-WP2-T1-S2 (M27-29): Choice of analytical software

Progress: The EU survey tool has been chosen - <https://ec.europa.eu/eusurvey/home/welcome>. Once the pilot survey has been finalised the survey will be adapted for the EU survey tool (stage 1).

JIP4-WP2-T1-S3 (M30): Finalise the first questionnaire (D-2.1)

Progress: The pilot survey has been sent to EFSA and ECDC for comments. Thereafter, stage 1 and stage 2 will commence.

JIP4-WP2-T2: Scoring of collected data and chosen indicators (NRL)

Task start month: M31-M36

Task Leader: 32-FHI

Deputy Task Leader: 30-RIVM

Task Participants: All OH-Harmony-Cap participants

Progress: The pilot survey has not yet been circulated among the survey population. We, have therefore not commenced the evaluation, assessment, and scoring of the collected data and chosen indicators. We expect that to be able to commence this task in October 2020

JIP4-WP2-T2-S1 (M31-M33)

Progress: See above

WP3: One Health Laboratory Interoperability Guidance

WP start month: M25-54

WP Leader: 26-Teagasc Deputy WP Leader: 36-INSIA

Participants: 1-ANSES, 9-BfR, 13-SSI, 21-APHA, 26-Teagasc, 27-ISS, 30-RIVM, 33-NVI, 34-PIWet, 35-INIAV, 36-INSIA, 39-SLV, 40-FOHM, 41-SVA

Shiga Toxin producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), *Cryptosporidium*, and AMR for *Salmonella* and *Campylobacter*.

JIP4-WP3-T1: Sampling and Testing

M25-36 (October 2019 – August 2020)

JIP4-WP3-T1-ST1; M25-M30: Establishing current practice (questionnaire)

Progress: Four questionnaires, on STEC, ETEC, *Cryptosporidium*, and AMR for *Salmonella* and *Campylobacter* have been drafted covering; [1] Sampling & testing; [2] Characterisation of methods and [3] Data management of harmonised reporting. The next stages include; [1] adapt these for the EU online survey tool; [2] undertake a pilot survey; [3] revise the questionnaires as required and [4] distribute via EFSA to the European Food and Waterborne Diseases and Zoonoses Network (FWD-



Net) (humans) and the national contact points of the National Reference Laboratories (NRLs) of 28 MS, Iceland, Norway and Switzerland (food, feed and animal testing) for completion

The current plan is to have stage 1 completed by the end of June, stage 2 completed in July, stage 3 undertaken in August and stage 4 commenced in September. Note, the questionnaires have been sent to EFSA (contact person, Frank Boelaert) and ECDC (contact person, Mike Catchpole) for comments. Comments have been received by Frank Boelaert and not Mike Catchpole. Note, both will be informed when we send out the surveys.

M31-M36: Summary report including

1. the current and best practice in designing statistically based sampling plans;
2. the most appropriate sampling methods;
3. all currently available testing (detection) methods;
4. how these are applied (application strategies)
5. The latest technologies/technological developments.

JIP4-WP3-T1-ST2 (M31-M36): Best practice

Progress: We have assigned writing tasks for the various sub-sections for our first technical report: Sampling and testing of Shiga toxin-producing *Escherichia coli* (STEC), Enterotoxigenic *Escherichia coli* (ETEC), *Cryptosporidium* and AMR in *Salmonella* and *Campylobacter* spp. in the European Union. The deadline for the drafting is August 21st.

WP4: Harmonisation of Protocols

WP start month: M25-M48

WP Leader: 27-ISS

Deputy WP Leader: 1-ANSES

WP4 participants: 1-ANSES, 9-BfR, 13-SSI, 21-APHA, 27-ISS, 32-NIPH, 30-RIVM, 33-NVI, 34-PIWet, 35-INIAV, 36-INSa, 39-SLV, 40-FOHM, 41-SVA

JIP4-WP4-T1: Collection of the laboratory protocols for model organisms

Task start month: M25-M33

Task Leader: 27-ISS

Deputy Task Leader: 1-ANSES

Progress:

During the kick-off meeting, it was agreed that besides collecting the protocols for the model organisms among WP4 participants, a similar request to ECDC Food and Waterborne Diseases and Zoonoses Network (FWD-Network) and to the EURLs for *E. coli*, AMR and Parasites would be issued. This letter aimed to reach the laboratories operating in these networks, both in the Public Health and Veterinary/Food Safety areas. WP4 participants collaborated in the preparation of the letter of request addressed to the Coordinator of ECDC FWD Program and the Directors of the three relevant EURLs. The letter of request was finalised in April (M28), thanks also to the contribution of the OH-EJP WP6 Communication team. The collection of protocols started in April among WP4 participants, and in May the request of collaboration in the collection protocols was sent to the ECDC FWD Network as well as the relevant EURLs. Because of the Covid-19 pandemic, we decided to postpone the request to the ECDC FWD Network and the EURLs, due to concerns that the request would be overlooked.



To date (August 31st), eleven Institutions operating in Public Health or Veterinary/Food Safety areas, and either participating in the OH-Harmony-CAP project or from other networks responded to our request. The documents shared so far consist in:

- 40 protocols on pathogenic *E. coli* (both ETEC and STEC)
- 12 protocols on *Cryptosporidium*
- 9 protocols on AMR in *Salmonella* and/or *Campylobacter*

The documents listed here include book chapters detailing lab procedures and one relevant publication.

The call for protocols will be reinforced periodically to obtain a wealth of protocols to be assessed in the following tasks.

JIP4-WP4-T2: Evaluation of the collected laboratory protocols

Task start month: M31-M39

Task Leader: 41-SVA

Deputy Task Leader: I-ANSES

Progress: A teleconference was held on August 18th between SVA, WP4 leaders and OH-Harmony-CAP coordinators to initiate this activity. Three working groups, one for each subtask on STEC/ETEC, *Cryptosporidium*, and AMR for *Salmonella* and *Campylobacter* composed of 4-6 participants with expertise and experience in respective subject has been formed. A first draft of an evaluation sheet has been produced. Also, the JIP4-WP4-T3 leader will be included in the process.

JIP4-WP4-T2-S1-S3 (M31-M39): Evaluation of the laboratory protocols for the detection and typing STEC/ETEC

Progress: A teleconference has been scheduled this month among WP4 leaders and OH-Harmony-CAP coordinators to initiate this activity. An STEC/ETEC working group has been formed.

JIP4-WP4-T2-S2 (M31-M39): Evaluation of the laboratory protocols for the detection and typing for AMR *Salmonella* and *Campylobacter*

Progress: A teleconference has been scheduled this month among WP4 leaders and OH-Harmony-CAP coordinators to initiate this activity. An AMR *Salmonella* and *Campylobacter* working group has been formed.

JIP4-WP4-T2-S3 (M31-M39): Evaluation of the laboratory protocols for the detection and typing of *Cryptosporidium*

Progress: A teleconference has been scheduled this month among WP4 leaders and OH-Harmony-CAP coordinators to initiate this activity. A *Cryptosporidium* working group has been formed



6.1.3.4.3 Progress of the research project: deliverables and milestones

6.1.3.4.3.1 Deliverables

JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category * (1 to 10)
OH-HARMONY-CAP	D-IA.2.2.OH-Harmony-Cap.1.1	Preliminary data management plan	M30	M32	M33		
OH-HARMONY-CAP	D-IA.2.2.OH-Harmony-Cap.2.1	Completed pilot survey	M30	M36	M36	Due to Covid-19 we have not be able to send out the pilot survey. Therefore, we have not be able to analyse the results	1
OH-HARMONY-CAP	D-IA.2.2.OH-Harmony-Cap.2.2	Technical report of the survey results	M36	M36	M36		2,4,6
OH-HARMONY-CAP	D-IA.2.2.OH-Harmony-Cap.3.1	Technical report on the current and best practice in sampling	M36	M36	M36		2,4,6

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of



surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity

6.1.3.4.3.2 *Milestones*

JIP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
OH-HARMONY-CAP	M-IA2.2.OH-Harmony-Cap.1	Kick-off meeting completed	M25	Yes		
OH-HARMONY-CAP	M-IA2.2.OH-Harmony-Cap.2	WP3-T1-ST1 completed	M30	No	M36	Due to Covid-19 we have not be able to send out the four questionnaires. Therefore, we have not be able to analyse the results.
OH-HARMONY-CAP	M-IA2.2.OH-Harmony-Cap.3	Preparing a list of methods for the detection, Characterisation and typing of the selected pathogens in the EU/EEA NRLs	M33	No	M36	Due to Covid-19, the request and collection of protocols started in May and not March. Note, the correct deadline should be M33 and not M30 according to ghannt chart.
OH-HARMONY-CAP	M-IA2.2.OH-Harmony-Cap.4	WP3-T1-ST2 completed	M36	No	M36	



6.1.3.4.3.3 *Follow-up of the recommendations and comments by the Ethics Advisors*

Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(1) Health and Safety: On the ethics checklist you have ticked a safety issue, therefore the beneficiaries must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	None received, none implemented
(2) Other – dual-use: On the ethics checklist you have ticked that this research involves some “dual-use items in the sense of Regulations 428/2009, or other items for which an authorisation is required”, therefore please provide a risk assessment and details on measures to prevent misuse of research findings must be provided.	Not relevant at present as no “dual-use items” were exchanged nor cultured.

6.1.3.4.4 **Impact of COVID-19 crisis on the project**

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Meeting Uppsala	M30	M33 ?		M30	M33?	Travel restrictions and social distancing due to Covid-19		



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Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Pilot Survey (WP2)	M30	M36	D-IA.2.2.OH-Harmony-Cap.2.1	M30	M36	Due to Covid-19 we have not be able to send out the pilot survey. Therefore, we have not be able to analyse the results		
WP3-T1-ST1 completed (WP3)	M30	M36	M-IA2.2.OH-Harmony-Cap.2	M30	M36	Due to Covid-19 we have not be able to send out the four questionnaires. Therefore, we have not be able to analyse the results.		
Preparing a list (WP4)	M33	M35	M-IA2.2.OH-Harmony-Cap.3	M33	M35	We had foreseen to finish the collection of protocols by September 2020. Due to Covid-19 we might need to postpone this milestone, to M35-M36, seeing the request of collaboration from networks other than Harmony project's participants was sent out in May and not in March.		

Comments:

In general, due to the Coronavirus (COVID-19) outbreak, the progress of the WP2, WP3, and WP4 have been limited since early March. The COVID-19 pandemic rapidly evolved (is still evolving), and multiple countries closed borders, public institutions and encourage people to work from home. With most working remotely, focusing on the well-being of loved ones and neighbours, and mainly addressing urgent matters related to work, such as our colleagues operating



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in public health and pandemic-associated investigations, delays were inevitable. As such, in late March it became clear that it would not be possible to send out surveys (WP2), questionnaires (WP4), and request of protocols (WP4). Also, regarding budget changes then we are awaiting feedback from project partners.

Our second OH- Harmony-Cap meeting was planned for Uppsala Sweden June 17th. For reasons, due to Covid-19, we have to postpone this. We were going to rescheduling to the fall however, it this does not seem possible. Therefore, we will arrange a conference call with the consortium in late October once we have received results from the WP2 and WP3 surveys.



6.1.3.4.5 Publications

Currently, we have no peer reviewed publications linked to this project

6.1.3.4.6 Additional output

Data Management Plan

Have you uploaded a first version of the project's DMP to the DMP group on the OHEJP website?

No

Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.

6.1.3.4.7 On-going and planned collaborations with national or European projects or networks

Currently, we are examining unpublished sequences encoding Stx1 and Stx2 in STEC in order to establish possible new subtypes and variants. This work is in collaboration with RKI, Germany, FDA, FSIS, CDC and NCBI, USE and with Health Canada. The work is specifically related to WP4.

Furthermore, we are developing databases with all relevant virulence genes from ETEC in collaboration with Swedish colleagues. The work is also specifically related to WP4.

6.1.3.5 JIP05-R2-IA2.1-MATRIX

6.1.3.5.1 Summary of the work carried out in the JIP

MATRIX started on January 1st 2020. The project aims to advance the implementation of One Health Surveillance (OHS) in practice by building onto existing resources, adding value to them and creating synergies among the sectors at the national level. One of the founding pillars of MATRIX is the knowledge that each country has different infrastructural and economic realities with respect to setting up functional OHS, and all MATRIX outputs will take into consideration these different realities.

The project officially launched on January 1st 2020. After members gathered thoughts and needs within their own institutions and partner institutions in the same member state (MS), a physical full consortium meeting was held February 6-7th in Lisbon, Portugal. All partner institutes were represented in the meeting and 39 individuals participated in the scheduled sessions. The sudden and severe emergence of COVID-19 has meant that most of the public health (PH) institutes involved in MATRIX have had to deal with significant re-prioritization of tasks, and have re-directed large proportions of their personnel resources to the pandemic response. Some institutes including Animal Health and Food sectors were also impacted by stay-at-home orders regionally or nation-wide. Another major obstacle has been the impediments to recruiting caused by the travel restrictions and an overall stall on organization processes. As such, delays in some tasks were unavoidable. However, some institutes were able to continue efforts in bridging across sectors, and the situation may have even been strengthened by the opportunity for laboratories and epidemiologists from the animal health (AH) and food safety (FS) sectors to help support the epidemic control work carried by the PH institutes in the respective MS. Some tasks were simply re-planned, or given a new focus in light of the new demands brought to light by this unprecedented situation.

In the kick-off meeting, each of the six work packages (WPs) within MATRIX presented their plans for the coming year. During six parallel sessions, the WPs agreed on several important steps to coordinate work between WPs and existing OHEJP projects – primarily to avoid overlap and duplication of work. WPs with similar tasks (WP1 & WP2 and WP4 & WP5 in particular) decided to share the tasks between



them and plan to hold regular meetings to discuss progress. Overall, the consortium agreed that as much information as possible should be collected from ongoing OHEJP projects. After the majority of OHEJP Project deadlines were extended for the first-call projects, SVA began to coordinate with the Project Management team that all relevant projects (ORION, COHESIVE, and NOVA) will find time to provide an overview of the current status of the projects to members of MATRIX. ORION conducted a webinar in June and the other webinars are in the process of being organized in the fall. Files will be shared through available platforms and final documents uploaded to the OHEJP official website under the closed MATRIX forum. The coordination team announced the intended plan for two physical consortium meetings per year as well as full consortium teleconferences every six weeks. However, the second 2020 physical meeting was cancelled and will be an online meeting due to Coronavirus restrictions.

COVID-19 has had a significant impact on work in all OH sectors across Europe and as a result, including the planned progress within MATRIX, especially for the first 6 months. The long-term consequences of the COVID19 pandemic are still unknown, however at present it is evident that at least two online consortium and several WP-specific meetings have been cancelled, new personnel has not been hired, causing some disruptions in the ability to work on MATRIX. In addition, one institute had to withdraw entirely from the project. The coordination team will meet and make plans for how to mitigate further impacts on the project.

On June 4th 2020, the coordination team held the first consortium-wide meeting since the kick-off meeting. During the meeting, timelines were readjusted as a result of the resource re-allocation during the pandemic and the Call 1 project extensions. One deliverable in WP2 and one deliverable in WP4 needed to be delayed until Y4 but the other deliverables are expected to be able to be completed as expected.

6.1.3.5.2 Progress of the project: description of activities

WP1 “Existing frameworks and OH capacity”

During the kick-off meeting, it was agreed that WP1 and WP2 should work closely together to gather information from ongoing EJP projects. The work in WP1 will initially focus on *Listeria* and *Salmonella* and then expand to include other pathogens/hazards. The inclusion criteria for the pathogens/hazards include the relevance for humans and animals, output compatibility, relevance for at least one partner country, data availability for all sectors, and data availability at comparable geographical levels. The WP participants also agreed on the importance to keep up-to-date with ECDC and EFSA tasks and projects.

WP1 and WP2 intended to have a meeting before the summer of 2020 to streamline future work, but a meeting has not been feasible so far. However, data collection was initiated and will continue over the next months and online meetings will be implemented during summer.

JIP5-WP1-T1: Generate an inventory of existing frameworks for surveillance of inter-sectorial hazards, by sector (AH, PH, and FS)

Within WP1, information is being collected on OH collaborations and existing operational OH frameworks in each country. This is based on information collected from those ongoing EJP projects, who have collected similar information (particularly NOVA, ORION and COHESIVE) and on information provided directly by each participating institute. The definition of inclusion and exclusion criteria for surveillance programmes has begun and will be continued within WP1.

WP2: Best-practices and multi-sectorial collaboration



Given the recent epidemiological situation regarding COVID-19 in Italy, the WP2 lead, IZSAM, has been involved in many activities in response to the outbreak. For this reason, there were some difficulties in meeting agreed upon deadlines decided at the kick-off meeting in Lisbon. However, WP2 started to collect information and outputs from other EJP projects, such as NOVA, ORION and COHESIVE. A new project named "WP2 Best-practices and multi-sectorial collaboration" has been created within the MATRIX closed group at onehealth.ejp.eu to share documents between WP2 members.

JIP5-WP2-T1: Mapping of the surveillance chain across all sectors for each hazard-track

This WP will begin to use the information gathered in WP1 to provide suggestions for best-practice data collection, analysis and dissemination of surveillance results. During the kick-off meeting, it was agreed that this WP could make good use of the ORION surveillance inventory (ORION WP2 Epi) to identify cross-sectorial frameworks that are already in place or could be used for a full OHS. This WP will not include individual projects (such as temporary local surveillance projects) unless there is scope within these for large-scale coverage or important new knowledge. A special focus of WP2 may be to use case-studies of different countries at three different levels of OHS: (1) no OHS is set up or working, (2) partial OHS is set up or working, (3) full or almost full OHS is set up or working. Indicatively, the level of OHS will be estimated taking into consideration the engagement in OHS of one or more of the three sectors (AH, PH, and FS). Moreover, each case study may be focused on one hazard track and one production chain, as a specific "case-hazard-production" (for instance, *Campylobacter*-poultry).

While this WP is waiting on preliminary results from WP1 on the inventory of existing surveillance frameworks to complete the assessment of the additional information to be collected, the leaders of Task 1 will collaborate with the leaders of WP2 Task 2 for agreements on deliverable sharing and templates.

JIP5-WP2-T2: Identify cross-sectorial surveillance chain linkages, particularly, which outputs should be shared and how they should be shared for OH orientated decision making

Not yet started.

JIP5-Task: WP2-T3: Propose best-practice guidelines and effective strategies for data collection, analysis, and dissemination aimed at multi-sectorial OH collaboration, for each specific hazard-track.

Not yet started.

WP3: Output-based surveillance evaluation

WP3 aims to review and build upon work in EJP projects and previous European funded projects to look at the practicalities of implementing output-based surveillance within a one-health context. Unfortunately, one of the task leaders and major contributor to this WP has had to withdraw from the consortium due to COVID-19. While progress has been slowed down by the COVID situation, and the loss of a partner (UCM-Visavet), this WP organized a teleconference meeting this Spring, and the WP leaders are working to redistribute the tasks of this former partner. It is anticipated that the tasks will be redistributed and/or re-planned without compromising the quality of expected deliverables. Attempts are being made to reallocate UCM's budget.

JIP5-WP3-T1: Inventory of previous work and current practice

Currently working on a literature search of papers relevant to the topic of output based surveillance. A mendeley group has been formed for the work package to enable collaboratively working on the task. One emergency meeting was conducted to address the withdrawal of one of the partners. Plans for meetings (teleconferences) every two months was delayed by the WP3 leader since her immediate obligations were assigned to COVID-19 duties. Progress on MATRIX activates was reprioritized around June.



JIP5-WP3-T2: Identification of operational partners and stakeholders

At the kick-off meeting, it was decided that this WP will start with a literature review and stakeholders' mapping exercise. The WP plans to use RISKSUR as input and use the information gathered in the SIGMA project for stakeholder mapping. The WP plans to set up a case study in year two, focusing on a topic of significant relevance to most of the EU region.

WP4: EUepiCap

In WP4, a generic benchmarking tool for characterizing, monitoring and evaluating surveillance capacities (the EUepiCap) which directly contribute to OHS will be developed. The surveillance will cover a pathogen/hazard that is OH relevant and must have a structure that is useful for all sectors in the OH chain. The development of the tool will be step-wise, starting from a semi-quantitative approach and aiming for a quantitative approach. The WP plans to develop a forecasting tool for the impact of alternative improvements of OHS – which can to some extent overlap with WP5. WP4 and WP5 will therefore keep strong collaboration and synergy, and a web meeting to coordinate the work in WP4 and WP5 was held on 28th February 2020.

JIP5-WP4-T1: Review of existing methods for surveillance systems evaluation

WP4 leaders began inventorying existing methods of evaluation of surveillance system (evaluation of surveillance functioning and evaluation of collaborations) and are going through the literature on these methods and case studies to identify indicators that would be relevant for the EUepiCap tool. They aim to identify indicators relevant for evaluation of collaborations at each step of surveillance (planning, data collection, data sharing, data analysis, result dissemination, etc.) at governance and operational levels.

Following the kick-off meeting, they have so far received an evaluation of surveillance systems for vector-borne diseases (from SVA).

WP4 started identifying information needed for evaluating the functioning and relevance of surveillance collaborations, and forecasting cost-effectiveness of any additional collaboration within a multi-sectoral surveillance system. The evaluation will take into account, contextual variables (e.g. importance of the hazard in different countries), results of evaluation of the functioning of surveillance system, surveillance effectiveness, economic data, and evaluation of collaborations. Existing data for those variables will be collected for each hazard from the literature and from consortium partners. A scientist was recruited by University of Surrey and recruitment is in progress at ANSES for MATRIX (both recruits will be working part time on MATRIX).

WP5: Outreach and roadmap

WP5 has two major tasks. First, it will produce a roadmap for developing and implementing national OHS activities targeted at different capacity levels. Discussions are being planned between COHESIVE and the leaders of this first task to determine synergies and possible continuation of tasks that are not able to be completed by COHESIVE. More specifics about coordinating these efforts will be decided as COHESIVE's deliverables start taking shape during their last year of activities, and the format and content of their outputs can be communicated to the OHEJP starting projects, such as MATRIX. The second major task is the implementation of a Knowledge-Integration Platform that supports knowledge exchange between different MATRIX partners with close connection to the overarching EJP platform. This platform will offer resources (e.g. tools/technologies/features) that support collaborative collection, exchange, management and creation of knowledge. The Knowledge-Integration Platform will further facilitate the dissemination and permanent access to the generated



project outcomes from all MATRIX WPs. This task will also make sure to reuse/build upon COHESIVE's outputs.

JIP5-WP5-T1: Perform a requirement analysis for national OH surveillance roadmaps

- This task shows high potential for synergies with WP4. Therefore, a strong collaboration between both WPs was agreed. To coordinate this, a WP4-WP5-web meeting took place on 28th February 2020. Also, it is agreed that WP4 and WP5 members can participate in WP-specific meetings. This already started in the WP4 meeting in July, where a member of WP5 actively participated.
- The planning on the execution of the requirement analysis for the national OH surveillance roadmaps started. Among other activities, the resources from other EJP projects like COHESIVE will be analysed.

JIP5-WP5-T3: Knowledge-Integration Platform

- The requirement analysis for the Knowledge-Integration Platform (KIP) started. Among other activities, the technical resources from other EJP projects like ORION, COHESIVE, and RADAR were analysed.
- As a first outcome it was decided that the KIP should support the following aspects:
 - Exchange of mathematical models from different OH sectors. To accomplish that it will be necessary that the dedicated KIP section supports harmonized information exchange formats, e.g. the Food Safety Knowledge Markup Language standard (see <https://foodrisklabs.bfr.bund.de/fsk-ml-food-safety-knowledge-markup-language/>). The extension of the FSK-ML towards PH/OH started. For that, web meetings that deal with FSK-ML with members of the RADAR project and the RAKIP initiative (who maintains FSK-ML) were conducted. Re-implementation of existing OH risk models as FSK-ML compliant files started with the purpose of validating the proposed PH/OH FSK-ML extension. Dedicated meetings that dealt with technical questions of example source-attribution models took place with domain experts.
 - Knowledge on uncertainty assessments. For this BfR will perform as a first step an internal pilot study that will create an internal knowledge base on uncertainty assessments. Based on the feedback from that pilot a dedicated section in the KIP will be implemented.
 - Supporting the WHO/OIE/FAO Tripartite Surveillance and Information Sharing Operational Toolkit (SISOT) – for this WP5 participated in several SISOT web meetings to find potential synergies and to align the work of WP5 with the WHO/OIE/FAO team.
 - A close collaboration between MATRIX and ORION was established, i.e. members of MATRIX participate regularly in relevant ORION calls.

JIP5-WP5-T4: Training and dissemination

- Presentation about MATRIX on the Cogwheel workshop (28th April 2020)
- Presentation about FSK-ML and its extension towards OH on the Annual Scientific Meeting (27th-29th May 2020)

WP6: Decision and collaboration dashboards

JIP5-WP6-T1: Country-based Identification of existing cross-sectorial OHs activities

This WP will create dashboards of OHS inputs and outputs, highlighting possible pitfalls and biases in multi-sectorial data analysis. Apart from data analysis and interpretation, the dashboards will also indicate which persons to contact in a specific situation such as an outbreak of a zoonotic disease. The WP will build on knowledge from particularly NOVA and COHESIVE and will ensure that synergies are created with the COHESIVE dashboards in order to avoid overlap. At the kick-off meeting, it was agreed



that the partners in the WP would communicate primarily via emails, focusing on sharing ideas, and during the second physical consortium meeting, planned for the fall, the group would revise the ideas and trace a strategy for the work during the rest of the year. With the cancelation of the physical meeting, the WP leader plans to call an online meeting early in the fall 2020.

WP0: Coordination

At the kick-off meeting, all participants were specifically informed about the dissemination strategy and reminded that deliverables are considered public unless an explicit request is made to make it private. Regarding general coordination of the project, it was agreed that:

- Teleconferences.** The coordination team will not provide any specific platform for web-meetings, and each WP leader should use the resources made available by their own institutes to host WP meetings. Each WP leader is responsible for arranging WP meetings when necessary. The MATRIX coordination team will host consortium and coordination meetings using SVA's license for Adobe Connect. WP leaders wanting to host public seminars or record sessions for public distribution using Adobe Connect should contact SVA to arrange.

- File sharing.** The MATRIX closed group will be based at the onehealthejp.eu website. However, because it is difficult to organize files here, this platform will not be used to share working files. Each WP leader is expected to manage file sharing according to their own needs and institutional resources. The MATRIX group at the OHEJP website will be used only to upload final documents (e.g. meeting minutes and deliverables).

- Meeting minutes.** The coordination team has created an online file to document all meetings, using the same file with the latest minutes on top. This allows all members to find all resources and links in one place.

- Other resources.** The coordination has created an online Google documents page with a link to all relevant resources as well as an updated address/contact list for all WP leaders and the whole consortium. It was agreed that participants are responsible for updating their contact information (including adding new members or removing others from their institution). All WP leaders need to use the latest contact list version. The coordination team will not keep track of the participation in the different WPs and institutions can change WP participation as necessary, making sure to keep the WP leaders informed. All relevant documents will also be added to the FORUM space on the MATRIX homepage at the OHEJP website. The presentations and minutes from the kick-off meeting were shared with the consortium on the Google documents page.

- Data management plan.** During the kick-off meeting, it was discussed how to set up the DMP and who should be responsible for this. There were no volunteers to take on this task, and it was agreed that the coordination team should assume the responsibilities.

One other Consortium-wide meeting was conducted on June 4th, 2020. Due to the significant impact of personal and institutional capabilities to work on MATRIX activities. The goal of the meeting was to meet again to remind each other of our work groups, check in to see any progress, and take the time to get all partners refocused on MATRIX activities.

Hazard tracks

The hazard tracks, which are a signature feature of MATRIX, will ensure that work in all WPs is directly relevant to specific pathogens/hazards. The tracks as follows were chosen based on institution work priorities and OH impact relevance: (1) *Campylobacter*, (2) *Salmonella* (3) *Listeria* and (4) Emerging threats. During the kick-off meeting, it was highlighted that not all WPs will have work that is focused



on track-specific outputs and therefore do not have to deliver work relevant to all tracks. The consortium agreed that it was not necessary to increase the number of tracks, as it is not guaranteed that work can be delivered for more than four tracks.

The Emerging Threats track was highlighted in the kick-off meeting. It was discussed whether this encompasses a general preparedness against emerging threats or a focus on specific new emerging pathogens. The consortium agreed that each WP may choose one of the above scopes, according to the specific contents of the WP and the interests of the WP participants.



6.1.3.5.3 Progress of the research project: deliverables and milestones

6.1.3.5.3.1 Deliverables

JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
MATRIX	D-WP0.1	Data Management Plan - draft	M36		TBD	On June 15 th , the Project leader received the email indicating the new DMP template will be promoted and disseminated to DMP leaders in June. The draft will be due at a date to be determined later.	3
MATRIX	D-WP1.1	Report on the commonalities and differences of the different operational frameworks in AH, PH, and FS	M36			Should be delivered on time in M36	1
MATRIX	D-WP2.1	Mapping of the surveillance chain for all hazard tracks, and cross-sectorial linkages	M36		M42	This deliverable will be delayed due to impacts from COVID	1



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M25-M36



JIP code	Project deliverable number	Deliverable name Original name, if different from the actual one	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted on time: Forecast delivery date	Comments Please mention: public or confidential, the Zenodo reference and other comments	Proposed category* (1 to 10)
MATRIX	D-WP4.1	Report on the selected set of criteria for evaluation of epidemiological capacities.	M36		M39	This deliverable will be delayed due to impacts from COVID	4

* Categories of Integrative activities (10 options): 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities (response); 7. Prevention: aligned use of facilities and models; 8. Other (please specify); 9. This is supportive to an integrative activity; 10. This is not an integrative activity



6.1.3.5.3.2 *Milestones*

JIP Code	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MATRIX	M-WP3.1	End of the first round of OHEJP projects: this will coincide with the end of the inventory and requirement analysis phase in most WPs	M36	No		The end of the first round of OHEJP Projects was extended due to Coronavirus. Therefore, our inventory and requirement analysis will attempt to obtain the resources from the projects directly instead of relying on the publicly available expected outcomes.

6.1.3.5.4 **Follow-up of the recommendations and comments by the Ethics Advisors**

Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(1) Non EU countries (Caribbean island of St. Kitts): On the ethics checklist you have not ticked non-EU countries, please edit and tick as the Caribbean island of St. Kitts. The beneficiaries must confirm that the research conducted outside the EU is in compliance with H2020 rules.	The Caribbean island of St. Kitts is intended to be provided a copy of the roadmap to utilize as an in country tool which will provide the roadmap proof of concept. However, they are not beneficiaries as they are not participating members of the project nor are they receiving any funding. Therefore, they were listed as "involved" but no potential ethic issues are raised.



Requirements of ethical reviewers in 2020	What measures and actions do you propose?
(2) Personal data processing: The beneficiaries must confirm that no personal data will be collected as part of the project; otherwise the GDPR (EU 2016/679) must be applied and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.	St. Kitts will not be a beneficiary of any funding or participation in the work packages.

6.1.3.5.5 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
JIP5-WP2-T1: Mapping of the surveillance chain across all sectors for each hazard-track	M30	M35	D-WP2.1	M36	M42	COVID-19 affected start time		
JIP5-WP4-T1. Review of existing methods for surveillance systems evaluation	M36	M39	D-WP4-D1	M36	M39	COVID-19 affected start time for collaboration efforts		

Comments:

In March 2020, the increasing rates of novel coronavirus COVID-19 resulted in a general lock-down of many European countries and importantly a drastic need for all public health agencies to shift their work focus towards this disease and its impacts. The emergence of COVID-19, also affected the work



undertaken in the veterinary and food safety sectors in Europe. As a result, some partner institutes in the MATRIX project have had delayed starts to tasks.

Specifically regarding WP2, since the epidemiological situation regarding COVID-19 was of particular concern in Italy during the first half of 2020, the WP leader IZSAM, had an unexpected effort to support all the activities in response to the emergency as well as required to follow stay-at-home orders and a general high impact to daily life. For this reason, some MATRIX related activities have suffered a delayed start and the deliverable D-WP2.1 required a 6 months extension. Some partners were able to continue or revise their collaborative, cross-sectoral work planned to be carried out under this WP, but coordination of these activities will depend on IZSAM being able to recuperate their leadership position in the WP.

The following specific impacts of the COVID-19 pandemic on MATRIX have already occurred:

- Minimal teleconferences or online meetings (consortium-wide or WP specific) were held since the kickoff meeting on February 5-6 through early June.
- The planned physical meeting between WP1 and WP2 leaders and participants was cancelled.
- MATRIX personnel at several institutes were reassigned to work full time on COVID-19 and may have been unable to register the time to work on MATRIX work intermittently during the first several months of the year. Recruitment of new MATRIX personnel was significantly delayed or indefinitely postponed for some partners. The allocated budgets may be impacted for the year, but we will be able to determine the full impact later this year.
- The work in WP1 and WP2 to gather existing OHS information from other EJP projects by the end of April was prematurely halted, due to the OHEJP Project from the first call being granted an extension of their deliverables.
- One partner institute (VISAVET UCM) was forced to withdraw their participation in MATRIX entirely. This particularly affects the content and timing of deliverables in WP3.
- The planned physical full consortium meeting in Teramo in October 2020 was canceled to follow travel restriction guidelines and social distancing recommendations. The Coordination team will be identifying the best method of continuing.

The long-term specific impacts on the project are still unknown, but overall it is expected that deliverables and milestones will be initially delayed, but we will make up time for most tasks along the project. Some tasks will be revised or their focus re-drawn, in particular in cases where partners were unable to hire new employees, and where tasks were planned to be lead by Visavet, who left the consortium. Timelines will be assessed at regular intervals during the upcoming years.



6.1.3.5.6 Publications

There are no MATRIX based publications at this time.

6.1.3.5.7 Additional output

E. M. Sundermann (BfR): Introduction on MATRIX; Cogwheel workshop, 28th April 2020, slides are available here: <https://onehealthjp.eu/wp-json/pm/v2/projects/74/files/20109/users/1602/download>

E. M. Sundermann et al. (BfR): FSK-ML and its extension towards OH, Presentation on the Annual Scientific Meeting (27th-29th May 2020), slides are available here: <https://onehealthjp.eu/wp-json/pm/v2/projects/74/files/20110/users/1602/download>

6.1.3.5.8 Data Management Plan

- Have you uploaded a first version of the project's DMP to the DMP group on the OHEJP website?

No Data Management Plan has been created for MATRIX at this time. The newest DMP training was attended by the MATRIX Consortium leader (who is the appointed person responsible for this task) on September 9th, 2020.

- Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.

When MATRIX began, the Data Management Plan was under review and we were instructed not to proceed with any DMP until the Project Management team agreed upon a template.

6.1.3.5.9 On-going and planned collaborations with national or European projects or networks

Several MATRIX members are also members of ORION, and will be able to transfer results and add value to them within MATRIX. WP1 leaders in MATRIX are also leaders of the work package on epidemiological knowledge sharing in OH in the ORION project. Leaders of MATRIX WP5 and WP6 are part of an initiative in ORION – called “supra-national One-health pilot” that is working closely with EFSA and ECDC to determine how the OH tools developed in ORION can be applied to the European Union level. We expect that work to benefit directly the roadmap and outreach work that we will develop in MATRIX's WP5.

WP1 is working closely together with WP2 of ORION, where FLI is also actively involved. Furthermore, the FLI is engaged in different working groups and projects with EFSA, working on surveillance and different animal diseases.

WP2 leaders in MATRIX are also leaders of the COHESIVE work package 4 on data platform to facilitate risk-analysis and outbreak control in this project and deputy leaders of task 2.1 about availability of food purchase data and barriers of the NOVA project WP2. The WP is also collaborating with the project BeONE in the evaluation and implementation of national level data sharing (WP3) as lead and deputy lead on several tasks.

In addition, WP2 leader (IZSAM) is the coordinator of the SIGMA Consortium, a project funded by the EFSA, which aims at finding solutions for facilitating the exchange of data between Member States and EFSA. Although the SIGMA project is limited to animal health issues, the data mapping solutions and the tools under development in this project can be of interest for MATRIX, as possible approaches applicable also in food safety.

The WP3 leader is also closely involved in COHESIVE WP2 (development of OH implementation guide) and COHESIVE WP3 (sharing of OH signals within and between countries) and is able to provide a point



of contact to all the WP leaders in COHESIVE. Discussions have already taken place regarding having a webinar to discuss the COHESIVE WP outputs developed up to this point.

WP5 is collaborating extensively with other projects. The extension of the FSK-ML format planned in WP5 is also relevant for the EJP RADAR project. In the RADAR project, a model repository was developed that is already based on the FSK-ML standard. We are in close communication with the responsible partners. In Task JIP5-WP5-T3, models compliant to the FSK-ML standard are implemented. Mathematical models and their exchange are relevant in multiple other EJP projects, e.g. EJP DiSCoVer and CARE. We are planning a meeting regarding this topic. The development of FSK-ML format and the including metadata schema to annotate knowledge started within the research project “Risk Assessment Modelling and Knowledge Integration Platforms (RAKIP)”. The RAKIP community, with partners from ANSES, DTU Food, and BfR, continue the collaboration to improve and extent the community-driven metadata schema. The work done in WP5 shows high potential for synergies with the work of the WHO/OIE/FAO SISOT-team (Surveillance and Information Sharing Operational Toolkit). We are in close contact with the team to align the work and potentially identify reciprocally beneficial tasks.

WP6 deals with the creation of “data for decision dashboards”. The WP is collaborating with a similar WP in the OHEJP project BeONE.

6.1.4 Task 4.3: Integrative support

6.1.4.1 Subtask 4.3.1: Alignment with strategic initiatives at EU level:

This subtask is responsible for the arrangement of so-called cogwheel workshops, activities aimed at allowing EJP partners (typically coordinators or WP leaders of JRPs or JIPs) to identify synergies, joint priorities and opportunities for collaboration with external actors/initiatives. All OHEJP projects are asked for input about which external initiatives to interact with in the cogwheel workshops. A list of relevant external projects is also provided by WP2.

In January, the fourth cogwheel workshop was arranged, targeting the EU project InfAct (Information for Action). A total of 40 partners and 28 countries are involved in the project, aiming at strengthening national and EU health information systems by establishing a sustainable research infrastructure, strengthening European health information and knowledge bases to reduce health information inequalities and supporting health information interoperability and innovative health information tools and data sources. For more information see deliverable 4.14.

The fifth cogwheel workshop was arranged in April, targeting five projects funded through the 9th call from the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) on diagnostics and surveillance; MAGITICS (machine learning for digital diagnostics of antimicrobial resistance), K-STaR (a K-mer -based approach for institutional antimicrobial resistance surveillance, transmission monitoring, and rapid diagnostics), OASIS (One Health antimicrobial resistance surveillance through innovative sampling), TRIuMPH (improving the tricycle protocol: upscaling to national monitoring, detection of CPE and WGS pipelines for One Health Surveillance) and IDx (an exploration of regulatory, corporate, relational, and technical barriers to uptake of diagnostics in the fight against antimicrobial resistance). For more information see deliverable 4. 18.

The sixth cogwheel workshop will be arranged during the autumn Y3.

There is a need for additional integrative activities, which can attract OHEJP partners outside the JIPs to take part of the results and outcomes. A proposal was developed by OHEJP WP4 and this was presented at the SSB meeting March 19, 2020. New activities are depending on a reallocated budget on a co-fund basis. The SSB meeting decided that a simulation exercise was of the highest priority. The planning for this will start as soon as the financing is in place. In addition, budget has been reallocated



into WP4 to finance the development of a web application of the decision tool produced by COHESIVE. This application will be connected to the MVNA website.

All JIPs have been assigned a contact point to EFSA and ECDC, respectively.

6.1.4.2 Subtask 4.3.2: Support function for integration of additional partners in ongoing JIP:

This subtask is responsible for so called integrative missions, aimed at helping OHEJP partners that are not originally partners of the JIPs ORION and COHESIVE to join in. No more partners have joined the first call JIPs this far during Y3. Many institutes are very occupied by duties related to the COVID-19 situation and resources to join new activities are scarce. Because of travel restrictions and a limited interest, already before the COVID-19 crisis, for Short Term Integrative Missions (STIMs) and Integrative Mentoring (IM) the budget allocated for these activities has been reallocated to other integrative efforts. ORION has initiated a supra-national pilot allowing people to spend a longer period with EFSA and ECDC. This initiative is very appreciated by the stakeholders. ORION and COHESIVE also work close to OIE and FAO.

6.1.4.3 Subtask 4.3.3: Scientific meetings to enhance and leverage integration:

WP4 supported WP3 in the successful organisation of ASM 2020.

A specific task for WP4 is to arrange Thematic Integrative Meetings (TIMs). These annual digital workshops aim to facilitate integration across the OHEJP domains, but within themes. One TIM was organised in April with the title "Practical use on NGS". Two topics were addressed; Topic 1: Getting from raw NGS data to outbreak detection and Topic 2: The future roles of NGS data producers and data collectors/users. The meeting attracted 79 participants.

6.1.5 Task 4.3: Organisation of call for additional JIPs for the period Y3-Y5

In late 2018 (Y1), the 2nd OHEJP call for projects was launched. The proposals were evaluated and three new JIPs were approved during Y2. In January Y3, the three JIPs CARE, OH-Harmony-CAP and MATRIX started. During spring Y3 the corona pandemic hit the world. The OHEJP reacted quickly and launched a call for another JIP dealing with the One Health aspects of the SARS-CoV2 virus on the agreed topic "Development and harmonisation of detection and characterization methods for SARS-CoV2 in humans, animals, and food and feed specimens". The proposal "SARS-CoV2 Research Integration and Preparedness" (COVRIN) was completed during June and has been evaluated and commented by the REA steering group. Discussions are now ongoing about how to adjust and improve the proposal.

6.1.6 Task 4.5: Open data management

Guidance on the development of project-specific data management plans (DMP) continues. One project, MADVir, has already finished and the final version of the MADVir DMP is uploaded in the OHEJP group. Since different templates have been used for the first call JRP/JIP DMPs, they have been time consuming to manage and evaluate. All first call final DMPs will be uploaded on the OHEJP website, as well as on Zenodo. For the second call projects a new tool will be used, which will facilitate for the DMP leaders to write and manage the DMPs, make the DMPs more unified, make it easier to administrate the DMPs and improve the degree of how the FAIR principles is fulfilled. The tool was launched in August and will allow harmonisation and centralisation of the data collected by OHEJP projects. The nomenclature used build on the One Health EJP Glossary, which was earlier developed. There have also been opportunities for training and there will be continuous supervision by the DMP function within WP4 about how to fill in and maintain the DMPs. In addition, this new software allows communication with the administrator. The projects are now in the progress of implementing their DMPs.



An access point for open data, “The One Health EJP Outcome Inventory” (OHOI), has been developed within WP5. The inventory functions as an entry point to the joint open resources and has links to the resources produced by JRP and JIPs. Deliverables and additional output from the OHEJP are taken into consideration when updating the OHOI. The OHOI can be accessed by the following links: Outcome section (<https://c1abo859.caspio.com/dp/e05d7000137f7c9dd846442f83fc>); Updates section (<https://c1abo859.caspio.com/dp/e05d70001b9da2f2fb0a4818b5f8>). A dialogue with the Communications Team is in place to link the inventory to the OHEJP website.

6.2 Deliverables and Milestones

6.2.1 Deliverables

Del. Rel. No	Deliverable title	Submission
D4.14	Report from 4th cogwheel workshop	M27
D4.15	Report from thematic meeting II	M30
D4.16	Report from supportive start-up meeting, 2nd round	M25
D4.17	2nd periodic report on JIPs	M27
D4.18	Report from 5th cogwheel workshop	M31
D4.19	Guidelines for evaluation of final reports	M34
D4.20	Report from 6th cogwheel workshop	M36

6.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS56	Four cogwheel workshops concluded and reported	M26	Achieved
MS57	Online questionnaire administered to new coordinators	M27	Achieved
MS58	Draft evaluation guidelines for JIPs presented to SSB	M32	Draft produced. Not yet presented to SSB. Next SSB meeting scheduled for September 18
MS59	All projects from 2nd call have started implementing the DMP	M34	Ongoing



MS60	External evaluators for JIPs recruited and internal assessment conducted	M36	Ongoing
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7 WP5 - Science to Policy translation to stakeholders

7.1 Work carried out to date

7.1.1 Task 5.1: Identification of the stakeholders and establishment of communication links

The third reporting year (M24-M32) was focused on consolidating the relation with the Key EU stakeholders ECDC and EFSA, and with other European and global stakeholders.

Exchange with the contact officer from EFSA continued to be very good, and the establishment of a new main contact for ECDC was finalised in January 2020. Contacts were established with the European Environment Agency (EEA) and the European Medicines Agency (EMA), as well as with the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation regional office for Europe (WHO-EURO). Contact representatives were appointed at these organisations to follow the progress of the OHEJP, and to join the Stakeholders Committee Meeting (SCM).

Communication with stakeholders was active by email, on the One Health EJP website, and by web-meetings. One of the major instrument is the SCMs.

The 5th SCM took place on the 26th of May 2020 as an online event. Representative from EFSA participated, and for the first time EEA, EMA, FAO and WHO-EURO were also represented. Unfortunately due to pressing commitments linked to the COVID-19 response, our contact representative at ECDC could not take part in the meeting. He was kept up to date and received all the informative material (presentations, meeting's minutes etc.). The EU funded projects JPIAMR and EU-JAMRAI also participated. The aims of the meeting – to give an update on current activities of the OHEJP, discuss the needs of the stakeholders and how they are complemented and addressed by the OHEJP, and to discuss the dissemination strategy and impact of the OHEJP outcomes – were reached well.

The SCM took place in conjunction with the OHEJP Annual Scientific Meeting 2020 to ease the participation of representatives of stakeholders. The representative of EFSA also gave a keynote talk at the Annual Scientific Meeting 2020.

To maximise impact of the OHEJP outcomes, targeted activities (workshops, seminars etc.) were seen as a suitable tool. This would require the identification of specific projects and outcomes of interest by the stakeholders. WP5 supports this interaction and improves the links between the projects and stakeholders' nominated contacts (Task 5.3).

In regards to dissemination, reports which are targeted to specific stakeholders or deal with a specific topic were considered optimal by the stakeholders (Task 5.4). In addition, outcomes are made available through the OHEJP Outcome Inventory (OHOI) (Task 5.4).

Liaison officers at EFSA were appointed for projects of interest of the 2nd call, and the importance of exchange with them was highlighted at the Project Leaders' forum, which took place on the 28th of May 2020.

WP5 was involved in the enlargement campaign of the OHEJP, which aims ideally at the representation of all EU countries in the consortium. A detailed list of organisations suitable for membership was drafted for all the EU countries not yet participating in the OHEJP, as well as for the countries which lack the representation of either an animal health or of a human health organisation.



7.1.2 Task 5.2: Identification of the research needs of EU stakeholders

WP5 has a number of tools in place to gather the needs of EU and international stakeholders.

Regular scanning of stakeholders' documents (publications, reports, regulations, press releases, speeches etc.) is performed, and identified needs are summarised in monthly documents stored in thematic groups of the OHEJP website, where they are available to consortium members and stakeholders. These documents help keeping the consortium up to date with stakeholders' needs, knowledge gaps, policy trends, new regulations, future risks etc. The websites scanned are those of: ECDC, EFSA, EMA, EEA, EC Press Corner, European External Action Service, DG-SANTE, European Parliament Think Tank, FAO (including regional offices), WHO (including regional offices), OIE (including regional offices), EMM Health and Food Safety, ProMED, Horizon Magazine, Avian Influenza and the Pandemic Threat News Pouch, One Health Initiative, One Health Commission, CDC Emerging Infectious Diseases Journal, Food Navigator, and CIDRAP.

WP5 dissemination activities are complementary to the general dissemination activities of the One Health EJP (WP1, Communications Team) and the results of the scanning of stakeholders' documents are available to support other means of dissemination (e.g. social media).

While the activity of scanning of stakeholders' documents helps keeping the consortium up to date with general needs of the stakeholders, it is an indirect way to identify needs. Direct, active dialogue with stakeholders is important and focuses on research and integrative needs in the area of foodborne zoonoses, antimicrobial resistance and emerging threats.

Following earlier discussions, a virtual helpdesk was established to allow easy communication about ad hoc needs. The helpdesk is mainly run by communication through email and can be complemented through a thematic group of the OHEJP website, allowing documentation of the steps taken to respond to the communicated needs if needed. Most importantly, personal communication via email is well established, and a number of web-meetings were organised to discuss the expectations of the stakeholders, and the support that the OHEJP can offer.

WP5 also contributes to the sustainability aspects of the OHEJP. Because not all of the stakeholders' needs can be addressed within the lifespan of the consortium, WP5 will give input of the Strategic Research Agenda together with WP2 and led by WP7.

7.1.3 Task 5.3: Linking of the scientific capacity available in the EJP with the stakeholders' identified needs: closure of knowledge gaps

The One Health EJP Outcome Inventory (OHOI, previously referred to as "capacity map") is a tool linking the stakeholders' needs with the scientific and integrative results of the consortium. It highlights outcomes of the OHEJP, supports dissemination of results of the various activities within the consortium (e.g. research projects, integrative activities), and depicts to some extent complementarity with activities outside the One Health EJP. The OHOI is a public online database accessible to all. As such it targets not just ECDC and EFSA and international stakeholders, but also national stakeholders, and supports internal and external collaboration and dissemination. The OHOI was seen as a valuable tool for dissemination of results of the OHEJP to national and international stakeholders, to other One Health initiatives, as well as within the OHEJP, to minimize the risk of duplication of work.

The OHOI is split between an [Outcome section](#) and an [Updates section](#).

The [Outcome section](#) lists the outcome of the consortium (databases, biobanks, computational methods, pieces of hardware, etc.), gives general information on the specific outcome, highlights the added value by depicting to a certain extent similar activities in place outside of the consortium, and links to specific resources if available. Most importantly it gives the contact information of the persons in charge, facilitating contacts in case more insights are desired.



The [Updates section](#) illustrates the progress of the different areas covered in the OHOI in a timely manner. It gives updates on the activities of JIPs and JIPs which are completed, in progress or planned, with reference to the source material where the information was acquired. If an update deals with a specific outcome, it links to the Outcome section of the OHOI. Contacts of the persons responsible for the update are also given.

Both the Outcome and the Updates section have a search function to ease navigation and to browse the inventory.

The backbone of the capacity map was implemented in the form of a relational database, using the commercial software Caspio. Dialogue with the Communications Team is in place to link it to the OHEJP website.

The OHOI was shaped following thorough discussion with the PMT and the key EU stakeholders, and it is currently accessible in its beta-version. Suggestions and comments are being taken into consideration and based on that the OHOI is being constantly improved.

WP5 is in charge of the management of the OHOI. WP5 updates the OHOI regularly, mostly taking information from the annual reports, deliverables and publications, but also from personal communications. After validation by the project leaders, the information is uploaded on the OHOI. Alternatively, project representatives can require an immediate editing/addition by contacting the WP5.

All projects within the One Health EJP are encouraged to use this platform to describe their approaches, skills, tools etc. and to include links to their specific activities and outcomes.

The deployment of the OHOI was advertised as news on the OHEJP website, at the SCM, at the ASM, the Project Leaders' forum and at the EFSA Summer School 2020, and WP5 is planning to present it in a number of conferences (in particular, ECDC's ESCAIDE and the 6th World One Health Congress).

Overall, the OHOI increases transparency of the consortium depicting complementarity of ongoing and completed activities as well as improving understanding where future approaches can build on work already performed, increasing thus the sustainability of the consortium. It supports closing of identified gaps and strengthens understanding on different levels (EU, national and institutional) as well as across domains and areas. It provides scientific support to enhance exploitation of results, as well as to follow up on the development of the activities (capacity building, better preparedness).

To avoid duplication of efforts within the OHEJP and to support dissemination, WP5 is also involved in the DMP Committee (Task 5.4), supervised by WP4, to coordinate and link the DMP with the OHOI.

WP5 is also in charge of more targeted means of dissemination: the targeted reports and the thematic reports, in which the identified needs of the stakeholders are analysed and linked with the OHEJP activities (Task 5.4).

Another way in which knowledge gaps are being closed, is through the allocation of resources to support specific stakeholders' needs with specific actions, as well as through the development of specific strategies addressing interests of national, EU and international stakeholders, as agreed following consultation with stakeholders.

Discussion on how to close specific knowledge gaps of the stakeholders is ongoing. Members of the Stakeholders Committee (Task 5.1) welcomed the idea of participating in dissemination workshops, to be organised in collaboration with WP3, WP4 and WP6. These workshops should focus on specific projects and outcomes identified by the stakeholders for being of particular interest. They will be organised in strict collaboration with JIPs and JIPs and will target specific stakeholders.

To close knowledge gaps as they emerge, additional funding can be allocated for targeted activities on a case by case base. WP5 coordinates, supervises and facilitates these activities. One example of timely support was the response to the request for assistance of the WHO-Global Outbreak Alert and



Response Network (GOARN). The OHEJP was invited to join the GOARN, and the vast network of the OHEJP was put at disposal of GOARN to disseminate its Request for Assistance aimed to the One Health community. Additionally WP5 is collaborating with GOARN and with the One Health Commission on a project to survey the value of One Health approach and of the One Health networks in response to the COVID-19 pandemic.

In addition to the vast array of WP5 mediated activities, bilateral collaborations between stakeholders and projects are in place. WP5 encourages stakeholders' representatives to participate in meetings and activities of JIPs and JRPs.

7.1.4 Task 5.4: Dissemination of new knowledge, tools and materials

WP5 implemented a variety of general (see the OHOI, Task 5.3) and tailored dissemination strategies in order to meet the needs of national, European and international stakeholders. This maximises the impact of the consortium's outputs and ensures the tools and results of the OHEJP will be useful in a timely manner.

The overall dissemination strategy is regularly discussed with stakeholders, particularly during the SCMs, and subjected to revision.

ECDC and EFSA are kept up to date about the scientific outputs of the consortium through targeted reports. These reports consist of a concise description of results and ongoing work of JIPs and JRPs, focusing on projects of highest interest for the stakeholders, as well as general announcements from the OHEJP consortium and scientific publications of all the projects. The documents also supports linkage with external resources, as well as with contact persons in case more insights on specific topics are needed.

Two targeted reports to Key EU stakeholders per year are produced and distributed. Even though these reports are targeted mainly to ECDC and EFSA, the 3rd targeted report was distributed also to EEA, EMA, FAO and WHO-EURO. The brief and clear nature of the 3rd targeted report to Key EU stakeholders was particularly appreciated by EFSA, as it was considered appropriate very suitable format for internal further distribution.

In addition to these regular reports, to highlight the responsiveness and timeliness of the OHEJP, WP5 produced the thematic report "Links between COVID-19 related needs of stakeholders and One Health EJP activities" (Extra deliverable D5.12). To minimise the risk of duplication of efforts and maximise added value, alignment of OHEJP activities to international research needs was analysed, evaluated and summarised. To maximise its visibility, the document was published on the OHEJP website and disseminated by a number of means, including to the Scientific Steering Board of the OHEJP.

WP5 collaborates with WP4 in the DMP committee. In particular, this collaboration between WPs ensures the complementarity between Outcome Inventory (OHOI) and the individual project DMPs.

Fostering communication with other EU projects is within the scope of WP5, aiming to avoid duplication of work, ensure complementarity, and eventually maximize the impact of the One Health EJP activities. To this aim WP5 facilitates active communication with the EU projects EU-JAMRAI and JPIAMR, which are part of our Stakeholders Committee and joined also the 5th SCM.

In addition, to maximize the visibility of OHEJP and WP5, presentations were given at the Scientific Steering Board (SSB) meeting and the EFSA Summer School 2020. A poster was presented at the Annual Scientific Meeting (ASM) 2020, attended by consortium members as well as external participants. WP5 will also be represented at a number of conferences and meetings, including the 6th World One Health Congress, ECDC's ESCAIDE 2020, and the PMC/POC meeting



7.2 Deliverables and Milestones

7.2.1 Deliverables

Del. Ref.	Deliverable title	Submission
D5.8	Third annual report on dissemination activities to international stakeholders	M36

7.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS70	New knowledge gaps and research needs identified n°2	M36	Regularly run during the year, monthly reports completed
MS74	Scientific support provided n°2	M36	Regular exchange during SC meetings and at other times as needed

8 WP6 - Education and training

8.1 Work carried out to date

8.1.1 Task 6.1: Short-Term Missions

The call to apply for Short Term Missions (STMs) in 2021 and to organise the following events in 2021 were all launched on 13/01/2020. All 2021 calls were given a deadline of 04.05.20, however, due to the impact of the COVID-19 crisis, the deadline was extended to 30.06.20. The validated procedures and protocols will be followed.

- A total of two Short Term Missions previously selected for funding successfully took place in 2020. WP6 monitored the progress of these missions through the individual reports submitted. Both mission expected outputs were impacted by the COVID-19 pandemic. The COVID-19 crisis also significantly impacted the success of the remaining three missions selected for funding. Two missions were postponed for later in the year, and the last mission was cancelled as the 'new' timing of the training no longer fit in with the planned work.

- STM selection procedures and protocols were reviewed, modified where necessary and validated for the participation in missions taking place in 2021.

8.1.2 Task 6.2: Workshop programme (satellite to Annual Scientific Meetings)

The ASM Satellite Workshop 2020 was selected to be organised by RIVM (the Netherlands) and titled 'Integrated approaches to zoonoses: a systems thinking primer'. This event was originally scheduled as a physical workshop to take place the day before the Annual Scientific Meeting in Prague on 26th



May 2020. However, this physical meeting could not take place due to the challenges caused by the COVID-19 crisis. The nature of this workshop requires it to be delivered as a physical meeting, and therefore alternative dates were explored to deliver the physical workshop as originally planned.

The workshop is targeted towards the consortium PhD students, junior and senior researchers and professionals who are working in the field of zoonoses, involved or interested in signalling, risk assessment and management, or interested in applying systems thinking to the research. This workshop plans to inquire how signalling and response is organised in EU member states and who plays a role in it. The workshop plans to use system thinking to open the perspective on opportunities to enhance the collaboration between sectors. The COVID-19 outbreak will be used as an example, to demonstrate that there will always be new and emerging zoonotic diseases that pose a serious threat to human and/or animal health. This kind of threat can happen in every country, requesting actions in the different sectors and collaboration between sectors.

The workshop explores deeper into stakeholder analysis and system mapping, two tools that are useful for disease management in general. The subject choice is left to individual delegates, and choices are AMR, foodborne zoonoses, one specific zoonosis, or all emerging zoonoses together at local, national or regional level.

As the workshop is limited to 30 participants, the following criteria were used to select the 30 delegates. Applicants increased their chances of being selected if they paired up with someone from their country; paired up with someone from a different sector from their country; paired up with someone with a different level of expertise (e.g. PhD student with junior/senior professional and vice versa). Delegates were selected and informed before the COVID-19 crisis.

- The ASM Satellite Workshop organiser selection procedures and protocols were reviewed, modified where necessary and validated for the event taking place in 2021.

8.1.3 Task 6.3: 'One health' Summer School for medical and veterinary science

The Summer School 2020 was selected to be organised by Wageningen Bioveterinary Research, in collaboration with UoS, Agreenium, Wageningen University, INRA, and the Netherlands Centre for One Health. The Summer School 2020 was titled 'Global One Health: From Research to Practice' and will be a follow-up from the previous summer school. The dates of this event are 17th-28th August 2020; however, the COVID-19 crisis caused challenges in delivering a physical summer school. WP6 worked with the local organising team to assess viable alternative options, and to deliver a successful and well-planned virtual summer school.

The Summer School organiser selection procedures and protocols were reviewed, modified where necessary and validated for the event taking place in 2021.

8.1.4 Task 6.4: Doctoral Training Programme

- WP6 continued to support and monitor the progress and dissemination activities of the PhD students.
-
- WP6 continued to provide guidance to the Principal Investigators (PIs) awarded the funding and their respective PhD students to ensure WP6 receives the relevant project progress updates, and to ensure the PhD dissemination and reporting procedure (provided by WP6) guidance document is adhered to. All dissemination activities were reported to the coordination team via the Internal Events Survey, and were also reported to WP6 and the Communications Team, who wherever possible, promoted these dissemination activities through our social media channels.



- WP6 hosted, organised and recorded a virtual Three Minute Thesis (3MT) PhD competition in M29 through Zoom, which was then livestream broadcasted during the virtual Annual Scientific Meeting that was organised due to the COVID-19 crisis. PhD students participated and showcased their projects in this competition, and then the recording was evaluated by three independent members of the ASM Scientific Committee, and finally a winner was selected and announced at the close of the virtual ASM. PhD students also submitted an abstract and presented a poster representing their PhD project.
- PhD students also continued to contribute content to their dedicated project page on the website and for the OHEJP social media channels to raise the profiles of themselves and their achievements, and to utilise the large network the OHEJP offers. Some PhD students also participated in the other WP6 activities to build a new generation of 'One Health' researchers e.g. applied for Short Term Missions in 2021.
- During the COVID-19 crisis, WP6 worked with the Communications Team to involve the OHEJP students in the social media challenge led by the European Commission in order to raise their profile in the European network of One Health researchers.
- WP6 evaluated and reported an inventory of non-refundable costs and delays due to the impact of COVID-19 on the 17 OHEJP PhDs taking place (16 PhDs funded by WP6 and 1 PhD funded by WP7). Of 17 projects, two suffered non-refundable costs, and 11 projects have confirmed that their projects will suffer delays in completion.

To report the progress of the project and to evaluate the COVID-19 impact, PhD students submitted a 9-month summary progress report to WP6 through the templates provided, to inform the content this Summary Progress Report Y4. The 9M PhD reports can be found here below.

8.1.4.1 Phd01-R1-AMR2-ECO-HEN

8.1.4.1.1 Progress of the research performed in the PhD project and key scientific results

During the reporting period (January to September 2020) the PhD work is being focussed on the in deep analysis of the surroundings of the trimethoprim resistance gene *dfrA36*, that was identified as the gene responsible of trimethoprim resistance in some isolates of *Escherichia coli* obtained from day-old chicks. In special, the plasmid harbouring this gene is under analysis.

The key scientific results for the reporting period are:

- **WP5. Reconstruction of plasmids spreading AMR genes from animals to eggshell's isolates.** The objective of this WP is to reconstruct the plasmids responsible for dissemination of AMR genes across isolates from different sources. It is widely recognized that the epidemiology of certain AMR genes (e.g. those conferring resistance to critically important antimicrobials in human medicine such as third-generation cephalosporins and colistin) is linked mainly to AMR gene spread via plasmids rather than via bacterial clones and thus knowledge on the AMR plasmids is essential to describe the flow of AMR in different ecological niches. M13-M14 (February/March – 2020).
- We have found a trimethoprim resistance gene in a large conjugative plasmid on, until now, four probably identical isolates belonging to the same sampling in day-old chicks. The gene, called *dfrA36*, was firstly described on the *Escherichia coli* chromosome isolated from calves in 2019. This information was presented in a poster at OHEJP ASM 2020.
- This conjugative plasmid is being used as an example of a plasmid entering in the farm with day-old chicks. Details of the laboratory work are the following:
- We are extracting the plasmids from the isolates with a commercial kit and performing an electrophoretic run for plasmids separation. Then, single bands are cut and plasmids recovered and studied by PCRs to detect the *dfrA36* gene. When performing the PCR analysis, we could



see that the different bands that we observed in the gel corresponded to the same plasmid, since we saw that the *dfrA36* gene was amplified from all the bands.

From now on, we want to continue isolating the plasmids from these bacterial isolates and digesting them with the *NotI* enzyme. Once the plasmid is digested and linearized, we want to perform a long read sequence in order to reconstruct the entire plasmid and study other possible resistance genes that the mobile genetic element could carry which is now complicated since we have the sequences divided into contigs and it is usually not possible to reconstruct complete plasmid sequences from short read sequence data.

Furthermore, we want to continue looking for this gene in other not yet sequenced isolates of the *E. coli* collection obtained from this farm, selected by having a similar resistance profile, especially simultaneous resistance to trimethoprim, sulphonamides and chloramphenicol, since *sul2* and *floR* are the closest resistance genes to *dfrA36* (as presented in the poster at OHEJP ASM 2020). PCRs for detecting *dfrA36* are being carried out on these isolates, and those that present the gene will be candidates for sequencing.

Deviation: WP5 was planned to be done in February-March 2020. Due to both problems in plasmid DNA extraction, as well as the inability to access the laboratory due to the coronavirus pandemic, the tasks of this WP have been delayed to July 2020.

- **WP6. Flow of AMR isolates between animals.** The objective of this WP is to follow the dynamics of AMR, both, isolates and associated platforms, from day-old chicks to pullets and laying hens. M15-M20 (April / September – 2020).
- T6.1. Checking on the isolates data base looking for non-sequenced isolates putatively needed for this WP.
- We are studying the resistance profiles of non-sequenced isolates, to identify relevant isolates for DNA sequencing to follow up the spreading of resistance genes and the putative platforms, especially plasmids. Clonal dissemination of resistance genes will be also considered.

T6.2. WGS of the isolates identified on T1.

- T6.3. Bioinformatic analysis of the WGS.
- T6.4. Data analysis and scientific manuscript preparation



8.1.4.1.2 Progress of the research project: milestones and deliverables

8.1.4.1.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD1-AMR2-ECO-HEN	D-E14-5.1.	A list of plasmids putatively present on <i>E. coli</i> isolates	M14 (March 2020)	M18 (July 2020)		Delay due to the coronavirus pandemic
	D-E14-6.1	A list of <i>E. coli</i> from animals isolates to be sequenced	M15 (April 2020)	M18 (July 2020)		Delay due to the coronavirus pandemic
	D-E14-6.2.	Manuscript	M20 (September 2020)			Under preparation
	D-E14-7.1.	A list of <i>E. coli</i> from eggs to be sequenced	M21			
	D-E14-8.1.	Manuscript	29			
	D-E14-9.1.	Doctoral thesis draft	36			



8.1.4.1.2.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD1-AMR2-ECO-HEN	M-E14-1	Updated list of phenotypic features of the <i>E. coli</i> isolates collection	M2 (March 2019)	Yes		
	M-E14-2	Training of the PhD-student on bioinformatic analysis of raw sequences	M4 (May 2019)	Yes		Achieved September 2019
	M-E14-3	New sequenced <i>E. coli</i> isolates from animals	M20 (September 2020)			
	M-E14-4	New sequenced <i>E. coli</i> isolates from eggs	M24 (January 2021)			

8.1.4.1.2.3 *Soft skills and Continuing Professional Development training*

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
VISAVET JOURNAL CLUB	Discussion of scientific publications	15/01/2020 05/02/2020 26/02/2020	VISAVET-UCM



Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Epidemiology workshop	Epidemiology and statics	03/03/2020 04/03/2020 02/06/2020 09/06/2020	UCM
ASM 2020 Conference	Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats	27-29/05/2020	OHEJP

- VISAVET JOURNAL CLUB consists of sessions in which a scientific article is presented and discussed among colleagues. It allows not only to learn to critically analyse an article, but also to expose your own opinion and argue with co-workers.
- The epidemiology workshop is part of the student's CPD. In the workshop, you learned how to design a scientific study depending on the objectives you want to achieve, also being able to assess whether the studies presented in the published scientific articles followed the correct statistical model. Also learned to use some very useful statistical software for future studies.
- The conference allowed the student to be in contact with professionals in the same field. Also, the student learned to make a poster for a communication, which will be very useful in her scientific career.



8.1.4.1.3 Publications and patents

- Irene Aldea, Alicia Gibello, and Miguel A. Moreno. First report of trimethoprim resistance gene *dfrA36* on an IncF-plasmid in *Escherichia coli* isolated from day-old chicks.
- Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 9.

8.1.4.1.4 Impact & relevance

During this nine-month period (January-September, 2020) the PhD project has not been in direct collaboration with external institutes; nevertheless, the PhD student is in close contact with VISAVET researchers working in other ongoing OneHealthEJP projects like ADONIS, DISCOVER and MATRIX for improving their bioinformatics skills.

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

Requirement (from ethical reviewers)	Measures and actions taken
<p>This project is focusing on laying hens. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the layers hens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so, please comment on any implications for the animal.</p> <p>Please state the 3Rs aspects of this work.</p> <p>Please describe how the animals' welfare are protected and considered (e.g. if the chicken is affected when taking samples, even if the work is dealing with faeces as these types of study can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals).</p>	<p>Dear reviewer,</p> <p>To the present moment, all the work has been performed with isolates stored in our laboratory and neither new farm visits nor new environmental sampling have been performed. Consequently there are no implications for animals regarding health or welfare.</p>



8.1.4.1.6 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Comments:

Laboratory work and full access to computer tools have been stopped or diminished from March to June due to coronavirus pandemic, and consequently, a delay of all the milestones and deliverables scheduled from March 2020 for approximately three months must be applied. Indeed, now it is not possible to know when the normal working pace will be restored.

8.1.4.1.7 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	Yes*
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No**
Other risks (please describe)	N/A

Additional information:

*The DTU supervisor, Dr. Valeria Bortolaia is no longer working at DTU.

** Not included those related to coronavirus pandemic



8.1.4.1.8 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Not Applicable

8.1.4.1.9 List of dissemination and communication activities

Name of the activity:	2 nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats (Presentation of poster and participation in 3MT competition)		
Date:	May 27 th - 29 th , 2020,		
Place:	Online meeting		
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)	750+	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

8.1.4.2 PhD02-R1-AMR2/3/6-LIN-RES

8.1.4.2.1 Progress of the research performed in the PhD project and key scientific results

Between January 2019 and February 2020, 1325 faeces samples (from cattle, pigs or poultry) and 148 nasal swabs samples (pigs) collected in Belgium were analysed on blood agar supplemented with linezolid to select resistant strains. One hundred forty-eight (148) (2 *Staphylococcus aureus* and 1 *Staphylococcus sciuri*, 143 enterococci (*Enterococcus faecium*, *faecalis*, *hirae*, *durans*, *gallinarum*, *asini*, *casseliflavus* and *saccharoliticus*), 1 *Pediococcus pentosaceus* and 1 *Lactobacillus johnsonii* (lost after isolation)) resistant to linezolid were isolated from these samples. Three strains isolated from human samples (urine, blood culture or skin lesion), 1 *S. aureus* and 2 enterococci were received from our Belgian partner (task 1.1). Fifty-two (52) strains are already completely sequenced (tasks 2.1 and 2.2). The three resistance genes, *cfr*, *optrA* and *poxtA*, were found as well as mutations in the 23S rRNA gene conferring resistance to linezolid at least once (task 2.1). More strains are waiting to be sequenced.

The Annual Work Plan provided to do the sampling and the collection of bacteria (task 1.1) for the Y2 and this task was finished on time. The task 2.1, NGS resistance analysis is in progress and the task 2.2, NGS subtyping of the strains and associated host specificity is in progress. The task 2.3 isn't started yet but will start soon. The task 2.4 scheduled for the next period (M33-M48) is removed because a very high number of isolates (~150) were found through selective monitoring performed for WP1 on samples gathered from official MRSA and Enterococci monitoring from food-producing animals in Belgium. Therefore, the project will focus on the NGS analysis of all these isolates and the investigation of putative risk factors associated with the numerous positive farms from which these isolates came from.

The task 3.1 is in progress and the task 3.2 is already started: establishment and test of the protocol and a first conjugation experiment was performed to assess the transferability of linezolid resistance genes.



8.1.4.2.2 Progress of the research project: milestones and deliverables

8.1.4.2.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD2-AMR2/3/6-PhD LIN-RES	D-E27-2.3	Genetic resistance profiles and subtypes of strains sequenced until M36	M36	M36	N/A	Resistance profiles are done (through PCRs and microdilutions). NGS is in progress. The determination of the species is also done. The determination of the subtypes is in progress.



PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
	D-E27-2.4	Results of in silico analysis of genetic scars of horizontal transfer or recombination events	M36	M36	N/A	
	D-E27-3.1	Results of in silico analysis of transferability	M36	M36	N/A	



PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
	D-E27-3.2	Poster or oral presentation at an international conference of the results of NGS analysis of linezolid resistant bacteria, including the in-silico analysis of transferability.	M36	M36	N/A	These results will be shown at the World One Health Congress 2020 in Edinburgh (30 oct – 3 nov 2020).

8.1.4.2.2.2 *Milestones*

PhD Project Reference	PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date
PhD2-AMR2/3/6-PhD LIN-RES	M-E27-3	Synthesis of genetic resistance, subtyping and transferability markers analysis of the linezolid resistant bacteria collected.	M36	no	M36	The transferability markers analysis is in progress. Genetic resistance and subtyping (MLST) is already assessed for the first genomes. Other NGS are scheduled soon.



PhD Project Reference	PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date
	M-E27-4	Poster or oral presentation at an international conference of the results of NGS analysis of linezolid resistant bacteria, including the in-silico analysis of transferability.	M36	no	M36	These results will be shown at the World One Health Congress 2020 in Edinburgh (30 Oct – 3 Nov 2020).



8.1.4.2.3 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Vulgariser sa recherche à l'écrit	Scientific vulgarisation	14 Decembre 2018	ULB
Vulgariser sa recherche à l'oral	Scientific vulgarisation	14 Decembre 2018	ULB
Galaxy live training 2019	NGS data analysis	4 April 2019	Sciensano
Formation sur l'encadrement d'équipe	Management	24-25-26 June 2019	ULB
Midi Cross-Experience - Infographie & data-visualisation	Data visualisation	13 Decembre 2019	ULB

8.1.4.2.4 Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 147.

8.1.4.2.5 Impact & relevance

All the collaborators participate to the scientific reflection. Building of the collections strains with the collaborators is in progress.



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

Requirement (from ethical reviewers)	Measures and actions taken
<p>Satisfactory ethics self-assessment</p> <p>Human biological samples</p> <p>The beneficiary must confirm that appropriate authorizations will be sought to collect the Human samples.</p> <p>Personal data processing</p> <p>The beneficiary must confirm that no personal data will be collected as part of the project; otherwise the GDPR (EU 2016/679) must be applied and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.</p> <p>Animal</p> <p>This project is focusing on laying hens. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the layers hens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals.</p> <p>Please describe how the animals' welfare are protected and considered (e.g. if the chickens are affected when taking samples, even if the work is dealing with faeces as these types of study can, this can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals).</p> <p>Please provide a statement on the 3Rs aspects of this work</p> <p>If Ethical Approval is required, please state which Research Ethics Committee this will be sent to</p>	<p>Since we gather a lot of samples through official sampling campaigns, we will finally not need to sample by ourselves animal or human samples.</p> <p>Then, no more ethical issues are linked to the LIN-RES project.</p>



8.1.4.2.7 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
N/A	N/A	N/A	D-E27-3.2	Jun 2020	Nov 2020	Conference re-scheduled to Nov 2020 due to COVID-19	N/A	N/A
N/A	N/A	N/A	M-E27-4	Jun 2020	Nov 2020	Conference re-scheduled to Nov 2020 due to COVID-19	N/A	N/A

Comments:

The deliverable D-E27-3.2 and milestone M-E27-4 were planned for June 2020 but have been rescheduled for November 2020.

8.1.4.2.8 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	No
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No



8.1.4.2.9 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Not Applicable

8.1.4.2.10 List of dissemination and communication activities

Name of the activity:	<i>One Health EJP ASM 2020 (presentation of a poster and participation in 3MT competition)</i>		
Date:	<i>27-29 May 2020</i>		
Place:	<i>Digital conference</i>		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
<i>Organisation of a Conference</i>	<i>No</i>	<i>Participation to a Conference</i>	<i>Yes</i>
<i>Organisation of a Workshop</i>	<i>No</i>	<i>Participation to a Workshop</i>	<i>No</i>
<i>Press release</i>	<i>No</i>	<i>Participation to an Event other than a Conference or a Workshop</i>	<i>No</i>
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>	<i>No</i>	<i>Video/Film</i>	<i>Yes</i>
<i>Exhibition</i>	<i>No</i>	<i>Brokerage Event</i>	<i>No</i>
<i>Flyer</i>	<i>No</i>	<i>Pitch Event</i>	<i>No</i>
<i>Training</i>	<i>No</i>	<i>Trade Fair</i>	<i>No</i>
<i>Social Media</i>	<i>Yes</i>	<i>Participation in activities organized jointly with other H2020 projects</i>	<i>No</i>
<i>Website</i>	<i>No</i>	<i>Other</i>	<i>No</i>
<i>Communication Campaign (e.g. Radio, TV)</i>	<i>No</i>		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number



<i>Scientific Community (Higher Education, Research)</i>	<i>750+</i>	<i>Media</i>	<i>0</i>
<i>Industry</i>	<i>0</i>	<i>Investors</i>	<i>0</i>
<i>Civil Society</i>	<i>0</i>	<i>Customers</i>	<i>0</i>
<i>General Public</i>	<i>0</i>	<i>Other</i>	<i>0</i>
<i>Policy Makers</i>	<i>0</i>		

8.1.4.3 PhD03-R2-AMR2.1-HME-AMR

8.1.4.3.1 Progress of the research performed in the PhD project and key scientific results

There has been a substantial delay to the commencement of this project due to recruitment issues and now the COVID-19 crisis. The first student who was recruited for the project in November 2019 changed his mind just after starting. This was followed by two further recruitment rounds. A suitable, highly qualified candidate, Mariel Aybar, was offered the position and has accepted but is current in lockdown in Peru and is therefore not in a position to start the project yet. A revised start date of September 2020 is proposed and the final months of the project after the One Health EJP concludes will be funded by Teagasc. Dr Burgess and Prof Morris are working with Ms Aybar to facilitate visas, fee waivers etc. Furthermore, Dr Burgess and Dr Morris have spoken with Geological Survey Ireland (GSI) regarding the identification of suitable sampling sites to ensure the project sampling campaign can be started once Ms Aybar takes up the position.

The proposed revisions to the project timeline and associated deliverables have been included in the AWP for Y4.



8.1.4.3.2 Progress of the research project: milestones and deliverables

8.1.4.3.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD3-AMR2.1-HME-AMR	1	Report on AMR bacteria present in soils of differing heavy metal content	M36	N/a	M46	This deliverable has not been achieved due to the delayed recruitment of the PhD student.



8.1.4.3.2.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD3-AMR2.1-HME-AMR	1	SOP in place for sampling culture based analysis	27	No	M38	Delayed due to late recruitment of PhD student
	2	Sequences of sufficient quality obtained from metagenomic analysis	35	No	M46	Delayed due to late recruitment of PhD student



8.1.4.3.3 Publications and patents

Not applicable yet.

8.1.4.3.4 Impact & relevance

This project provides Teagasc and NUIG an exciting opportunity to further build their strong collaborative relationship examining antimicrobial resistance in the environment and its relationship to food production. It is in line with the research objectives of both research groups and will provide valuable data on the impact of environmental pressures on antimicrobial resistance dissemination. Such data is currently very sparse. Furthermore, the project partners will collaborate with other OHEJP projects and partners regarding the comparison of the isolates obtained during this project. This will facilitate building stronger collaborative linkages between the participants and the opportunity to build future research consortia.

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

Requirement (from ethical reviewers)	Measures and actions taken
Biological samples The beneficiary must confirm that no Human and/or Animal samples will be collected for further analysis. Environmental and Health and Safety (H&S) Aspects Considering the area of work, the beneficiary must re-confirm that there are no environmental or H&S Aspects	No human or animal samples will be collected as part of this project. There are no environmental or H&S aspects in relation to this project. All samples and lab work will be undertaken using SOPs in place in the partner institutes and all isolation work will take place in appropriate BSL2 labs.

8.1.4.3.6 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
1	Dec 2020	Oct 2021	D1	36	46	Recruitment delay	0	49,146€ (Y2 budget + 66% of Y3 budget)

Comments:

COVID-19 has had a significant impact on this project. Prior to the pandemic we reported issues with recruiting a suitable PhD candidate. A suitable candidate was identified in March and the position offered but at the time our research labs were closed, as were embassies for procurement of the



appropriate study visa. As mentioned in section 1 the candidate is currently in Peru. Due to the ongoing COVID-19 related restrictions in Central and South America Ms. Aybar has not been able to apply for a study visa. Ms Aybar continues to engage with the Irish Embassy to expediate this process.

8.1.4.3.7 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	Yes
Other risks (please describe)	

Additional information:

As outlined there has been a significant delay to the project start and associated delay in work plan execution and reporting. Nonetheless, Dr Burgess and Prof Morris believe the project remains achievable with the right candidate and slightly tighter timelines. This has been made possible through collaboration with other relevant projects as outlined in Section 8.

8.1.4.3.8 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

- An Initial meeting has taken place with Geological Survey Ireland and through collaboration with the Tellus survey (<https://www.gsi.ie/en-ie/programmes-and-projects/tellus/Pages/default.aspx>) this will enable suitable high and low metal content soils across Ireland for resistome analysis.
- This project is complementary to the EPA funded AREST project (<https://www.nuigalway.ie/medicine-nursing-and-health-sciences/medicine/disciplines/bacteriology/research/arest/>) which is examining antimicrobial resistance in the environment. Prof Morris is the coordinator of this project and Dr Burgess is a participant. The culture based methodologies being employed in AREST will be particularly relevant for HME-AMR. Ms Aybar will have the opportunity to collaborate with these colleagues for methodologies and isolate characterisation.
- Dr Burgess and Prof Morris both contribute to Ireland's National Action Plan for Antimicrobial Resistance which is currently being updated and the results of this project will contribute to achieving the objectives of that plan.



- HME-AMR is complementary to a project led by Dr Orla O'Sullivan as part of the SFI funded VistaMilk project (<https://vistamilk.ie/>) which is examining the soil resistome in different sites across Ireland. Dr Burgess and Dr O'Sullivan will collaborate to ensure synergy of the projects and avoid duplication.

8.1.4.3.9 List of dissemination and communication activities

Name of the activity:	OHEJP ASM 2020		
Date:	27-29 May 2020		
Place:	Online		
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 (poster)
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)	750	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

8.1.4.4 PhD04-R2-AMR2.1-KENTUCKY

8.1.4.4.1 Progress of the research performed in the PhD project and key scientific results

Objectives.

Salmonella enterica serovar Kentucky (*S. Kentucky*) is a common causative agent of gastroenteritis in humans. It is one of most notorious *Salmonella* serotypes, as it is strongly associated with antimicrobial resistance (AMR). We hypothesize that the success of the MDR *S. Kentucky* ST198 is either due to (i) understudied accommodation of genetic elements encoded within mobile genetic elements (MGE) and their interaction with the core and accessory genome, or (ii) altered expression of a core genome-encoded virulence factor.

During the first nine months of the project, we selected four *S. Kentucky* strains for full genome sequencing using hybrid assemblies of short and long sequence reads. There were the following isolates from routine NRC practices in Sciensano: S16BD08730, S18BD00684, S18BD03994 and S18BD05011, either being *S. Kentucky* ST198::*bla*_{CTX-M-14b} (i.e. chromosome-encoded), CIP^R, CTX^R or Kentucky ST198 *pbla*_{CTX-M14-b} (i.e. plasmid-encoded), CIP^R CTX^R. These sequenced strain will form the basis for detailed investigation of these mobile genetic elements.

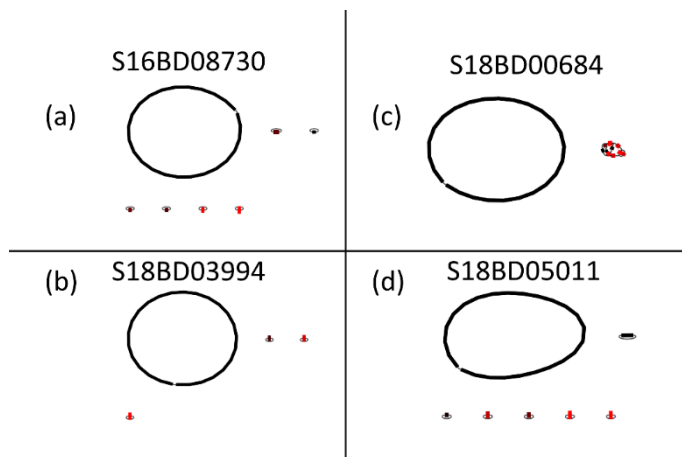
Methodology.

Short read sequencing libraries were prepared with an Illumina Nextera XT DNA Library Preparation Kit and sequenced on an Illumina MiSeq instrument with a 250-bp paired-end protocol (MiSeq v3 chemistry) according to the manufacturer's instructions. Trimming of the short reads was performed with Trimmomatic (version 0.32). First the Illuminaclip option was used to remove the Nextera adapter sequences. Then a sliding window approach of four bases and trimming when the Phred score dropped below 30 was employed. Lastly, the leading and trailing bases of a read were removed when the Phred score dropped below 3. Lastly, all reads that were smaller than 50 bp were removed.

The MinION long read sequencing library was prepared by using the 1D ligation sequencing kit (SQK-LSK108, Oxford Nanopore) according to the manufacturer's protocol for genomic DNA without barcoding. In total there were two MinION flowcells used, and libraries with 8 and 12 barcodes were loaded on them respectively (EXP-NBD103, Oxford Nanopore). From each isolate 1 µg of DNA was used at the start of the protocol. The optional steps of shearing the DNA to 8kb fragments with Covaris G tubes, while the DNA repair step was not performed. The sequencing was carried out on a R9.4 flowcell (Oxford Nanopore) and sequenced for 48 hours.

For the Flongle long read sequencing libraries, the adapted 1D ligation protocol for Flongles was used with the SQK-LSK109 sequencing kit. From each isolate 500 ng of DNA was used at the start of the protocol. DNA repair was no longer optional in SQK-LSK109 and therefore this was performed for the Flongle runs. In the SQK-LSK109 there are two washing buffers the SFB and LFB of which the latter enriches for DNA fragments >3,000 bp. Both these washing buffers and the inclusion or exclusion of the shearing step were used on separate Flongle flowcells. Moreover, on the Flongles no barcoding was performed.

Basecalling and demultiplexing of the Nanopore sequences was performed with Guppy (3.2.4). Then all Nanopore reads with a quality score lower than 7 or a length lower than 1000 were removed with NanoFilt. For the output of the sequencing runs and the theoretical coverage of each sample see table S14. The statistics of the Illumina reads was determined with FastQC and of the Nanopore reads was determined with NanoStat. Raw sequencing data and the de novo assemblies were submitted to NCBI Sequence Read Archive (SRA) and NCBI Genbank.



Results.

The chromosomes of each isolate were assembled in one contig and each isolate also showed one or multiple plasmids of which most were reconstructed in one contig (Fig 1). One plasmid from isolate S18BD00684 was split in multiple contigs, i.e. it was not possible to bridge a repetitive region in this plasmid due to the lower average read size in the MagCore DNA extracts. All isolates were determined to be of sequence type (ST) 198.

Figure 1. Visualization of hybrid assemblies of isolates S16BD08730 (a), S18BD03994 (b), S18BD00684 (c) and S18BD05011 (d).

The chromosomes of isolates S16BD08730, S18BD03994 and S18BD05011 share the AMR genes *aac(3)-Id*, *aph(3'')-Ib*, *aph(3')-Ia*, *aph(6)-Id*, *sul1*, *tet(A)* and *aac(6')-Iaa*. All these AMR genes except for *aac(6')-Iaa* were localised very close to each other and by aligning this region to the NCBI nucleotide database it was determined that these genes were part of a *Salmonella* genetic island 1 K (SGI1-K). Two isolates (S16BD08730 and S18BD03994) carried the ESBL gene *bla_{CTX-M-14b}* in the chromosome, while one isolate (S18BD00684) contained another ESBL gene, *bla_{TEM-1B}*, in the chromosome. Moreover, the latter isolate also contained the ESBL gene *bla_{CMY-2}* on a plasmid. Isolate S18BD05011 contained no ESBL genes on its chromosome, but *bla_{CTX-M-104}* and *bla_{TEM-1B}* were localised on two different plasmids, contigs 2 and 3, respectively. Initially, ResFinder assigned *bla_{CTX-M-14b}* to isolate S18BD05011 with an identity of 99.89%, but with the CARD database [43] it was determined that a point mutation at position 824 corresponds to the *bla_{CTX-M-104}* gene. Upon further inspection, a **region of 2850 bp including the ESBL gene was found to be similar in the chromosome of S16BD08730 and S18BD03994 and in the plasmid (contig 2) of S18BD05011**. In these regions, there was only a 1 bp difference, resulting in either the *bla_{CTX-M-14b}* (S16BD08730 and S18BD03994 on the chromosome) or *bla_{CTX-M-104}* (S18BD05011, on a plasmid) variants. The ISEcp1B transposase, which is part of the IS1380 family, was detected in this region adjacent to the ESBL gene (Figure 2). In the NCBI database there were no exact matches, but with a literature search, a description of this 2850 bp fragment was found in Lei et al. 2020 in the chromosome of a *S. Kentucky* isolated from Chinese poultry.

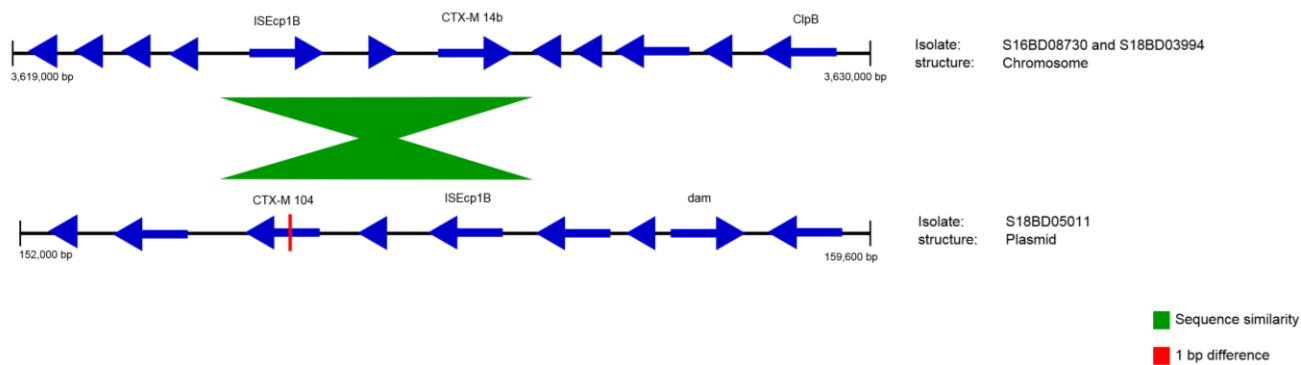


Figure 2. Similarity between the chromosomes of S16BD08730 and S18BD03394 and the plasmid of S18BD05011 (contig 2). The location and orientation of the genes is indicated with blue arrows. There is a 1 bp difference between *bla*_{CTX-M14b} and *bla*_{CTX-M104}.



8.1.4.4.2 Progress of the research project: milestones and deliverables

8.1.4.4.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD4-AMR2.1-KENTUCKY	D1	Contract signed and candidate enrolled	M22	M25/30	NA	Due to consequences of the Covid-19 crisis, the original candidate (Jasper Van der Peet) had to return permanently to The Netherlands in M28. He was replaced by Alaa Albasiony, who will pick up activities in M32.
	D2	Global collection of S. Kentucky strains completed	M25	M25	NA	
	D3	Training in long-read sequencing completed	M25	M26	NA	Finished before Covid-19 crisis
	D4	Protocol for long-read sequencing designed and implemented	M32	M26	NA	Earlier start possible through collaboration with Full Force project



8.1.4.4.3 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD4-AMR2.1-KENTUCKY	M1	Quality training completed at Sciensano	M23	Y		Will need to be repeated for the new candidate.
	M2	S. Kentucky strain and genome collection completed	M30	Y		
	M3	Completion of hybrid assemblies of four S. Kentucky strain	M36	Y		Bioinformatics shifted to earlier work due to lab closure by Covid-19 crisis.

8.1.4.4.4 Soft skills and Continuing Professional Development training

Given the change of personnel, Alaa Albasiony will start only In M32 and did not yet start any courses. However, we already planned a language course to start In October.

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Professional French	Language Course	01/10/20	Sciensano



8.1.4.4.5 Publications and patents

No publication and patents thus far.

8.1.4.4.6 impact & relevance

The Kentucky project units **Sciensano, INRAE and** Laboratory of Food Microbiology of **KULEUVEN (BEL)**, which clearly have complementary expertise. Sciensano holds the National Reference laboratory of *Salmonella*, and has experience in short- and long read sequencing (see section 1 of this report). **Prof. Abram Aertsen's** group uses analytical genetics and live (single-)cell biology approaches to study the spread, establishment and adaptive phenotypic impact of mobile genetic elements. **Benoît Doublet** and his team have long-term expertise in plasmid biology, and will study the transfer dynamics of these elements. It is clear these groups, which have never collaborated before, will greatly learn from each other and will exchange knowledge, strains and experiences along the way. The final goal is to improve our methodologies and understanding of transfer dynamics of these mobile elements, and its impact on antimicrobial resistance.

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

Requirement (from ethical reviewers)	Measures and actions taken
Environmental and Health and Safety (H&S) Aspects The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	I confirm that the PhD candidate has followed the necessary training in quality and biosafety to perform his work.

8.1.4.4.8 Impact of COVID-19 crisis on the project

1. Due to the Covid-19 crisis, all laboratory activities were suspended for two months at Sciensano, KU Leuven and INRAE. The PhD student shifted to bioinformatics (see section 1), so milestones M2 and M3 were already reached, while wet lab research was postponed. However, as we are still early in the project, the timing of deliverables and milestones still seems realistic.
2. The planned research visit to INRAE (M32) is very uncertain at this time, although crucial for buildup of expertise in conjugation and plasmid transfer methodology.
3. The Kentucky project only foresees budget for personnel cost, so the budgetary impact is limited. If the program will be extended with two months to compensate for the lab closures, we would need a budgetary injection of €6.000 to pay the salary of the PhD candidate.
4. A new candidate will start in August 2020, and will build on the work of Jasper Van der Peet, who worked four months on the project.

8.1.4.4.9 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	NO



Description of risk	Yes/No
Loss of technical training staff delaying progress of the work	NO
Delay in work plan execution	YES
Conflicts between the collaborative partners that support the PhD	NO
Lack of commitment between the collaborative partners that support the PhD	NO
Delay in duties, tasks or reporting	NO
Poor working relationships within the PhD project team	NO
Change in PhD student circumstances requiring temporary leave	YES (see above)
Other risks (please describe)	NO

Additional information:

1. Due to lab closure during the Covid-19 pandemic, research activities were suspended during two months (M27-28).
2. Also due to consequences of the Covid-19 crisis, the original candidate (Jasper Van der Peet) had to return permanently to The Netherlands in M28. He was replaced by Alaa Bassiounie, who will pick up activities in M30.

8.1.4.4.10 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Long-read sequencing was performed according to methodology derived from the Full Force project (JRP-19).

8.1.4.4.11 List of dissemination and communication activities

No dissemination and communication activities thus far

8.1.4.5 PhD05-R2-AMR2/6.1/ET5-METAPRO

The incorporation of the PhD candidate was in March 2020 and short after started the coronavirus crisis, where our lab became part of a network to diagnose CoVID-19 in elderly homes in Madrid. The candidate was actively involved in this activity and due to all the restrictions applied in our country no progress could be made in his PhD project until June. AWP3 has been updated with the planning for the rest of the year, and the AWP for the rest of the years have been adapted to these new circumstances



8.1.4.5.1 Progress of the research project: milestones and deliverables

8.1.4.5.1.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD5-AMR2/6.1/ET5-METAPRO	D1	Sampling questionnaire	DEC 2019		SEP 2020	

8.1.4.5.1.2 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD5-AMR2/6.1/ET5-METAPRO	M1	Spanish sampling design	DEC 2019	No	SEP 2020	
	M2	Spanish sampling execution	MAR 2020	No	DEC 2020	
	M3	Metagenome sequencing	JUN 2020	No	MAR 2021	
	M4	Enterobacteria isolation and WGS	SEP 2020	No	MAR 2021	



	M9	Analysis of the Spanish genomic and metagenomic data	DEC 2020	No	DEC 2021	
	M5	United Kingdom sampling design	DEC 2020	No	JUN 2021	



8.1.4.5.2 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Workshop “Aspectos epidemiológicos y estadísticos de un trabajo de investigación”	Epidemiology and Statistics	03-04/03/2020, 02/06/2020, 09/06/2020	Faculty of Veterinary Medicine, Universidad Complutense de Madrid
qPCR technician and analyst at Lab UCM COVID-19	COVID-19, qPCR	15/03/2020 – 01/06/2020	Universidad Complutense de Madrid
Course “Textos científicos con LaTeX”	LaTeX software	27/05/2020 – 16/09/2020	Universidad Complutense de Madrid
Biosafety seminar	Microbiology and Biosafety	14/07/2020	Faculty of Veterinary Medicine, Universidad Complutense de Madrid
One Health EJP Summer School 2020	One Health	17-28/08/2020	Wageningen University
Teaching of Practical classes in the Veterinary Medicine Degree	Microbiology and Immunology	Academic Course 2019/2020	Faculty of Veterinary Medicine, Universidad Complutense de Madrid
VISAVET Journal Club	Microbiology	Academic Course 2019/2020	VISAVET

8.1.4.5.3 Publications and patents

None

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 98.

8.1.4.5.4 Impact & relevance

This PhD project connects three major laboratories focused on the fight of Antimicrobial Resistance, having all of them participated in other European Projects in the past. This offers a possibility to maintain the great relationship that has been always present between institutes. This fact is highlighted by the various exchanges of students that have had part and how successful they have been. In addition, since the PhD project supervisors study AMR from different perspectives, this new collaboration brings the opportunity to combine them all together to get the most out of it. But it is



not only limited to that. Being part of the EJP networks allows to connect the partners and the student with different backgrounds and perspectives that are often needed.



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

Requirement (from ethical reviewers)	Measures and actions taken
<p>Biological samples</p> <p>The beneficiary must confirm that no Human and/or Animal samples will be collected for further analysis.</p> <p>Environmental and Health and Safety (H&S) Aspects</p> <p>Considering the area of work, the beneficiary must re-confirm that there are no environmental or H&S Aspects</p>	<p>Biological samples</p> <p>The intention is to use sewage from the different origins; therefore, the samples will not be collected directly from humans or animals.</p> <p>Environmental and Health and Safety (H&S) Aspects</p> <p>Our research will not cause any harm to humans, animals or the environment; and will not involve endangered fauna or protected areas.</p>

8.1.4.5.6 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Spanish sampling design	December 2019	September 2020	Sampling questionnaire	December 2019	September 2020	Incompatibility to access potential sampling points		



Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Spanish sampling execution	March 2020	December 2020				Incompatibility to plan sampling		
Metagenome sequencing	June 2020	March 2021				No sampling performed		
Enterobacteria isolation and WGS	September 2020	March 2021				No sampling performed		
Data Analysis	December 2020	December 2021				No sampling performed		
UK sampling design	December 2020	June 2021				No sampling performed		

Comments:

All the Annual Work Plans have been updated with the new estimated timeline of completion of the PhD project.



8.1.4.5.7 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

Additional information:

Due to a late incorporation of the candidate and the CoVID-19 crisis the PhD project start date had to be postponed and the work plan has been also compressed. Any other special situation not predicted could cause a major delay in the work plan execution



8.1.4.5.8 Interactions with on-going JRP/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

FARMED: Fast Antimicrobial Resistance and Mobile-Element Detection using metagenomics for animal and human on-site tests (<https://onehealth.ejp.eu/jrp-farmed/>). Since the objectives and the techniques of both projects are similar, the candidate will take part actively in the tasks performed at UCM associated to this project.

EFFORT: Ecology from Farm to Fork Of microbial drug Resistance and Transmission (<http://www.effort-against-amr.eu/>). The group participated actively in the EFFORT project and its activities and resources are the foundation of the present research project.

JPIAMR: Joint Programming Initiative on Antimicrobial Resistance. The lab is part of the NEAR-AMR (<https://www.jpamr.eu/near-amr/>) and of the GAP-ONE (<https://www.jpamr.eu/gap-one/>) projects. The candidate can benefit of these networks to extend the search of plazomicin resistance determinants in other parts of Europe and Africa or to estimate the economic burden of plazomicin resistance.

AVANT: Alternatives to Veterinary Antimicrobials (<https://avant-project.eu/>). The student will also be involved in the development and test of alternatives to antimicrobials.

8.1.4.5.9 List of dissemination and communication activities

Name of the activity:	<i>OHEJP Annual Scientific Meeting 2020 (poster)</i>		
Date:	<i>27th–29th May 2020</i>		
Place:	<i>ONLINE</i>		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
<i>Organisation of a Conference</i>		<i>Participation to a Conference</i>	<i>X</i>
<i>Organisation of a Workshop</i>		<i>Participation to a Workshop</i>	
<i>Press release</i>		<i>Participation to an Event other than a Conference or a Workshop</i>	
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>		<i>Video/Film</i>	
<i>Exhibition</i>		<i>Brokerage Event</i>	
<i>Flyer</i>		<i>Pitch Event</i>	
<i>Training</i>		<i>Trade Fair</i>	



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			
	Num ber		Num ber
Scientific Community (Higher Education, Research)	750	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	<i>OHEJP Three Minute Thesis Competition</i>		
Date:	<i>28th May 2020</i>		
Place:	<i>ONLINE</i>		
<i>Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories</i>			
	<i>Yes / No</i>		<i>Yes / No</i>
<i>Organisation of a Conference</i>		<i>Participation to a Conference</i>	
<i>Organisation of a Workshop</i>		<i>Participation to a Workshop</i>	
<i>Press release</i>		<i>Participation to an Event other than a Conference or a Workshop</i>	<i>X</i>
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>		<i>Video/Film</i>	
<i>Exhibition</i>		<i>Brokerage Event</i>	
<i>Flyer</i>		<i>Pitch Event</i>	



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)	750	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

8.1.4.6 PhD06-R1-ET5-PEMbo

8.1.4.6.1 Progress of the research performed in the PhD project and key scientific results

WP1: Establishment of reference sequences from the main French clonal groups

Task-1.1: Culture, cloning and extraction of *M. bovis* strains (M1-M6)

We selected ten representative *Mycobacterium bovis* genotypes among the genetic diversity of the *M. bovis* strain genetic population in France. Strains were first cultured in liquid medium to recover them from the collection (stored at -20°C) before the cloning step on solid medium. A single colony was picked, constituting the clone. Each clone was enriched in liquid medium prior to DNA extraction to obtain sufficient and concentrated DNA. This step was performed in the first 3 months of the PhD project (October-December 2019).

In January 2020, we performed the first DNA extractions using the NucleoBond® AXG kit (Macherey-Nagel as described in one of our previous studies (Branger et al 2020). Unfortunately, we encountered technical problem at this stage as the obtained DNA concentrations resulted too weak for long read sequencing. We introduced several new technical steps in order to ameliorate the protocol and adapt it as best as possible for mycobacteria DNA extraction (longer bacterial lysis periods, more concentrated reagents...) albeit none of them proved successful enough. We were at this stage when the COVID-19 crisis began and lockdown started.

Meanwhile, Ciriac performed a bibliographical review in order to assess if any other available DNA extraction protocols suited for obtaining mycobacterial DNA of high quality as required existed. We



have now established a new protocol (bet beading/phenol-chloroform) that we will soon put into practice in the next weeks.

Task-1.2: Sequencing and *de novo* assembly (M7-M18)

Genomes of *M. bovis* strains will be sequenced by two complementary technologies, PacBio and Illumina (pe-250x2), in order to take advantage of the long readings and the low error rate obtained by each method respectively.

We expect to send genomic DNAs for sequencing (genomic sequencing will be done by Geoscreen, Lille, France) during this summer.

WP2: Genome sequence analysis

Task-2.2: Genomic markers analysis (M13-M24)

Ciriac performed genomic analysis on previously available genomes. He participated to a trainee from the 19 to 21 November, "Linux et script pour la bioinformatique", organized by the CNRS in Montpellier. The goal of this trainee was to get familiar with the Linux environment and to learn the fundamental requirement for Python coding. Ciriac is now able to write simple scripts to analyze WGS data.

His first steps in bioinformatics focused on the presence and distribution of an insertion sequence, IS6110, in the genomes of *M. bovis*. IS6110 is a very useful genetic marker of the *Mycobacterium tuberculosis* complex employed for TB direct detection by PCR and for genotyping. Ciriac adapted scripts to find the number and localization of IS6110 in the bacterial genomes. These insertion elements were localized in genomic contigs using an approach that include Mauve contig reorder. Each result was confirmed with blast alignment and

He highlighted a wide diversity in the copy number and the localization of this sequence in the different genetic families of *M. bovis* in France. This study was presented as a poster at the ASM meeting (Figure 1). These multicopy strains were grouped on specific nodes and these genotypes are mostly found in French bovine tuberculosis endemic regions of. He also showed an increase of the proportion of IS6110 multicopy strains isolated during the last 20 years, suggesting that this kind of strains are the main causative agent of current French TB.

At present, Ciriac is studying the insertion localization in the genomes these strains in order to evaluate if they could lead to phenotypic behaviors that could explain a better fitness or transmissibility and thus their epidemiological success.

During this first study, a mutation of IS6110 in two strains (one in BCG group and the other one in the GB35 group) and a deletion of a part of this sequence in all members of the F9 family. These mutations are currently being confirmed by Sanger-based sequencing. The putative consequences of these mutations in the genotype of these strains will also be evaluated *in silico*.



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8.1.4.6.2 Progress of the research project: milestones and deliverables

8.1.4.6.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD6-ET5-PEMbo	D-E5-1	First steering committee report	M26		September 2020	Delay due to the COVID crisis. Previously planned the 25 march 2020

8.1.4.6.2.2 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD6-ET5-PEMbo	M-E5-1	PacBio + Illumina sequencing for obtaining reference genomes	M30	No	M32	Delayed due to unexpected technical problems and COVID crisis



8.1.4.6.3 Soft skills and Continuing Professional Development training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Journée d'accueil des nouveaux doctorants	Introduction of the doctoral school; explanation of the good progress of the thesis during the next three years.	02/10/2019	ABIES
UZH training for working in the NSB3 lab	To learn how to work in a confined type 3 biosecurity lab	31/10/2019	ANSES
MOOC Bioinformatique: algorithmes et génomes	To develop skills in bioinformatics and genome data analysis.	1/11/2019→9/12/2019	MOOC Fun platform
Linux et script pour la bioinformatique	To develop skills in bioinformatics, especially Python.	19/11/2019→21/11/2019	CNRS Montpellier
Bases en épidémiologie des maladies animales et zoonotiques	To learn basic notions on epidemiology. To learn how to set up an epidemiology study. To learn how to use different statistics tests ...	01/12/2019→19/12/2019	MOOC Fun platform
UZH training for working in the molecular biology lab	To understand UZH's molecular biology lab organisation and to evaluate my skills	19/12/2019	ANSES
ADOC - Construire et activer son réseau	To develop skills in professional presentation and elevator pitch. ADOC give tips to improve your networking.	20/01/2020	Paris Est university



Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Developing Fluency in English: Intermediate - Advanced level in English (B1-C1) session 2	To enhance the students' awareness appropriate vocabulary, pronunciation, intonation and improve their overall confidence in oral communications.	4/02/2020→21/04/2020	Paris Est university
Doc 'Avenir 2020	<p>This was ABIES PhD candidates' annual day, organised by doctoral students to discuss about their future career, and to learn different techniques to help them applying and getting a suitable job.</p> <p>This year, the main subject was about networking and developing a good professional profile</p>	12/02/2020	ABIES
Devenir acteur de la science ouverte : ouvrir ses publications et les déposer dans HAL	<p>To understand concepts and practices in relationship to "open science".</p> <p>To learn how to apply your rights for the deposit of publications in an open archives</p> <p>To learn how to use the HAL interface.</p>	08/07/2020	ABIES

8.1.4.6.4 Publications and patents

No publications



Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 76.

8.1.4.6.5 Impact & relevance

The aim of this thesis project, a collaborative study between ANSES, Animal Health Laboratory (Maisons-Alfort) and INRAE, Infectiology and Public Health laboratory (Nouzilly), two French EJP Partners, is to better understand the complex biology of *M. bovis* through the study of the complete genomes of a large panel of isolates of interest. The first two parts aimed at obtaining reference sequence (WP1) and identifying large genomic events (WP2). This part of the project will be carried out at ANSES. The third part of the project will consist on studying phenotypic traits that the genetic events disclosed by the genomic studies through analyses of antigenic variability, lipidomics and proteomics studies. This part of the project will be carried out at INRAE.

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

Requirement (from ethical reviewers)	Measures and actions taken
Biological samples The beneficiary must confirm that no Human and/or Animal samples will be collected for further analysis. Environmental and Health and Safety (H&S) Aspects The beneficiary must confirm that authorisations for relevant facilities (e.g. security classification of laboratory) have been obtained, and that safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project. Non EU countries (putative collaboration with Argentina) The beneficiary must provide details on the material which will be imported to/exported from EU and confirm that the adequate authorisations have been obtained. Please provide more information on Nagoya Protocol Compliance	No human or animal samples will be collected for further analyses Authorisations for relevant facilities have been obtained, and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project. Whether any collaboration was established with one of the potential mycobacterial groups in Argentina, INTA-Castelar, the <i>M. bovis</i> French strains to be sent will be so only if a MTA between the two interested parties (Anses-INTA) was signed beforehand and the <i>ad hoc</i> import licence to send them to Argentina was appropriately established. Concerning the Nagoya Protocol in the French territory, within the Framework of the n° 2016-1087 law of the 8 August 2016 for reconquering biodiversity, nature and landscapes, which is the implementation of Nagoya Protocol, the decree n°2017-848 of the 9 May 2017 fixates the rules to have access to the genetic resources situated in the national territory and to share the advantages resulting in their use. Argentina complies with the Nagoya protocol by the law 2726 of the 26 November 2015.



8.1.4.6.7 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Task-1.1	M6	M11	M-E5-1					

Comments:

Ciriac should have been in the last phase of lab manipulations (T-1.1) for starting genome analysis (T-1.2).

We have encountered some technical difficulties (as you could see in the first part of this report) before the lab's lockdown; we have not been able to shift from T-1.1 to T-1.2 before the expected working month.

Thus, the working program will be slightly delayed, although we are almost sure that we will be able to catch up time later on in Ciriac's project.

8.1.4.6.8 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No



8.1.4.6.9 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Not Applicable

8.1.4.6.10 List of dissemination and communication activities

Name of the activity:	<i>Poster presentation "Occurrence of variable insertion sites and copy numbers of IS6110 in genomes of Mycobacterium bovis field strains reveal high disparity among different genetic families" in One Health Annual Scientific Meeting 2020.</i> Participation in the 3MT competition		
Date:	27-29 May 2020		
Place:	Virtual		
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	Yes
Training	No	Trade Fair	No
Social Media	Yes	Participation in activities organized jointly with other H2020 projects	No
Website	No	Other	



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)	750	Media Twitter Research Gate	600 views, 33 interactions, 3 retweets 19 views
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

8.1.4.7 PhD07-R2-ET2.1-MACE

8.1.4.7.1 Progress of the research performed in the PhD project and key scientific results

The original intended start of the project was M21, however due to delay in finding a suitable candidate, the start date of the project was M24. This means the original deliverables for Y2 and Y3 could be potentially delayed by 3 months, the project will still finish before the end of WP6 of the OHEJP.

The relevant literature has been flagged (MACE.Y2.A). However, the main focus of the student has been to conduct an elicitation with surveillance and One Health experts at an international conference. This exercise will allow to capture in the mathematical model developed in this project more realistic considerations regarding “willingness to pay” and “willingness to accept” of different control and surveillance strategies. This task relates to MACE.Y5.3; and was originally scheduled for later in the project (milestone MACE.Y5.A; M53), however a unique opportunity presented itself to conduct this task with the support of the organizers of the 4th International Conference on Animal Health Surveillance (ICAHS). This exercise has never been done for surveillance in a One Health context, and will have unique policy relevant impact, which will be shared with key stakeholders. This means work on the review (MACE.Y2.1) has been postponed.

Progress has been made towards deliverables MACE.Y3.1 and MACE.Y3.2. Coding of the model has started, but not finished (MACE.Y3.A). Simulations still need to be finished (MACE.Y3.B) Possible delay of up to 3 months, as mentioned above, is expected due to delayed start of the project.

An abstract was accepted in the BSP conference, however the conference was canceled due to COVID



8.1.4.7.2 Progress of the research project: milestones and deliverables

8.1.4.7.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD7-ET2.1-MACE	MACE.Y2.1	Draft of review of surveillance and control tools for CE	M24		M32	This deliverable has been delayed to focus on the elicitation.
	MACE.Y3.1	Draft of publication with the control scenarios	M32		M36	Progress has been made with the model, possible delay of 3 months (due to delayed start of project).
	MACE.Y3.2	Model of CE validated in Argentina	M32		M36	Progress has been made with the model, possible delay of 3 months (due to delayed start of project).

8.1.4.7.2.2 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD7-ET2.1-MACE	MACE.Y2.A	Relevant literature on surveillance and control of CE identified	M23	YES		Relevant literature identified.



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PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
	MACE.Y 2.B	Training in Mathematical modelling	M24	Partial	M36	Partial training, some activities delayed due to COVID
	MACE.Y 3.A	Fitted model of CE to data from Rio Negro	M30	NO	M36	Progress in collating the data for model fitting
	MACE.Y 3.B	Simulations of different control scenarios	M32	NO	M36	Simulations to be run once model is fitted
	MACE.Y 5.A	Online polls with stakeholders completed	M53	Partial		Poll finalised, conference delayed to M34



8.1.4.7.3 Soft skills and Continuing Professional Development training

N/A

8.1.4.7.4 Publications and patents

No publications yet. Awaiting results of milestone MACE.Y5.A to finalise draft. Collaboration with stakeholders in Brazil (regarding mortality due to CE) is currently in the final draft stages.

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 47.

8.1.4.7.5 Impact & relevance

This project has allowed collaboration with partners within the consortium (namely between UoS and ISS), as well as supporting stakeholders in South America. The PhD student has engaged in activities in Peru, Argentina, Brazil and Uruguay, supporting ministry of health officials through data analysis of current surveillance and control programmes. As this is the beginning of the project, the outcomes are fairly limited thus far, however the engagement with the stakeholders has been excellent. We are extending our network of collaborations and partnerships to maximize the impact of the work developed, currently engaging with partners in the East Mediterranean region.



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

Requirement (from ethical reviewers)	Measures and actions taken
<p>Non EU countries (Argentina and Peru)</p> <p>-The beneficiary must provide details on the material which will be imported to/exported from EU and confirm that the adequate authorisations have been obtained.</p> <p>-As low middle income countries are participating in the study, the beneficiary must confirm that fair benefit-sharing arrangements with local stakeholders are ensured during the project (cf the Global code of conduct for research in resource-poor settings – www.globalcodeofconduct.org)</p> <p>This project states that the beneficiary will collect “data” from sheep and dogs.</p> <p>Further details are needed on the researcher’s interaction and ‘use’ of a legal animals (e.g. sheep and dogs). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals.</p> <p>Please describe how the animals’ welfare are protected and considered (e.g. if the dogs or sheep are affected when collecting data. Are any animals restricting for data collection etc).</p> <p>Please confirm if there are any impacts on the animals.</p> <p>Please provide a statement on the 3Rs aspects of this work</p> <p>If Ethical Approval is required please state which Research Ethics Committee this will be sent to, in the EU and non-EU countries.</p>	<p>The project is using historical data that has already been collected through the ongoing control and surveillance programme in Rio Negro, Argentina. The data is collected following standard protocols that have been approved in the country (Argentina). Animal welfare is managed through the guidelines approved in the country (Argentina). There are no physical materials transferred to the University of Surrey/EU, we only receive the data in silico (i.e. csv/excel files).</p> <p>The lead of the group in Argentina, Prof. Edmundo Larrieu, is registered as an external supervisor, which ensures fair benefit-sharing of all the outputs. Outcomes are also communicated with the local authorities (Echinococcosis surveillance and control programme in Rio Negro).</p>



8.1.4.7.7 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			MACE.Y2.B	M24	M36	Some training activities have been cancelled		

Comments:

Some of the training has taken place, but other training is scheduled in the future, due to the COVID disruption

8.1.4.7.8 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	No
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	



8.1.4.7.9 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

We are collaborating with the Programa de control de la hidatidosis en la Provincia de Río Negro, Argentina (Control program of hydatid disease in the province of Río Negro, Argentina) and with the Comisión Zoonosis in Uruguay and their Programa de Equinococosis quística (Programme of Cystic Echinococcosis).

8.1.4.7.10 List of dissemination and communication activities

<i>Name of the activity:</i>	Annual One Health European Joint Project (OHEJP) Scientific Meeting 2020. (Presentation of poster and participation in 3MT competition)		
<i>Date:</i>	May 2020		
<i>Place:</i>	virtual		
<i>Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories</i>			
	<i>Yes / No</i>		<i>Yes / No</i>
<i>Organisation of a Conference</i>	<i>no</i>	<i>Participation to a Conference</i>	<i>yes</i>
<i>Organisation of a Workshop</i>	<i>No</i>	<i>Participation to a Workshop</i>	<i>No</i>
<i>Press release</i>	<i>No</i>	<i>Participation to an Event other than a Conference or a Workshop</i>	<i>No</i>
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>	<i>No</i>	<i>Video/Film</i>	<i>No</i>
<i>Exhibition</i>	<i>No</i>	<i>Brokerage Event</i>	<i>No</i>
<i>Flyer</i>	<i>No</i>	<i>Pitch Event</i>	<i>no</i>
<i>no Training</i>	<i>no</i>	<i>Trade Fair</i>	<i>No</i>
<i>Social Media</i>	<i>No</i>	<i>Participation in activities organized jointly with other H2020 projects</i>	<i>No</i>
<i>Website</i>	<i>No</i>	<i>Other</i>	<i>No</i>



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)	750	Media	0
Industry	0	Investors	0
Civil Society	0	Customers	0
General Public	0	Other	0
Policy Makers	0		

8.1.4.8 Phd08-R2-ET5.1/4.1/FBZSH3-DESIRE

8.1.4.8.1 Progress of the research performed in the PhD project and key scientific results

Description of project activities:

1. The mobile application has been launched in September 2019. Data collection is currently slow, so it needs to be increased before modelling or data analysis can commence. This was already anticipated, and we also discussed a plan B in case data collection with the mobile application will not result in sufficient coverage of the Netherlands.
2. Initial laboratory analysis of pathobiome of brown and black rats has been performed on available samples. This is ongoing. Depending on the results, we will perform new pathobiome studies and focus on additional pathogens.
3. A field study has been set-up, including various environmental variables that can be used for risk analysis and potentially risk mapping. This data is currently being collected..
4. «The PhD student will assess various methods that are used for greening of cities, in view of the effect on rat populations. » Though a start has been made with this activity by interviews of professionals of municipalities, a more detailed assessment of the various greening activities in cities still has to be done.

The PhD candidate has joined the Wageningen University International graduate school for Animal Sciences (WIAS) for courses and training. Courses that have been followed thus far:

- Advanced statistics course : Design of Experiments
- Geostatistics
- Presenting with impact
- Brain friendly working and writing

Furthermore, the PhD proposal for internal evaluation within the graduate school has been finalised

Finally, the (first) field study has been designed and started May 2020. Also, laboratory training has started



8.1.4.8.2 Progress of the research project: milestones and deliverables

8.1.4.8.2.1 Deliverables

Not Applicable

8.1.4.8.2.2 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD8-ET5.1/4.1/FBZSH3-DESIRE	M1	Pathobiome analysis: list of pathogens and protocols	M27	Yes		A first overview of pathogens has been created based on samples that were already available. These results are currently analyzed, after which this will be published.



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PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
	M2	Field study set-up	M30	Yes		The field study has started in May



8.1.4.8.3 Publications and patents

No peer reviewed publications or patents In Jan 2020-Sep 2020

8.1.4.8.4 Impact & relevance

Though the PhD study only started 9 months ago, the collaboration between the involved partner institutes (RIVM, WBVR/WUR, FLI) has already increased. The PhD supervisors of RIVM and WBVR meet on a regular basis, offering opportunity to discuss additional subjects and new collaborations as well. The intensified collaboration between the RIVM and FLI was exemplified by the PhD student joining a field project of the FLI in the third month of her PhD. This allowed the PhD student to gain insight already in the work practices of the FLI.

While setting up the field study, meetings with partner institutes such as the Utrecht University and Wageningen University have been organized to discuss collaboration on smaller student projects that will benefit this PhD study. Furthermore, meetings with professionals of different Dutch cities have been held to collect their needs in the subject of urban wildlife/rats (related to urban greening).

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

Requirement (from ethical reviewers)	Measures and actions taken
Environmental and Health and Safety (H&S) Aspects Health and safety procedures conforming the relevant guidelines of the RIVM and the national legislation are followed. The work procedures have been discussed with our safety officer. The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project. Animals This project states that the beneficiary will capture and study rats. The beneficiary also indicated they may use / trap other rodent species or wild species living in urban environments. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. rats). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the rats are affected when collecting data.	<p>The appropriate health and safety procedures will be followed during this project by the PhD student and involved staff. RIVM has standard guidelines for working in the laboratory. For the fieldwork, the procedures have been discussed with a biosafety officer of the RIVM.</p> <p>In the Netherlands, wild rats are not included in the Nature Conservation Act, that lists wild animal species that are protected in the Netherlands. Thus, people are allowed to kill rats as long as it is done with tools that are approved for this.</p> <p>However, in this study rats are captured (with snap traps) for scientific purposes and are thus considered experimental animals. Permission was granted from the Central Authority for Scientific Procedures on Animals under the project number AVD3260020172104. The PhD student has been given permission to perform the necessary procedures.</p> <p>Furthermore, rats collected from regular pest control activities will be used instead of or complementary to rats that are specifically captured for the study, whenever possible.</p>



Requirement (from ethical reviewers)	Measures and actions taken
<p>Are any animals restricting / handled for data collection etc).</p> <p>Please confirm if there are any impacts on the animals.</p> <p>Please provide a statement on the 3Rs aspects of this work</p> <p>If Ethical Approval is required please state which Research Ethics Committee this will be sent to, in the EU and non-EU countries.</p>	

8.1.4.8.6 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			MACE. Y2.B	M24	M36	Some training activities have been cancelled		

Comments:

Only participation in the OHEJP ASM physical meeting was cancelled, but these costs have been reported elsewhere. Thus far, the progress of the PhD study has not been affected by CoViD-19 crisis.

8.1.4.8.7 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	No
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No



Description of risk	Yes/No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	

Additional information:

Thus far, none of these are applicable.

8.1.4.8.8 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

The PhD student and PI's are involved in a grant proposal about "Ticks and the city". If this is granted, this would result in extensive collaboration between the projects. In the selection of the field sites, this has already been taken into account.

8.1.4.8.9 List of dissemination and communication activities

Name of the activity:	<i>WIAS annual conference (participant, no presentation)</i>		
Date:	<i>13&14 February 2020</i>		
Place:	<i>Lunteren (The Netherlands)</i>		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
<i>Organisation of a Conference</i>	<i>No</i>	<i>Participation to a Conference</i>	<i>Yes</i>
<i>Organisation of a Workshop</i>	<i>No</i>	<i>Participation to a Workshop</i>	<i>No</i>
<i>Press release</i>	<i>No</i>	<i>Participation to an Event other than a Conference or a Workshop</i>	<i>No</i>
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>	<i>No</i>	<i>Video/Film</i>	<i>No</i>
<i>Exhibition</i>	<i>No</i>	<i>Brokerage Event</i>	<i>No</i>
<i>Flyer</i>	<i>No</i>	<i>Pitch Event</i>	<i>No</i>
<i>Training</i>	<i>No</i>	<i>Trade Fair</i>	<i>No</i>



<i>Social Media</i>	<i>No</i>	<i>Participation in activities organized jointly with other H2020 projects</i>	<i>No</i>
<i>Website</i>	<i>No</i>	<i>Other</i>	<i>No</i>
<i>Communication Campaign (e.g. Radio, TV)</i>	<i>No</i>		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Num ber		Num ber
<i>Scientific Community (Higher Education, Research)</i>	<i>150</i>	<i>Media</i>	<i>0</i>
<i>Industry</i>	<i>5</i>	<i>Investors</i>	<i>0</i>
<i>Civil Society</i>	<i>0</i>	<i>Customers</i>	<i>0</i>
<i>General Public</i>	<i>0</i>	<i>Other</i>	<i>0</i>
<i>Policy Makers</i>	<i>5</i>		

Name of the activity:	NCOH Science café (participant, no presentation)		
Date:	29 October 2019		
Place:	Utrecht (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	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)	70	Media	0
Industry	0	Investors	0
Civil Society	0	Customers	0
General Public	0	Other	0
Policy Makers	10		

Name of the activity:	OHEJP Annual Scientific Meeting (participation in the 3MT competiion)		
Date:	27-29 May 2020		
Place:	online		
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	Yes



Exhibition	No	Brokerage Event	No
Flyer	No	Pitch Event	No
Training	No	Trade Fair	No
Social Media	Yes	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)	750	Media	0
Industry	0	Investors	0
Civil Society	0	Customers	0
General Public	0	Other	0
Policy Makers	0		

8.1.4.9 PhD09-R2-FBZSH3/AMR2.1-UDOFRIC

8.1.4.9.1 Progress of the research performed in the PhD project and key scientific results

Literature review

The project involves the building of a theoretical understanding by the student, evidenced by a literature review to be completed by August 2020. The first section of the literature review is focussing on *Campylobacter* transmission and its impact on industry and human health. Also, *Campylobacter* genetics, including virulence mechanisms, mutations and gene transfer. The second section will be focussed on *Campylobacter* susceptibility to antimicrobials with a specific focus on Fluoroquinolones (FQ). The review will summarise FQ's historic use, mechanisms of action and their relationship with *Campylobacter*. Currently, progress has been made on *Campylobacter* and its impacts with a special focus on the statistics behind associated diseases in humans and poultry. The review also includes a summary of the major *Campylobacter* virulence factors, including subsections on motility, adhesion and invasion, toxicity and iron acquisition. Finally, initial progress has also been made in *Campylobacter* genomics, with literature being summarised in the genetic structure and diversity of the genus.

Data collection and identification of data gaps

A review of available datasets from *Campylobacter* research and national surveillance in broilers at APHA was undertaken. The datasets consisted of a collection of *Campylobacter* isolates from broiler



chicken sources, and various accompanying meta-data, e.g antimicrobial resistance phenotype, genotypic data (including AMR, MLST), and farm and production data. In the first instance data characterisation has been carried out on the dataset from 2012-2015 as the data within this time period was deemed the most complete. The reviewed datasets provide information that includes phenotypic resistance information, genomic data (including some whole genome sequence data) and epidemiological meta-data. Initially an understanding of the origins of the isolates is being built and plan is being drawn up to align older phenotypic AMR data with that derived from more modern methodologies. Work is being carried out on the data from this period to characterise and to identify trends or sources of FQR amongst *Campylobacter*. Initial observations show that FQR in *Campylobacter* is increasing year-on-year with certain clonal complexes showing heightened levels of resistance. Farming method and the abattoirs at which samples were taken appear to also have an influence. Further analysis will be carried out to investigate these initial findings.

Training in WGS and bioinformatics

Training in Whole Genome Sequencing (WGS) and bioinformatics has been undertaken earlier than planned in lieu of microbiological practical techniques. This is due to COVID preventing access to lab-based training. Bioinformatics training has involved the building of a database of known antimicrobial resistance (AMR) genes for *Campylobacter*, and initial training to assemble genomes (using sequence files from the 2012-2015 dataset), training to identify multi locus sequence types using *Srst2* and detecting AMR genes and mutations using *APHA SeqFinder*. The first set of samples has been identified and will be used as a training set to learn the basic bioinformatics processes including downloading large WGS datasets.

General information

The UDoFRiC team has had regular meetings via Webex with an initial meeting carried out on 6th April to brief the team on the data involved in this project and to plan the first steps of the project to be completed by the student. On the 11th June a second team meeting was held between all supervisors and student in order to discuss the findings thus far, with assistance and insight offered from the supervisors involved. After the discussion, a plan was put forward in order to determine the next steps of the project.

Next steps

The relationship between MLST clonal complexes and increased incidence for FQR will be investigated further as will the effect of farm and abattoir location, age, year, production type, *Campylobacter* species and co-resistance to other antibiotics (tetracycline).

The analysis of the development of FQR in broilers over time will be extended beyond 2012-16 by collation of phenotypic and genotypic data from broiler chicken isolates collected in 1994/95 and in 2007-2009



8.1.4.9.2 Progress of the research project: milestones and deliverables

8.1.4.9.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD9-FBZSH3/AMR2.1-UDOFRIC	1	Literature review of FQ in <i>Campylobacter</i>	August 2020	August 2020		On course for completion by current deadline
	2	Completion of 9-month review	September 2020	September 2020		N/A

8.1.4.9.3 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD9-FBZSH3/AMR2.1-UDOFRIC	1	Completion of literature review	August 2020			Layout and structure of the literature review has been established between the supervisor and student. The first section of the literature review is focused on <i>Campylobacter</i> and its impact, diversity and virulence. A second section will be soon formatted focusing on fluoroquinolones and their relationship with <i>Campylobacter</i> . Student is on course to complete this deliverable by the established deadline.



PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
	2	Completion of data collation and identification of data gaps	September 2020			<p>Analysis of sub-section of available data(<i>Campylobacter</i> from 2012-2015) of the available data is currently being carried out. Investigations into the variables of data collected with heightened levels of FQR is currently being completed.</p> <p>Initial findings indicate a relationship between FQR and <i>Campylobacter</i> species, clonal complex groups, abattoirs at which samples were taken and bird age.</p> <p>Analysis findings will inform on further data needs for phenotypic and genotypic data and FQR, and if this data can be generated from the wider archive of datasets and isolates available.</p>
	3	Completion of training in bacteriology and MIC	September 2020	No	Dec 2020	Due to current isolation guidelines set out within the UK because of COVID-19 the student has been unable to partake in lab-based training.
	4	Completion of 9 month review	September 2020			n/a



8.1.4.9.4 Publications and patents

N/A

8.1.4.9.5 Impact & relevance

The UDoFRiC project combines the collaborative experience of the APHA, ANSES and Warwick University. It combines the expertise of microbiologists, epidemiologists, and bioinformatics throughout various institutions throughout the UK and France.

The project is supervised by Dr John Rodgers who leads the National reference lab for *Campylobacter* in animals, Dr Muna Anjum who leads bacterial characterisation workgroup and is the AMR research lead along with Dr Manal Abu Oun at the Animal and Plant Health agency (APHA).

Professor Noel McCarthy is the lead of Evidence in Communicable Disease Epidemiology and Control at Warwick University. The project also includes a year at the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) under the supervision of Dr Isabelle Kempf who is a researcher microbiologist specialized in AMR; Leading the Mycoplasma Bacteriology Antimicrobial Resistance Unit in ANSES Ploufragan and Dr Katell Rivoal, who works in the Hygiene and Quality of Poultry and Pork Products research unit and is leading scientific projects on zoonotic pathogens (*Salmonella*, *Listeria*, and *Campylobacter*) in poultry productions.

The study will interrogate archives of *Campylobacter* from UK surveillance activity in broilers from 1994 to 2018 (isolate, phenotype, MLST, WGS, production metadata), to determine the acquisition and diversity of resistance to FQ over time (temporal trends). In addition, data from French broilers and from other potential sources of *Campylobacter* exposure to people (livestock/environment) will be interrogated wherever possible (APHA/ANSES and Public access archives and databases).

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

Requirement (from ethical reviewers)	Measures and actions taken
Environmental and Health and Safety (H&S) Aspects The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project. Animals This project is focusing on broiler chickens. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. broiler chickens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the chickens are affected when taking samples, even if the work is dealing with faeces as these types of study can, this	Environmental and Health and Safety (H&S) Aspects In the UK, the PhD will be fully trained to deliver all tasks safely and in compliance with the APHA General Health and Safety policy. This will ensure that all work is risk assessed and adequate training, supervision, facilities and equipment will be provided to protect the student and staff. As a minimum we will comply with the Health and Safety at Work etc. Act 1974 and the Management of Health and Safety at Work Regulations 1999. In France, the PhD student will be trained in hygiene and security (H&S) by the PhD supervisor, and by the agents in charge of H&S in the Anses Ploufragan Laboratory. The student will have all necessary protective equipment (laminar flow hood,



Requirement (from ethical reviewers)	Measures and actions taken
<p>can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals).</p> <p>Please provide a statement on the 3Rs aspects of this work</p> <p>If Ethical Approval is required please state which Research Ethics Committee this will be sent to.</p>	<p>masks, safety goggles, protective gloves...) and he will work in laboratory (L2 level) and animal facilities (A2 level), according to the French Labour Code, and conforming to relevant local/national guidelines/legislation.</p> <p>Animals</p> <p>All our animal facilities are under Directive on the protection of animals used for scientific purposes 2010/63/UE. When using conventional chickens, in case of breeding research, directives 98/58/CE, 1999/74/CE and 2007/43/CE must be applied.</p> <p>For experiments planned for the UDOFRIC thesis, we will proceed to obtain agreements from our ethic committees, and requirements of the national legislation or rules will be followed. Approval by the relevant ethics committees is required prior to the start of any animal experiment. Ethical committee on animal experiments is ANSES/ENVA/UPEC (Registered under the number 16 with the French ministry of research (Chairman: Luc Hettinger). Procedures are evaluated by this ethical committee ANSES/ENVA/UPEC and approved by the French ministry of research. Only approved experiments will be conducted.</p> <p>Where relevant planned experiments will also be reviewed by local Ethics Committees at APHA and the University of Warwick.</p> <p>Animal welfare laws: The study will be carried out in compliance with National Animal Welfare Regulations i.e. Ministerial Regulation of 1st February 2013 on the protection of animals used for scientific and educational purposes, which meets the 2010/63/EC Directive of the European Parliament and of the Council regarding 'the protection of animals used for experimental and other scientific purposes'.</p> <p>Replacement</p>



Requirement (from ethical reviewers)	Measures and actions taken
	<p>We are committed to use alternatives to animal studies wherever reasonable. The fitness of the susceptible and resistant strains will be studied <i>in vitro</i> (growth in culture medium and survival on different surfaces) but currently, the <i>in vivo</i> fitness (colonization, competition between colonizing strains, transmission between animals) necessitates animal models. However, by regularly consulting the Federation of Laboratory Animal Science Associations (FELASA) and the European Centre for the Validation of Alternative Methods (ECVAM), we will make sure that we can actively respond to the introduction of other validated alternatives and that we can actively contribute to improved protection and respect for the welfare of animals.</p> <p>Reduction</p> <p>The <i>in vitro</i> models as outlined above will help us to generate valuable information and limit animal experiments. Our experience of the <i>Campylobacter</i> models will also be used to reduce the numbers of included chickens, based on statistical study, to obtain scientific and statistically sound results.</p> <p>Refinement</p> <p>Animals will be placed on litter, and will have appropriate enrichment (straw, perching material...). <i>Campylobacter</i> does not induce suffering. The animals will be observed daily to detect any sign of discomfort. They will be offered water and conventional feed ad libitum. In case of severe injury, birds will be euthanized. Sampling of feces will be limited according to statistical studies (number of sampling per bird).</p>



8.1.4.9.7 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Completion of training in bacteriology and MIC	Sept 2020	December 2020	3	Sept 2020	December 2020	COVID-19 isolation guidelines has prevented lab based training	n/a	n/a

8.1.4.9.8 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	Yes
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

Additional information:

Please see section 6. Due to the COVID-19 lab based training has been delayed.



8.1.4.9.9 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Not Applicable

8.1.4.9.10 List of dissemination and communication activities

Name of the activity:	3 Minute thesis competition at One Health EJP Annual Scientific Meeting 27-29 may 2020		
Date:	15 May 2020		
Place:	Online		
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	Yes
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)	750 registered delegates	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

Name of the activity:	<i>OneHealthEJP #ScienceFromHome social media challenge</i>		
Date:	<i>12 May 2020</i>		
Place:	<i>Online</i>		
<i>Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories</i>			
	<i>Yes / No</i>		<i>Yes / No</i>
<i>Organisation of a Conference</i>	<i>No</i>	<i>Participation to a Conference</i>	<i>No</i>
<i>Organisation of a Workshop</i>	<i>No</i>	<i>Participation to a Workshop</i>	<i>No</i>
<i>Press release</i>	<i>No</i>	<i>Participation to an Event other than a Conference or a Workshop</i>	<i>No</i>
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>	<i>No</i>	<i>Video/Film</i>	<i>No</i>
<i>Exhibition</i>	<i>No</i>	<i>Brokerage Event</i>	<i>No</i>
<i>Flyer</i>	<i>No</i>	<i>Pitch Event</i>	<i>No</i>
<i>Training</i>	<i>No</i>	<i>Trade Fair</i>	<i>No</i>
<i>Social Media</i>	<i>Yes</i>	<i>Participation in activities organized jointly with other H2020 projects</i>	<i>No</i>
<i>Website</i>	<i>No</i>	<i>Other</i>	<i>No</i>



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)		Media	
Industry		Investors	
Civil Society		Customers	
General Public	1,667 (@OneHealthEJP twitter followers)	Other	
Policy Makers			

8.1.4.10 PhD10-R2-FBZSH3/AMR2.1-WILBR

8.1.4.10.1 Progress of the research performed in the PhD project and key scientific results

Due to the Covid-19 pandemic in UK and due to the March start of this PhD, only desk based tasks have commenced. A literature review is currently being undertaken on the role of wild birds in dissemination and persistence of antimicrobial resistance (AMR) in the farm environment. The review includes sections on identifying the current situation regarding AMR in different environments; what is currently known about wild birds in relation to carriage and dissemination of AMR; and evaluation of different methodologies for identifying AMR, both by phenotype and genotype, in bacteria.

Due to Covid-19 restrictions we have not been able to recruit outdoor farms for this study. Hence, faecal samples have not been collected or processed from pigs or wild birds present on farm. However, some historical *E. coli* isolated from gull faeces, collected from an outdoor pig farm, during a longitudinal study in the Oh-EJP project ARDIG are being considered for inclusion in this PhD project as a result of the delays caused by COVID-19.

A poster was submitted to the OHEJP ASM 2020 meeting, and an oral presentation on the PhD was presented virtually in the 3MT competition for the OHEJP AGM 2020, as part of communicating their research. The student also took part in a workshop at University of Surrey on microbiomes in order to learn from other institutes taking part in metagenomic research, which may be included later on in the WILBR project.



8.1.4.10.2 Progress of the research project: milestones and deliverables

8.1.4.10.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD10-FBZSH3/AMR2.1-WILBR	1	Literature review of contribution of wild birds to AMR in the farm environment	M32			
	2	Completion of 9-month review	M33			

8.1.4.10.2.2 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD10-FBZSH3/AMR2.	1	Completion of literature review	M32			
	2	Recruitment of farms for longitudinal study	M33			



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M25-M36



PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
1-WILBR	4	Completion of 9- month review	M33			



8.1.4.10.3 Publications and patents

No publications or patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 150.

8.1.4.10.4 Impact & relevance

The supervisory team brings together leading experts in veterinary, wildlife and environmental AMR, with expertise spanning veterinary and molecular microbiology, bioinformatics, microbial ecology and evolution, as well as wildlife disease.

William Gaze is working with the United Nations Environment Project on AMR in the environment, having recently authored the UNEP Frontiers report on AMR and the environment. He is currently located within two interdisciplinary units, Exeter's Centre for Environment and Human health, and the Environmental and Sustainability Institute, which is also part of the University of Exeter

Beside his work as a researcher within SVA, Stefan Börjesson is involved in the Swedish AMR monitoring program and is also a senior lecturer in clinical microbiology with an emphasis on AMR in a One-health perspective at Linköping University at the Department of Clinical and Experimental Medicine.

Muna Anjum leads the Bacterial Characterisation Workgroup and is the AMR Research Lead at the APHA working at the interface of molecular and veterinary microbiology, within the One Health remit. As lead for the AMR research, she is also involved in supporting national AMR surveillance activities and APHA's response to national outbreaks, and identifying new and emerging threats. She is a member of the DEFRA Antimicrobial Resistance Coordination Group, which advises and reviews the DEFRA activities on antimicrobial usage in animals and AMR in microorganisms from feedstuffs, animals and food, the APHA lead for the Defra AMR in the Environment group, and works closely with colleagues in Public Health England in various research projects and national activities.

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

Requirement (from ethical reviewers)	Measures and actions taken
The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	The student will receive extensive training in microbiological procedures that will be carried out, and is required to read and work to the standards stipulated within APHA risk assessments when going on farm surveys and carrying out microbiological testing. Farm surveys will be undertaken alongside other scientists/vets.



Requirement (from ethical reviewers)	Measures and actions taken
<p>Please comment on any implications for the animal.</p> <p>Please state the 3Rs aspects of this work.</p> <p>Please describe how the animals' welfare are protected and considered</p>	<p>Faecal samples will be collected directly from the ground and farm environment; therefore no direct animal handling is required and is outside of the Animal (Scientific Procedures) Act 1986. Animal welfare on farm is covered by the animal welfare act 2006, and additional controls are required by other farm assurance schemes, which pig farms also adhere to.</p>

8.1.4.10.6 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			2	M33	M35	COVID-19	not yet known	not yet known

Comments:

The project has been delayed by COVID-19 as the student by this point should have been recruiting farms in preparation for longitudinal sampling. The recruitment and sampling have both been delayed and if these restrictions persist it may have a major impact on the proposed PhD. The original plan was to sample across five time points in three years, (2x first year, 2x second year, 1 x third year) but this is unlikely to happen as a result of COVID-19. A new estimated completion date is hard to predict, as it is reliant on external factors, such as lockdown restrictions being lifted to allow access to the farms.

8.1.4.10.7 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No



Description of risk	Yes/No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	None

8.1.4.10.8 Interactions with on-going JRP/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Possibility of collaboration with FARMED EJP project as both have a desire to use Hi-C technology.
This PhD overlaps with the ARDIG project, where wild birds on farm have already been sampled.

8.1.4.10.9 List of dissemination and communication activities

Name of the activity:	<i>Animal Microbiome Workshop</i>		
Date:	<i>28/02/2020</i>		
Place:	<i>University of Surrey</i>		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
<i>Organisation of a Conference</i>		<i>Participation to a Conference</i>	
<i>Organisation of a Workshop</i>		<i>Participation to a Workshop</i>	Yes
<i>Press release</i>		<i>Participation to an Event other than a Conference or a Workshop</i>	
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>		<i>Video/Film</i>	
<i>Exhibition</i>		<i>Brokerage Event</i>	
<i>Flyer</i>		<i>Pitch Event</i>	
<i>Training</i>		<i>Trade Fair</i>	
<i>Social Media</i>		<i>Participation in activities organized jointly with other H2020 projects</i>	
<i>Website</i>		<i>Other</i>	



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			
	Num ber		Num ber
Scientific Community (Higher Education, Research)	27	Media	
Industry	1	Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

<i>Name of the activity:</i>	<i>One Health EJP ASM 2020 (Presentation of poster and participation in 3MT competition)</i>		
<i>Date:</i>	<i>27-29/04/2020</i>		
<i>Place:</i>	<i>Online</i>		
<i>Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories</i>			
	<i>Yes / No</i>		<i>Yes / No</i>
<i>Organisation of a Conference</i>		<i>Participation to a Conference</i>	<i>Yes</i>
<i>Organisation of a Workshop</i>		<i>Participation to a Workshop</i>	
<i>Press release</i>		<i>Participation to an Event other than a Conference or a Workshop</i>	
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>		<i>Video/Film</i>	
<i>Exhibition</i>		<i>Brokerage Event</i>	
<i>Flyer</i>		<i>Pitch Event</i>	
<i>Training</i>		<i>Trade Fair</i>	
<i>Social Media</i>		<i>Participation in activities organized jointly with other H2020 projects</i>	



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			
	Num ber		Num ber
Scientific Community (Higher Education, Research)	750	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

8.1.4.11 PhD11-R1-FBZ4/5- EnvDis

8.1.4.11.1 Progress of the research performed in the PhD project and key scientific results

Description of the Work. To develop a general tool to assess the risk of infectious diseases (in particular zoonosis) when we have information of relevant environmental factors.

During the first 6 months Laura has reviewed about 56 papers, taking notes and compiling the relevant information as well as populating a table with quantitative values related to the growth and survival rates of *Salmonella* in the main modes of transmission that will be used in the final thesis.

Laura begun to do some basic exercises to understand the logic of programming, i.e. creating and using vectors, functions and control structures.

Laura is also getting familiar with programs that she will have to use: GitHub for a common repository of the code; access and usage of the HPC (Eureka) to run heavy codes.

Laura is now learning key concepts in Infectious Disease Modelling (see training)

Training

Laura has also signed up for a few relevant trainings, including:

- Introduction to Infectious Disease Modelling and Its Applications (SIDM) at the London School of Hygiene & Tropical Medicine, UK (15-19/06/2020)
- Infectious Disease Modelling Specialization from Imperial College, UK.

Outputs

Poster presentation: "Understanding the main environmental drivers for salmonellosis using mechanistic modelling" at the Annual One Health European Joint Project (OHEJP) Scientific Meeting 2020.

Participation in the 3-minute thesis contest under the OHEJP activities.



8.1.4.11.2 Progress of the research project: milestones and deliverables

8.1.4.11.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD11-FBZ4/5-EnvDis	D-XX-6.1	Mandatory Trainings	June 2020	Within June		<p>Attendance to following trainings:</p> <ul style="list-style-type: none"> Welcome to your Doctorate RDP 12/02/2020 UoS Postgraduate Research Showcase IoD/Doctoral College 23/01/2020 Demonstrating in Laboratories RDP 29/01/2020 Assessment and Feedback DHE 03/02/2020 Workshop on Git Dean Roe 04/02/2020 Introduction to Teaching in Higher Education DHE 07/02/2020 Infectious disease modelling Giovanni Lo Iacono 18/02/2020 Introduction to HPC Pritesh Tailor 24/02/2020 Presentation skills ELSP/ James Green 12/02/2020 Presentation skills ELSP/ James Green 19/02/2020 Programming with R LinkedIn Learning Ongoing News and updates meeting with the NTD group University of Surrey Biweekly



PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
						<ul style="list-style-type: none"> News and updates meeting with the vHive group University of Surrey Weekly
	D-XX-6.2	Presentation of findings (e.g. conferences, internal school seminars)	December 2020	May 2020		During the first 6 months Laura has reviewed about 56 papers, taking notes and compiling the relevant information as well as populating a table with quantitative values related to the growth and survival rates of <i>Salmonella</i> in the main modes of transmission that will be used in the final thesis.
	D-XX-6.4 (Optional)	Attending Relevant Training courses (this is optional, and it might happen in year 2 depending on the student's needs.)	December 2020	June 2020		<p>Laura has also signed up for a few relevant trainings, including:</p> <ul style="list-style-type: none"> - Introduction to Infectious Disease Modelling and Its Applications (SIDM) at the London School of Hygiene & Tropical Medicine, UK (15-19/06/2020) - Infectious Disease Modelling Specialization from Imperial College, UK.



8.1.4.11.2.2 Milestones

PhD Project Reference	PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date
PhD11-FBZ4/5- EnvDis	M-XX-6.1	Review of the literature on <i>Salmonella</i> , <i>Leptospirosis</i> , environmental epidemiology and modelling approaches;	June/2020	Yes		During the first 6 months Laura has reviewed about 56 papers, taking notes and compiling the relevant information as well as populating a table with quantitative values related to the growth and survival rates of <i>Salmonella</i> in the main modes of transmission that will be used in the final thesis.
	M-XX-6.2	Collection of the available data from different sources, MEDMI, APHA, CEH etc. (Objective 1);	December/2020	Partially		Data from MEDMI are available to collaborators. It would be easier to access the data from their physical hard-drive (remote transfer is difficult due to stringent security measures). Following Covid10 lockdown this has not been possible. Laura is however working on



						other aspects of the PhD, so this has not been a big problem. We envisage we will get the data by the end of the year has planned.
	M-XX-6.3	Generate specific hypotheses about the underlying mechanisms envisaged in Figure 1 in the case for support (Objective 2-3);	December/2020	Partially		Laura is still working on this by reviewing the literature. We envisage we will reach the milestone by the end of the year has planned.
	M-XX-6.4	Interaction with colleagues at PHE and Gianni Lo Iacono who have estimated the probability of <i>Salmonella</i> cases, knowing recent environmental parameters at a certain location shown in Figure 2 and 3 in the case for support. (Objective 2-3)	December/2020	Partially		Laura is constantly in contact with Prof Nichols from PHE. She is also in contact with Dr Emma Gillighan who is working on the estimation of the probability of <i>Salmonella</i> cases, knowing recent environmental parameters. Because of Covid19, however, Dr Giliighan has not been able to work on this. I hope that we will reach the milestone by the end of the year has planned, but this might need to be changed. We need to discuss this with Prof Nichols and Dr Gilligham to know their plan.



8.1.4.11.3 Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 63.

8.1.4.11.4 Impact & relevance

The project involves the University of Surrey, Public Health England (PHE) and Zoetis. Laura's project builds on current work being done at PHE by Dr Gilligham and Prof Nichols and myself (Lo Iacono). Essentially PHE will focus on a phenomenology of Salmonella in England and Wales and Laura's project will complement this by using mechanistic models. The project fits also very well with research interests of Dr Kanellos in Zoetis and the activities led by Prof Cook in vHive (<https://vhive.buzz/>). Following initial meetings with partners we are now planning to extend the collaboration by applying for extra funding focusing on the use of big data (e.g. weather data) to understand and measure the impact of the environment on Infectious diseases.

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

Requirement (from ethical reviewers)	Measures and actions taken
There appear to be no ethical issues if all data are already in the public domain	I confirm that all data are already in the public domain

8.1.4.11.6 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Comments:

Hopefully things will be back in track by the end of the year as planned.

Laura, however, was waiting for PHE to provide some data and now this action is suffering delays due to the COVID-19 urgent management undertaken by PHE. Despite this delay, Laura still can work without the data, focusing on preliminary work that needed to be done anyways: e.g. literature review, designing the modelling approach (i.e. selecting route of transmission and identify suitable environmental factors based on the literature rather than data), simulating the dependence of *Salmonella* on these factors.



It was our intention, as well, to visit, work closely and discuss further with the PHE collaborators (Prof Nichols, Dr Emma Gillingham) different approaches to the project. This will need to wait.

8.1.4.11.7 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	NO
Loss of technical training staff delaying progress of the work	NO
Delay in work plan execution	NO
Conflicts between the collaborative partners that support the PhD	NO
Lack of commitment between the collaborative partners that support the PhD	NO
Delay in duties, tasks or reporting	NO
Poor working relationships within the PhD project team	NO
Change in PhD student circumstances requiring temporary leave	NO
Other risks (please describe)	YES, The major difficulty that Laura is experiencing has been a lack of concentration due to the lack of a daily routine since the lockdown.

8.1.4.11.8 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Not applicable now although we are exploring a few initiatives.

8.1.4.11.9 List of dissemination and communication activities

Name of the activity:	Understanding the main environmental drivers for salmonellosis using mechanistic modelling" at the Annual One Health European Joint Project (OHEJP) Scientific Meeting 2020. (Presentation of poster and participation in 3MT competition)
Date:	May 2020
Place:	virtual



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
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)	750	Media	0
Industry	0	Investors	0
Civil Society	0	Customers	0
General Public	0	Other	0



Policy Makers	0		
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8.1.4.12 PhD12-R2-FBZSH9-AptaTrich

8.1.4.12.1 Progress of the research performed in the PhD project and key scientific results

This is the 9-month report. During these 9 months, two problems occurred:

Firstly, administrative problems did not allow to start the thesis as expected (VISA problem). This problem has been fixed.

Secondly, the COVID-19 situation did not allow access to the laboratory from the second week of March to the third week of May (8 weeks). Since, then, the access to the laboratory is possible but restricted to limit the number of persons at the same time in the same place. Although priority is given to PhD student, the situation is not yet back to normal.

Activities:

The PhD student has been trained at Mc Gill institute (extra EJP-partner) for the SELEX (Systematic Evolution of Ligands by Enrichment) method (cell-SELEX, protein-SELEX). Then the protocols to adapt the SELEX method to *Trichinella spiralis* whole Muscle Larvae (ML) has been set up at Anses.

T. spiralis ML has been recovered from infected mice and fixed in ethanol. They are now used for the whole larvae-SELEX method defined.

Selection of a DNA-based aptamers set specific for *T. spiralis* whole ML has begun using a DNA library by SELEX method (Systematic Evolution of Ligands by Enrichment). Intact *T. spiralis* whole ML are incubated with the DNA library for multiple rounds (up to date 3 cycles were performed) with a progressive decrease in ML number and interaction time at each round.

As it is very important to select aptamers against early and late *T. spiralis* expressed proteins, recombinant stage specific proteins were expressed and protein-SELEX method will begin to be applied on these recombinant proteins in a close future.

At each round of the two SELEX methods, the quantity of the different aptamers selected is evaluated by electrophoretic gel, and qPCR using the number of melting peaks to quantify. The number of cycles to obtain less than ten aptamers by selection will be defined.

During each cycle round, a PCR step allows to amplify the aptamers in each set including a FAM Tag



8.1.4.12.2 Progress of the research project: milestones and deliverables

8.1.4.12.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD12-FBZSH9-AptaTrich	D-1	<i>T. spiralis</i> ML are now fixed in ethanol	24	27		
	D-2	Aptamers set on whole larvae is selected	29	Ongoing	32	
	D-3	Aptamers set on stage specific proteins is selected	36	ongoing	36	

8.1.4.12.2.2 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD12-FBZSH9-AptaTrich	M-1	The PhD student has been trained for SELEX	22	26		
	M-2	The protocols for SELEX are ready	24	27		



8.1.4.12.3 Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 24.

No publications

8.1.4.12.4 Impact & relevance

Up-to-date, the extra-partner from McGill institute helped to recruit the perfect candidate and trained him to the SELEX method. The protocols were set up at Anses and are available for the three partners (Anses, BfR and McGill).

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

Requirement (from ethical reviewers)	Measures and actions taken
Human biological samples The beneficiary must confirm that appropriate authorizations will be sought to collect the Human samples. Non EU countries (Canada) The beneficiary must provide details on the material which will be imported to/exported from EU and confirm that the adequate authorisations have been obtained. This project is focusing on laying hens. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the layers hens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the chickens are affected when taking samples, even if the work is dealing with faeces as these types of study can, this can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals). Please provide a statement on the 3Rs aspects of this work If Ethical Approval is required please state which Research Ethics Committee this will be sent to.	Human biological samples All serums have already been sampled with the authorizations that must be obtained in accordance with Canadian laws, and within the framework of the NRC in place at the McGill Institute. Non EU countries (Canada) Both the French and German NRLs have defined <i>Trichinella</i> positive and negative pig sera already available in their respective repository and the Canadian reference center has defined some <i>Trichinella</i> positive Human sera in its repository. Each project participant will test the effectiveness of the test on its own samples. Therefore, no shipment is planned. If, however, the evolution of the project leads to shipments, the Nagoya protocol will be respected. This project will not focus on laying hens. On the other hand, we will have to use mice to obtain larvae. Their number will be very limited since each mouse contains thousands of larvae. The experiments on mice were approved by an ethical review



Requirement (from ethical reviewers)	Measures and actions taken
	committee (C2EA-16 Comité d'éthique ComEth ANSES/ENVA/UPEC, under the approval number: saisine 12-0048, ComEth 13/11/12-4).

8.1.4.12.6 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Aptamers selection on <i>T. spiralis</i> whole larvae	M29	M32	D-2 & M-3	M36	M39	Lab closed during the crisis	11250	11250
Production of stage specific proteins and apply protein-SELEX	M36	M39	D-3 & M-3	M36	M39	Lab closed during the crisis		

Comments:

For now, as it is still the beginning of the thesis, we estimate that the work was delayed for 3 months.

8.1.4.12.7 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No



Description of risk	Yes/No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

8.1.4.12.8 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Not applicable

8.1.4.12.9 List of dissemination and communication activities

Name of the activity:	Poster presentation at OHEJP ASM 2020 (Presentation of poster and participation in 3MT competition)		
Date:	27-29-05-2020		
Place:	Online		
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	Yes
Training		Trade Fair	
Social Media		Participation in activities organised jointly with other H2020 projects	
Website		Other : Poster presentation	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)	750	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

8.1.4.13 PhD13-R2-FBZ8/AMR2-VIMOGUT-AMR

8.1.4.13.1 Progress of the research performed in the PhD project and key scientific results

Since the start of the PhD project, the student has followed several courses and seminars to learn about the background of microbiome analysis and microbial ecology. These included KNVM: Microbial Ecology, Baseclear: Animal Microbiome Expert, WUR-VLAG Graduate School: Intestinal Microbiome of humans and animals, Utrecht University: Analysis and interpretation of Microbiome and Metagenomics data. Furthermore, she has received in-house training on the use of 16s barcoding and further molecular biology techniques, completing Milestones 1 and 2.

Samples that were collected before the start of the project have now been sequenced using 16s barcoding as part of Milestone 4. Analysis of this data was performed successfully and reconfirm the findings of a preliminary dataset. The maturation of the chicken gut occurs in three successional stages in which the richness of the composition of the community greatly increases over the first 21 days. While all animals were eventually colonised by ESBL producing *E. coli*, those with a greater diversity in the second stage were colonised later indicating a possible window of opportunity for intervention strategies. Currently, there are technical difficulties in the analysis when the preliminary and novel datasets are merged. The student is addressing these difficulties with a collaborator from iDiv Germany. It is expected that this analysis, and with it Milestone 5, will be finalised by October 2020. Upon completion, a manuscript will be prepared describing the relationship of the chicken gut microbiota and the colonisation of the gut by ESBL *E. coli*, as described in Milestone 7/Deliverable 1.

For Milestone 3, a visit was planned to APHA for training on the in vitro chicken gut model that is operational there. However, due to difficulties to obtain a UK visum and the fact that the supplier of the Bio-reactor system that was obtained by WBVR provides a basic cultivation course, this was considered sufficient training. The design and implementation of the system was discussed with APHA via Skype and APHA will be included in the progress of the set-up of the system at WBVR.

The Bio-reactor system has now been delivered to WBVR and initial test runs to calibrate the system have been executed. The student has participated in a course hosted by Applikon on the Luculus software that controls the system. The Basic cultivation course for Bio-reactors was postponed by Applikon due to the Covid-19 crisis. This is currently scheduled to take place in August or September



2020. Test runs to determine a reliable colonisation of the model by ESBL E. coli, as described in Milestone 6, have been postponed until after the Basic Cultivation course has taken place.



8.1.4.13.2 Progress of the research project: milestones and deliverables

8.1.4.13.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD13-FBZ8/AMR2-VIMOGUT-AMR	D1	Manuscript on preliminary findings for the relationship between chicken gut microbiome maturation and ESBL colonisation.	Month 36	-	March 2021	Unforeseen challenges with the data analysis described for Milestone 5 are currently being addressed. This is the final work for this manuscript.



8.1.4.13.2.2 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD13-FBZ8/AMR2-VIMOGUT-AMR	M1	Molecular technique training and 16S barcode sequencing.	Month 22	Yes		
	M2	Attend course on analysis for 16S barcode sequencing.	Month 24	Yes		
	M3	Visit APHA for training on <i>in vitro</i> chicken gut model.	Month 24	Yes		The visit to APHA was not possible due to visa requirements. However, the training on the <i>in vitro</i> chicken gut model was successfully accomplished by taking courses with Applikon® Biotechnology and online meetings with APHA.
	M4	Perform 16S barcode sequencing on currently collected samples.	Month 27	Yes		



PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
	M5	Perform analysis of initial 16S experiment	Month 30	No	October 2020	Initial analysis was presented in OH-EJP ASM poster. Challenges with the merging of a preliminary and novel dataset are currently addressed as a final part of this analysis.
	M6	Perform initial test runs on <i>in vitro</i> gut model to determine CFU for reliable ESBL colonisation.	Month 24	No	December 2020	The work on the <i>in vitro</i> chicken gut model has been delayed considerably due to the Corona-crisis. Some standardisation and initial test runs in the <i>in vitro</i> system have been performed but the model is not completely in use yet.
	M7	Write manuscript on initial results relationship between chicken gut microbiome maturation and ESBL gut colonisation.	Month 36		March 2021	See deliverable 1: Unforeseen challenges with the data analysis are currently being addressed. This is the final work for this manuscript.



8.1.4.13.3 Publications and patents

So far, no manuscripts have been submitted for publication.

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 31.

8.1.4.13.4 Impact & relevance

Although the planned training at APHA has not yet been possible, communication on progress with the *in vitro* gut model occurs. Now that the employment contract of the PhD student was extended her residence permit can be extended until the end of the PhD project which will hopefully prevent further difficulty for obtaining a UK visum and will allow a visit to the APHA when the Covid-19 crisis subsides.

As several partner institutes within the consortium work on the topic of intestinal health using *in vitro* gut models and microbiomics, it is expected that when the PhD student progresses in her project, she will enjoy further interaction with partner institutes on the use of these techniques.

The analysis of the microbiome data sets has started a novel collaboration between WBVR and the German Centre for Integrative Biodiversity Research (iDiv) with Dr. Stephanie Jurburg.

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

Requirement (from ethical reviewers)	Measures and actions taken
<p>This project is using broiler chickens at conventional farms. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the broiler chickens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the the broiler chickens from sampling etc.</p> <p>Please describe how the animals' welfare are protected and considered (e.g. if the chickens are affected when taking samples, even if the work is dealing with faeces as these types of study can, this can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals).</p> <p>Please provide a statement on the 3Rs aspects of this work</p> <p>If Ethical Approval is required please state which Research Ethics Committee this will be sent to.</p>	<p>During the VIMOGUT project, sampling at conventional broiler farms and possibly slaughter houses will be carried out.</p> <p>If samples are taken at slaughter houses, the intestinal tract of animals will be collected after slaughter and evisceration are carried out. No changes are made to the standard procedures of the site and no additional animals are slaughtered for the benefit of the research.</p> <p>For the sampling at farms, only conventional broilers will be used with no restrictions or manipulation of the animals' diets.</p> <p>Animals will not be sacrificed for the research carried out for VIMOGUT unless approval has been given by the local animal welfare committee (IvD) at WUR and the national board on animal experiments (CCD), as per local guidelines. Sampling at conventional broiler farms will include the collection of fresh droppings for which animals may be briefly isolated to relieve themselves. Handling of the animals will only be performed after careful instruction and under the constant supervision of a trained veterinarian.</p>



Requirement (from ethical reviewers)	Measures and actions taken
	As per local law in the Netherlands, the 3Rs are always considered when animal experiments or on-site sampling is performed. The <i>in vitro</i> model that is set up during VIMOGUT is part of the strategy to replace the need for animal experiments in microbiota research. To refine the experiments and reduce the number of animals that are sacrificed, fresh droppings will be used for this study instead of caecal content of the animals, unless there is a clear need for the use of caecal content. As mentioned above, permission will be sought from the local animal welfare committee IvD and the national board on animal experiments CCD.

8.1.4.13.6 Impact of COVID-19 crisis on the project

Comments:

The COVID-19 crisis has had some effects on the feasibility of meeting deadlines of the milestones as described above. While the work on *in vivo* microbiome has been able to be continued, the work on the *in vitro* model has had significant delays due to the temporary closure of the laboratory facilities and the postponement of an essential course to properly commence this part of the work.

While the situation in the Netherlands is progressing well, it is currently unclear if the crisis will have an impact on the feasibility to collect samples at commercial broiler farms in year 4.

8.1.4.13.7 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No



Description of risk	Yes/No
Other risks (please describe)	No

8.1.4.13.8 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Currently not applicable.

8.1.4.13.9 List of dissemination and communication activities

Name of the activity:	<i>OH-EJP 3-minute thesis competition at OH-EJP ASM 2020 (winner of 3MT competition)</i>		
Date:	<i>28-05-2020</i>		
Place:	<i>Online</i>		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
<i>Organisation of a Conference</i>		<i>Participation to a Conference</i>	Yes
<i>Organisation of a Workshop</i>		<i>Participation to a Workshop</i>	
<i>Press release</i>		<i>Participation to an Event other than a Conference or a Workshop</i>	
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>		<i>Video/Film</i>	
<i>Exhibition</i>		<i>Brokerage Event</i>	
<i>Flyer</i>		<i>Pitch Event</i>	Yes
<i>Training</i>		<i>Trade Fair</i>	
<i>Social Media</i>		<i>Participation in activities organised jointly with other H2020 projects</i>	
<i>Website</i>		<i>Other</i>	
<i>Communication Campaign (e.g. Radio, TV)</i>			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			



	<i>Num ber</i>		<i>Num ber</i>
<i>Scientific Community (Higher Education, Research)</i>	750	<i>Media</i>	
<i>Industry</i>		<i>Investors</i>	
<i>Civil Society</i>		<i>Customers</i>	
<i>General Public</i>		<i>Other</i>	
<i>Policy Makers</i>			

Name of the activity:	Poster presentation at OHEJP ASM 2020		
Date:	28-05-2020		
Place:	Online		
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	Yes
Training		Trade Fair	
Social Media		Participation in activities organised jointly with other H2020 projects	
Website		Other : Poster presentation	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

	Nu mbe r		Nu mbe r
Scientific Community (Higher Education, Research)	750	Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			

8.1.4.14 PhD14-R2-FBZ4-ToxSauQMRA

8.1.4.14.1 Progress of the research performed in the PhD project and key scientific results

The present research project aims to answer to the scientific question: “What is the attribution of the traditional raw pork products in the human *Toxoplasma* infection? “ based on three main areas of study consisting of (i) a thorough investigation of *T.gondii* predilection sites in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst) (WP1); (ii) evaluate the impact of the manufacturing process (including different incorporation rates of nitrites and NaCl) and the conservation of dry sausage on the viability of *T. gondii* (WP2); (iii) a quantitative microbiological risk assessment analysis to be conducted for the various raw pork products (dry sausage, dry ham, etc) (WP3). The 3-year project spans over four Annual Periods (Y2-Y5). The present 9-month report focuses on the progress and activities from WP1 and WP2.

Within the WP1 (Investigation of *T.gondii* predilection sites in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst)), we have worked on two different tasks: WP1-T1: Experimental infection of pigs and WP1-T2: Predilection sites of *T.gondii* in pigs with the help of external partners: Institut du Porc (IFIP) and Université de Champagne-Ardenne (URCA).

For WP1-T1, the main focus was to be able to collect meat massively contaminated with *Toxoplasma gondii*. Therefore an infection with a high dose (1000) of parasites was carried out (strain ME49) in pigs. These tests were carried out with 2 parasitic forms that may be at the origin of contamination in pigs: the oocyst (ingestion from the environment) and the tissue cyst (ingestion from infested meat). Three pigs were inoculated with oocysts and 3 others with tissue cysts. Serological monitoring of the serum of these pigs was carried out by Modified Agglutination Test (MAT) on a weekly basis until D30, then every 15 days until the end of the protocol (3-4 months/80-90 kg).

However due to several logistic problems (late delivery/lack of viability of parasitic forms, last-minute change of the experimental facilities), the inoculation of the 3 pigs with the tissue cysts form of the parasite has failed twice. Four-weeks post-infection, they revealed negative by MAT, while the 3 pigs inoculated with oocysts revealed positive. Therefore an experimental infection of mice with viable oocysts of *T. gondii* has been set up, in order to produce our own tissue-cysts for experimentally infection of the remaining 3 pigs. Eight weeks later, the mice were culled, the brain has been collected, homogenized, the tissue cysts of *T.gondii* identified and counted. A dose of 1000 tissue cysts /pig has



been inoculated orally. Serological monitoring of the serum of these pigs was carried out by Modified Agglutination Test (MAT) on D2, 5, 7, 9 p.i. and at the end of the protocol, since the Covid19 shut-down was set up. At the end of the protocol the 3 pigs were confirmed by MAT to be positive for *T.gondii* infection.

After euthanasia of the MAT positive pigs, 4 tissues /pig were collected for analysis: heart, breast, shoulder and ham as tissues used in the manufacture of dry sausage. For each of the anatomical regions studied, the different muscles (4 for breast, 7 for shoulder and 13 for ham) were pooled. Some 40 supplementary muscles per carcass were individually collected, representing the most important anatomical regions. One hind leg was collected for each pig for dry ham production.

Concerning the WP1-T2, the main focus was to identify the predilection sites of *T.gondii* presence in pig tissues. Therefore, mouse bioassays and quantitative PCR was performed on a part (200g) of the pooled sample per region. The remaining parts of the pools were used for industrial (salting, smoking, etc) processing (WP2).

The analysis of tissue samples (by qPCR and mouse bioassays) is still under way, therefore more detailed results will be presented in the 12-month Report.

Within the WP2 (Assessment of the persistence of *T. gondii* during the production and storage of dry sausages and dry ham), we have worked on two different tasks: WP2-T1: Manufacture of dry sausages and dry ham and WP2-T2: Assessment of the persistence of viable *T. gondii* in pork delicatessen with the help of external partners: Institut du Porc (IFIP), Université de Champagne-Ardenne (URCA) and INRAE Corte (Corsica).

For WP2-T1 the manufacture of dry sausages was carried out on a pilot scale by IFIP, according to a protocol representative of those used by a commercial factory. Briefly, after mincing, the muscle pool was divided into seven portions (corresponding to the 7 combinations of nitrites (as sodium NaNO₂ nitrites) and NaCl concentrations that was to be compared: 120 (maximum dose of nitrites mentioned in the Code of Practice), 60 and 0 ppm of nitrites combined with the usual dose of 26 g/kg NaCl or a reduced dose of 20 g/kg NaCl or 0 g/kg NaCl. For each of the tested formulations, 3 dry sausages was collected at different dates (D0, D2, D10, D20, D30 and D50). On each analysis date, IFIP has carried out a physico-chemical monitoring (pH, Aw, weight loss) and a count of the lactic flora from a dry sausage per formulation, in particular to check that the process is running properly. A total of 168 dry sausages was required for this study (3 dry sausages × 7 recipes × 6 analysis dates for *T. gondii* monitoring as well as 1 dry sausage × 7 recipes × 6 analysis dates for physico-chemical and bacteriological analyses).

The dry hams were meant to be sent and manufactured by INRAE Corte (Corsica), using two traditional salting techniques (a long one : 2.5 days/kg and a short one: 1 day/kg). However due to a local strike in the harbour of Marseille, the hams arrived with 10 days of delay, causing the sanitary quality of meat to be questionable in terms of manufacturing. Therefore, a long salting technique has been applied only, with 300g of product that was taken at D30 and D90 due to Covid19 shut-down.

Concerning the WP2-T2 the main focus was the analysis of the dry sausages for the presence of viable *T. gondii* by bioassay in mice that has been performed in the animal facility of URCA. The presence of *T. gondii* DNA in the inocula was quantified by URCA using a qPCR and will be quantify in the next months by ENVA by MC-qPCR.

Key scientific results:

1. Successful experimental infection of pigs with both parasitic forms (oocysts and tissue cysts)
2. Successful collection of meat samples in spite of Covid19 shut-down
3. Complete artificial digestion of meat samples from “oocysts” pig group
4. Manufacturing of 168 dry sausages and their analysis by bioassays



5. Manufacturing of long-salting dry ham and the analysis by bioassays

Challenges:

1. Failure in oral infection with tissue cysts form of the parasite
2. Covid19 shut-down
3. Strike in harbour of Marseille

Adaptations:

1. Repeating the oral infection with tissue cysts form of the parasite for 3 times
2. In house production of tissue cysts
3. The Covid19 shut-down that forced us to adapt our sampling schemes and planning



8.1.4.14.2 Progress of the research project: milestones and deliverables

8.1.4.14.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD14-FBZ4-ToxSauQMRA	D-PhD-ToxSauQMRA-WP1	Final report on the experimental infection of the pigs (serological monitoring, rectal temperature, weight) and predilection sites of <i>T.gondii</i>	M36		M39	Due to Covid19 sanitary shut down a 3 months delay has to be taken into account (for the moment)



8.1.4.14.2.2 *Milestones*

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD14-FBZ4-ToxSauQMRA	M-PhD-ToxSauQMRA-01	Pig infection experiment is terminated. Weekly/monthly weight and rectal temperature are collated and presented in a graph. Samples are collected and prepared for testing and dry sausage and ham processing.	M27	Yes (partially)	M33	Due to Covid19 sanitary shut down and the need to repeat 3 times the tissue-cyst oral infection, 6 months delay has to be taken into account. Only ½ of the samples are already collected. On contrary all sausages and ham have been processed.
	M-PhD-ToxSauQMRA-02	Serological testing of pigs is finalised and weekly levels of Igs are collated in a graph.	M30	Yes		



PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
	M-PhD-ToxSau QMRA-03	Testing of tissue samples prior to processing is finalised and quantitative PCR, Magnetic-capture PCR and mouse bioassay data are collected in tables.	M36	No	M39	Due to Covid19 sanitary shut down a 3 months delay has to be taken into account (for the moment)
	M-PhD-ToxSau QMRA-05	Testing of dry sausage samples is (almost) finalised and quantitative PCR and mouse bioassay data are starting to be collected in tables	M36	No	M39	Due to Covid19 sanitary shut down a 3 months delay has to be taken into account (for the moment)



8.1.4.14.3 Publications and patents

For the moment Filip DAMEK, presented his PhD project and the results up to date, as a poster, in the Annual Scientific Meeting of EJPs (27-29.05, Prague).

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 40.

Moreover, during this period, Filip DAMEK published the article: "Detection of *Toxoplasma gondii* in retail meat samples in Scotland" Jacqueline Plaza, Filip Dámek, Isabelle Villena, Elisabeth A.Innes, Frank Katzer, Clare M.Hamilton Food and Waterborne Parasitology (2020) (<https://doi.org/10.1016/j.fawpar.2020.e00086>) in relation with his previous work but still in the field of Toxoplasma.

8.1.4.14.4 Impact & relevance

From the beginning, the PhD project was built as an interdisciplinary project involving partners from the Vet (ANSES, RIVM), Med (URCA, RIVM) and industrial (IFIP) area. The topic itself touches all three domains, fitting perfectly into the OneHealth approach. Therefore the PhD student is working in an inter-disciplinary environment, gathering together vets, researchers, doctors, pharmacists, engineers, technicians with a broad spectrum of activities such as parasitology, food-product manufacture, risk assessment analysis, statistics, epidemiology. Precisely, the experimental infection of pigs has been performed by JRU BIPAR, the sausages were manufactured by Institut du Porc (IFIP), and tested both by JRU BIPAR and URCA. Later on the results will be interpreted with the help of statisticians (ANSES) and the QMRA model will be run and performed in RIVM. The PhD project and the PhD student are playing perfectly the role of a pivot within this interdisciplinary environment. Without them this part of the research would not have been possible.

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

Requirement (from ethical reviewers)	Measures and actions taken
Environmental and Health and Safety (H&S) Aspects The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project. Animals This project is focusing on pigs. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the pigs). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the pigs are	Due to the Covid19 crisis, a delay of 3 months has to be taken into account, so the estimated completion date is December 2022 All new personnel working in the ANSES laboratories has to undergo a ½ day training/visit of the laboratories with the local Health and Safety correspondent. During this visit, the personnel are highlighted the specific dangers and critical points for each section of the lab and are informed about the relevant local and national guidelines that needs to be followed. The visit ends with the signature of a training sheet summarizing all this points. One copy being kept by the personnel. The entire experimental infection has been already approved by the local Ethical Committee (ANSES – ENVA –UPEC) and the



Requirement (from ethical reviewers)	Measures and actions taken
<p>affected when taking samples, even if the work is dealing with faeces as these types of study can, this can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals).</p> <p>Please provide a statement on the 3Rs aspects of this work</p> <p>If Ethical Approval is required please state which Research Ethics Committee this will be sent to.</p>	<p>Ministry of Research under the number 18-035 (n° APAFIS: 2018032908554996) where all these aspects (3R, welfare, etc) has been detailed.</p>

8.1.4.14.6 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable
WP1-T1: Experimental infection of pigs	M25	M31	M-PhD-ToxSauQMRA-01	M27	M33	See 1 below	n.a.	n.a.
WP1-T2: Predilection sites of <i>T.gondii</i> in pigs	M36	M39	M-PhD-ToxSauQMRA-03	M36	M39	See 2 below	n.a.	
			D-PhD-ToxSauQMRA-WP1					
WP2-T2: Assessment of the persistence of viable <i>T. gondii</i> in pork delicatessen	M42	M45	M-PhD-ToxSauQMRA-05	M45	M48	See 2 below	n.a.	n.a.

Comments:



Due to Covid19 sanitary shut down and the need to repeat 3 times the tissue-cyst oral infection, 6 months delay has to be taken into account. Only ½ of the samples are already collected. On contrary all sausages and ham have been processed.

Due to Covid19 sanitary shut down a 3 months delay has to be taken into account (for the moment), the time needed for the collection and processing of all samples by various techniques. During the shut-down no lab work was permitted.

8.1.4.14.7 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	Yes (see below)

Additional information:

Filip DAMEK is an excellent PhD student, dynamic and pro-active. From the beginning of the PhD project we had to deal with several unexpected problems, but this has not discouraged him, demonstrating he can adapt and is well suited to finalise this project successfully. The challenges included:

1. A last-minute change of the animal facilities: the experimental infection facilities of INRAE Tours cancelled our reservation 2 days before the beginning of the project with very few explanations. We had to find an emergency solution in order to stick to the scheduled time-plan. The ENVA/Anses animal facilities were luckily available to host our animals.
2. A lack of viable parasites: when checking the positivity of our animals at day 30 p.i. we were surprised to realise that all animals in the tissue-cysts group were negative, while the animals from the oocysts group were positive, forcing us to repeat the oral infection. A second infection failed again, most likely to a lack of viable parasites provided by our external partner. A third infection was finally successful, when we fed orally the pigs with mice-brain homogenate following an oral infection performed in ENVA/Anses animal facilities.
3. Strike in Marseille harbour: after collecting the hams from the experimentally infected animals and sent to INRAE Corte in Corsica for short/long time salting, a strike has been declared in Marseille harbour (<https://www.leparisien.fr/economie/la-corsica-linea-annule-toutes-ses-traversees-mercredi-jeudi-et-vendredi-14-01-2020-8236179.php>), blocking our shipment for several days. In fact the hams arrived 12 days after leaving our lab, in a degraded sanitary condition. Therefore only a long salting procedure has been performed.



4. Covid19 shut-down: from 17/03 to 11/05 no lab activities were available postponing all sample collection and treatment. Beginning with 11/05 Filip had an access to the lab, but the research activities are restarting very slowly with multiple difficulties due to the sanitary constrained situation (no more than 1 person/room, social distancing, etc)

8.1.4.14.8 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Filip DAMEK has been involved in the EJP JRP Toxosources, as part of the Anses team, participating at the kick-off meeting (3-4.02, Copenhagen, Denmark) and since then participating at all the videoconferences of the various WPs, where Anses is involved in. Filip visited the RIVM (24.02.20 to 28.02.20) to get acquainted and discuss plans with the QMRA team.

Similarly, part of the Anses team, Filip DAMEK is involved in the national research project n° 0917003490 financed by the French Ministry of Agriculture through the France Agri Mer agency with the title: Study of the tropism and persistence of *Toxoplasma gondii*: from pork carcass to sausage, gathering the above mentioned partners (Ifip, URCA, Inrae Corte), representing in the same time the fundament/basis of his PhD programme.

8.1.4.14.9 List of dissemination and communication activities

Name of the activity:	<i>Electronically poster defence within the Annual Scientific Meeting of OHEJP and participation in the 3MT competition</i>		
Date:	<i>27-29.05.2020</i>		
Place:	<i>Prague</i>		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
<i>Organisation of a Conference</i>	<i>No</i>	<i>Participation to a Conference</i>	<i>Yes</i>
<i>Organisation of a Workshop</i>	<i>No</i>	<i>Participation to a Workshop</i>	<i>No</i>
<i>Press release</i>	<i>No</i>	<i>Participation to an Event other than a Conference or a Workshop</i>	<i>No</i>
<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>	<i>No</i>	<i>Video/Film</i>	<i>No</i>
<i>Exhibition</i>	<i>No</i>	<i>Brokerage Event</i>	<i>No</i>
<i>Flyer</i>	<i>No</i>	<i>Pitch Event</i>	<i>No</i>
<i>Training</i>	<i>No</i>	<i>Trade Fair</i>	<i>No</i>



<i>Social Media</i>	<i>No</i>	<i>Participation in activities organized jointly with other H2020 projects</i>	<i>No</i>
<i>Website</i>	<i>No</i>	<i>Other</i>	<i>No</i>
<i>Communication Campaign (e.g. Radio, TV)</i>	<i>No</i>		<i>No</i>
<i>Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories</i>			
	<i>Number</i>		<i>Number</i>
<i>Scientific Community (Higher Education, Research)</i>	<i>750</i>	<i>Media</i>	
<i>Industry</i>		<i>Investors</i>	
<i>Civil Society</i>		<i>Customers</i>	
<i>General Public</i>		<i>Other</i>	
<i>Policy Makers</i>			

8.1.4.15 PhD15-R2-FBZ5-TRACE

8.1.4.15.1 Progress of the research performed in the PhD project and key scientific results

Work package 1 Whole genome sequencing.

During the first 9 months of the project work was done to establish a sample (pre)processing (enrichment) to make whole genome sequencing possible. Different methods were used and DNA depletion treatments were explored. mRNA of 18S (host) and 16S (bacterial) origin was successfully removed. HEV whole genome sequences were successfully generated using above enrichment methods and specific primers. Currently work is done to analyse sensitivity. HEV culture preceding sequencing may be considered to increase the amount of HEV virus (work on a culture method is part of BIOPIGEE project).

Work package 2 HEV phylodynamics.

Using HEV whole genome sequences data generated in WP1, we will construct a timed-phylogeny of HEV based on core-genome SNP analysis. From this analysis we can infer time-points of evolutionary divergence of different HEV types. Coupled to geographic location data of the isolates we will also include a spatial dimension to the phylogeny which will inform us on the geographical origin and spread of HEV types over time. Comparison of sequences over time will also be used to identify sequences that differ between time periods in which we observed a different epidemiology of HEV, giving indications of probable increased colonization success in pigs, increased environmental survival and/or increased virulence.

Work package 3 Identification of HEV virulence genes and HEV quasispecies.

Work on this work package is planned to start in month 34



Work package 4 Data analyses and data evaluation

Work on this work package is planned to start in month 34



8.1.4.15.2 Progress of the research project: milestones and deliverables

8.1.4.15.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD15-FBZ5-TRACE	1	Sample processing protocol for HEV RNA positive target samples of different origin and associated deep sequencing procedure for HEV from such samples	36		42	Due to the Coronavirus crisis a 6 months delay is expected
	2	Report/publication on HEV dynamics including information about the geographical origin of predominant virulent strains and identification of genetic traits changed over time.	36		42	Due to the Coronavirus crisis a 6 months delay is expected
	3	Identification of virulence genes and elucidation of the relationship between quasispecies and changing virulence of circulating HEV strains	48		54	Due to the Coronavirus crisis a 6 months delay is expected



PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
	4	Report explaining HEV strain shifts and HEV disease outcomes related to HEV circulation and HEV variability. Evaluation of results for future anticipation and intervention as possible.	60		64	Due to the Coronavirus crisis a 4 months delay is expected
	5	Evaluation of results for future anticipation and intervention as possible.	60		64	Due to the Coronavirus crisis a 4 months delay is expected

8.1.4.15.2.2 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD15-FBZ5-TRACE	1	Detailed outline PhD plan	24			



8.1.4.15.3 Publications and patents

No publications have been produced yet.

8.1.4.15.4 Impact & relevance

One Health EJP SRA 1 Updated List and Descriptions of Priority Research and Integrative topics: “risk factors and infection dynamics”. Development and harmonisation of deep sequencing-based methods for detection and tracing of foodborne zoonotic agents and emerging threats have been identified as a main research item within the updated EJP One Health SRA. Data from this project will lead to improved surveillance and more harmonized data analyses on the foodborne zoonosis HEV. This will contribute to broader and flexible actions to detect actual hazards, main reservoirs, trends and routes of transmission as well as common approach and timely analysis and data sharing which will be needed more and more with ongoing globalization

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

Requirement (from ethical reviewers)	Measures and actions taken
<p>The beneficiary must confirm that appropriate authorizations will be sought to collect the Human samples.</p> <p>Please reconfirm that this work is not using or interacting with animals directly, please reconfirm this.</p>	<p>In this case the appropriate authorization regarding the collection of human HEV sequences is RIVM itself. In addition, we confirm that this research is not using animal s directly.</p>

8.1.4.15.6 Impact of COVID-19 crisis on the project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
HEV Whole genome sequencing	48	54	D1	48	54	Covid 19	NA	2.5kEuro
HEV Phylodynamics	48	54	D2	48	54	Covid 19	NA	2.5kEuro
Identification of HEV quasispecies	60	64	D3	60	64	Covid 19	NA	2kEuro



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M25-M36

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Analyses HEV bioinformatics data	60	64	D4	60	64	Covid 19	NA	1kEuro

8.1.4.15.7 List of critical risk

Description of risk	Yes/No
Loss of PhD supervisor(s)	N
Loss of technical training staff delaying progress of the work	N
Delay in work plan execution	Y
Conflicts between the collaborative partners that support the PhD	N
Lack of commitment between the collaborative partners that support the PhD	N
Delay in duties, tasks or reporting	N
Poor working relationships within the PhD project team	N
Change in PhD student circumstances requiring temporary leave	N
Other risks (please describe)	N

8.1.4.15.8 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

This PhD project relates to the EJP OH project BIOPIGEE: HEV viability screening method developed in BIOPIGEE may be used In TRACE as applicable.

8.1.4.15.9 List of dissemination and communication activities

Not Applicable



8.1.4.16 PhD16-R2-FBZ2/AMR6.1-Codes4strains

8.1.4.16.1 Progress of the research performed in the PhD project and key scientific results

Background and aim.

One of the main objectives of the PhD project is to develop a novel nomenclature system, potentially applicable to all bacterial pathogens. However, our first focus is on a highly antibiotic resistant bacterial pathogen, *K. pneumoniae* (Kp). Secondly, we will focus on a foodborne bacterial pathogen, *E. coli* (Ecoli).

The main strain nomenclature approach, which is currently widely applied to bacterial pathogen surveillance, is based on core genome Multilocus Sequence Typing (cgMLST). It relies on sets of predefined gene loci, the sequence variants of which are given unique identifiers (allelic numbers). Resulting allelic profiles are given unique identifiers (cgST) or are grouped based on their similarity, generally using the single-linkage clustering method. However, one of the main limitations of the cgMLST classifications is that they suffer from instability: in case of discovery of 'intermediate' genotypes, the fusion of pre-existing groups can happen, due to the inherent mathematical property of single-linkage. This introduces nomenclatural confusion and requires manual management of group delineation. A strain naming approach that avoids the genotype identifier instability problem was introduced by Vinatzer and colleagues (1), and is known as LIN (Life Identification Number) code. This approach provides a multi-level numerical code for each genome based on its similarity (Average Nucleotide Identity, ANI) to the closest genome already encoded. As it does not rely on single-linkage clustering, this provides a stable, definitive code to each genome. However, ANI is not an accurate metric and is not reproducible enough for strains that are very closely related, and is therefore not recommended for epidemiological surveillance or outbreak investigation studies. Until today, cgMLST and LIN codes have been implemented separately.

The aim of the PhD project is to develop a novel NGS-based genotyping approach taking the best of the above worlds, i.e., combining the advantages of cgMLST (reproducibility, standardization) with those of the LIN code approach (complete stability). That is, the development of the cgMLST-based LIN code systems, where the pairwise distance will be based on the number of allelic mismatches, rather than ANI.

D2.1. Pilot genome dataset

We first used a pilot dataset of *Klebsiella pneumoniae* genomes. We used the publicly accessible database of *Klebsiella* genomes (<https://bigsd.b.pasteur.fr/klebsiella/klebsiella.html>), which comprises data submitted by more than 400 labs worldwide. A set of genomes were removed after quality filtering. The pilot dataset of genomes was defined to comprise 751 isolates. This dataset represents the non-redundant diversity and population structure of *Klebsiella pneumoniae*, including the different phylogroups and some strain sets derived from outbreaks (M2.1).

D3.1. cgMLST schemes for Kp and Ecoli

Klebsiella pneumoniae:

As a metric for the LIN code system, we intend to use pairwise distances between the allele profiles. We first had to make a choice of which cgMLST scheme to use. In 2014, Bialek-Davenet et al (2) defined a cgMLST scheme of *Klebsiella pneumoniae* that included 634 highly conserved and syntenic genes. Here, we re-evaluated this cgMLST schema with our dataset, and removed five loci that were of low prevalence among this set of genomes; resulting in a cgMLST scheme that comprises 629 loci. In addition, the loci templates were extended to include entire coding sequences of the corresponding genes (M3.1).



To define the allelic profiles, the 629 loci were scanned for presence and allelic identity in the 751 genomes. Pairwise cgMLST distances were then computed as the number of distinct alleles, ignoring missing data in pairwise comparisons, i.e., the number of mismatches was divided by the proportion of loci called in both strains.

Escherichia coli:

We have not yet chosen the cgMLST scheme for this species yet. One obvious candidate, given its widespread use, is the one from EnteroBase.

D3.2. LINcodes algorithm defined and implemented on full dataset

We used the pilot *K. pneumoniae* dataset to develop a LIN code algorithm. We have implemented the LIN code attribution algorithm using the Python programming language (**M3.2**). The developed LINcoding software tool takes as input a file of allelic profiles and a set of thresholds, defining a LIN code scheme. Then, the tool returns two files:

- LIN DataBase: a file containing the LIN codes associated with each allelic profile, allowing to create new LIN codes from future profiles;
- Genome LIN code list (genome names and their LIN code)

In the course of our experiments and literature searches, we noticed that this method has a significant problem of dependency on the order of entry of isolates. In other words, according to their encoding order, the same set of allelic profiles can lead to LIN codes resulting in categorizations by different prefixes. We therefore sought to minimize this problem by defining an optimal input order. As a result, we use the input order guided by a minimum spanning tree algorithm.

To implement the LINcodes approach to the full dataset of the *K. pneumoniae* species, an important issue was the definition of the set a thresholds. We aimed to define thresholds taking into account the phylogenetic structure of the species, and to maximize its usefulness in population biology and epidemiology. The codes must also be intuitive enough to be largely adopted by epidemiologists. We are now finalizing a prototype comprising 10 thresholds based on our current understanding of the phylogenetic structure of the species. In short, the distribution of pairwise similarity values among 7000 available genomes was used to determine discontinuities in the genetic structure of the species.

References:

1. Marakeby H, Badr E, Torkey H, Song Y, Leman S, Monteil CL, Heath LS, Vinatzer BA. 2014. A system to automatically classify and name any individual genome-sequenced organism independently of current biological classification and nomenclature. PLoS ONE 9:e89142.
2. Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Jones L, Delannoy-Vieillard AS, Garin B, Le Hello S, Arlet G, Nicolas-Chanoine MH, Decre D, Brisse S. 2014. Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. Emerg Infect Dis 20:1812–20.



8.1.4.16.2 Progress of the research project: milestones and deliverables

8.1.4.16.2.1 Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD16-FBZ2/AMR6.1-Codes4strains	D2.1	Pilot genome dataset	12	12		None
	D3.1	cgMLST schemes for Kp and Ecoli	3		12	The E. coli scheme will be chosen at later stage, probably based on EnteroBase. We chose to fully evaluate the approach on K Pneumoniae first, before turning to E. coli
	D3.2	LINcodes algorithm defined and implemented on full dataset	6	6		None



2.1.1.1.1.1 Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD16-FBZ2/AMR6.1-Codes4strains	M2.1	Pilot genome dataset defined	12	Yes		
	M3.1	cgMLST schemes defined for Kp and Ecoli	3	Yes		For K. pneumoniae only. Implementation to E. coli will be fast once the approach is fully defined on K. Pneumoniae
	M3.2	LINcodes algorithm defined	6	Yes		



8.1.4.16.3 Publications and patents

No publications

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 71.

8.1.4.16.4 Impact & relevance

The project will define, implement and evaluate a novel bioinformatics strategy to classify and name strains of pathogenic bacteria, from the level of deep lineages down to shallower levels of diversity that differentiate epidemiological related strains from non-related ones. It will be first tested on Ec and Kp, two important ubiquitous 'One Health' pathogens. However, the general applicability of the approach means that in the future, the classification and nomenclature of strains of other pathogens could benefit from the PhD project outcomes.

By facilitating in the future, communication on bacterial strains across sectors and countries, the project is highly relevant to multiple topics and objectives of One Health EJP: antibiotic resistance clonal dissemination, emerging pathogens, cross-sector transmission, public health and basic microbiology integration.

The project will deliver a novel nomenclature system of bacterial pathogens genomes that will be stable by design, unlike existing systems based on SNPs and cgMLST single linkage groupings. This has a far-reaching impact on possibilities to integrate efforts of agencies (e.g. at the international levels, ECDC, EFSA, PulseNet international) to detect, monitor, understand and control the spread of pathogens.

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

N/A

8.1.4.16.6 Impact of COVID-19 crisis on the project

N/A

Comments: No impact, as our bioinformatics work was not affected.



8.1.4.16.7 **List of critical risks**

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	No
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No



Description of risk	Yes/No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No

8.1.4.16.8 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

The novel method developed in the PhD project will find natural dissemination ways via the existing networks of collaborations in which the main investigators are involved: MedVetKlebs, klebNET, SpARK, kleb-GAP and Nor-Kleb-Net for Kp; and E. coli surveillance networks and national and international levels (e.g., French NRC & NRL @Pasteur and ANSES; ECDC; PulseNet international).

The novel nomenclature system will be compared with existing nomenclatures (dictionaries of nomenclature correspondence between LIN codes and current SNP/cgMLST nomenclatures for wider communication and backwards-compatibility) and will need in the future to be integrated in existing platforms that serve nomenclatures of bacterial strains (e.g., SnapperDB, EnteroBase, BIGSdb-Oxford and PulseNet international for E. coli; BIGSdb-Pasteur for K. pneumoniae). Future interactions with 'dev-op' specialists (application developers) will be established with free software such as EnteroBase, BIGSdb and Innuendo; or commercial software such as BioNumerics or SeqSphere.

8.1.4.16.9 List of dissemination and communication activities

Name of the activity:	<i>One Health EJP Annual Scientific Meeting 2020</i> Poster : A new approach for typing bacterial strains, based on the joint use of cgMLST and LIN codes, and its application to <i>Klebsiella pneumoniae</i> species		
Date:	27 th _ 29 th May 2020		
Place:	Digital Conference		
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 2020 Oral : Three Minute Thesis (3MT) competition : Codes4strains		
Date:	27 th _ 29 th May 2020		
Place:	Digital Conference		
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	



<i>Non-scientific and non-peer-reviewed publication (popularised publication)</i>		<i>Video/Film</i>	
<i>Exhibition</i>		<i>Brokerage Event</i>	
<i>Flyer</i>		<i>Pitch Event</i>	Yes
<i>Training</i>		<i>Trade Fair</i>	
<i>Social Media</i>		<i>Participation in activities organized jointly with other H2020 projects</i>	
<i>Website</i>		<i>Other</i>	
<i>Communication Campaign (e.g. Radio, TV)</i>			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Num ber		Num ber
<i>Scientific Community (Higher Education, Research)</i>		<i>Media</i>	
<i>Industry</i>		<i>Investors</i>	
<i>Civil Society</i>		<i>Customers</i>	
<i>General Public</i>		<i>Other</i>	
<i>Policy Makers</i>			

8.1.5 Task 6.5: One-Health Continuing Professional Development (CPD) Module

The first Continuing Professional Development (CPD) module was planned to be a two-day event on 12th-13th March 2020 organised by RIVM, with the theme 'Outbreak Preparedness'. The COVID-19 crisis caused this event to be postponed just days before it was to take place.

A virtual interactive workshop will now be delivered in M35 by RIVM. This event will be offered to those who were originally selected for participation. Depending on the final numbers confirmed, the application call may be launched once more.

Sharing best practices and experiences of One Health approaches stands at the centre of this first CPD module, providing a learning platform aimed at knowledge integration and strengthening of One Health collaboration across Europe. The module was targeted towards Early Career Researchers (<5years post-PhD) and students from within the One Health EJP consortium. The module is planned to accommodate 25 delegates who were selected before the COVID-19 crisis. This outbreak preparedness module aims to provide:

1. an understanding of the basic principles of infectious disease control, as well as monitoring and evaluation;
2. a platform to share experiences and lessons learned from past outbreaks;



3. an understanding of the Dutch zoonoses response structure, as an example of a One Health approach in prevention and response;
4. insight into relevant aspects of risk perception and communication to professionals and the public;
5. an interactive experience in risk assessment and response to a zoonotic disease.

The outbreak preparedness module consists of a mixture of lectures and interactive working group sessions.

The first CPD deliverable report (D6.5) has been requested to be postponed again. The deadline had already been delayed from M24 to M28, due to a lack of CPD applications, extension of deadline, and hence a later event. A revised deliverable deadline for D6.5 of M36 will be requested.

The second CPD module call was closed and the local organising team was selected in M27. The second CPD module will take place in M38 organised by BfR (Germany), with the theme "Digital innovation for One Health practitioners". The second CPD deliverable report (D6.9) has been requested to be postponed from M36 to M40.

The CPD module organiser selection procedures and protocols were reviewed, modified where necessary and validated for the event taking place in Y4. No applications to organise this event were received and the call has been extended.

8.1.6 Task 6.6: Communications workshop and media training

Successful planning and organisation of the Communication and Media workshop has taken place for planned delivery in M34. The Bulgarian Food Safety Agency will be hosting and organising this two-day event in collaboration with UoS. The dates of this event are 5th-6th October 2020; however, the COVID-19 crisis has caused challenges in delivering a physical workshop. This event was planned and re-organised as a virtual event and will still be delivered in M34. Deliverable report D6.2 will be delivered as planned in M36. WP6 and local organisers have planned a high-quality interactive programme to be delivered on the Google Meets online platform.

8.2 Deliverables and Milestones

8.2.1 Deliverables

Del. Rel. No	Deliverable title	Submission
D6.5	Report of the first CPD module in one health	M29 – postponed due to Covid-19 situation
D6.6	Report n°1 of the annual short term missions completed also uploaded onto the EJP webpage.	M26

8.2.2 Milestones



Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS79	Launching of annual call for Short Term Missions n°2	M28	The call was launched in M25

9 WP7 – Sustainability

9.1 Work carried out to date

9.1.1 Task 7.1: Gathering Stakeholders' Needs and Expectations

A SWOT analysis has been completed and delivered: the analysis of strengths, weaknesses, opportunities and threats of the present and future of the OHEJP is considered as a main support to the OHEJP sustainability (Deliverable 7.1).

Due to the response rate (20%), the results of the SWOT can provide a partial picture. Nevertheless, the responses were sharp and detailed, and the outcomes represent a useful indication. Therefore, the outcomes of the SWOT analysis need to be followed up by future activities by WP7 as well as other WPs of the OHEJP. A synthetic analysis of the indications shows:

A). Strengths and opportunities.

Whereas the identification of relevant themes has been lengthy due to some redundancy, respondents have clearly and sharply identified clusters of strengths and related opportunities:

- 1) One health implementation through collaboration across sectors
- 2) The OHEJP develops solid international collaboration opportunities between researchers
- 3) The OHEJP offers a multidisciplinary base for construction of consortia for participation in other calls
- 4) Training opportunities, implementing dissemination in the broader sense
- 5) Last but not least, the OHEJP produces capacity building on One Health

To summarize, the establishment and activity of an EJP consortium on One Health is generally perceived as a winning strategy. The strengths and related opportunities, therefore, must be taken into account for envisaging strategies for the sustainability beyond the due EJP term.

B) Weaknesses and threats.

A critical overview of weaknesses and related threats is of high interest and relevance for the OHEJP sustainability. Beyond the already mentioned redundancies, the major weakness clusters (which can also become threats) are:

- Related to the OHEJP concept

- 1) Imbalance towards animal health and food safety with respect to the human health sector This is a usual problem with OH initiative. The involvement of the medical expertise is all-important, but still lagging behind. A strong effort should be devoted to fill this gap for any future concept of the OHEJP to be successful. Also applies to the following point.



2) Lack of the “environment” component and “ecological/complex” approach. Poor consideration of specific overarching drivers such as climate change. Actually, Environmental issues are increasingly present -albeit still to a limited extent- in several OHEJP activities, such as PhD projects. What is still missing is a consistent vision of “environment” with the goals and conceptual frame of the OHEJP. Such vision can be particularly relevant in regard of such topics as emerging threats (early warnings, comprehensive surveillance systems) or AMR (risk assessment).

3) Lack of effective stakeholder involvement. In particular the agencies have so far (time of SWOT) only been “assisting” the activities, but their actual contribution has been very limited, definitely less than it was foreseen when the OHEJP was planned. This might be critical for EJP long-term sustainability. A step forward can be to distinguish: non optimal approach to involvement of OHEJP institutional stakeholders with regard to specific activities, including both projects and training; and lack of effective involvement for other stakeholders. An overall strategy/governance of such involvement within the OHEJP is needed.

4) Lack of measurement of the added value/products/deliverable of the OHEJP Consortium and non-optimal interaction with OHEJP Project owners at national level

In general, a lack of understanding by Project owners of the added value of the OHEJP products and networking is a critical issue for financial sustainability and commitment.

- Related to the management of the OHEJP

1) The administrative superstructure that makes it difficult to use resource. Whereas the complex and cumbersome administrative structure is mainly inherent to the EJPs in general as envisaged in H2020, it cannot be overlooked that this is perceived as a problem by many respondents. The lack of flexibility of the administrative procedures within the consortium might also contribute to the deficient stakeholder involvement. Thus, an effort to reduce its impact is definitely worthwhile, also because it influences the following point.

2) Difficult co-ordination, notwithstanding the strenuous efforts by the individuals in charge. Consequences: too many structures, with difficult and lengthy decision-making and managing of internal calls. This latter concern may have been caused, at least partially, by the large overlaps between the actions taken by different projects funded internally. This aspect cannot be overlooked as it represents a weak link of the strong scaffold of the OH-EJP and is perceived as a problem by many respondents. Thus, a streamlining of the structure has to be contemplated.

3) Getting more comprehensive at EU level; and get a more global breath. Indeed, greater involvement and participation of EU Countries involves dealing with the co-funding issue but is of evident importance for the long-term sustainability of the OHEJP. The more global approach for a EU-targeted project could target primarily the acceding/neighbouring Countries and pivot on training activities.

The main threats, actually, stem from the weaknesses of the OHEJP if they will not be adequately addressed. In addition, there is a widespread awareness that BREXIT would be a risk and a rather serious one. Other threats are definitely less likely: the possibility of real competitors on the OHEJP topics is low. On the other hand, a closer connection with project owners is required in order to maintain the focus on the OHEJP topics, because these could be challenged by other priorities. Indeed, the rampaging Covid19 (appeared after the SWOT) could boost the attention toward a OH approach to emerging infectious threats. Of course, to exploit this opportunity a fast and effective EJP response is needed.

9.1.2 Task 7.2: Strategic Research and Innovation Agenda (SRIA) 2021-2030)

In Y3 WP7 has started a plan to implement the SRIA in collaboration with WP2, considering the results of the SWOT analysis and the demands of stakeholders as well as the inputs of the OHEJP Consortium. Work to be completed in Y4.



9.1.3 Task 7.3: Making the EJP sustainable through other funding and/or legal basis

In Y 3 WP7 has started a plan in collaboration with WP2, to assess the new opportunities for OHEJP sustainability provided by Horizon Europe and Partnerships. As well as the drivers, that modulate the demands of stakeholders. This task will be completed in Y 4.

9.1.4 Task 7.4: Making the bridges between EJP's beneficiaries and stakeholders sustainable

For the PhD project SUSTAIN, interviews were collected from experts of the Swedish Veterinary Agency, the Swedish National Food Agency and the Swedish Public Health Agency. Due to the covid-19 pandemic all traveling was suspended, which entailed the restructuring of the face-to-face interviews in Sweden to online interviews. The interviews are part of a qualitative study on the institutionalisation of One Health. The interviews were transcribed, analysed and will be compared with interviews conducted with experts in Italy. Additionally to the qualitative study, a literature search was conducted and evolved into a bibliometric analysis of One Health literature. This analysis resulted in the development of the article "The state of one health research across disciplines and sectors – a bibliometric analysis" (<https://doi.org/10.1016/j.onehlt.2020.100146>).

It was planned to disseminate the findings at two conferences. The World One Health Congress in Edinburgh was postponed from June to the 30.10. – 03.11.2020 due to the covid-19 pandemic. The One Health EJP Annual Scientific Meeting 2020 was transformed to an online event, where a poster on the bibliometric analysis of One Health was presented.

Besides this, teaching activities at the Roskilde University on global health governance and supervision of bachelor and master semester projects were performed.

9.2 Deliverables and Milestones

9.2.1 Deliverables

Del. Rel. No	Deliverable title	Submission
D7.1	Report of the end user Stakeholders' Needs and Expectations	M36

9.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS98	Organisation of the Workshop on Stakeholders and users' Needs and Expectations	M27	Workshop postponed due to Covid-19 situation

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