One HEALTH EJP

D1.6 Summary Progress Report Year 2

WP1 Coordination

Responsible Partner: ANSES
Contributing partners: all partners

GENERAL INFORMATION

European Joint Programme full title	Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards
European Joint Programme acronym	One Health EJP
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1   Publishable summary

1.1   Summary of the context and overall objectives of the project

1.1.1   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

1.1.2   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.

1.1.3   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.

1.2 Work performed during the reporting period (M13-M21) and main results achieved

1.2.1 WP1

Following retirement of the former Coordinator André Jestin on 31st December 2018, a new organisation of the Coordination level was born as of 1st January 2019, based on what was previously agreed among the consortium members:
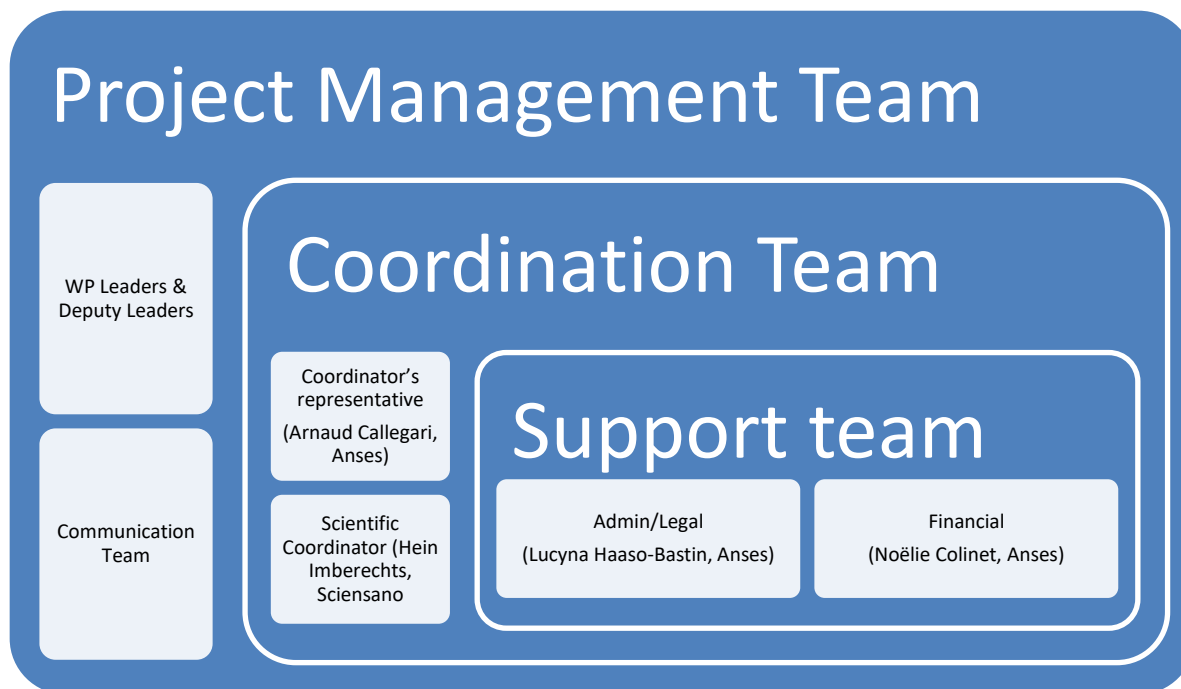
The Coordination Team continues with the management of the EJP by having weekly conference calls for the day-to-day management of the project. Also regular conference calls and two face-to-face meetings (30 January and 21 May, the latter together with External Scientific Advisory Board in Dublin) with the Project Management Team were held in order to follow up with all WP leaders and deputy leaders.

The meetings were set up with the Scientific Steering Board, the Programme Management Committee, the Programme Owners Committee and with the External Scientific Advisory Board.

On 14th February 2019, the meeting with the Research Executive Agency was organised in order to inform them on the amendment and periodic reports. The second meeting was held on 5th July 2019 and gathered the OHEJP Coordination team and the REA's Steering Board.

The Support Team has managed finances and the financial report of year 1 as well as the first amendment to the Grant Agreement.

The Coordination Team together with the Project Management Team has prepared the periodic report.

The Communication Team based at University of Surrey has implemented the following activities:

- The One Health EJP website was redesigned, rebranded and relaunched in M13. This has been ongoing to ensure that the website content is continually up to date and closely monitored.

- The One Health EJP was rebranded to ensure the alignment of all material disseminated by the One Health EJP. This includes all One Health EJP templates (minutes, agendas, deliverables etc.), all One Health EJP merchandise and all One Health EJP flyers and programmes.

- A selection of One Health EJP merchandise has been created and disseminated at One Health EJP events, including the Annual Scientific Meeting and the first One Health EJP Summer School. Merchandise includes pens, notepads, post-it notes and lanyards.

- The One Health EJP Communications Strategy has been further developed and the first version of this document was disseminated. This strategy outlines how the communications should be conducted throughout the One Health EJP; including the communication objectives and procedures, digital strategy, the events strategy and a detailed description of the branding and communication tools available to members of the consortium.

- The deliverable D1.5- First Annual Report for the Stakeholders has been completed and submitted on 1st October 2019. This document is targeted to the One Health EJP stakeholders, including the general public. It details the key objectives of the One Health EJP, progress of each Work Package and the dissemination activities of the One Health EJP.

- Flyers for the One Health EJP ASM Satellite Workshop and the Summer School were created and disseminated to advertise these events and encourage participation. These flyers and tools were aligned with the branding outlined in the One Health EJP Communication Strategy. This ensures that the One Health EJP is easily recognised and that the events generate interest.

- The One Health EJP ASM was the first platform available for the Communication Team to disseminate One Health EJP information. The Communications Team managed a Communication Booth at this event which provided delegates with One Health EJP merchandise, the Summer School flyer, One Health EJP highlights flyer, WP6: Education and Training information and the One Health EJP brief and flyer. The Communication Officer and Communication Manager were also available to offer support to One Health EJP consortium members with any requests.

1.2.2   WP2

In the first month of 2019 the updated SRA was delivered (D2.7). In addition to the updated SRA, which was delivered as a confidential document, 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: Summary version of the updated Strategic Research Agenda. This additional deliverable based on D2.7 and intended for public dissemination has been added to the list of deliverables. In addition, the repository of EU projects/initiatives that is available to all partners through the OHEJP website has been updated. Strategic interactions with some related EU projects and initiatives, such as JPIAMR and JAMRAI, were conducted and an analysis was made of other the relevant EU-projects/initiatives.

1.2.3   WP3

The second year has been dedicated to the supervision of the 1st call JRPs in the first round of projects.

During Y2, WP3 has also carried out the evaluation of 2nd call proposals for JRPs in parallel with WP2, and prepared assessment summaries for the SSBs decision meeting in September 2019. Relevant guidelines for final reporting and evaluation of those reports have been produced together with WP4.

The first ASM, ASM2019, was successfully organized, and the organisation of ASM2020 was started.

1.2.4 WP4

During Y2, WP4 has successfully carried out the evaluation of 2nd call proposals for JIPs in parallel with WP3, and prepared assessment summaries for the SSBs decision meeting in September 2019. Relevant guidelines for final reporting and evaluation of those reports have been produced together with WP3. Supervision of and interaction with the JIPs has continued, with the 1st periodic report identifying a series of suggestions for improvements that have been taken on board. More emphasis has been put on internal integration, both in the formulation of the 2nd call and through the reallocation of additional funding for the integration of additional partners in ongoing projects, where at least 4 new partners are joining forces with one of the JIPs (COHESIVE). The JIPs have also had extensive external contacts, and WP4 is supporting this development by giving a thematic/cogwheel workshops, for example on institutional experiences of implementing NGS as a tool for surveillance.

WP4 has also taken responsibility for launching an initiative on data sharing, to bring together legal expertise from partners to explore the possibilities to have more harmonised approached to the implementation of GDPR. This initiative has been well received both by the stakeholders and by the Programme Managers.

WP4 has also continued to support partners in their practical learning about how to develop data management plans, and to push the need for institutional support and development in this respect. Practical guidance has been given in webinar, report, forum and workshop format. In addition, the OHEJP publication policy has been developed by WP4.

1.2.5 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 (FAO, OIE, WHO). The third Stakeholder Committee meeting was held in connection to ASM 2019, and the fourth is prepared to take place in October/November 2019.

A help desk was established to easy communication on specific support needs of key EU stakeholders.

It was concluded that the needs of the Key EU stakeholders had been well taken into account in the strategic priorities defined in WP2 and addressed in the second round of proposals managed by WP3 and WP4.

The dissemination activities of WP5 were established and further developed to disseminate new scientific data and results to the different stakeholder groups in a targeted manner.

The capacity map was implemented and content updated to support interaction and collaboration between consortium members and stakeholders.

1.2.6 WP6

During Y2, WP6 has ensured the following events activities:

- The second call of the doctoral programme was launched, and a further 12 projects were selected in this second call. A total of 16 PhD grants have been selected for funding, which is 4 more than originally anticipated.

- The first Short Term Mission (STM) call was launched in M13, and a total of 4 Short Term Missions were selected for funding in 2019.

- The Summer School 2019 call was closed in M13 and the validated procedure was followed to select the organisers. The Summer School this year was selected to be organised by University

of Surrey (UoS) in collaboration with Public Health England (PHE) and Wageningen Bioveterinary Research. The Summer School 2019 was titled 'Approaches towards One Health Operationalisation' and will be hosted by UoS and Chatham House from 18th August to 30th August 2019, in addition to pre-online learning material and follow up tasks and projects.

- The ASM Satellite Workshop 2019 call was closed in M14 and the validated procedure was followed to select the organisers. The event was co-organised by SVA, Sciensano, and the University of Surrey, and the workshop theme was 'Digital Innovation and Data Management'. The workshop was hosted by Teagasc at the ASM in Dublin on 21st May 2019.

- The Continuing Professional Development (CPD) module call was closed in M16, and the validated procedure was followed to select the organisers. The organisers were selected in M18. The first CPD module will be a two-day event in early 2020 organised by RIVM, with the theme 'outbreak preparedness', with a further longer CPD event planned later in 2020.

- All protocols were reviewed, modified if necessary and validated for the selection procedure for the organisers of the WP6 events taking place in 2020.

- The Communication and Media workshop call was launched in M14 and the deadline was in M18. The validated procedure was followed to select the organisers. The Bulgarian Food Safety Agency will be hosting and organising this two-day event in 2020.

- The calls of all other WP6 activities in 2020 were launched in M14-M17. These are the Summer School, Satellite Workshop, CPD module and Short Term Missions.

- The ASM Satellite Workshop 2020 call was closed in M19 and the validated procedure was followed to select the organisers. The event will be organised by RIVM (the Netherlands) and the workshop theme will be 'New and Emerging diseases'.

- The Summer School 2020 call was closed in M19 and the validated procedure was followed to select the organisers. The event will be organised by Wageningen BioVeterinary Research (the Netherlands) which will build on the curriculum from the first summer school.

- The Short Term Mission 2020 call was closed in M20 and the validated procedure was followed. A total of six applications were submitted and will be sent for independent review in M21.

- The CPD module 2020 call closes in M21 and the validated procedure will be followed.

1.2.7 WP7

WP7's activities were mainly dedicated to the SWOT analysis. The objective of the SWOT is to collect stakeholders' needs and views which will help us to find the best scenario to main the EJP sustainable.

The main messages from SWOT analysis are of appreciation for the high-quality scientific work done and the consistent networking at EU level, as well as the potential to produce transferable outcomes regarding One Health practice. In the meanwhile, the analysis highlighted three main points to achieve a long-term sustainability, namely: a greater involvement of medical expertise (human health); a greater attention towards the environmental aspects of One Health; and an effort towards networking and knowledge exchange/transfer at global level, i.e. beyond the EU. The SWOT analysis will be followed up in Y3.

Following a preparatory meeting between the WP7 chair (ISS) and Vice-chair (Institut Pasteur) held at Institut Pasteur (Paris) on June 2019, two events were organized:

- A physical meeting between ISS and OIE held at OIE headquarters (Paris) on September 17, 2019. Indeed, following a discussion among ISS partners, the OIE was identified as one main

stakeholders in order to achieve a more global impact. During the meeting ISS presented the OHEJP activities; an OIE officer acting as contact point with OHEJP was identified.

- A physical meeting of WP7 involving representatives from ISS, Institut Pasteur, BfR, ANSES, RIVM, SVA as well as Sciensano held on September 27, 2019 in Brussels. The aim of this meeting was to discuss and fine-tune the short- and medium-term plan of activities, with a special towards Horizon Europe, further involvement of global stakeholders (OIE, FAO, WHO), involvement of national stakeholders beyond the EU (eg, Countries of the Mediterranean Basin).

1.3 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.

2 WP1 - Coordination and Management

2.1 Work carried out to date

2.1.1 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 (Ethical review report for Y1, 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 first amendment to the Grant Agreement including budget adjustments, clarification of articles 11, 12 and 15 of the Grant Agreement used by some Beneficiaries, withdrawal and fusion of partners and update of the work programme. The 1th Amendment to the Grant Agreement (AMD-773830-13) has been modified twice at the request of the REA. The final version of the Amendment (AMD-773830-32) was submitted on 5th June 2019 and approved by the European Commission on 22nd July 2019.

2.1.2 Task 1.2: Project management

The CT, consisting of the Coordinator, Deputy Coordinator and Support Team, has 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 organised weekly teleconferences to monitor the project's progress and to ensure the timely implementation of the AWP year 1. 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) have had monthly teleconference 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. They also validated the deliverables which have been prepared and submitted during this period. This has been facilitated with some face-to-face meetings, shared monitoring tools, regular phone meetings and mail exchanges.

2.1.3 Task 1.3: Organisation of EJP management and governance meetings

One Scientific Steering Board (SSB) meeting at RIVM in Bilthoven, The Netherlands was held on 21 March to present the activities of the first year and the planning for year two. Under the presidency of Karin Artursson (SVA) and Burkhard Springer () the first amendment to the Grant Agreement, the Periodic Technical Report and the Periodic Financial Report were presented and validated. An overview of the ongoing JRP and JIP was given, as well as the details regarding the second call of JRP and JIP that was launched in October 2018. Finally, the SSB agreed on the extension procedure for projects and more specifically on that of project MoMIR-PPC.

The second SSB meeting was held on 19 September at UCM in Madrid. The participants agreed on the selection of the JRP and JIP proposals for co-funding, based on the individual evaluations of the full proposals by external experts, and a thorough discussion on the impact that the project might have for the objectives of the OHEJP.

On 9 May 2019 the first Programme Management Committee meeting was held at ANSES in Paris. The General Directors or CEOs of the beneficiaries were informed about the monitoring of all WPs of the OHEJP (including the amendment, and first year technical and financial report) and of the comprising JRP and JIP, as well as on the second call actions. Also, a discussion was held that focused on a possible way to extend the activities started under this OHEJP, for instance as a EU Co-fund Partnership under Horizon Europe. The participants agreed that the added value of the actual OHEJP, especially the networking among peers and the alignment of methodologies for reference tasks and outbreak management are important and should not get lost after the OHEJP deadline of December 2022.

The first face to face meeting with the External Scientific Advisory Board (ESAB) was held in Dublin on 23 May 2019, in parallel with the first Annual Scientific Meeting. Three members were present in person and three others connected through Skype. The participants were very much supportive of the OHEJP, which they found well organized and with very interesting objectives, i.e. the support of the specific network aiming at the alignment and harmonization of activities. The main recommendations were reach out to new partners, also outside Europe, to look for a process to react on urgencies and to link with existing initiatives (cfr ECDC and EFSA) in order to avoid overlap. The ESAB also supports all initiatives to look for an extension of the OHEJP after 2022.

On 19 June 2019 a meeting with the Programme Owners Committee was held at ANSES, Paris. The objectives of that meeting was, beside informing the ministries and food agencies of the respective EJP beneficiaries on the activities done during the first year, to discuss the OHEJP sustainability process.

2.1.4 Task 1.4: Communication tools

Subtask 1.4.0: Communication Groups: The Communication Officer had regular contact with the Communication Contact Person network that was established in the first year of the One Health EJP. This network has been responsible for the dissemination One Health EJP news and information within their respective institutes. A CCP mandate was developed to detail the role of members of the CCP network. The Communications Officer ensured that each of the CCPs understood their role and were happy to continue to be part of this network following the implementation of the mandate. The Communications Team has organised a meeting with the CCPs scheduled for M21 providing the first opportunity to discuss the CCPs' role and impact in greater detail and to establish good working relationships.

Subtask 1.4.1 Web site/interface: The website was continuously monitored to ensure that the information, events and news was up to date. New designs were also implemented to several of the pages, including the Work Package 6 pages. New pages added to the website included: a publications page, a WP6 landing page, a stakeholders page and pages for the 2019 Summer School and ASM Satellite Workshop events. The content for the PhD projects and 2019 STMs were also updated. All One Health EJP events and related events were documented on the 'Events' page of the website. The website also has a private space where members of the consortium can log in and view internal documentation. The private space has 90 groups, including a group for each institute, each Work Package, each Joint Research or Joint Integrative projects, a stakeholders group, a group for the external advisory board, ethics committee external advisory board, an ethics committee, groups for each of the Work Package 3, 4 and 6 funding calls, for the Data Management plan and a group for all One Health EJP consortium members. They groups are controlled and monitored by assigned administrators to ensure security and the to ensure the smooth functionality of each respective group. The private space of the website currently has 425 members (June 2019). The private space has a forum facility where updates and reminders can be posted to members in each group. Additionally, these groups are designed for uploading and storing files for access to members.

The traffic to the website is monitored using Google Analytics, this allows the tracking of successful pages on the website, success of newsletter, it also allows the tracking of the audience and the browsers they use. The Communication Officer can also track the success of social media posts and adverts which advertise specific pages. This enables us to tailor the user experience and the Communication Strategy, in addition to improving the website.

Subtask 1.4.2 Internal communications: The Consortium newsletters were published in February, June and September 2019. These newsletters detailed key events and highlights from the previous three months, updates from the key meetings and events such as the ASM, updates from the One Health EJP Joint Research and Joint Integrative projects and highlighted the funding opportunities from the Education and Training activities. Project Leaders and PMT members were encouraged to contribute to these newsletters and thus far, ten of the research projects have provided updates for the Consortium Newsletter. These newsletters were sent to members internal to the consortium including, PMT, SSB, PMC, Institute Reps, Project Leaders and CCPs (approximately 310 people) using MailChimp. MailChimp allows the Communication Officer to monitor the number of times each newsletter is opened and forwarded on to those that may be interested in the One Health EJP updates. Additionally, this newsletter was uploaded on to the 'Newsletters' page of the One Health EJP website. The Consortium Newsletter does not contain any internal information, however it targeted towards One Health EJP members and the scientific community, therefore it was shared on the One Health EJP Twitter and LinkedIn social media accounts to disseminate updates from the One Health EJP. The success of this strategy can also be monitored using a combination of the social media analytics and website analytics.

Subtask 1.4.3 External communications: Press releases: A press release for the One Health EJP ASM was published during the first ASM by Irish Department of Agriculture, Food and the Marine and was published on the One Health EJP website and the Teagasc website. Furthermore, the Summer School was advertised on the University of Surrey website and a collaborative press release for the Summer School, including the University of Surrey, WUR, Chatham House and PHE was made available on the One Health EJP website.

Subtask 1.4.4 External communications: Professional social networks: The One Health EJP Twitter and LinkedIn accounts are now well established and have generated a significant amount of interest between M13 and M21 compared to M1 to 12. The research for the digital strategy and subsequent complementation in the Communications Strategy has been highly successful and the One Health EJP has in excess of 70,000 impressions on Twitter each month and over 7,000 impressions on LinkedIn each month. The number of followers on these platforms has also increased on a monthly basis. The ASM in May 2019 resulted in the greatest numbers of impressions and engagement on Twitter that have been reported by the One Health EJP since Year 1. Twitter had over 40,000 impressions in the three days of the ASM, 162 retweets and 532 likes, highlighting that events are successful platforms for One Health EJP Communication activities.

Subtask 1.4.6 External communications: Merchandise and branding: Merchandise for the One Health EJP was developed to be disseminated at all of the One Health EJP events and meetings. This merchandise included: pens, notepads, post-it notes, lanyards, USB sticks, document folders, in addition to One Health EJP flyer, brief, WP6 updates and One Health EJP highlights. A One Health EJP banner was also printed to be displayed at One Health EJP events. A WP5 and WP6 poster is also available to display at events to increase awareness of the Work Packages (all WP Leaders were encouraged to create a poster if they deem it necessary). Over the duration of Year 2, the One Health EJP merchandise has been expanded and refined considerably. There were also Bluetooth speakers available as gifts for speakers at our events.

Subtask 1.4.7 Annual and final report: The annual report for Year 1 was completed following input from the Communications Team at the University of Surrey, WP1 and PMT. This report submitted on October 1st is now available on the front end of One Health EJP website.

2.1.5 Task 1.5 Ethics

Based on the Periodic Technical report, the first Ethics Report was prepared, discussed with the Ethics Advisors on 19 February during a skype meeting and was finally submitted on 11 March (D1.23).

In the report, the advisors mention: "the One Health EJP Coordinator in collaboration with the Ethics Advisory Board has put in place an appropriate process for ethical review and oversight".

It details all recommendations that the Ethics Advisors made and how the Project Leaders have dealt with those, per JRP or JIP. No significant issues were identified. A general remark was that ethical approval codes including the name of the approving body and date of issue should be registered. As for GDPR issues, the beneficiaries should provide the contact address of the Data Protector Officer of the institution in charge of processing the data obtained.

The next follow up of the ethics principles for the projects that started in 2018 will be done at the end of 2019, beginning of 2019. As for the JRP and JIP of the second call, an ethics self-assessment was requested together with the full proposals. These will be discussed when the proposals for co-funding will be known, after September 2019.

2.2 Deliverables and Milestones

2.2.1 Deliverables

Del. Ref.	Deliverable title	Submission
D1.4	Annual report on the internal and external newsletter produced during the first year	13 Feb 2019
D1.5	Complete version of annual report for stakeholders n°1	1st Oct 2019
D1.6	Summary Progress Report Y2	1st Oct 2019
D1.7	Annual Work Plan for the 3rd Year	1st Oct 2019
D1.23	Ethical review report for Y1	11 Mar 2019

2.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS3	SSB Meeting n°2	14	The 2nd SSB meeting was held at RIVM headquarters in Bilthoven on 21 March. Minutes are available upon request.
MS4	SSB Meeting n°3	20	The 3rd SSB meeting was held at UCM in Madrid on 19th September. Minutes are available upon request.
MS13	PMC/POC/ESAB & Annual scientific meeting	21	The 1st PMC meeting was held at ANSES headquarters at Maisons-Alfort (FR) on 9 May. The 1st ESAB meeting was held at TEAGASC Conference Center in Ashtown (IE) on 23 Mai. Minutes of these meetings are available upon request. ASM was held in Dublin (IE) from 22nd to 24th May 2019.

3 WP2 – Integrative strategic research agenda

3.1 Work carried out to date

3.1.1 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.

3.1.2 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 second year, as defined in the deliverables, the repository has been updated and downloaded on the website including new relevant EU-projects/initiatives as for example eNOTICE (European Network of CBRN Training Centers), HealthyLivestock (Tackling Antimicrobial Resistance through improved livestock Health and Welfare) and DISARM (Disseminating Innovative Solutions for Antibiotic Resistance Management). Moreover, the last (6th, 7th and 8th) JPIAMR Joint calls were also included for OHEJP consortia consultation. Regarding potential strategic interactions, teleconferences have been organized with JPIAMR (Joint Programming initiative on Antimicrobial Resistance) and JAMRAI (Joint Action Antimicrobial Resistance Healthcare-Associated Infections) to look for synergies. Moreover, the WP leaders and researchers of JIP/JRP have also interacted with ongoing EU-projects trying to enhance the research, optimising resources and avoiding duplications.

3.2 Deliverables and Milestones

3.2.1 Deliverables

Del. Rel. No	Deliverable title	Submission
D2.7	Updated strategic research agenda	Submitted as a confidential deliverable on 22nd July 2019
D2.10	Summary version of the updated Strategic Research Agenda	Submitted as a public deliverable on 22nd July 2019

3.2.2 Milestones

N/A

4 WP3 - Joint research projects

4.1 Work carried out to date

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

During the first year, the following guidelines were drafted and submitted:

- Guidelines for project leaders to report on JRP (D3.1)

- Guidelines for the submission and selection of project proposals (JRP and JIP) (D3.3)

- Guidelines for the monitoring of JRP and JIP (D3.5)

The instructions that describe in detail the process of proposal evaluation by external experts and the selection of the proposals for co-financing were prepared in 2018. In short, these guidelines describe that WP3 and WP4 teams contact independent experts who have to register and detail their expertise through an online tool. In a first step, experts are allocated to panels that group related projects and they individually evaluate the projects assigned to that panel. Subsequently, during a teleconference the panel members discuss and align their individual assessments, and agree on recommendations that may be useful for the candidate Project Leaders, as well as to PMT and SSB. Also the criteria for evaluation are explained and accessory documents for evaluators (confidentiality agreement, protection of personal data, declaration of conflict of interest and a template for reporting) are included. This procedure was validated by the PMT in January 2019 and submitted as D3.6.

The last instructions regarding the monitoring of the JRP deal with the evaluation of the final project reports, when the projects have come to an end. These instructions are very similar to those regarding the evaluation of the proposals (see D3.6), i.e. the identification of the external experts and their assignment to panels, clarification of the criteria, templates for reporting and additional documents. However, there is only an individual evaluation expected, no consensus meeting. The deliverable D3.9 has been uploaded on the participant portal on 31 July 2019.

A schematic overview of the process of the proposal submission, its evaluation and the monitoring of the project clarifies the coverage of the various guidelines:

	Proposal writing			Project		
PL JRP	D3.3 Submission			D3.1 Reporting	D3.1 Reporting	D3.1 Reporting
PL JIP	D3.3 Submission			D4.1 Reporting	D4.1 Reporting	D4.12 Reporting
WP3 / WP4				D3.5 Monitoring	D3.5 Monitoring	D3.5 Monitoring
Experts		D3.6				D3.9
						D4.19
		Evaluation proposal				Evaluation final report

The possibility to extend ongoing JRP, as well as JIP, with a limited number of months and without additional budget, was largely discussed by the PMT. The procedure anticipates the submission of a formal request (template) and the extension with maximum 6 months. Note that the projects, both JRP and JIP, of the second call will all last 2,5 years and are excluded for extension, due to the time needed for the external evaluation of the final reports.

As for the enlargement of on-going projects by inclusion of new partners, another procedure is available. Both beneficiaries and non OneHealth EJP members may join existing JRP or JIP, under certain conditions, i.e. the original work plan cannot be modified, the extra work will be funded by external budget (not eligible to the OHEJP) and the newly added partners will not own any result generated by the project.

Finally, the procedure for inclusion of new OneHealth EJP beneficiaries in an on-going project with clear integrative activities is under discussion. Since integrative activities are the core business of OneHealth EJP because they support the harmonization and alignment of the consortium's capacity, protocols, databases, biobanks, surveillance strategies etc. an additional budget can support such enlargement of an existing project. The request for such project enlargement should be submitted in June 2020, i.e. when an estimation of the unspent budget, which was allocated to the 2-year projects that started in 2018, will be known.

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

The Project Leaders reported on their activities run during the first 12-months using the templates that WP3 team sent out in November 2018. The complete first periodic report of JRP (outcomes 2018) is deliverable D3.7 that was submitted in February 2019.

In summary, the 11 Joint Research Projects planned the submission of 77 deliverables, but only 32% of them were uploaded to the private area of the OHEJP website at the end of 2018. A likely explanation is that the project leaders were not yet familiar how to use the private space. Also, not all of the so-called deliverables in fact deliver documents (see further).

About one-fifth of the expected deliverables were postponed to the second year. The delays were mainly due to recruitment and leave of staff, delays in the availability of samples or equipment or to minor reorganization of the activities in agreement with the project consortium partners. For most projects, these delays are unlikely to have a major impact. Two projects MoMIR-PPC and TOX-Detect

reported more considerable delays and needed modification of their work plan. The Project Leaders of both JRP were contacted and the current SPR of year 2 is the opportunity to evaluate the project's progress.

About thirteen percent of the project deliverables should be considered as milestones. To avoid this confusion, the difference between milestones and deliverables was clarified on 25 February 2019 in a separate mail to the candidate Project Leaders for the second call projects.

Finally, the 11 joint research projects planned to achieve a total of 77 milestones. Seventy-three percent of the milestones were finalized while 27% had been delayed.

According to the procedure of extension at no cost, for a maximum of six months, the following 2 year projects have requested such an extension: IMPART, RaDAR, ListAdapt, METASTAVA, AirSample and MedVetKLebs. Both PMT and SSB agreed with these six extensions, following an electronic vote in July-August. In addition, MoMIR-PPC has received an extension of 1 year, as explained elsewhere.

A preliminary report of the activities and outcome of the eleven JRP follows.

The management issues with which TOX-Detect had to cope in the first year, were discussed in several meetings in the beginning of 2019 (see details in the report). Although some delay in the work is noticed mid-2019, also due to technical issues (MALDI-ToF and problems with expression constructs), the Project Leader is confident that the project will deliver as expected.

As for MoMIR-PPC, the AWP for Y1 and Y2 have been re-written and it is clear that precious time was lost looking for new partners (finally, NDRVMI from Bulgaria has taken over from DTU-Vet). Also the need to request ethical permissions for both animal experiments and sampling from volunteers has taken more time than anticipated. For these reasons, it seems acceptable that project MoMIR-PPC has been granted one extra year, without additional budget.

At the present time, 70% of the planned deliverables have been finalized. The remaining 30% have mainly be postponed to the end of the year, with the exception of MoMIR-PPC, which was granted an extension of 1 year. Of these finalized deliverables, 70% was uploaded on the private project groups of the OHEJP website. The project leaders are in the process of uploading the remaining deliverables and are frequently communicating about this with WP3.

4.1.2.1 IMPART

4.1.2.1.1 Summary

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

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

For WP3, the delivery of the Sensititre plates was seriously delayed. Consequently, the distribution of the Sensititre plates (consisting of three different panels) to the partners took place in March 2019 including the necessary software for partners using a Sensivision reading device. The minimum

inhibitory concentration (MIC) testing of bacterial isolates started in May 2019. The list of bacteria to be tested was discussed during the physical meeting in Dublin and this list was shortened and finalised in June 2019. The MIC testing will be performed by the participants from May to November 2019. The data analysis in order to set the ECOFFs is postponed to the first half of 2020.

In WP4, a first draft protocol for disk diffusion was developed based on available descriptions and literature. For optimization and standardization, ten *C. difficile* strains were selected. The experiments are not finished at the moment. The collection of isolates was completed and all isolates were confirmed as *C. difficile* using different techniques. Furthermore, the MIC was determined. The ring trial will be organized after the completion of a method recommendation for the participating partners. Inhibition zone diameter distributions and proposing cut-off values for *C. difficile* will be determined after the completion of a method description.

Regarding the communication within IMPART a kick-off meeting was held at Schiphol for all consortium members in February 2018 and a mid-term meeting in May 2019. The final meeting will be held in spring 2020. Emails are 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. In addition, extra Skype meetings were organized to discuss the pre-ring trials of WP1 and WP2. Furthermore, IMPART activities were presented on both Cogwheel meetings organised in 2018 with EFFORT and COMPARE. IMPART will keep on looking for synergies with other research projects in order to avoid duplicate research.

4.1.2.1.2 Project-specific milestones and deliverables

4.1.2.1.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
IMPART	D-JRP1-1.3	Evaluation of the pre-ring trial	11	13		The evaluation of the pre-ring trial has been postponed to January 2019.
IMPART	D-JRP1-2.3	Evaluation of the pre-ring trial	11	13		The evaluation of the pre-ring trial has been postponed to January 2019.
IMPART	D-JRP1-1.4	Protocol for the final ring trial	15	17		Draft protocol uploaded on OHEJP website (final protocol will be uploaded asap)
IMPART	D-JRP1-2.4	Protocol for the final ring trial	15	17		Draft protocol uploaded on OHEJP website (final protocol will be uploaded asap)

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
IMPART	D-JRP1-4.1	Collection of inhibition zone diameter distributions	15		21	Delay due to our problems to hire a technician for the project work as well as to a 3-month delivery problem of a gas mixture, which is required for our anaerobic workstation, and, finally, the late supply of some isolates by the project partners (all problems occurred in year 1).
IMPART	D-JRP1-1.5	Notifications of shipment of the samples for the final ring trial	16	17		Notification uploaded on OHEJP Website
IMPART	D-JRP1-2.5	Notifications of shipment of the samples for the final ring trial	16	17		Notification uploaded on OHEJP Website
IMPART	D-JRP1-4.2	Publication in an open-access peer-reviewed journal	18		30	Delay results from the delayed progress of Tasks 4.1-4.4
IMPART	D-JRP1-5.4	Protocols and video tutorials online on IMPART EXTRANET	18	17		Protocols uploaded on IMPART Group EXTRANET.
IMPART	D-JRP1-1.6	Evaluation of the final ring trial	19		21	Expected September 2019

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
IMPART	D-JRP1-2.6	Evaluation of the final ring trial	19		24	The evaluation of the final ring trial has been postponed to December 2019

4.1.2.1.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
IMPART	M-JRP1-4	Established disk diffusion method (WP4)	8	Yes	14	Advertised technician position could not be recruited on time. Delay in gas delivery by manufacturer.

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
IMPART	M-JRP1-7	Mid-term video meeting to validate the protocol (WP1, WP2 and WP5)	13	Yes	17	A physical meeting with the participants in the IMPART consortium was held at the OH-EJP conference in Dublin in May 2019. Participants not present at the conference attended through Skype. Work so far was presented together with draft protocols for the final ring trials for WP1 and WP2.
IMPART	M-JRP1-8	Performing final ring trial (WP1 and WP2)	17	WP1: No WP2: No	WP1: 18 WP2: 21	WP2 final ring trial is delayed and will be performed in month 21.
IMPART	M-JRP1-9	MIC data collection complete (WP3)	18	No	24	Delivery date postponed due to delay in delivery of the Sensititre plates. (6 months extension requested for the project, submitted 14-06-2019)
IMPART	M-JRP1-10	Proposal of cut-off values based on inhibition zone diameter distributions (WP4)	18	No	30	The analysis of the data will be performed in 2020

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
IMPART	M-JRP1-4	Established disk diffusion method (WP4)	8	Yes	14	Advertised technician position could not be recruited on time. Delay in gas delivery by manufacturer.

4.1.2.1.3 Description of the project activities per task

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

4.1.2.1.3.1.1 JRP1-WP1-T1: Describe existing methods to be evaluated in a ring trial

Task started in July 2018 and was finalized in December 2018, see annual report 2018

4.1.2.1.3.1.2 JRP1-WP1-T2: Preparation of the samples for the pre-ring trial (WP1 and WP2)

Task started in July 2018 and was finalized in November 2018, see annual report 2018

4.1.2.1.3.1.3 JRP1-WP1-T3: Performance of the pre-ring trial and evaluation (WP1)

Pre-ring trial was performed with 3 participants (RIVM, WBVR and NVI).

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

Samples were prepared in Anses Fougères Laboratory in June 2019 (week 24). Each of the 12 participants received 6 samples (meat or caecal content) to be analyzed for detection of mcr-producing Enterobacteriaceae according to the protocol send out at draft stage for comments in May 2019 and final version in early June. Shipment was organized to make sure all the participants will receive within 24h their samples under monitored positive cold conditions.

4.1.2.1.3.1.5 JRP1-WP1-T5: Performing the final ring trial

Each partner who accepted to take part in WP1 (11 labs, 9 countries) are performing the ring trial during week 25 and 26, 2019.

4.1.2.1.3.1.6 JRP1-WP1-T6: Analysis of the results and reporting

Analysis of the result and dissemination of the outcome to the participants will be taken in charge by Anses Fougeres Laboratory and is planned to be achieved in September 2019.

4.1.2.1.3.2 WP2: Selective isolation, detection and characterization of carbapenemase-producing Enterbacteriaceae

4.1.2.1.3.2.1 JRP1-WP2-T1: Describe existing methods to be evaluated in a ring trial

Task was finalized in December 2018, see annual report 2018.

4.1.2.1.3.2.2 JRP1-WP2-T2: Preparation of the samples for the pre-ring trial (WP2)

Task was finalized in November 2018, see annual report 2018.

4.1.2.1.3.2.3 JRP1-WP2-T3: Performance of the pre-ring trial and evaluation (WP2)

The pre-ring trial was performed among only three laboratories (RIVM, WBVR and NVI) in late November 2018. The pre-ring trial focused on testing several conditions to narrow down different

possibilities to test in the final ring trial among all 11 participating laboratories in the IMPART WP2 consortium.

The aim of the pre-ring trial was to test the following conditions:

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

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

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

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

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

The ready-to-use selective agar plates and media to prepare in-house were distributed a few days before the pre-ring trial (arrived at the three participating laboratories on Monday November 19th 2018). The samples were sent from Anses Fougères on Tuesday November 20th 2018 and arrived at:

- WBVR: afternoon November 21st 2018

- RIVM: afternoon November 21st 2018

- NVI: evening November 21st 2018

All samples were labelled with a LETTER (A-I) and a NUMBER specific for each laboratory. Parallels were distributed of each sample and the pre-enrichment of the samples started on the day of arrival.

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

The samples will be prepared at Anses in week 36 and shipped to the participants on September 10th 2019.

4.1.2.1.3.2.5   JRP1-WP2-T5: Performing the final ring trial

The final ring trial for WP2 is delayed and will be performed in week 37, September 2019. The delay is due to difficulties finding a date that suited all participants before the summer holidays.

4.1.2.1.3.2.6   JRP1-WP2-T6: Analysis of the results and reporting

The task is postponed to M24, December 2019.

4.1.2.1.3.2.7   JRP1-WP2-T7: Publication in peer-reviewed journal

The task is postponed to M30, June 2020.

4.1.2.1.3.3   WP3. Establishing epidemiological cut-off values (ECOFFs)

4.1.2.1.3.3.1   JRP1-WP3-T1: Inventory, prioritizing and inclusion criteria

This task was finalized in July 2018, see annual report 2018 and refined after the physical meeting in Dublin in June 2019.

4.1.2.1.3.3.2 JRP1-WP3-T2: Production of MIC data

4.1.2.1.3.3.3 JRP1-WP3-T3: Collection and quality control of MIC data

The collection and quality control of MIC data has not started yet.

4.1.2.1.3.3.4 JRP1-WP3-T4: Analysis of the data and publication of ECOFFs

The analysis of MIC data and publication of ECOFFs has not started yet.

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

4.1.2.1.3.4.1 JRP1-WP4-T1: Establishment of a disk diffusion method for antimicrobial susceptibility testing of C. difficile

Disc diffusion was mainly based on Erikstrup et al., 2012. We investigated the effect of different factors to optimize the method and method description: Different inoculum densities (McFarland (McF) 1.0, 1.5, 3.0), solid media (Brucella blood agar (BBA), Wilkins-Chalgren agar (WCA), Schaedler Anaerobe agar (SA), Columbia blood agar (CBA)), liquid media (Schaedler bouillon (SB), Tryptose Peptone Glucose Yeast (TPGY) bouillon, Brucella bouillon (BB) and Anaerobic conditions.

We determined optimal conditions that:

1. resulted in highly reproducible results and

2. can be implemented even by laboratories without the need of expensive equipment investments.

4.1.2.1.3.4.2 JRP1-WP4-T2: Assembly and characterization of C. difficile strain collection

Until December 2018, in total 476 *C. difficile* isolates were collected from Germany, The Netherlands, Sweden and Portugal. The strains were isolated from human, animal, food and environmental sources and represent at least 70 different PCR-ribotypes (RT) with RTs 014, 027 and 078 being the most prevalent ones. All isolates were furthermore characterized according to their toxigenic profile, and MICs for clindamycin, erythromycin, metronidazole, moxifloxacin, tetracycline, vancomycin, rifampicin, imipenem were determined.

4.1.2.1.3.4.3 JRP1-WP4-T3: Performance of a ring trial study

The task is postponed to M23, November 2019.

4.1.2.1.3.4.4 JRP1-WP4-T4: Producing inhibition zone diameter distributions and proposing cut-off values for C. difficile

We started to determine inhibition zone diameters of all isolates from the strain collection (Task 4.2) using the draft method description resulting from Task T4.1. The laboratory work is expected to be finished until M24. Afterwards, the publication of the results and proposed cut-off values in a peer-reviewed journal is planned until M30.

4.1.2.1.3.5 WP5: Coordination of the four work packages and knowledge dissemination both

4.1.2.1.3.6 internally within and externally beyond the IMPART consortium

4.1.2.1.3.6.1 *JRP1-WP5-T1: Organization of IMPART*

WP leaders have been in contact through Skype meetings every two weeks.

4.1.2.1.3.6.2 *JRP1-WP5-T2: Communication within IMPART*

All documents that have been produced in IMPART frame were disseminated through the private group IMPART on the OH-EJP website.

Draft protocols, information on planning of ring trials and questionnaires were sent out through emails whenever needed by the WP leaders.

Presentations and posters linked to the IMPART work have been made available as soon as possible on the IMPART private group on the OH-EJP website.

Taking advantage of a majority of the scientists involved in IMPART were present OH-EJP ASM in Dublinin May 2019, the mid-term Video-conference became a face to face meeting. One external partner was not attending and a Skype connection was set for them to be able to participate to the discussions.

4.1.2.1.3.7 JRP1-WP5-T3: Communication beyond IMPART

EURL-Antimicrobial resistance (DTU Danemark) offered a 40 min slot on their annual workshop for an update on the IMPART preliminary result to be shared with the European NRL (Lyngby, 26 April 2019, Kees Veldman, Jannice Slettemeaas and Sophie Granier) During the OH-EJP ASM in Dublin, two posters were displayed to update on findings within the context of IMPART.

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

The final physical meeting is planned now in Spring 2020 at Schiphol airport in Amsterdam. The frequency of the Skype meetings for the work package leaders will remain the same until M30: every two weeks.

4.1.2.2 *ARDIG*

4.1.2.2.1 Summary

The ARDIG project has continued to progress and after 18 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. Representative from each participating partner institute met at the Annual Scientific Meeting held in Dublin in May 2019 for the annual meeting where detailed discussion on progress as well as partner updates were presented. Several work package specific meetings are planned over the next few months, as well as an AMR genomics workshop. There have also been regular communications within the consortium by email. Furthermore, for each WP, questionnaires have been designed by the WP leads (WPLs), and all relevant partners have responded.

WP1 (**Comparison of AMR and antibiotic sales/usage data collected through existing national surveillance and research programs and assessment of risk factors**). Most partners have already participated in the detailed questionnaire/survey designed by BfR, the WPL, which included

information on available data sources in each country/institute. The remaining outstanding partners are currently investigating the possibility of gathering the information required from their national surveillance programmes. However, the data collected so far has already been summarized in deliverable D-JRP2-1.1 that was submitted as a report in M12 (December 2018). Based on the report a publication is being prepared and further discussions are planned on how trends will be analysed across the data set.

WP2 (**Longitudinal studies of AMR persistence).** Both Med and Vet Partners have commenced with selecting isolates from archived retrospective studies and collected through prospective longitudinal studies designed within ARDIG. Prospective studies include a number of livestock (veal calves, pigs and poultry) that vet partners from different Institutes have designed and are engaged in as part of ARDIG; further details are provided in the report. Partners have discussed at the annual meeting in May how the sampling plan from different Institutes can be harmonised, as well as questionnaires provided to farmers and plates that will be used for selection of *Escherichia coli* (selective and non-selective plates). A prospective human study involving five institutes that was planned for January 2019 has started and will commence for one year. The isolates being collected by each partner will be *E. coli* isolated from urinary tract infections from a local hospital and General Practitioner.

WP3 (**AMR characterization, transmission of plasmids and fitness of MDR isolates**). Partners have been progressing with using a combination of molecular techniques, including whole genome sequencing, for AMR characterization of isolates. The isolates sequenced to date, on which some analysis has been performed, includes isolates from national archives as well as those collected through longitudinal prospective studies. For most partners this has involved characterization of isolates by WGS (short reads), although other techniques such as PCR and Pulse Field Gel Electrophoresis have also been used. Although most partners have been characterising the AMR profiles of isolates, they are attempting to identify/characterise the circulating plasmids which may be involved in AMR transmission. For this several partners have also used long read sequencing (MinIon and PacBio) for circularisation of plasmid genomes, so these can be defined more accurately.

Based on a questionnaire designed by WPLs, which partners participated in, an AMR genomics workshop will be organized in October 2019 where ARDIG partners will consider ways of harmonizing methods for analysis WGS data.

4.1.2.2.2 Progress of the project: project-specific milestones and deliverables

4.1.2.2.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
ARDIG	D-JRP2-3.1	Prevalent AMR genes and platforms in enterobacteria from humans, animals, food and environment.	20	September 2019	December 2020	All partners have started to assess and report on the AMR gene content of their isolates, especially those already collected in prospective studies and from historical collections. However, as isolates are still being collected in several of the prospective longitudinal studies for up to another 12 M, their analysis will not be complete by September 2019. Also, a workshop to harmonise AMR gene analysis within ARDIG is being planned for October.

4.1.2.2.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
ARDIG	M-JRP2-3	Preliminary molecular characterization of AMR genes from isolates collected in WP1 and WP2.	12	Yes		All partners have started molecular characterisation of isolates. WP3 provides details of the work.
ARDIG	M-JRP2-6	Assessment of AMR genes and platforms in enterobacteria collected from humans, animals, food and environment.	18	No	36	See comments above.

4.1.2.2.3 Description of the project activities per task

4.1.2.2.3.1 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.

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

APHA

The APHA contributions to WP1 are as follows:

• A literature and online search was performed to identify data sources for benchmarking or ongoing monitoring of AMR and AMU.

• The resulting articles were assessed for relevance and whether they included data of interest.

• All relevant stakeholders were contacted to obtain additional information on the content of the databases.

• Completed detailed questionnaire on English/Welsh AMR and AMU data sent by BfR.

• Attended meetings with the industry to request more detailed AMU data.

• Reviewed the report produced by the BfR on AMR and AMU data sources.

• Provided all available data requested for AMR and AMU in the agreed format and template.

UoS

The UoS has collected/is collecting:

• Animal data (from a local farm): antibiotic use, farm characteristics and AMR in *E. coli* strains isolated from chickens, pigs and cattle faecal samples collected over a 12-month period (Jul 2018-Jun 2019).

• Human data (from four local NHS hospital trusts): AMR in *E. coli* strains isolated from urinary tract infections and blood bacteraemia samples collected over a 12-month period (Jan-Dec 2019) or previously (2017-2018). Patient data (including age, gender, region and antibiotic use) will be also provided. Ethics approval by HRA is in place to collect patient data and two of the sites have already confirmed capacity and capability to provide this information.

UCM

The UCM forms part of the National Surveillance Network for animal AMR, and collaborates with the National action plan on AMR and AMU. Thus, all data collected at a National level are available for ARDIG. Further, UCM has collected data on AMR and AMU from 20 pig farms and 20 poultry farms, that will be used as further input for the project. As for human data, UCM has contacted the Public Health Institute, to receive data on AMR and AMU. For this purpose, a collaborative work is envisaged.

ANSES

Data from the long-term National Monitoring Network for AMR in Diseased Animals (RESAPATH) issued each year were included into ARDIG. They consist of AMR data collected per year and per animal species/infection type/bacterial species/antibiotic. AMR data collected in French livestock according to the Directive 652/EU (slaughterhouse and retail) were also included into ARDIG. Data from AMU

were provided by the National Agency for Veterinary Products and consist of sales data converted into animal exposure indicators.

NVI

Data from the Norwegian Veterinary prescription registry has been extracted for the selected species and validated against sales data for the years 2015 -2018. Further work to aggregate the data for the integrated analysis will be performed within the next month.

Data from the Norwegian surveillance program has been extracted for the years 2014 to 2018 and will be available for the integrated analyses across the consortium countries within a month.

WBVR

In coordination with BfR, AMU and AMR data for the Netherlands have been aggregated in the supplied format by BfR and submitted for inclusion in the aggregated analysis. The possibility for inclusion of AMR data of clinical isolates from livestock from the Netherlands is still under investigation.

RKI

The RKI has extracted human AMU (hospitals, ambulatory) and AMR data from its surveillance systems according to the template (years 2014-2017) in order to construct the joint ARDIG database. A data transfer/sharing agreement has been drafted and is being reviewed by the BfR. The approval of this transfer/sharing agreement is required to be able to use the AMR/AMU data from RKI.

BfR

For WP1, and based on the results of the previous year, a manuscript describing monitoring antimicrobial resistance and drug usage in human and animal sector and food borne antimicrobial resistance in the involved countries has been developed and will be shortly submitted to the Infection and Drug Resistance Journal. In addition, these results have been already presented in an international congress as an oral communication and they are scheduled to be part of others. In relation to data collection, participating partners are still providing country data on antimicrobial resistance from *E. coli* and on antimicrobial usage in animal and human sector together with food borne antimicrobial resistance. Several teleconferences and face to face meetings have been carried out in order to discuss about data sharing. A data sharing agreement contract has been elaborated to achieve legal requirements demanded from RKI and it will be signed by all members. Data analysis of the collected data have been started. In addition, significant efforts have been taken over in order to join forces with other projects and overcome obstacles.

The AMU-AMR data collection on human, animals and food from partner countries continues until the end of July.

Some difficulties collecting, comparing and analyzing routinely and not routinely data reported to Europe have been encountered. In some cases, the location of staff responsible for AMU-AMR data has been tedious. Some efforts have been taken in order to try to get further data that facilitate the comparison and analysis. A collaborating member of the JIACRA project has been contacted who has confirmed us that they are facing up similar obstacles. Some efforts are being carried out to try to join forces.

IP

The surveillance of AMR and AMU in France is currently evolving with an increased role of Santé Public France making the collect of data cumbersome. We have been able to collect AMU data from 2014 to 2016, but 2017 data are still missing. From the published ONERBA data, we have collected aggregated data concerning antibiotic resistance among *E. coli* isolates from UTI and bloodstream infections.

However, getting line by line information on the analysed *E. coli* isolates is more complicated as anticipated and we are still trying to obtain them.

PHE

WP1 collaborators contributed to the development of the survey capturing information on accessible AMR/AMU data sources (monitoring / surveillance systems) in participating countries for animals and humans and fed into ARDIG's 12M report. In addition, information on AMR and AMU data sources for England was provided via the survey.

Approvals from PHE's Office for Data Release were successfully sought for transfer of AMR and AMU data from England to the WP1 Lead's team based at the National Agency for Risk Assessment in Berlin, Germany. AMR data for clinical *E. coli* isolates (urine/blood) from humans extracted from PHE's national laboratory surveillance system (SGSS) were transferred to Berlin in March 2019 (Please see summary table below).

Clinical E. coli isolates (urine/blood) from humans extracted from PHE's national laboratory surveillance system (SGSS), England, 2014-2017

Year	Number blood isolates	Number urine isolates	Total
2014	5721	189200	194921
2015	8887	274017	282904
2016	9396	280603	289999
2017	10138	270613	280751
Total	**34142**	**1014433**	**1048575**

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

A meeting will be planned in summer 2019 to discuss the data analysis plan and to subdivide tasks within the main WP1-T2 partners.

So far it has not been possible to collect data from all countries so no trend, association and risk factor has been detected yet. However, substantial differences have been already identified between data collected from the human and animal sectors. In addition, there seems to be a limited amount of AMR data in the food sector from some countries that may hamper a part of the analysis.

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

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

NVI

Isolates from a previous study focusing on cephalosporin resistant Enterobacteriacecae have been characterized. All broiler flocks raised on ten broiler farms were sampled during the period from May to October in 2016 and a total of 42 positive isolates were obtained (one isolate per flock). These isolates were subjected to sequencing using Illumina technology in order to study a possible on-farm persistence/transmission between bathes of animals on the same farm or broiler house. In total, 11

different E. coli STs were identified. blaCMY-2/IncK2 plasmids were the most common gene/plasmid combination (present in nine different STs). A possible clonal persistence of ESC-resistant *E. coli* at house level was shown for only a minor proportion of the included houses. Isolates from the same house belonging to the same ST could differ by a considerable number of SNPs, shown for ST38 isolates found in 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.

WBVR

For the Netherlands, longitudinal data from pig farms were not expected to be included in ARDIG. However, as longitudinal data from pig farms is collected in several countries or is available in the form of historical studies, an attempt is made to include samples from a study in the Netherlands in 2011-2013, performed by Utrecht University in collaboration with WBVR.

ANSES

Extended-Spectrum-Cephalosporins (ESC)-resistant Enterobacteriaceae have been isolated from veal calves in France have been included to be studied within ARDIG. Two studies were set up to investigate the trends in ESBL/AmpC prevalence and antimicrobial usages (AMU) in veal calves during the fattening process. In a first study, ten fattening farms were selected and visited twice. A total of 50 animals per farm were sampled for ESC-R carriage and other AMR phenotypes upon arrival and 5-6 months later before slaughter. A second study was then set up to get further insights into the dynamic of ESBL/AmpC spread over the fattening period. Three farms out of the ten from the first study were visited 11 or 12 times at regular intervals of 15 days. A total of 15 calves per farm were sampled and processed as for the first study. In the two studies, the number and types of treatments during fattening were collected.

IP

We are analysing ESBL *E. coli* isolates from a longitudinal follow-up of patients in a long-term hospital by systematic rectal swabs. These isolates were obtained through the i-bird project (https://research.pasteur.fr/en/project/i-bird-individual-based-investigation-of-resistance-dissemination/) coordinated by our collaborators (D. Guillemot, J. L Herrmann and L. Opatowski). 420 MDR *E. coli* isolates have been obtained from 115 patients including 323 ESBL producing isolates. Isolates will be sequenced to infer their diversity, intra-host evolution and more importantly transmission (WP3). In relation with WP3, we are also analysing ESBL plasmid transfer within the hospital.

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

APHA

Two sampling visits were carried out in 2019 in the pig farm selected for longitudinal studies. At each visit 360 individual faecal samples and approximately 200 pooled faecal and environmental samples were collected and E.coli was isolated from these samples. Culture for E.coli was carried out on both non selective and antibiotic (ciprofloxacin and cefotaxime) selective culture media. The proportion of E.coli resistant to these antibiotics was calculated performing bacterial counts. The proportion of resistant E.coli was very low, and no significant changes were observed between time points. Minimum inhibitory concentrations were calculated against a panel of antibiotics relevant for public health (EU harmonised monitoring panel). The levels of resistance in E.coli isolated from non antibiotic selective media was low. Resistance was observed in some isolates against tetracycline, sulphametoxazole, chloramphenicol and ampicillin. These resistances are not uncommon in indicator E.coli isolated from

pigs. Wildlife samples carried resistant E.coli, and, although the directionality of this transfer is unknown, it is possible that certain E.coli strains present in wildlife had been acquired from the pig population on farm.

UoS

The UoS has just finished the collection of *E. coli* isolates from healthy chickens and pigs (5 faecal samples per animal per month) over a 12-month period (Jul 2018-Jun 2019) in collaboration with a local farm. *E. coli* isolates from cattle faecal samples (5 per month) have been collected over a 3-month period (Aug-Oct 2018). Ethics approval to collect animal samples is in place. Phenotypic and genotypic characterization of collected isolates is currently in progress.

UCM

The UCM collects random E. coli isolates from pig farms that are visited every 6 months. All E. coli are isolated and identified using the international EFSA procedure, including the use of specific selective media without antibiotics and harmonised MIC determinations. The isolates are subjected to PFGE routinely, and sequenced in case of possible outbreaks. Further, in depth studies have been performed in several poultry and pig farms, where specific plasmids and mcr-genes have been identified and characterised. Salmonella has been isolated in several farms, and a publication regarding these data is under way.

NVI

Recent data from monitoring in broilers have demonstrated absence of cephalosporin resistant Enterobacteriaceae in Norwegian broiler production. A study in pigs will therefore replace the planned broiler study. A pig study was planned in 2018 but, recruiting pig herds for this study has been a great challenge. Attempt to recruit pig farms will continue, and a change of study design will probably be needed.

WBVR

A longitudinal study that was planned on 5 broiler farms in the Netherlands is well underway and is expected to finish before the end of 2019.

For veal farms, a new study has started in the Netherlands which is carried out on national funding by Wageningen Livestock Research and WBVR. Sampling on two veal farms from this study are matched to the ARDIG format. Sampling for this purpose has started and will continue throughout 2019.

ANSES

In the two studies, ESBL-producing E. coli were collected from MacConkey agar for the culture of the dominant flora and onto selective ChromID ESBL agar (bioMérieux) for the specific selection of ESC-resistant isolates from the subdominant flora. After incubation at 37°C for 24 h, one presumptive *E. coli* colony was arbitrary selected from each plate and isolates were identified using MALDI-TOF. In the first study, ESBL-producing *E. coli* rates have significantly decreased in all 10 farms (arrival: 67.7%; departure: 20.4%)(Gay et al, Frontiers in Microbiology 2019). Feeding milk containing antimicrobial residues to veal calves is hypothesized to explain the high ESBL loads in animals at the entrance on farms. In the dominant flora, proportions of resistances to amoxicillin, tetracyclines, streptomycin and sulfonamides were very high (>60%) at arrival of animals in the farm and had significantly increased at departure. Proportions of resistances to other beta-lactams than amoxicillin were overall low and significantly decreased during the fattening process. Resistance to quinolones also significantly decreased from arrival to departure. A total of 11 isolates were resistant to colistin (MICs ranging between 6 and 16 mg/L) of which 9 were detected in animals upon arrival (originating from 7 different farms), and 2 in animals at departure (both originating from the same farm). The proportion of multi-resistant isolates significantly increased from 60.2% upon arrival to 67.2% at departure of animals. The

proportion of isolates susceptible to the seven selected antibiotics was 23.3% upon arrival and 7.3% at departure. Only two isolates displayed co-resistances to all seven antibiotics. The second study is still on going. Preliminary results show that the three farms differed by the prevalence of ESC-resistant *E. coli* since a total of 84 ESC-R-, 15 ESC-R- and 76 ESC-R-positive *E. coli* were recovered from farm A, B and C, respectively. The same decrease in ESBL-producing *E. coli* rates as in the first study was observed albeit with different dynamics most probably depending on the types and number of antibiotic treatments per farm.

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

UoS

The UoS is collecting *E. coli* isolates from human urinary tract infections (10 from hospital and 10 from GPs per month, per hospital trust) over a 12-month period (Jan-Dec 2019), in collaboration with three local NHS hospitals. The UoS has also gathered a set of *E. coli* isolates (10 per month) from human blood bacteraemia cases through a local hospital over a 12-month period (Jul 2017-Jun 2018).

NVI

E. coli isolates from humans with UTI in a large centrally located hospital in Norway, and from GP in the same area, can be available for the project (the 20 first isolates of each category, from each month in 2019). The isolates are sent us monthly and they are stored at NVI, together with relevant/requested data. Currently there are some uncertainties about funding for WGS of the isolates. Plans for further investigations of the isolates must be made at a later stage, depending on the possibilities for low-cost sequencing.

IP

During the period of 2019-20, we have set up and started the systematic longitudinal collect of *E. coli* isolates responsible for UTI in collaboration with the team of Thierry Naas at the Bicêtre hospital. We are collecting each month ten isolates from emergency, considered as community acquired and ten isolates from different wards (hospital isolates).

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

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

UoS

The UoS has characterised/is characterising:

• Animal isolates: AMR profile of pathogenic avian *E. coli* isolates from local poultry veterinary practices and commensal *E. coli* isolates from healthy chickens, pigs and cattle. A subset of these isolates is being/will be further characterised by WGS.

• Human isolates: AMR profile of pathogenic *E. coli* isolates from urinary tract infections and blood bacteraemia has been/will be provided by the corresponding hospital microbiology laboratory. Presence of ESBL and MCR genes has been detected by multiplex PCR in a set of 245 UPEC strains isolated during 2017 in three different hospitals. 94 of these isolates have been sequenced, and AMR genes, phylogeny, and plasmid content have been analysed. Further characterisation of human isolates is being/will be performed by WGS.

The UoS has prepared questionnaires to harmonise methods and results for WP3 between partners. The completed questionnaires have been summarised and discussed in WP3-specific meetings, and the involved partners have agreed to organise a workshop to analyse WGS data using a single pipeline to allow comparison of results.

UCM

In UCM, plasmid characterisation is being performed for several years now. In Ardig, we have implemented routine hybrid sequencing for all isolates having a Public Health relevance: all isolates are sequenced using Illumina and MinIon. Further, we have started a collaboration with the Sanger Center in Cambridge, and have currently remote access to their database, the largest microbiological database world-wide. This has allowed us to track clones identified in humans, animals or the environment back to their origin in different parts of the world, combining genomic data with Google-Earth algorithms. The idea is to, eventually, be able to identify the origin of these emerging isolates and to hinder it onsite, before world-wide spread. Further, UCM will support the workshop to be held in APHA, and will participate in the OH-EJP workshop in Croatia.

Finally, UCM has been able to receive a **PhD from EJP-OH**, in collaboration with two further partners of ARDIG, UoS and IP: metagenomics and genomic approaches for the prevention of the spread of plazomicin resistance in humans, animals and the environment. This will further strengthen the collaborative work within ARDIG.

NVI

A collection of more than 260 cephalosporin resistant Enterobacteriaceae has been sequenced by NVI using short-read NGS (archived isolates). The strains were isolated from broilers between 2012 and 2016 and can be available for the project.

BfR

To better understand the *qnr* PMQR pathway as well as the distribution of *qnr* genes, *Escherichia (E.) coli* isolates recovered in 2017 in Germany from bovine and swine origin were phenotypically and genotypically characterized. Antimicrobial resistance was determined by broth microdilution according to CLSI guidelines. MIC values were evaluated using EUCAST epidemiological cut-off values. *E. coli* from bovine as well as from swine origin, resistant to quinolones were subjected to six different *qnr*-PCRs to identify the respective variants, XbaI-PFGE, S1-PFGE and whole genome sequencing (WGS). Of 3,425 *E. coli* tested, 351 isolates from bovine and swine origin were classified as quinolone-resistant (MIC$_{NAL}$ ≥16 mg/L and/or MIC$_{CIP}$ ≥0.06 mg/L). The most abundant *qnr*-variant in bovine as well as swine originated isolates was *qnrS*, followed by *qnrA*. PFGE profiling with XbaI-digestion demonstrated a rather high heterogeneity. The highly diverse PFGE pattern did not indicate an association to a predominant *E. coli* clone spreading via vertical transmission nor to the origin of the sample matrix-wise. Also, S1-PFGE plasmid profiling showed a variety of extrachromosomal elements of various sizes. Also, WGS of those isolates confirmed the high genetic diversity of the quinolone-resistant *E. coli* strains. So far, quinolone-resistance could not be attributed to a specific lineage of *E. coli* or to the matrix-origin of the isolate. Further analysis is needed for better understanding the plasmid diversity within *qnr*-harboring *E. coli* and the prerequisites of their spread.

Out of more than 800 colistin-resistant commensal *E. coli* isolates from the national monitoring program on antimicrobial resistance in commensal *E. coli* from food and livestock of Germany, the presence of mobile colistin resistance determinants was determined. All *mcr-4* (n=14) and *mcr-5* (n=4) carrying isolates were further investigated by XbaI- and S1-PFGE to characterize their genomic restriction profile and the content of extrachromosomal elements (plasmids). Based on these data the occurrence of a clonal lineages harboring *mcr-4* or *mcr-5* coding plasmids could be excluded. Further whole genome sequencing of the isolates was performed to determine their genetic background in

detail. The sequence-based results confirmed the heterogenicity of the *E. coli* isolates but indicated the presence of closely related *mcr-4* or *mcr-5* coding plasmids. Both plasmid types (*mcr-4* or *mcr-5*) exhibited a highly conserved core genome which seems to be sometimes targeted by sequence insertions encoding proteins of unknown function. So up to now, its impact for the plasmids is unknown. Stability testing of the plasmid replication in *E. coli* showed that the plasmids were replicated stable in *E. coli* for at least 500 bacterial generations without colistin selection pressure. Re-sequencing of the plasmids indicated no alterations in the genome sequence of the plasmids. *In vivo*-filter mating studies with the prevailing *mcr-4* and *mcr-5* plasmids showed that none of the plasmids was self-transmissible. With except of one *mcr-5* carrying plasmid that seems to harbor a complete set of transfer genes for conjugation, all other plasmids only harbor transfer gens that might be necessary for mobilization. Further analyses were performed in the next month to determine if the *mcr-5* plasmid with a complete set of transfer genes will be self-transmissible at any circumstances.

In order to identify further bacteria that might also involved in the spread of *mcr*-genes PCR investigations with a diverse set of bacterial isolates from wastewater treatment plant of German slaughterhouses were performed. *mcr*-determinant could be detected in bacteria of the genus *Kluyvera* spp., but also in *Acinetobacter baumannii* and *Klebsiella pneumoniae*. The isolates will be subjected to whole genome sequencing in the next month for further characterization of their genetic background.

ANSES

In the first study, the ESBL phenotype was largely due to the presence of CTX-M group 1 enzymes, which were identified in 71.5% of the animals upon arrival, and in 61.2% upon departure to the slaughterhouse (Gay et al, Frontiers in Microbiology 2019). Of the 241 *bla*CTX-M-group1-carrying *E. coli* upon arrival, 200 harbored $bla_{CTX-M-1}$ (200/241, 83.0%), 23 $bla_{CTX-M15}$ (23/241, 9.5%), 12 $bla_{CTX-M-32}$ (12/241, 5.0%); 4 $bla_{CTX-M-55}$ (4/241, 1.7%) and 2 $bla_{CTX-M-3}$ (2/241, 0.8%). PFGE profiles performed on a subset of 10 ESBL-producing isolates per farm upon arrival showed a wide variability without any clustering. At departure, $bla_{CTX-M-1}$ was also the most frequently identified gene (51/60, 85%) followed by $bla_{CTX-M-55}$ (4/60, 6.7%), $bla_{CTX-M-15}$ (3/60, 5.0%), and $bla_{CTX-M-3}$ (2/60, 3.3 %). At departure, the PFGE profiles were much more similar than upon arrival so that a high degree of clonality was observed inside each farm. As an example, the PFGE distribution in farm E at departure, where three distinct PFGE profiles were observed, highlights the epidemiological success of certain ESBL *E. coli* clones more than others during the fattening process. Nonetheless, since different CTX-M enzymes were also produced by the same clone, not only a clonal but also a plasmid dissemination has likely occurred, which illustrates the complexity of ESBL spread at farm level. Similarly, the emergence of CTX-M-2 enzymes before slaughter was most likely due to the dissemination of a single clone within farm C since all but one CTX-M-2 enzymes were identified in this farm. Altogether, depending on the farm presenting ESBL-positive isolates, from 1 (farm H, 1 ESBL producing *E. coli* isolate) to 7 (farm B, 26 ESBL-producing *E. coli* isolates), distinct PFGE profiles were observed at the end of the fattening process. Of note, none of the successful clones identified at departure for the slaughterhouse was shared between farms, proving a specific and local evolution. *E. coli* belonged to phylogroups A (n = 134, 39.8%), B1 (n = 78, 23.1%), B2 (n = 9, 2.7%), and D (n = 116, 34.4%) upon arrival, and to phylogroups A (n = 50, 51.0%), B1 (n = 15, 15.3%), B2 (n = 1, 1.0%), and D (n = 32, 32.7%) at departure to slaughterhouse. The *mcr-1* gene was identified in 18 isolates, while one isolate carried both the *mcr-1* and *mcr-3* genes. At departure for slaughterhouse, only 4 animals from 2 different farms still carried a colistin-resistant *E. coli* (MICs ranging between 2 and 4 mg/L). The *mcr-3* gene was detected in all four isolates and was co-harbored with the $bla_{CTX-M-55}$ gene. In the second study, molecular characterization of ESBL genes is still on going.

APHA

A total of 386 *E. coli* isolates from 5 pig age classes (gilt, farrowing, dry, weaners and grower/finisher) as well as an environmental source (seagull faeces) from 3 time-points across a 12 month study duration on a low antimicrobial usage farm have been successfully sequenced using Illumina short read sequencing. These isolates were derived from both non-selective and antibiotic-containing selective media, with the diversity of sequence types (ST) heavily influenced by selection media. A total of 54 unique sequence types were observed from non-selective media while in contrast only 7 sequence types were observed from selection media containing ciprofloxacin, with the majority (69%, n=107) of these belonging to ST 744. Similarly from selection media containing cefotaxime, only 8 sequence types were identified and the majority (75%, n=27) belonged to ST 88.

Resistance gene content of all isolates sequence was analysed using the APHA SeqFinder pipeline, 250 isolates were identified as containing non-chromosomal AMR genes presenting a total of 63 unique AMR gene profiles. The top 10 resistance gene profiles account for 58% (n=146) of isolates containing non-chromosomal resistance genes and of these patterns only one was identified as containing an ESBL (*bla*CTX-M-15). The most frequently observed resistance pattern (n=28) was *aadA5*, *strA*, *strB*, *bla*TEM-1b, *intI1*, *mphA*, *sul1*, *sul2*, *tetAB*, *tetB*, *dfrA17,* with the next most frequently observed resistance pattern (n=23) differing by one gene (*strA* replaced by *aph3-Ia*); although both these resistance patterns were only observed in a single sequence type, ST 744.

IP

Our main activity in the framework of this WP was to further analyse the emergence and the dissemination of carbapenemase producing *E. coli* (CP-*Ec*). Compared to the previous report we have performed experiments for the revision of our manuscript entitled "Stepwise evolution and convergent recombination underlie the global dissemination of carbapenemase-producing *Escherichia coli*" also accessible on BioRxiv. We have performed a statistical analysis to support our conclusion that carbapenemase genes are acquired preferentially in specific backgrounds mutated in the porin genes *ompC* and *ompF* and in *ftsI* encoding penicillin binding protein 3. In addition, we have systematically analysed the antibiotic resistance gene content of CP-Ec and shown that carbapenemase genes are preferentially acquired in MDR backgrounds and are associated with ESBL from the CTX-M family. Based on these new results we have proposed hypotheses regarding the evolutionary trajectories of CP-Ec strains.

With regard to sequencing, we have sequenced 723 CP-*Ec* isolates from the French NRC and 53 ESBL isolates from the i-bird cohort. We are planning now to sequence UTI isolates from the longitudinal study performed at the Bicêtre Hospital and additional ESBL isolates from the i-bird collection. We will more in depth analyse the plasmid content of these isolates and their transmission.

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

NVI

For a more complete analysis of circulating ESBL plasmids in broilers a subset of isolates have been sequenced using long-read sequencing (PacBio). The isolates were sampled in 2016 and originate from a study were all broiler flocks raised in Norway were screened for cephalosporin resistant Enterobacteriacecae during the period May to October. Approximately 10% of the flocks were positive, and blaCMY-2 was the dominating gene responsible for cephalosporin resistance. However, a minor proportion were classical ESBLs all containing the blaCTX-M-1 gene, located on IncI plasmids. All isolates have been sequenced using Illumina technology, in addition a subset of isolates (one isolate pr sequence type) have been sequenced with PacBio. Preliminary data on the reconstruction of plasmids shows a diversity on IncI plasmids and also a variability with regard to self-mobility by

conjugation. All plasmids will be fully reconstructed and will subsequently be subjected to further plasmid studies.

WBVR

Molecular characterisation of plasmids through WGS using short-read and long-read platforms has been carried out on MCR positive isolates from the Netherlands on > 130 isolates dating from 2010-2018. Analysis of these plasmids will be finalised shortly.

In WP3-T1, MCR was discovered for the first time on a novel plasmid type, IncI1, and characterisation of the transfer in vitro was carried out to confirm if these plasmids have the potential to spread to different bacterial populations. Several isolates were shown to carry MCR on colE plasmids which are non-conjugative, however, these were shown in vitro to be mobilisable by a range of different plasmid types.

A new method for the analysis of phase variation systems using long-read sequencing was tested. The shufflon phase variation system of IncI1 was analysed which has effects on the selection of recipient specificity during conjugation between bacterial cells. The system was shown to be constantly active during various growth phases, resulting in a continuous genetic reorganisation of the region, resulting in a high variation of potential recipient cells that can be targeted at any time. This work was recently accepted for publication (Brouwer *et al.* 2019 PMID 30885787).

ANSES

As mentioned above, in the first study, not only a clonal but also a plasmid dissemination has likely occurred in the studied farms. ESBL and *mcr-1* plasmid characterization is on going using S1 PFGE, Southern blot and short and long read sequencing.

APHA

Long-read Oxford Nanopore sequencing has been performed on a total of 15 isolates to date, which has resulted in the complete circularisation of 17 plasmids, of which 8 were identified as containing resistance genes, however further work into characterisation of these plasmids is ongoing. Long-read sequencing has also allowed the identification of a conserved transposon integrated into the chromosome of multiple ST 744 isolates of approximately 27kb and containing varying AMR genes, accounting for the variation in resistance patterns observed.

A large amount of clonal spread of isolates has been identified on farm, most notably in regard to ST 744 isolates, where a single clone was identified 5 times in time-point 1, 3 in time-point 2 and 12 in time-point 3. This spread has been observed both between all age classes of pigs and between pigs and the environmental seagull samples. Likewise persistence of these clones throughout all time-points has been observed with low (<14) SNP counts between clones from time-point 1 and time-point 3 (12 months). This indicates that clonal transfer and persistence is an important factor in AMR spread and persistence on farm.

Further planned work includes the sequencing of isolates taken from a farm unit associated with the farm previously sampled, from which animals are transferred when undergoing antimicrobial treatment. This will allow comparison of strain diversity, AMR gene content and spread within these animals in the presence of antimicrobials to their non-treated counterparts.

UoS

The UoS is currently analysing WGS data to come up with a set of circulating plasmids carrying high priority AMR genes. Transfer and fitness of candidate plasmids will be further characterised *in vitro* and using *in vitro* animal gut models.

The University of Surrey has set up both chicken and porcine *in vitro* gut models and these are currently undergoing extensive validation using a metagenomics approach.

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

This work has not started yet.

4.1.2.2.3.4 WP4: Project coordination and management.

4.1.2.2.3.4.1 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.

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

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

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

For Year 1 ARDIG 9M and 12M reports were submitted in full and in a timely. In addition ARDIG submitted their Data Management Plan in full and in a timely manner.

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

A teleconference meeting is being planned by the end of June to make a final decision on what type of analysis to perform with the data collected in WP1.

A teleconference meeting is being planned in July to make a final decision on the dates for a WGS analysis workshop for WP3. The teleconference will also discuss the types of training/analysis that will be undertaken in the workshop.

4.1.2.3 *RaDAR*

4.1.2.3.1 **Summary**

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

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

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

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

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

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

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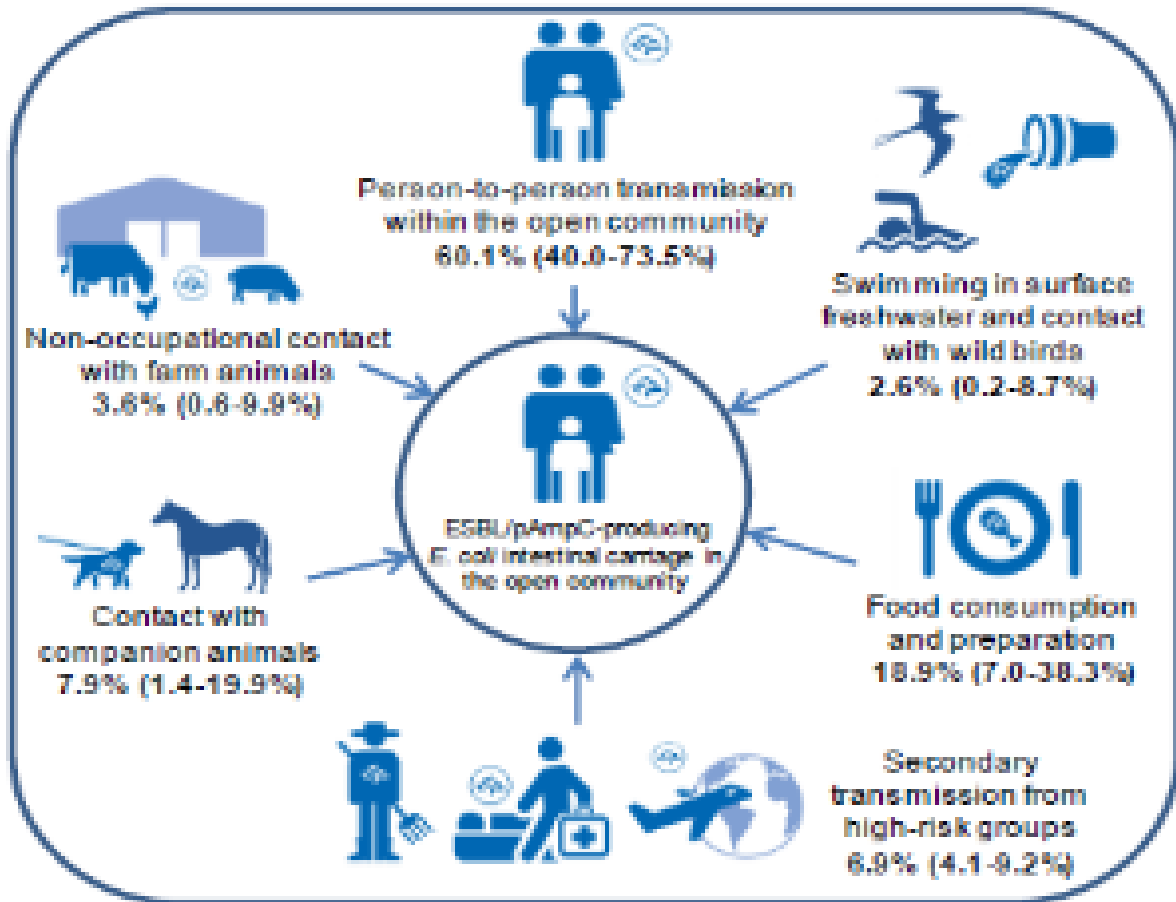
Figure. Summary results of ESBL source-attribution (submitted to Lancet Planetary Health)

4.1.2.3.2 Project-specific milestones and deliverables

4.1.2.3.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
RaDAR	D-JRP3-4.1	Model repository of state of the art ML methods for risk profiling available	12	to be updated		to be updated
RaDAR	D-JRP3-1.2	Establishment of a database of field (meta-)genomic data	15	18		Plasmid database & WGS dataset from environmental samples completed (see Deliverable document D-JRP-1.2)
RaDAR	D-JRP3-1.4	Test and parameterization of the assembly pipeline for metagenomics data	18		24	Preliminary data obtained from plasmid assembly/reconstruction tools & plasmid annotation/characterization programs
RaDAR	D-JRP3-1.5	Biological annotations of plasmid identified in the pipeline	18		24	

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
RaDAR	D-JRP3-1.6	WGAS-based method for genomic data analysis	18		30	
RaDAR	D-JRP3-1.7	Development of regression model for genomic data analysis	18		30	
RaDAR	D-JRP3-5.1	Report on structured expert judgement	17	to be updated		to be updated
RaDAR	D-JRP3-6.1	Publication on final evidence network	18	to be updated		to be updated

4.1.2.3.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
RaDAR	M-JRP3-12	Formulate CM/NGM (CVI, NCOH) and SD (RIVM) transmission models	13	No		Delayed due to delay in recruiting PhD student. Extension to milestone requested

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
RaDAR	M-JRP3-13	Produce case study (collected datasets) baseline results for PK/PD (ANSES)	15	Yes	18	Datasets have been collected to estimate unknown phamarcokinetic and pharmacodynamic parameters. Simulations of case studies has begun.
RaDAR	M-JRP3-14	Test and validation of assembly pipeline on synthetic and reference genomic data	15		18	Integration of plasmid tools for pipeline
RaDAR	M-JRP3-15	Establish connection between the PK/PD model and the on-farmmodel (ANSES, APHA)	16	Yes		Connections between the PK/PD and farm model formed part of a poster presented at the OHEJP ASM.
RaDAR	M-JRP3-16	Establish connection between the PK/PD model and the on-farmmodel (ANSES, APHA)	16	Yes		
RaDAR	M-JRP3-17	Calibrate models using data library (CVI)	17	No		Delayed due to delay in recruiting PhD student. Extension to milestone requested

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

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
RaDAR	M-JRP3-25	Analysis of field genomic data with WGAS-based method	18	no	30	
RaDAR	M-JRP3-26	Analysis of field genomic data with regression model	18	no	30	

4.1.2.3.3 Description of the project activities per task

4.1.2.3.3.1 WP0: Coordination and communication

4.1.2.3.3.1.1 *JRP3-WP0-T1: Coordination and project management (M1-M24)*

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

4.1.2.3.3.1.2 *JRP3-WP0-T2: Consortium meetings (M1-M24)*

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

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

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

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

4.1.2.3.3.1.3 *JRP3-WP0-T3: Annual reports (M1-M24)*

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

Submitted in January 2019

4.1.2.3.3.2 WP1. New genomic information to feed AMR transmission models

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

Complete plasmids and associated metadata available in public databases have been retrieved and curated to create a comprehensive plasmid database. The analysis of this database gave a global overview of plasmid diversity and classification and will provide information on plasmid host range and transmission routes. The manuscript summarizing these results is currently being prepared and should be submitted for publication. This database will be used for further bioinformatics developments (Task 1.2). New environmental datasets have been collected. This includes whole genome datasets (mostly short read sequencing data) ; Salmonella surveillance network (2000-2016) from all food, animal and environmental sectors, a total of 2839 isolates. This dataset have a particular focus on colistin resistance and the plasmidome in relation with certain serovar (S. infantis).

Quinolone resistant *E. coli* (QREC) from animals (poultry, pigs, wild birds, foxes) in Norway; cephalosporin resistant *E.coli* (containing blaCMY-2) from poultry in Norway. Two metagenomics

dataset have also been included ; The pig faecal metagenomics data collection obtained in the frame of the EFFORT H2020 project (https://academic.oup.com/jac/article/74/4/865/5289505 ; ENA: PRJEB22062)

The metagenomics dataset of urban sewages under the H2020 COMPARE project (doi: 10.1038/s41467-019-08853-3.; ENA: ERP015409). Other metagenomics dataset relevant to the AMR dissemination problem that have been made publicly available recently might be included.

The curated database will be used in JRP3-WP1-T2, JRP3-WP1-T3, JRP3-WP1-T4 for testing and validating the methods.

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

Plasmid assembly is particularly challenging from short read sequencing reads due to the presence of numerous repetitive elements. An automated pipeline will be developed by integrating plasmid assembly programs, annotation tools and reference-based identification. PlasmidSPAdes will perform plasmid *de novo* assembly based from the coverage and the assembly graph. All the reconstructed plasmids will be annotated using Prokka and resistance genes searched against the Resfinder database. Finally, our comprehensive database will be integrated into the MOB-suite program for plasmid characterization

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

Data obtained in JRP3-WP1-T1 will be run through the *in silico* validated pipeline (JRP3-WP1-T2). Output will be examined and curated by microbiological experts and phenotypic characteristics such as MIC values will be used for verifying the genomic findings.

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

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

Whole-genome association studies (WGAS) to identify SNP's and Indels statistically associated to AMR traits whether plasmid-borne or not.

Regression model that will focus on the presence/absence of gene belonging to the accessory genomes (chromosomal and plasmid-borne).

4.1.2.3.3.3 WP2. Pharmacodynamics and transmission models

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

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

Datasets at the pig individual level have been collected in order to estimate unknown parameters linked to fecal bacteria shedding, the transit of AMD in digestive tract and the pharmacodynamic impact of AMD on selection of resistant bacteria. Simulations have been performed but sensitivity and uncertainty analyses remain to be done.

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

Delayed. This task requires the result of sensitivity analysis of JRP3-WP2-T1-ST1 and model of JRP3-WP2-T1-ST3 development.

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

The model framework has been completed. A joint poster demonstrating the linkage between the farm model, the PK/PD model and the exposure assessment being developed in WP3 (JRP3-WP3-T2-ST2) was presented at the OHEJP ASM in Dublin. Work has begun on development of the model, but resources issues are currently delaying progress. We are currently recruiting to resolve this issue.

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

Meetings have been held with the Veterinary Medicines Directorate and the Pig Health and Welfare Group to determine relevant on-farm intervention measures to consider in the scenario analysis

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

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

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

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

This task has been delayed, as the PhD-student on this project only started working in September 2018. We are performing a systematic review with the research question: "What is the difference in estimated importance of transmission routes of antimicrobial resistant bacteria according to different methodologies?" We were also involved in a submitted paper to estimate the relative contribution of different acquisition routes of ESBLs of humans and to estimate of the reproduction number among humans.

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

Delayed due to delay in task JRP3-WP2-T2-ST1, we first aim to understand the source attribution before we estimate the effect on intervention measures.

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

This task has not yet started.

4.1.2.3.3.4 WP3. Transmission through the food chain.

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

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

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

Additional work is ongoing to technically facilitate usage of the inventory. The structure of the model inventory, as described in the annual report 2018, has been realized on a BfR test server. This server is currently hosting an alpha version of the web application in order to gather feedback on functionality, design and user experience of already implemented features. With respect to the feature list described in the annual report 2018, the latest version of the application is capable of:

- saving and displaying model and model results

- changing model parameter and executing models

- uploading new models from FSK files and script files

- sorting and displaying models, that can be filtered by details

- searching dynamically for models and model details

- providing restricted accessibility for unauthorized users

- providing a simple and intuitive user interface

- displaying and executing model code in the web browser

The following features still are planned to be implemented:

- storing assessments of models

- sending notifications on new models and comments

- creating dynamic plots / charts

- providing a guide to annotate and create FSK files

After the implementation of the missing features and release of an optimized first beta version, it is planned to migrate the application to a stand-alone server. However, the model inventory is already accessible on https://nolar.bfr.berlin.

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

A comparative Exposure Assessment model of ESBL-producing *E. coli* through meat consumption (https://doi.org/10.1371/journal.pone.0169589) has been transferred to FSK standard. Currently we are transferring the model on the spread of ESBL/AmpC *E.coli* in the broiler production chain (https://doi.org/10.1111/risa.13145) to the FSK standard.

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

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

An interactive web application has been added to the chicken production model (using Shiny R software) in order to see how a model in such a form can be handled in the repository. Furthermore we have presented the model to representatives of the poultry processing industry and got feedback on ideas to improve the chicken processing model. Thus the concept for an improved chicken production chain model aims at changing conceptual assumptions of the model like the assumption that there is always only one carcass in one processing step (while in reality in certain processing steps several animals are in one process stage like in the scalding or defeathering step or during immersion chilling).

For the primary production (hatchery, transport, fattening at farm) we were looking for additional data to develop the model further. The objective is, to adapt the model on the spread of ESBL/AmpC *E.coli* in the broiler production chain to estimate the level of colonisation of animals with ESBL/AmpC *E.coli*. Further work is needed on this.

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

Development is underway of two sub-models for the pork chain. Firstly, an existing consumer-phase model describing inactivation, growth, and cross-contamination of ESLB 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. These modifications are implemented for compatibility with WP6 - the pork production chain model will be the case study for the evidence synthesis work. Furthermore, first steps have been made for transferring the consumer phase model to the FSK repository (JRP3-WP3-T1-ST2). The second sub-model is the pork slaughterhouse model. Here we are in the process of amending the existing EFSA Salmonella in Pork model. Since also this model will be integrated in WP6, we greatly simplify the model structure, and replace Salmonella specific parameter values with a parametrisation more appropriate for ESBL E. Coli. 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.

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

Laboratory experiments regarding the association between the cooking time and temperature of the eatable parts of the blue mussels are finished. Further, blue mussels have been contaminated with *E. coli* and the concentrations were analysed on blue mussels before and after the contamination as well as after different cooking times. The temperature was measured simultaneously. The experiments were repeated three times.

The next step will be to perform an experiment to assess possible survival of ESBL producing *E. coli*. The model will use the data collected under these experiments. The deliverable of this task will be postponed with 3 months.

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

This task builds on the work performed in WP2 and WP3-T2. During the second half of the year we will determine the potential for deriving or creating generic methods for exposure assessment for different

production types, with a focus on the chicken and pork production chain. Discussion will address how different bacterial species, plasmids and genes might be covered in this generic approach. Main work will be performed during the final period of the project. The deliverable of this task will be postponed by 6 month.

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

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

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

This task has been completed, see annual report 2018

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

This task has been completed, see annual report 2018

4.1.2.3.3.5.1.3 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

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

This task has been completed, see annual report 2018)

Remark:

When applying for the project, a system was sought with which the results of the investigations could be made publicly available. The system to be used should consider the FAIR principle ("Findable, Accessible, Interoperable, and Re-usable"). This can be realized with a GitHub repository.

Since the results of the investigations are now to be made available according to a data management plan (DMP) to be created, the establishment of a Github repository was postponed to a later point in time after clarification of the exact design of the DMP. Nevertheless, the repository can be set up at short notice, as all preparations has been completed.

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

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

For the evaluation we need data sets that have already been evaluated an published. These data sets should then be located in the AMR area. So far we have been able to obtain two such data sets through cooperation.

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

Task completed.

The workbench for the evaluation of the ML algorithms uses publications on microbiological resistance in journals with peer review system. It is guaranteed that the data used for the publication can be

accessed (in the concrete case the data were made available by the authors under restrictions). The data are evaluated with different ML algorithms and the results are compared with the results of the original work regarding the determined influencing variables and their impact. On the basis of these criteria a similarity measure can be developed.

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

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

4.1.2.3.3.6 WP5: The burden of disease caused by AMR exposure

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

Completed in Y1 (see 12M report).

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

Completed in Y1 (see 12M report)

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

We are creating a mathematical framework to determine the extent at which infections with AMR bacteria add to the burden of infections and to what extent there is replacement of infections with AMS-bacteria by AMR-resistant bacteria. In addition, We are performing a review to estimate the parameters for a model we created to estimate the burden of UTIs taking into account that many individuals with an AMR-UTI would get an UTI with an AMS bacterium if resistance would have been absent.

4.1.2.3.3.6.4 *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")*

A paper was submitted to Lancet Planetary Health on the source attribution of ESBL-carriership among humans in the general population. Main result was that at least 60% of carriership can be attributed to human-human transmission, and roughly 20% due to food sources. This work was presented at One health EHP Annual Scientific meeting, Dublin).

Methodology was explored for attribution based on metagenomics and including human as a reservoir.
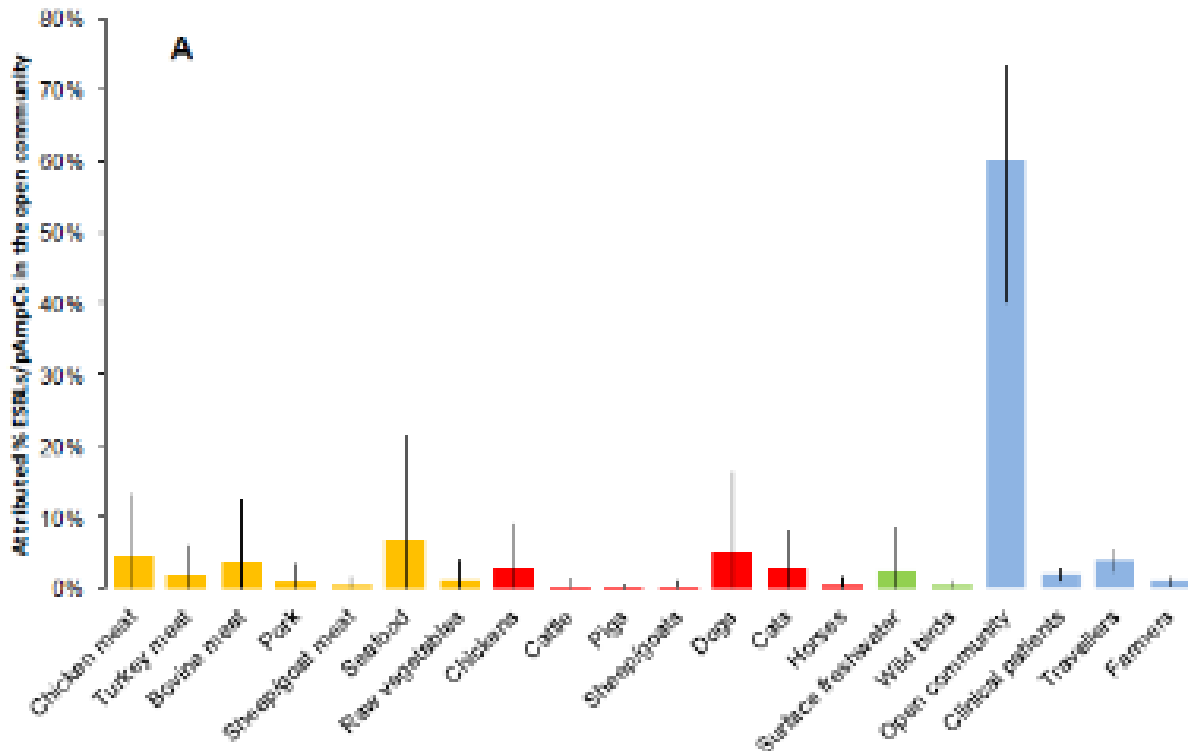
Figure. Attributable fraction of sources contributing to human carriership of ESBL *E. coli*.

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

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

4.1.2.3.3.7 WP6: Integration of information by Bayesian evidence synthesis

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

This task has been completed, see annual report 2018

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

This task has been completed, see annual report 2018

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

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

– Updated source attribution calculation and updated data on dose-response relation for ESBL E.coli carriership have been included under the evidence synthesis framework. QMRA and EPI estimations of carriership are now more in agreement, constructively contributing to an overall estimation of human ESBL E.coli carriership. Slaughterhouse contamination model needs yet to be included.

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

We started with defining the final model structure, modules and parameters. Important aspect is to include QMRA modules further down-stream (i.e. farm level modules from WP2).

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

A second annual meeting is planned for December 2019 or January 2020.

4.1.2.4 MAD-Vir

4.1.2.4.1 Summary

The MAD-Vir project is progressing as planned. The MAD-Vir project have just held a successful meeting in connection with the OHEJP 1st ASM in Dublin (21st of May 2019). All participated except ANSES, VRI and IZSAM.

A second ring-test has been sent to APHA, INIA and PIWET, which consist of purified and non-purified QCMD Panel samples. The test is ongoing however; preliminary results from INIA show problems with the detection of the purified samples. This could indicate that the samples have not been stored correctly during shipment. This is currently under investigation. APHA is not using the harmonized standardized S.O.P for the PanVirus array but instead uses an in-house protocol. Therefore, a direct comparison of the results of the ring-test generated from APHA cannot be compared to the results generated from the other Institutes (INIA, PIWET and SSI) that uses the harmonized protocol. So far, it has not been possible to implement the harmonized S.O.P at APHA.

So far, each partner in the project have select different samples from their biobanks to be tested at SSI with the PanVirus microarray. A common standardized sample pre-treatment/inactivation protocol has been presented by SSI and it has been decided that all participant should follow this protocol. In the 1st round of analysis the PanVirus array v2 detected 87% (52/60) of the samples tested. The samples that were not detected by the microarray were predominantly positive for West Nile virus (WNV) lineage 2, which indicated that probes for WNV lineage 2 on the PanVirus array v2 needed optimization. A new version the PanVirus array v3.1 was designed and the non-detected samples were re-analyzed. The re-analysis detected the correct virus in 7 of the 8 samples, which in total give a 98% (59/60) detection of the samples in the 1st round.

In the 2nd round of analysis the PanVirus array v3.1 detected between 87%-100% of the PCR confirmed positive samples except for samples from UoS, ANSES (Fish) and APHA in which the PanVirus array only detected 18%, 37% and 60% respectively. Investigation of the samples from UoS, APHA and ANSES (fish) showed that the common standardized sample pre-treatment protocol were not followed which could explain the reduced detection of these samples. ANSES (fish) has pre-treated samples (using the standardized protocol) for analysis in the 3rd round of analysis at SSI.

The 3rd round of analysis is ongoing.

4.1.2.4.2 Progress of the research project: milestones and deliverables

4.1.2.4.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
MAD-VIR	D-JRP4-2.1	Collection of samples (maximum 1500 samples in total from year 2017 to 2019)	M18	M18		

4.1.2.4.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)
N/A		No Milestones after M12		

4.1.2.4.3 Description of the project activities per task

4.1.2.4.3.1 WP1: Coordination and management (M0-M20)

Project management and coordination of the project is proceeding according to the plan.

4.1.2.4.3.2 WP2: Sample collection (M0-M20)

Each partner in the project has selected different samples of interest from their biobanks to be tested with the PanVirus microarray in the 1st, 2nd and 3rd round at SSI. A common standardized sample pre-treatment/inactivation protocol was followed when possible.

To this date SSI have received 761 samples for testing, PIWET has tested 21 samples, INIA has tested 3 samples and APHA 0 samples (Table 1). The samples are from wild life (rabbit, wild boar, duck, pheasant, stork, tortoise, hare, pigeon, springbook, deer, crow, pteropus vampyrus, partridge, eagle, owl, hedgehog and mouse), farm animals (sturgeon, trout, salmon, cat, goat, cow, chicken, horse, turkey, pig, sheep, and elephant), aedes, ticks, other insects and human samples

Table 1: Number of samples analyzed by PanVirus microarray

INSTITUTE	NO. OF SAMPLES						
	SSI 1st round	SSI 2nd round	SSI 3rd round	PIWET	INIA	APHA	Total
PIWET	14	25	30	21			90
INIA	5	20	25		3		53
IZSAM	10	20	25				55
OIK	16	20	30				66
ANSES	8	37	14				59
VRI	3	20	25				48
IZSLER	12	46	25				83
UOS	0	20					20
APHA	0	17				0	17
SSI	43	76	175				294
TOTAL	111	301	349	21	3	0	785

4.1.2.4.3.3 WP3: Diagnostic and surveillance (M0-M20)

4.1.2.4.3.3.1 JRP4-WP3-T1: Technology transfer (M0-M2)

This task has been completed, see annual report 2018

4.1.2.4.3.3.2 JRP4-WP3-T2: QA-validation (M0-M20)

SSI has sent 12 virus positive samples (purified or non-purified inactivated samples) to INIA, PIWET and APHA for a second ring testing in 2019 (Table 2). All the samples were Quality Control for Molecular diagnostics (QCMD) samples from different test panels. The ring-test is ongoing.

Table 2: Second Ring-test of selected samples from different QCMD test panels

SAMPLE NO.	VIRUS CONTENT	VIRUS TYPE	RING-TEST NO.2			
			SSI	PIWET	INIA	APHA
1	Human coronavirus OC43 (Bovine viral diarrhea virus 2, Bovine polyomavirus 1, Bovine parvovirus 2, Border disease virus C413)*	RNA	+	n.a	-	n.a
2	Human parainfluenza virus 2 (Bovine viral diarrhea virus 2, Bovine parvovirus 2, Border disease virus C413)*	RNA	+	n.a	+	n.a
3	Enterovirus (Pestivirus type 1, Bovine viral diarrhea virus 2, Bovine viral diarrhea virus 1, Border disease virus C413)*	RNA	+	n.a	-	n.a
4	Human parvovirus B19	DNA	+	n.a	+	n.a
5	Human Herpesvirus type 5	DNA	+	n.a	+	n.a
6	Human Herpesvirus type 1	DNA	+	n.a	+	n.a
7	Zika Virus (French Polynesian)(Pestivirus type 1, Bovine viral diarrhea virus 1)*	RNA	+	n.a	-	n.a
8	Human Metapneumovirus A	RNA	+	n.a	+	n.a
9	Human adenovirus type 4	DNA	+	n.a	n.a	n.a
10	HHV6 type A, HHV6 type B	RNA	+	n.a	+	n.a
11	Human rhinovirus A90 (Pestivirus type 1, Bovine viral diarrhea virus, Border disease virus C413)*	RNA	+	n.a	+	n.a
12	Enterovirus 68 (Bovine viral diarrhea virus 2, Border disease virus C413)*	RNA	+	n.a	+	n.a

*many of the samples also contain Pestivirus type 1, Bovine viral diarrhea virus and Border disease virus C413 that we will notified the company about n.a; not analyzed

As an additional quality control the molecular EQA on Neurotropic disease virus (2018) and Orthopox virus (2019) organized by EVD-LabNet were tested at SSI using the PanVirus array v3.1 (Table 3 and Table 4).

TABLE 3: TESTING OF THE MOLECULAR EQA ON NEUROTROPIC DISEASE VIRUS (2018)					
QCMD-SAMPLE	Material	Virus content	Copies/vial	Detection by RT-PCR	Detection by PanVirus array v3.1
1	plasma	Toscana linage A	$3,50 \times 10^5$	+	+, + Chikungunya
2	Plasma	Toscana linage B	$2,48 \times 10^5$	+	+
3	Plasma	TBE	$1,01 \times 10^5$	+	+
4	Plasma	WNV linage 1	$1,44 \times 10^5$	+	+
5	Plasma	WNV lineage 2	$9,92 \times 10^5$	+	+
6	Plasma	Usutu	$1,27 \times 10^4$	+	+
7	plasma	neg ctrl.		-	-
8	Plasma	neg ctrl.		-	-
9	Plasma	neg ctrl.		-	-
10	Plasma	neg ctrl.		-	-

TABLE 4: TESTING OF THE MOLECULAR EQA ON ORTHOPOX VIRUS (2019)					
QCMD-SAMPLE	Material	Virus content	Copies/vial	Detection by Orthopox PCR	Detection by PanVirus array v3.1
1	plasma	Monkey pox	25.7	+	+
2	Plasma	Monkey pox	29.2	+	+
3	Plasma	Monkey pox	29.6	+	+
4	Plasma	Neg ctrl.	-	-	-
5	Plasma	Monkey pox	28.2	+	+

6	Plasma	Monkey pox	34.9	+	+
7	plasma	Monkey pox	36.3	+	+
8	Plasma	Neg ctrl. (ORF)	29.5	-	+
9	Plasma	Cowpox	29.8	+	+
10	Plasma	Cowpox	28.0	+	+
11	Plasma	Neg ctrl. (HSV-1)	29.9	-	+
12	Plasma	VACV	28.2	+	+
13	Plasma	Neg ctrl. (VZV)	32.8	-	+

The PanVirus array v3.1 performed similar to the different in-house RT-PCR assays for neurotropic disease virus (Table 3) and similar to the Orthopox PCR assay (Table 4).

4.1.2.4.3.3.3   JRP4-WP3-T3: Analysis of samples (M0-M20)

Analysis of the 3rd round is ongoing. Of the samples tested in the 1st and 2nd round at SSI 285 of the samples were expected to contain a known virus content and 120 of the samples had an unknown viral content. The sensitivity of PanVirus array v3.1 on PCR positive samples varies between the different Institutes (Table 5).

TABLE 5: SUMMARY OF RESULTS FROM THE 1ST AND 2ND ROUND OF ANALYSIS AT SSI							
INSTITUTE	Samples with known viral content	Samples detected with PanVirus array	Samples confirmed positive with PCR	The sensitivity of the PanVirus array on PCR confirmed samples	Samples with co-infections	Samples with unknown virus content	Samples positive for an unknown virus
NPHC	24	21	22	96%	10	14	2
ANSES	14	10	10	100%	2	11	0
ANSES (FISH)	19	7	19*	37%	7	1	0
VRI	17	17	17	100%	4	6	0
UOS	11	2	11*	18%	2	9	3
APHA	10	6	10*	60%	3	7	7

PIWET	20	13	15	87%	15	19	16
INIA	20	16	18	89%	12	5	0
IZSLER	45	40	41	98%	34	0	0
IZSAM	29	21	24	88%	8	1	0
SSI	76	68	69	99%	14	47	9
TOTAL	285	221	256	86%	111	120	37

 * assumed PCR positive, but not tested due to lack of assay at SSI

The PanVirus array analysis of samples from ANSES (Fish), UoS and APHA showed a detection rate of 37%, 18% and 60% respectively, whereas the PanVirus array analysis of samples from all other Institutes showed a detection rate from 87%-100%. When looking into the samples from ANSES (Fish), UoS and APHA in more detail, these three Institutes did not use the harmonized Standardized sample pre-treatment protocol that has been distributed among the MAD-Vir partners. Because the PanVirus array S.O.P uses an unbiased metagenomic amplification protocol, sample pre-treatment is essential in order to remove competition from genomic DNA. If this is not performed, the sensitivity of the PanVirus array is significantly reduced (Table 5).

4.1.2.4.3.4　WP4: Data Sharing (M0-M20)

The common EJP website (https://onehealthejp.eu) is used for the MAD-Vir project. The private MAD-Vir group is used for all microarray results and other documents such as minutes, newsletters, summary reports, deliverables, presentations etc.

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

The MAD-Vir group had a face to face satellite meeting in connection with the OHEJP 1st ASM in Dublin in May 2019. Participants list can be seen at the website in the MAD-Vir group folder. Currently no other face to face meetings or tele-conferences are planned.

4.1.2.5　*Tox-Detect*

4.1.2.5.1　Summary

The second meeting of the ToxDetect project has been organized on March 20-21, 2019. 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 update the reference strain collection including new strains of *S. aureus* and *B. cereus* to focus on the new targets designated during the second meeting. Moreover, it has been decided to launch MaldiTof analysers. All partners were invited to prepare biomasses according to the commonly approved protocol and to send them to Anses platform MaldiTof (Nancy). The Maldi Tof platform received strains and associated MTA (if relevant) and results are expected during autumn for a MaldiTof reference spectra library available by the end of this year.

Due to the need of formal administrative authorizations to work on toxin construction in Germany, a delay has been observed for the development of recombinant toxins for staphylococcal enterotoxins

M, N and O. As the development of these standards is a pre requisite for the method development, a delay has also been pointed out in the dedicated work package.

Preliminary studies were performed to select relevant targets for *B. cereus*. Thus, the consortium choose to work on sphingomyelinase, cytK2 and HlyII.

Due to internal reorganization in Sciensano leading to lack of availability of the scientist in charge of the WP dedicated to mass spectrometry, it has been commonly decided to move this WP leardership in another partner. Pasteur institute took the lead of the WP at the beginning of this year.

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

4.1.2.5.2   Progress of the project: project-specific milestones and deliverables

4.1.2.5.2.1   Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
Tox-Detect	D-JRP4-1.2	Libraries of MALDI-ToF reference spectra	3	No	24	It was proposed to postpone the analysis of strains due to a need of preparation and signature of MTA between all partners. MTA were signed and Biomasses were sent to Maldi-TOF platform (Partner 01). Analysis are in progress and will continue during summer. All data will be assessed by the end of year 2, and then transferred to partners.
TOXdetect	D-JRP5-0.2	Intermediate report	18	No	20	Will be dispatched on M19 (this report)
TOXdetect	D-JRP5-0.3	Report of the meeting 1	19	19	20	Will be dispatched on M20

4.1.2.5.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
Tox-Detect	M-JRP4-04	Exchange of libraries of MALDI-ToF reference spectra	3	No	12	Additional analysis by Maldi-TOF is necessary before exchange of libraries. TC organized with coordinators and WP Leader on 16th May 2018 Protocol extractions have been discussed, defined and shared among partners on November 2018. Partners have to perform extraction before sending extracts to MALDI-ToF platform (early 2019). MTA were signed and Biomasses were sent to Maldi-TOF platform (Partner 01). Analysis are in progress and will continue during summer. All data will be assessed by the end of year 2, and then transferred to partners.

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
Tox-Detect	M-JRP4-05	Reference materials available	5	No	18	Reference strains are available for WPs depending on the availability of MTA and results of M-JRP4-04. Reference materials available after MTA signature
Tox-Detect	M-JRP5-06	High content analysis methods developed	18	no		Methods are currently under developed. Delay for both ELISA and MS based methods are explained in the text.
Tox-Detect	MS2.2	RT-PCR assays developed	14			Protocols tested and shared among partners. Ongoing
Tox-Detect	MS2.4	RNAseq data analysed	24	-	-	Protocol of RNA extraction and experiments are in progress

4.1.2.5.3 Description of the project activities per task

4.1.2.5.3.1 WP0. Coordination, management and communication

4.1.2.5.3.1.1 *JRP4-WP0-T1: General coordination and management of the project (administrative and financial) (M0-M36)*

This task takes place over the first, second and third annual period of the EJP.

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

- Face to Face meeting on Task 3.1 at Pasteur Institute (France) (17 January 2019)
- Face to Face meeting at Sciensano (Belgium) WP 3 organisation (08 February 2019)
- TC WP3 on organisation 15 February 2019 with partners of WP3
- TC WP4 on 12 March 2019
- TC WP3 on 14 March 2019
- TC WP1 on 15 March 2019
- Face to Face meeting on Task 3.1 at Sciensano (Belgium) (29 April 2019)
- TC WP4 on 06 June 2019

4.1.2.5.3.1.2 *JRP4-WP0-T2 to JRP4-WP0-T5: Organisation of four face-to-face meetings with all partners (M0-M36)*

15 participants representing all Tox-Detect partners were present during meeting (on 20 and 21 March 2019). All participants presented their institutions, activities and involvement in the Tox-Detect project.

The meeting was split into two half days. General discussion dealing with EJP projects took place after the presentation of Fanny Baudoin from EJP general coordination. Briefly, she presented Guidelines, specific rules, budget, communication tools and spoke about the possible 6-month extension. F Baudoin also highlighted the need to be present in the EJP meeting which took place in May in Dublin (IE). Unfortunately, noboby from the ToxDetect consortium was able to join this conference.

4.1.2.5.3.1.3 *JRP4-WP0-T6: mandatory reports on network activities: interim activity report, final report*

This task is in progress

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

4.1.2.5.3.2.1 JRP4-WP1-T1: Constitution of S. aureus strains collection (M1-M3)

As it was decided to focus on toxins SEM, SEN and SEO, strains of Staphylococcus aureus have been proposed by BfR partner to be included in the list previously established. MALDI-ToF mass spectrometry analyses are in progress and will continue during summer.

4.1.2.5.3.2.2 JRP4-WP1-T2: Constitution of B. cereus strains collection (M1-M3)

The list of strains was established. Some minor changes have been done regarding the strains chosen by IP partner for the project. The table of selected strains has been updated (21 strains sent for MALDI-ToF mass spectrometry analysis as expected). Moreover, Bacillus pseudomycoides CIP 105701 was added as a control in WP3-T2 (development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from B. cereus), because it is not supposed to express any toxin.

4.1.2.5.3.2.3 JRP4-WP1-T3: Constitution of C. perfringens strains collection (M1-M3)

Some of the selected strains have been replaced or not studied by MALDI-ToF mass spectrometry due to problem of cultivation. Analyses are in progress and will continue during summer.

4.1.2.5.3.2.4 JRP4-WP1-T4: Transfer of libraries of MALDI-ToF reference spectra (M3-M3)

Libraries of MALDI-ToF reference spectra are being made. All data will be assessed by the end of year 2, and then transferred to partners.

4.1.2.5.3.3 WP2 Characterization of toxins/virulence factors

4.1.2.5.3.3.1 JRP4-WP2-T1: Characterization of candidate toxin and/or virulence genes using toxicity tests (M4-M24)

This task was launched on M4. The growth conditions of the strains as well as the cytotoxicity assays were discussed between partners. 21 B. cereus strains and 40 C. perfringens strains were selected on the 28/05/2018. After analysis of the selected reference strains, cytotoxicity assays were performed on a selection of strain supernatant.

Protocols for the production of culture supernatants of B. cereus and C. perfringens (vegetative and sporulating) have been implemented and optimized by ANSES and INRA. The protocols and data were shared and discussed during a TC on the 29th of August 2018.

The protocols were used to obtain culture supernatants of B. cereus and C. perfringens for the strains of ANSES (C. perfringens) and ANSES and Pasteur (B. cereus). These supernatants were tested in a biological simplicate for the B. cereus strains and in biological duplicates for the strains of C. perfringens in cytotoxicity test by ANSES (V. Fessard).

All the data were presented during the 2nd Toxdetect meeting in march 2019.

ANSES and Pasteur will provide new supernatants strains of B. cereus and C. perfringens to test strains in duplicates (or triplicates) for cytotoxicity.

In summary, no cytotoxicity was observed in Caco2 cells treated with supernatants from C. perfringens strains. However, an increased IL-8 secretion was seen following treatment with vegetative Clostridium perfringens strains.

For B. cereus strains, the cytotoxicity varied with strains. No correlation could be highlighted between toxicity and strain origin so far.

4.1.2.5.3.3.2 JRP4-WP2-T2: Assessment of virulence and toxin gene expression using RT-PCR and transcriptomic assays (M4-M24)

4.1.2.5.3.3.2.1 JRP4-WP2-T2-ST1: Optimization of growth conditions to be used for gene expression analysis (M4-M10)

This task was launched on M5.

The protocols of bacterial growth and RNA extraction were optimized for B. cereus. After analysis of the selected reference strains, various growth conditions are currently been tested.

Culture conditions for C. perfringens were the same as those used for the production of culture supernatants for cytotoxicity assays. The optimal growth conditions for C. perfringens allowing for toxin production, and especially the Cpe toxin, was not found. It was thus decided to focus particularly on this matter.

4.1.2.5.3.3.2.2 JRP5-WP2-T2-ST2: Development of RT-PCR assays and transcriptomic analysis (M10-M24)

The protocol of RNA extraction was optimized for C. perfringens. Total RNA was extracted for both defined conditions: vegetative and sporulation.

RNA-seq partner's contracts and discussion of the number of selected strains to be sequenced are made and one recipient has already been identified.

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

First of all, it should be noted that the leadership of WP3 has been modified to take into account an internal reorganization at Sciensano and the fact that Mirjana Andjelkovic is no more in charge of the Mass Spectrometry team. She has been replaced by Julien Masquelier, who will take care of all the proteomics experiments planned in WP3 but who could not take the lead on WP3. For this reason, Julia Chamot-Rooke (IP) was asked to become WP3 leader and she accepted. A meeting has been organized at IP the 17/01/2019 to discuss this reorganization. A part of the budget allocated to Sciensano for leading WP3 will be reattributed to IP (J. Chamot-Rooke).

4.1.2.5.3.4.1 JRP4-WP3-T1: development of Mass Spectrometry-based methods for the detection of new enterotoxins (eg SEG, SEH, SEI) from S. aureus (M4-M27)

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

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

4.1.2.5.3.4.2 JRP4-WP3-T2: development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from B. cereus (M4-M27)

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

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

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

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

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

Next step will be to try top-down experiments to evaluate if we can characterize intact proteoforms for the various toxins we now expect.

This information has been shared among the partner in charge of the immuno-enzymatic assays (INRA, M. Gohar) in WP4.

4.1.2.5.3.4.3 JRP4-WP3-T3: Development of Mass Spectrometry-based methods for the detection of toxins and/or virulence factors from C. perfringens (M4-M27)

The methodological adjustments of the LC-MS/MS analyses are planned this autumn with the supernatants that will be provided by the ANSES of Maisons-Alfort. The Q-Exactive HF-X mass spectrometer (ThermoScientific) that will be used is being installed at the PFEMcp (proteomic component of the Metabolism Exploration Platform) and will be operational in July. A one-year postdoc is being recruited to carry out methodological developments and analyses, in coordination with the other two platforms involved in the project.

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

This task will be discussed later on.

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

4.1.2.5.3.5.1 JRP4-WP4-T1: Development of quantitative immunoassays for five known S. aureus and B. cereus toxins and virulence factors (M4-M32)

Progress in this work package for S. aureus has been significantly delayed due to 1) poor results from the proposed cell-free toxin production attempt and switch to recombinant toxin production in E. coli (requiring a "Work with genetically modified organisms" approval process by the German authorities) and 2) the consortium decision to change the originally proposed targets from SEG, H and I to SEM, N and O; the BfR was granted approval for the recombinant work by the authorities in December 2018; as the cell-free toxin production effort was originally to include inoculation of animals with the cell-free material, cancellation of this attempt necessitated the contracting of an independent company for producing monoclonal hybridoma cultures using recombinantly produced material; final approval by the BfR of the selection of the appropriate company was obtained in June 2019; the company estimates that, upon delivery of the purified toxin antigens, final delivery of the hybridoma cultures will require 5-8 months; in the meantime as a fallback measure, polyclonal sera will be produced at the BfR in order to ensure that design of the immunoenzymatic assays will not be further delayed.

Regarding Bacillus cereus, the unavailability of the strains reference library and of their genomic sequences has delayed the work until late 2018. For one of the three toxins, sphingomyelinase, chosen during the 2018 kickoff meeting, the genetic construction is finished and more than 10 mg of pure protein has been produced.

Rabbit polyclonal anti-sphingomyelinase antibodies should be available in September, after which the ELISA assay will be developed. For the two other toxins, a WP4 meeting held on June, 6th recommended to develop ELISA essays on HlyII and CytK2. The genetic constructions just started for these two toxins.

4.1.2.5.3.5.1.1 JRP4-WP4-T1-ST1: Selection of 5 target genes and construction of genetic tools for proteins overexpression (M4-M7)

For S. aureus, SEM, N and O genes were amplified and expression constructs were prepared (addition of affinity tag and protease cleavage sequences); sequencing constructions will be performed.

For Bacillus cereus, genetic constructs have been achieved for sphingomyelinase cloned from a foodborne outbreak B. cereus strain. HlyII and CytK2 were chosen as the two other targeted toxins and genetic constructs have just been started for these toxins

4.1.2.5.3.5.1.2 JRP4-WP4-T1-ST2: Proteins production (M8-M18)

For S. aureus, cloning and sequencing of prepared constructs are in progress

A little more than 10 mg of B. cereus sphingomyelinase has been prepared. Production of the two other toxins will be performed once the genetic constructs will be achieved.

4.1.2.5.3.5.1.3 JRP5-WP4-T1-ST3: Development of specific Ab (poly/monoclonal) (M12-M24)

For S. aureus, production of specific Ab is pending awaiting recombinant toxins from BfR.

Production of rabbit polyclonal anti-sphingomyelinase from B. cereus has started and the antibodies will be delivered in September.

4.1.2.5.3.5.1.4 JRP5-WP4-T1-ST4: Design of immunoenzymatic assays (M17-M32)

For S. aureus and B. cereus production and characterization of specific antibodies/sera is pending.

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

WP2 delivery of new virulence factor is expected.

4.1.2.5.3.6   WP5 Inter-laboratory ring trial scheme

This WP has its first deliverable in M25.

4.1.2.5.3.7   WP6. Dissemination, protection and exploitation of results

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

All necessary information was disseminated among partners through mailing or teleconferences, based on the Consortium Agreement. As ToxDetect coordinator, Anses served as a link between all partners and EJP coordination and sent e-mails to update all partners on project activities.

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

In this task, ToxDetect activities were disseminated to the public, either within the scientific community or to national/international decision makers and institutions relevant to the topic of ToxDetect.

In the first 12 month reporting period, partners presented on network activities at international conferences, symposia and meetings highlighting ToxDetect contents:

- 3 oral presentations;
- At several occasions, ToxDetect was presented in meetings with both scientific and political background, where decision makers or national / international authorities were represented with audiences of up to 100 persons.

Liaison with other National and/or EU projects and stakeholders: a liaison with other projects aims at exploring synergies with related EU activities and to avoid unnecessary duplication of work. In the current reporting period, a liaison with the EU-project EuroBioTox as well as a liaison with PhD works were established. In these projects, there is an overlap of experts and institutions that deal with bacterial toxins. Scientists representing projects take part in the respective project meetings strengthening interactions and cooperations. In this context, the ToxDetect consortium has to move to other toxins to avoid overlapping and multiple national and/or EU fundings (see 12 month report).

Publications and patents

The possibility to file patents and/or to publish ToxDetect results in scientific journals was discussed within the consortium partners during the second ToxDetect meeting organized in March 2019.

Table 1: Dissemination of information on ToxDetect project to the public

Type of activities (presentation, poster, website, brochure etc)	Speaker (name, institution)	Title of presentation	Date	Place	Type of audience (scientific, public, political, national authority…)	Size of audience
Oral presentation : Workshop dedicated to the toxin detection in EU projects	JA Hennekinne, Anses	ToxDetect	24 May 2018	Bern, CH	Scientific, national authority	10
Oral presentation : presentation of the ToxDetect project in the annual EU NRL for CPS workshop	JA Hennekinne, Anses	ToxDetect	1 June 2018	Maisons-Alfort, FR	Scientific	30
Oral presentation : presentation of the ToxDetect project in FoodMicro conference	JA Hennekinne, Anses	ToxDetect	3 September 2018	Berlin, DE	Scientific	100

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

For WP5: teleconference in July 2019

For WP2: teleconference in September 2019

For WP3: teleconference in September 2019

For WP4: teleconference in September 2019

For WP1: teleconference in November 2019

4.1.2.6 *NOVA*

4.1.2.6.1 Summary

On project management level, the main accomplishments during spring 2019 have been to finish a more detailed data management plan and to organise a second annual meeting.

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

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

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

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

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

4.1.2.6.2 Project-specific milestones and deliverables

4.1.2.6.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
NOVA	M-JRP6-6	Surveillance strategies to implement into the models agreed	12	Will be updated in final report	18	The discussion is focusing on how to integrate the management part of the surveillance in the models. There will be no uniform approach to this. Each model has to take into account the legislation of how to handle the problem. The major focus in the models will thereby be on the performance of the actual monitoring.
NOVA	D-JRP6-0.2	Documentation of consortium assembly and steering committee meeting	20	Will be updated in final report		

4.1.2.6.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
NOVA	M-JRP6-9	Development of method to handle time and GTIN.	17	Yes		
NOVA	M-JRP6-9	Development of method to handle time and GTIN.	17	Yes		

4.1.2.6.3 Description of the project activities per task

4.1.2.6.3.1 WP0: Coordination and project management

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

Monthly meetings with WP leaders have been held. The coordinator (and other project participants) has also attended the annual scientific meeting of the OHEJP and has taken part of collaboration meetings with the representatives from the ORION and COHESIVE projects. The deputy coordinator has presented research from NOVA at the OHEJP Programme Managers' Committee meeting in May, and the WP leader of WP3 has presented the NOVA project at the 2019 stakeholder meeting organised in Brussels by Sciensano, also in May.

During spring, project management has included work to deliver a data management plan for the project. Experience from creating such a plan was very limited or absent among the project participants. Therefore, this required attendance of educational sessions organised by the OHEJP management, and additional meetings to plan and complete this work process. With support from the OHEJP management, a structure was made to document the diversity of data and requests for specific data management within the different work packages and partner institute. This structure also facilitates regular updates of the plan.

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

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

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

No major tasks to report.

4.1.2.6.3.2 WP1: Food chain surveillance mapping

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

Given the work on a Med-Vet glossary of the participants of ORION project WP2 Integration in which members of NOVA project WP1 also participated in the first year, to complete the deliverable of this WP, a collaboration has been established with the respective ORION team, as well as COHESIVE. After further collaboration with participants of NOVA WP2-5, additional terms are being added in the glossary and defined. The work towards a joint publication (NOVA-ORION-COHESIVE) has been also initiated recently.

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

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

regarding this exercise and its use. Based on the overall mapping exercise, it was agreed to draft a questionnaire (in progress) addressing the OHEJP participants, in order to collect information and identify perceived opportunities and barriers in food-borne disease surveillance.

4.1.2.6.3.3   WP2: Analysis of food purchase data

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

Within this task, the availability of purchase data for outbreak investigations has been explored through two questionnaires and has been described in detail in D.2.1.1. The second deliverable will focus on barriers on use of food purchase data in collaboration with WP2 Task 2. During the next month, the course of action will be decided.

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

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

4.1.2.6.3.3.2.1 JRP6-WP2-T2-ST1:

Identify existing use of CPD for outbreak investigations, including through a survey of EU public health institutes, conducted in cooperation with JRP6-WP2-T1. In the second year, we have initiated the formation of a network consisting of stakeholders from across Europe that have experience or interest in working with CPD in relation to outbreak investigations. A formal survey among the stakeholders is being prepared.

4.1.2.6.3.3.2.2 JRP6-WP2-T2-ST2:

Develop Best Practice descriptions for CPD for outbreak investigations. The design of a framework for reporting outbreak investigations using CPD and utilising the outputs from WP2-T2 has been initiated. A Short Term Mission (STM) hosted by SSI is planned for the last week of June with the aim of the finalising the framework for publication.

4.1.2.6.3.3.3   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 to our knowledge has not previously been done. In the second year, the electronic web module in which Danish users can be invited to sign up and give consent for their purchase data to be used (described in D.2.3.1) has now been finalised and we are currently testing the system for errors before it can be launched during the fall of this year.

4.1.2.6.3.3.3.1 JRP6-WP2-T3-ST1:

Achieve supermarket CPD to study the ways to structure data on foods and run simulation studies for the risk of outbreak/sporadic foodborne infections. Model description for running simulations on CPD is currently underway, with the aim to have running model by year 3.

4.1.2.6.3.3.3.2 JRP6-WP2-T3-ST2:

Build a research infrastructure to achieve consent from sporadic cases of *Salmonella* and *Campylobacter* and healthy controls in order to use CPD to conduct analytical studies of foods posing

a risk for sporadic *Salmonella* and *Campylobacter* infections. We are finalising the protocol for a planned case-control study with this aim.

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

This task aims to use electronic food purchase data at the institutional level for investigation of nosocomial foodborne outbreaks. A systematic review of foodborne outbreaks within health institutions have been conducted and an MS is being prepared. The study was presented at the OHEJP annual scientific meeting by Ides Boone, RKI ("Healthcare-associated foodborne outbreaks in OECD countries and a special focus on Germany"). A questionnaire has also been drafted to describe the availability, usability, and traceability of food/catering data in healthcare settings in order to support foodborne outbreak investigations. This questionnaire will be administered in the summer of 2019

4.1.2.6.3.3.5   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 will commence in the second half of 2019. The integration of an existing food risk mapping model with FoodChainLab is running parallel with improvements of the existing model.

4.1.2.6.3.4   WP3. Syndromic surveillance

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

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

This task has been completed, see annual report 2018

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

This task has been completed, see annual report 2018

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

Tasks 2 and 3 were launched at the end of Year 1. Task 1 showed that the three countries participating in Tasks 2 and 3 do not have the same level of progress in the implementation and use of Sys monitoring tools. We therefore put in place different strategies depending on the degree of progress. However, we chose the same case study in all three countries: the monitoring of Salmonella and Campylobacter from animal and food production to human population. We also proposed to harmonize the methods used (detection algorithms) sharing experience between countries.

In Sweden, two time series of cases were created:

A.   The number of chicken slaughter batches tested for Campylobacter weekly, and the percentage of positives

B.   The weekly number of reported cases of Campylobacter in humans.

Our individual analysis of each time series included the traditional process of evaluation of a time series for syndromic surveillance: retrospective analysis with a full assessment of temporal effects. An epidemiologist, Wonhee Cha, has been employed to work on task 2 and task 3 (from September 2018).

A single case study has been chosen in France: the monitoring of Salmonella and Campylobacter from animal and food production to human population. For France, we will develop univariate surveillance modules for Salmonella in feed, cattle, poultry and pig, as no operational systems are currently available (task 2), despite that several sources of data were identified in task 1. The time series for bovine have been prepared. Data from human surveillance are still under acquisition.

In Norway, a syndromic surveillance system called Sykdomspulsen (NorSySS) was established in 2012. This is used to monitor the infectious status and possible outbreaks of gastro-intestinal; we have a univariate analysis on the human gastro-intestinal diagnosis. Univariate analysis will be carried out on Salmonella in poultry and pig productions and Campylobacter in slaughter chicken productions (data will be obtained from the Veterinary Institute). A statistician, Gunnar Rø, has been employed to work on task 2 and task 3 (from May 2019).

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

For the three countries, Task 3 consists in analysing the different data sources jointly. Several strategies will be explored:

1.   Study the correlation between these different time-series: the strength of the correlation between two series and the nature, in particular the time lag of the correlation. Using traditional statistics, attempt to use this correlation as a predictor variable when detecting aberrations

2.   Compare this with Bayesian statistics, where the "value of evidence" of both series are combined to determine a level of outbreak alarm.

3.   Use weather data to correlate both series to yearly temperatures (Sweden and Norway).

4.1.2.6.3.5   WP4: Spatial risk mapping

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

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

Spatial patterns have been assessed for first time in Spain (currently at province level). High priority geographical areas for implementing Salmonella surveillance programs have been identified to accomplish the D-4.2. Identification of periods with higher probability of detection of infection identified in high prevalence regions and temporal evidences for an association with human cases (month 24).

Clustering in AMR distribution are being explored for Salmonella from surveillance programs (using poultry as a proof of concept).

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

The developed disease spread model has enabled a better understanding of *S. Dublin* infection spread in Swedish dairy herds, as it related to herd population dynamics and time-varying trade patterns between farms. The model is being improved using more sophisticated approaches (Bayesian) and including between-herd transmission models and spatial and environmental factors to identify optimal surveillance strategies to accomplish the D-4.4. Evaluation of optimal surveillance strategies (M24).

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

Data on pig movements and pig feed suppliers are being recorded from Spain and are being explored to accomplish the D-4.5. Characterization of the spatial network structure of the pig industry in a Mediterranean scenario and Salmonella data mapped and analyzed (month 24).

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

Data on salmonella on extensive pig industry and wild boar are being explored to identify hot spot areas with higher potential for Salmonella transmission and to accomplish the D-4.8 Cartographic map of hot spot areas for Salmonella transmission between wild boars and extensive farming (month 24).

4.1.2.6.3.6 WP5: Evaluation of surveillance programs & cost efficiency

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

The building of a model to simulate spread of Salmonella through the GB pig industry has been started. Work has also been completed to generate key parameters for the model. Bayesian approaches have been applied to abattoir survey data and serological monitoring data to estimate the prevalence of Salmonella within farm and at slaughter. An approximate Bayesian computation approach has also been developed and applied to longitudinal data from GB pig farms, estimating the transmission rate and ensuring that the resulting model realistically represent on farm prevalence of Salmonella.

About the Salmonella model in poultry industry, we explored the usefulness of mathematical models in understanding the transmission of Salmonella in Danish poultry farms which was presented in Dublin during One Health workshop. The structural model is formulated. The data was obtained from the poultry industry. The estimations of epidemiological parameters of the formulated model are based on Bayesian methods. We will use the model to estimate the performance of the current Salmonella surveillance in the Danish broiler production industry. This work is in cooperation with the industry and authorities.

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

The task is performed together with a research group focusing on national surveillance of zoonoses in the animal production using gene-based methods in general.

At present time, we work on gathering existing metagenomic data from different part of the animal production. The data will be used as the "truth" in the evaluation of surveillance programs based on metagenomics, wherein we will simulate results from different surveillance strategies.

Currently, we are discussion which scenarios to use to mimic endemic and emerging occurrence of foodborne zoonoses/genes in the population (data).

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

A retail-to-DALY model for pig meat products was simplified to be able to accommodate data from Denmark, France, the Netherlands, Sweden and the United Kingdom. Data collection in these countries is in progress or finished. Model and data will be used to optimise the distribution sampling capacity using the concept of risk-based sampling.

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

The coordinator and WP leaders will have monthly one-hour (or longer, if needed) video conferences. The dates for these will be booked in August 2019.

An annual assembly with an opportunity for face-to-face meetings across and within WPs will also be held. Considering a potential extension of the project into 2021, it is being discussed if we are able to have two more annual assemblies, or if we want to postpone our annual meeting 2020 into the beginning of 2021. A decision will be made during the fall 2019. A teleconference for all participants has been suggested and will potentially be held in November 2019 or early 2020, depending on the decision about the next face-to-face meeting.

4.1.2.7 *LISTADAPT*

4.1.2.7.1 Summary

In the first months of 2019, The LISTADAPT JRP project closed the WP1 dedicated to the collection of strains (and their genomes). The achieved collection is constituted of three compartments. The first compartment (C1) regroups strains isolated in environment (soil, river, farm environment) as well as strains from animals (both healthy and animal presenting clinical symptoms). The second compartment (C2) is constituted of strains from five ready-to-eat food categories. It collects genomes from LISTADAPT partners from previous studies or from sequencing activities carried out in the context of NRL's activities. The last compartment is composed of strains involved in human sporadic cases. A meeting was organized in April with LISTADAPT partners and external labs to present the diversity characterization of these compartments.

The LISTADAPT partners involved in phenotypic characterization (WP3) received the second set of 100 strains (those from environment) in April (4 months later than expected). A second meeting will be organized in October 2019 to cross-compare the different phenotypic results obtained with the 200 strains. All explored phenotypes made good progress except for bacterial adhesion in relation to the problem encountered by INRA partner related to recruited staff.

The second sequencing batch of strains was realized in beginning of 2019, the third batch in July. All strains from first and second batches were assembled and annotated. A final batch will be sequenced in September. LISTADAPT partners decided to make the LISTADAPT genomes available in an ENA umbrella bioproject that will be made public with the publication of genome announcement papers related to environmental strains.

As, the collection of strains and the sequencing were delayed, a 6 months prolongation was asked. This will help to perform the latest task of WP4 (JRP7-WP4-T3-ST2 and JRP7-WP4-T4) and to better discuss within the consortium the results gathered during the project.

The Data Management Plan of LISTADAPT was successfully deployed with the notable creation of a Zenodo repository (10.5281/zenodo.2617258).

The LISTADAPT leader will leave the project during the summer of Y2 for another position in ANSES. Two options are considered. In the first (the most likely situation), the project lead will be transferred

to the successor of the current leader (the person being considered is a senior researcher that as strong relation with all project partners and with LISTADAPT project). In the second situation, the lead will be transferred to a scientist involved in the project. The final decision will be done in accordance with the global EJPOH coordination. The current leader will continue to play its role in the event of a delay

4.1.2.7.2 Project-specific milestones and deliverables

4.1.2.7.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
List Adapt	D-JRP7-3.1	Resistance profiles to biocide and antibiotics for the 200 L. monocytogenes strains.	12	16		
List Adapt	D-JRP7-5.2	Minutes of the training sessions	10		18	The training sessions of 2019 are inserted (the one of April and the one done in the beginning of July)
List Adapt	D-JRP7-4.1	Bibliographic study on catalogues of genes	13	16		The list of genes involved those already identified by COMPARE and additional bibliographic study including ANSES and DTU works.
List Adapt	D-JRP7-4.2	Report on prevalence and distribution of clonal complexes among the reservoirs	13	17		Presented at EJP OH conference in May
List Adapt	D-JRP7-0.3	Plan for dissemination and Exploitation of results	14	16		The dessimination plan was validated during face-to-face meeting in April

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
List Adapt	D-JRP7-1.4	Report on strain collection and strategy for selection of strains for sequencing.	14	14		The strategy was presented during the EJP OH conference in May
List Adapt	D-JRP7-2.3	Annotation of the Listeria monocytogenes assembled genomes from 2nd batch sequencing	15	17		Please update
List Adapt	D-JRP7-4.3	Software chosen for bioinformatics analysis	16	16		Validated during the April LISTADAPT meeting
List Adapt	D-JRP7-2.4	Annotation of the Listeria monocytogenes assembled genomes from Ad hoc Whole genome sequencing	17	22		The last batch will be sequenced in September 2019

4.1.2.7.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
LISTADAPT	M-JRP7-2	Selection of the 200 strains of Listeria monocytogenes from genomic analyses in WP2	3	Yes		100 strains have been selected based on their genomic characteristic and context of isolation. These strains correspond to strains isolated along the food production chain.

For the left 100 (from environment), the selection was based on the prevalence of CC in C1 compartment. The strains were send to partners in May? |
| **LISTADAPT** | M-JRP7-6 | WGS raw data produced | 6 | Yes | | The sequencing was reported of 2 months (related to report of M.2.2) |
| **LISTADAPT** | M-JRP7-7 | Face-to face meeting -2018 | 8 | Yes | 14 | A face-to-face meeting has been organized in April 2019 |

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
LISTADAPT	M-JRP7-14	Selection of some representative Listeria monocytogenes strains for adaptation to biocides	12	Yes	12	15 strain selected
LISTADAPT	M-JRP7-15	WGS raw data produced	13	Yes		
LISTADAPT	M-JRP7-16	Second batch Lm genomes assembly completed	14	Yes		High quality assemblies have been produced
LISTADAPT	M-JRP7-17	The database includes MLST data (CC and ST) of all the strains	14	Yes		The database has permitted the first communication of results in MAY. Abstracts for ISOPOL conference ar also based on the database
LISTADAPT	M-JRP7-18	Second batch Lm genomes annotation completed	15	Yes		-
LISTADAPT	M-JRP7-19	Ad hoc batch Lm genomes assembly completed	16	No	21	The third bacth was send in June. The last batch will be sequenced in September (M21)

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
LISTADAPT	M-JRP7-20	Bioinformatic analysis done for all the strains	16	No	24	Full characterisation of genomes are delayed because of the delay associated to sequencing
LISTADAPT	M-JRP7-21	Bioinformatic analysis done for all the strains	17	No	24	Full characterisation of genomes are delayed because of the delay associated to sequencing
LISTADAPT	M-JRP7-22	Ad hoc batch Lm genomes annotation completed	17	No	22	Delay
LISTADAPT	M-JRP7-23	Face-to face meeting -2019	17	No	22	A second one will be carried out in October 2019

4.1.2.7.3 Description of the project activities per task

4.1.2.7.3.1 WP0: Coordination

Numerous tele-conference meetings have been organized from month 1 to 9 (see WP5). A two-day physical meeting between LISTADAP was organized in April. During the meeting the global strategy of sharing genomes was discussed as well as the communication objectives of the WP1.

4.1.2.7.3.2 WP1: Constitution of a strain collection representative of the different reservoirs of Listeria monocytogenes

4.1.2.7.3.2.1 *JRP7-WP1-T1: Strain collection (M1-M12)*

This task has been completed, see annual report 2018

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

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

This task has been completed, see annual report 2018

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

This task has been completed, see annual report 2018

A final campaign of sampling is ongoing from month 3 to 6 in year 2, in order to increase the number of countries for strains from soils and small animals (slugs and snails).

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

This task has been completed, see annual report 2018)

4.1.2.7.3.3 WP2: Whole genome sequencing of Listeria monocytogenes strains

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

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

This task has been completed, see annual report 2018

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

The second and third batches of strains have been prepared at month 3 and 6 of Y2 respectively, for a total number of 500 strains.

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

This task has been completed, see annual report 2018.

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

4.1.2.7.3.3.2.1 JRP7-WP2-T2-ST1: First batch Whole genome sequencing for available Lm strains (M3-M6)

This task has been completed, see annual report 2018.

4.1.2.7.3.3.2.2 JRP7-WP2-T2-ST2: Second batch Whole genome sequencing for additional Lm stains (M13-M14)

The WGS sequencing of the second batch of strains has been performed in the beginning of Y2.

4.1.2.7.3.3.2.3 JRP7-WP2-T2-ST3: Ad hoc Whole genome sequencing (M3-M14)

The WGS of the third batch of strain has been done in month 6. A final additional batch is planned for month 9.

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

In year 2, all the assembly genomes of second batch were produced showing a high quality (median number of 21 contigs). Annotation of this high quality genomes was carried accordingly. Figure below shows the phenogenetic tree for first and second batches.
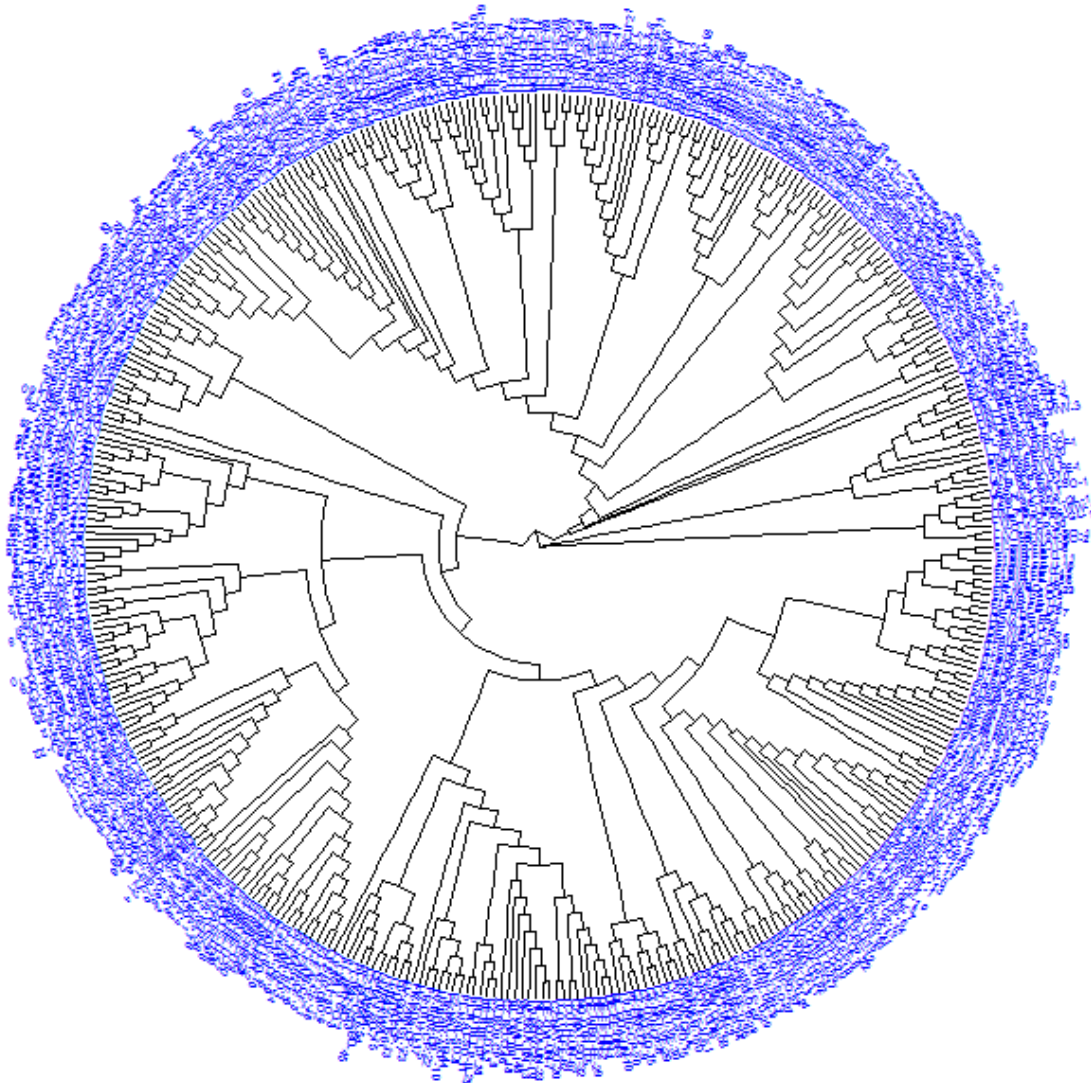
Figure. Phylogenetic tree based on mash-distance for the first and second batch of strains sequenced in LISTADAPT.

4.1.2.7.3.4   WP3 Phenotypic characterisation of Listeria monocytogenes strains

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

The selection of the set of 100 strains representative of C1 compartment was carried out in month 3 of Y2. The selection was established according to MLST. The most prevalent clonal complexes were selected. The strains were received by LISTADAPT partners in month 5.

4.1.2.7.3.4.2 *JRP7-WP3-T2:The effects of biocides on Listeria monocytogenes strains adaptation (M3-M22)*

4.1.2.7.3.4.2.1 JRP7-WP3-T2-ST1: Antibiotics and biocides resistance profiles of Listeria monocytogenes strains (M3-M22)

The characterization of MICs values for 14 antibiotics and 8 biocides of the first set of 100 food strains was achieved in month 3 of year 2. The characterization for the second set of strains is undergoing in Y2 and will be terminated for month 10.

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

The assessment of the ability to adapt to 4 biocides after repeated daily exposure to sublethal concentrations and to develop cross resistance against antibiotics has been carried out from month 1 to 5 of year 2 for f 15 Lm strains presenting with various antimicrobial resistance profiles.

4.1.2.7.3.4.2.3 JRP7-WP3-T2-ST3: The effect of biocides on Listeria monocytogenes strains in biofilm (M12-M24)

34 isolates have already tested on three disinfectants (seven different concentrations). The disinfectants used were Didectyl Dimethyl Ammonium Chloride, Sodium hypochlorite and Hydrogen peroxid.

4.1.2.7.3.4.3 *JRP7-WP3-T3: Bacterial adhesion and biofilm formation of Listeria monocytogenes strains (M3-M24)*

Some delay is expected for the delivery of the adhesion phenotype (see above "1. Summary of the work"). The delivery for the end of Y2 is not guaranteed at that stage.

4.1.2.7.3.4.4 *JRP7-WP3-T4: Survival and persistence of Listeria monocytogenes strains in different ecological niches (M3-M24)*

4.1.2.7.3.4.4.1 JRP7-WP3-T4-ST1: Survival of L. monocytogenes in food products and gastro-intestinal environment (M3-M24)

The growth in broth in response to lactate and acetate, both frequently used preservatives, have been carried out for 130 isolates (including all isolates in the culture collection from food). The study was performed with two concentrations of each additive and three temperatures in the range from 4-12 °C.

The characterization of survival in gastro-intestinal conditions will be carried out at the end of Y2.

4.1.2.7.3.4.4.2 JRP7-WP3-T4-ST2: Survival of L. monocytogenes in soil microcosm (M3-M16)

This phenotype characteryation has been terminated. 219 isolates (200 strains from LISTADAPT phenotype collection and 19 other strains of interest) was screened for soil survival. With a majority of isolates, a sharp decline was observed and less than 2% of the initial population was detected after 36 h of incubation in soil while a minority of isolates had survival rates of over 5%.

4.1.2.7.3.5 WP4: Identification of genetic traits in Listeria monocytogenes underlying adaptation to the ecological niches

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

The distribution of clonal complexes among the reservoirs has been done and presented in EJP general meeting. This characterization helped to determine the two set of strains for phenotypic studies. The final distribution would marginally evolve with the last batch of strains sequenced.

4.1.2.7.3.5.2 *JRP7-WP4-T2: Literature search of genes or genetic mechanisms responsible for virulence, adaptation and survival (M22)*

The list of genes involved in virulence, adaptation and survival was adapted from the H2020 COMPARE project. The characterization will be carried out when the last genomes will be sequenced (month 9 of year 2).

4.1.2.7.3.5.3 *JRP7-WP4-T3: Biostatistic analysis of annotated genomes (M6-M21)*

4.1.2.7.3.5.3.1 JRP7-WP4-T3-ST1: Identification of statistically relevant methods and development of analysis (M6-M16)

During the workshop of Y1, a list of relevant tools for identifying markers of adaptation to niches (environment, food industry) was established (see Table 4). LISTADAPT partners has identified two alternative methods (DBGWAS, and machine learning method from DTU). Within the full lists, at least three methods will be tested (Machine learning, GWAS based on presence/absence matrix, and Treewas for SNP).

For research of genes identified in JRP7-WP4-T2, LISTADAPT partners have chosen to use ABRICATE method (https://github.com/tseemann/abricate).

4.1.2.7.3.5.3.2 JRP7-WP4-T3-ST2: Processing of all isolates (M22-M30)

The final analysis is postponed to year 3 considering the delay for other task.

4.1.2.7.3.5.4 *JRP7-WP4-T4: Comparative analysis phenotypical data / genotypical data (M24-M30)*

Because of the delay for gathering phenotypical data, this task is postponed for Y3.

4.1.2.7.3.6 WP5: Trainings and dissemination

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

This task has been completed, see annual report 2018

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

This task has been completed, see annual report 2018

Two additional trainings have been organized in year 2. The first was done in parallel of the meeting in April and helped to train 20 participants to R package methods.

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

The LISTADAPT Proficiency Testing 2019 is planned for the end of Y2. This PT seeks to assess participants' sequence procedures and specifications in relation to the bacterial cultures tested as well particpants bioinformatic procedure. The PT will be based on the Lm strains from environement

JRP7-WP5-T4: Dissemination (M1-M30)

The LISTADAPT partners in year 2 disseminated their results during

- EJP OH general meeting in Dublin: two oral presentations and one poster;

- IAFP EU in Nantes: a poster ;

- ISOPOL Toronto conference: five abstracts submitted for oral of poster.

Two genomes announcement papers are planned for strains from C1 compartment.

4.1.2.8   *Metastava*

4.1.2.8.1   Summary

We refer to the detailed documentation of the project progress at M12 in the annual report. The present document only shortlists updates compared to that overview.

WP1: Tasks JRP8-WP1-T1 and JRP8-WP1-T2 have been previously delivered. Task JRP8-WP1-T3 (documentation of publicly available datasets) in ongoing. We keep the documentation of public datasets "live" but shifted the focus to datasets we are producing ourselves in Metastava due to the limited metadata available for most public datasets. Task JRP8-WP1-T4 is scheduled for the last 6 months of the project , and is heavily linked to task JRP8-WP2-T1; a joined effort to document existing guidelines and norms was started at the Metastava progress meeting in Brussels in February.

WP2: Task JRP8-WP2-T1 is linked to task JRP8-WP1-T4 where we have started an effort to document currently available norms and guidelines for diagnostic metagenomics and NGS QC in general. A shared file will be set up for these purposes. Task JRP8-WP2-T2: external controls for metagenomics QC: datasets have been produced for veterinary representative sample matrices (tissue, swab, fecal, serum) and first line data analysis is ongoing. Similar effort for human samples was initiated. Task JRP8-WP2-T3 data on batch effects collected, analysed, and conclusions made. Deliverable document to be finalized and made accessible. Task JRP8-WP2-T4 (applying QC metrics to parallel projects) awaits conclusions from JRP8-WP2-T1. Task JRP8-WP2-T5. several partners participated in a COMPARE PT. In addition a Metastava PT was agreed on, samples selected and prepared, and a data generation and analysis workflows agreed. Scheduled to ship summer 2019 with finalization in September 2019.

WP3: task JRP8-WP3-T1all samples listed and documented (D3.1). task JRP8-WP3-T6. procedure for analyzing the data has been agreed (D3.2 document still to be finalized on template and submitted). Tasks JRP8-WP3-T2 to JRP8-WP3-T5 ongoing.

WP4: In addition to initiatives reported in annual report: participation in discussion OHEJP-IRIDA-INNUENDO about WGS data. Stakeholder dissemination activities included presentations @ OHEJP Programme managers committee (9.5.2019), a national (Belgium) stakeholders info meeting about OHEJP(5.6.2019), OHEJP programme owners committee (19.6.2019). General dissemination in addition to 12M report: 2 posters at ASM OHEJP.

WP5: Heavy management workload with Data management v.1; application for 6 months extension; 21 month progress report.

4.1.2.8.2 Project-specific milestones and deliverables

4.1.2.8.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
Metastava	D-JRP8-1.3	List of sequence datasets	10		22	Partially delivered (public datasets). Partly to realize (partner's datasets from parallel projects). Focus will be on own generated data due to better metadata documentation. Reporting and sharing of valuable reference datasets towards end of project.
Metastava	D-JRP8-2.3	Report on batch and contamination effects in metagenomics	12		19	Data generated. Analysis done. Report in draft stage. Deliverable to be submitted.

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
Metastava	D-JRP8-3.2	Procedure for analyzing analytical sensitivity and robustness datasets	12	17		Due to delay of D-JRP8-3.1 . agreed, procedures and ref database shared. Deliverable report to be sumitted.
Metastava	D-JRP8-5.3	Half term report	13	13		Submitted 12 mth report
Metastava	D-JRP8-2.2	Report and guidelines for the use of exogenous process controls in metagenomics	18		24	Additional data planned to extend to human sample matrices. Guide document should include both vet and med.

4.1.2.8.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
Metastava	M-JRP8-M2	Public and own dataset identified	8	No	24	Partially delivered (public datasets). Partly to realize (partner's datasets from parallel projects). Focus will be on own generated data due to better metadata documentation. Reporting and sharing of valuable reference datasets towards end of project.
Metastava	M-JRP8-M4	Procedure for analysing analytical sensitivity and robustness datasets agreed	12	17		
Metastava	M-JRP8-M5	Progress meeting	12	13		Feb.2019. Brussels live meeting.
Metastava	M-JRP8-M6	Proficiency test panel ready for shipping	18	18		Samples ready. to start PT in summer 2019

4.1.2.8.3 Description of the project activities per task

4.1.2.8.3.1 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

4.1.2.8.3.1.1 *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)

4.1.2.8.3.1.2 *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)

4.1.2.8.3.1.3 *JRP8-WP1-T3: identifying available sequence datasets (M1-M12)*

Shift to Metastava -produced data as reference due to better metadata quality. Postponed listing to end project.

4.1.2.8.3.1.4 *JRP8-WP1-T4: Propose a standardised framework for the description of the application scope and analytical properties of a metagenomics assay (M18-M24)*

Planned

4.1.2.8.3.2 WP2: Quality assurance tools for the validation and interpretation of metagenomics

4.1.2.8.3.2.1 *JRP8-WP2-T1: The development of quality metrics to evaluate the significance of the outcome of a metagenomics experiment (M10-M24)*

Linked with wp1-t4: effort started to document existing guidelines, references, norms for diagnostic metagenomics, and for diagnostic use of NGS in general. Shared document to point to references.

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

All data for vet. Sample matrices generated and first line analysis done. Now extended to also human relevant matrices and will report conclusions including vet & med data later in project

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

Done but deliverable report to be submitted

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

Awaiting WP2-T1

4.1.2.8.3.2.5 *JRP8-WP2-T5: Metagenomic proficiency test (M13-M24)*

Ready for shipment. Planned for summer 2019

4.1.2.8.3.3 WP3: evaluation of the analytical properties of metagenomics workflows

4.1.2.8.3.3.1 JRP8-WP3-T1: analytical sensitivity, HEV (M1-M24)

Data collection ongoing

4.1.2.8.3.3.2 JRP8-WP3-T2: analytical sensitivity, norovirus (M1-M24)

Data collection ongoing

4.1.2.8.3.3.3 JRP8-WP3-T3: analytical sensitivity, large DNA viruses (M1-M24)

Data collection ongoing

4.1.2.8.3.3.4 JRP8-WP3-T4: analytical sensitivity, STEC (M1-M24)

Data collection ongoing

4.1.2.8.3.3.5 JRP8-WP3-T5: analytical sensitivity, detection of ABR genes (M1-M24)

Data collection ongoing

4.1.2.8.3.3.6 JRP8-WP3-T6: bioinformatics and statistical analysis of analytical performance experiments (M1-M24)

Done. Methodology agreed and described in procedure. Database version shared for standardisation. Deliverable report to be formally submitted.

4.1.2.8.3.4 WP4: Concertation with ongoing efforts and dissemination.

4.1.2.8.3.4.1 JRP8-WP4-T1: concertation with ongoing initiatives (M1-M24)

In addition to M12 report: discussion OHEJP-IRIDA-INNUENDO about WGS data.

4.1.2.8.3.4.2 JRP8-WP4-T2: formal dissemination (M1-M24)

2 posters at OHEJP ASM

4.1.2.8.3.4.3 JRP8-WP4-T3: dissemination of recommendations to stakeholders (M1-M24)

OHEJP Programme managers committee (9.5.2019), a national (Belgium) stakeholders info meeting about OHEJP(5.6.2019), OHEJP programme owners committee (19.6.2019).

4.1.2.8.3.4.4 JRP8-WP4-T4: Organization of a scientific meeting (M20-M24)

To be planned

4.1.2.8.3.5 WP5: Project management

4.1.2.8.3.5.1 JRP8-WP5-T1: Consortium agreement (M1-M6)

This task has been completed, see annual report 2018

4.1.2.8.3.5.2   JRP8-WP5-T2: Internal communication (M1-M24)

Feb 2019: Live Metastava progress meeting Brussels

7.06.2019: Teleconference on finalisation of Proficiency test organisation

23.04.2019: General Assembly teleconference: progress and 6 month extension decision.

Additional targeted mailings and bilateral teleconference & phone calls

4.1.2.8.3.5.3   JRP8-WP5-T3: reporting and liaising with the EU (M20-M24)

Unplanned additional tasks: Data management plan (v01 done); application for 6 month extension; modifications in the work plan regarding the role of WBVR; the present 21 month progress summary.

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

To be planned teleconferences per WP or specific issues like discussing the proficiency test outcome.

A scientific output meeting/project meeting will be planned in the extension period if obtained. Ideally we will maximize the presentation of our output during the 2020 OHEJP annual scientific meeting as this would reach a large audience. Remark: no specific budget for own scientific meeting.

4.1.2.9   AIR Sample

4.1.2.9.1   Summary

General: The project is proceeding according to the Second-Year plan, there is a good sense of collaboration and active dialogue by e-mail, exchange of protocols, Skype meetings and phone calls. Four new newsletters have been circulated in 2019, one successful hands-on workshop on metagenomics bioinformatics has been organized in Oslo. The outcome of the Oslo workshop are expected to propose a metagenomics test that consists of DNA extraction from airfilters, and a subsequent simple bioinformatics solution to DNA sequence analysis.

Company agreement: The project has re-negotiated the agreement with Sartorius (Germany) to continue with supporting the partners with gelatine filters and airsampling device worth of ca. € 20000 into the second year of the project. All partners were in contact with their local Sartorius office, obtained the equipment and implemented the protocol.

Lab work: Overall, the project has successfully moved into the evaluation phase (below):

Harmonization ->Implementation > **Evaluation >** Validation.

We have thus completed the method harmonization and implementation phases. During the summer 2019, we will focus on farm sampling, sample analysis and data generation. All protocols, including a consensus real-time PCR have been fully implemented by the partners. The results of the farm sampling will be discussed at the second annual meeting planned on October 15-16, 2019, Brno, The Czech Republic.

Deliverables:

1.   The SOPs for harmonized protocols have been prepared and implemented.

2.   A video clip has been prepared for free-access, online video demonstration of the airsampling protocol.

3.   A manuscript describing the outcome of last year farm sampling has been prepared and submitted to Food Microbiology (manuscript enclosed).

4.   The second manuscript describing the outcome of 2019 farm sampling will be planned at the second annual meeting in Brno.

5.   A novel device has designed and printed in 3D to conduct airsampling directly on the selective enrichment, Bolton broth, for Campylobacter. A comparative study is planned during the summer sampling to assessing the efficiency of sampling directly in the enrichment broth, rather than transferring the gelatine filters into the broth tube. A patent search conducted in Denmark has indicates novelty of this approach against the existing prior art.

Dissemination activities: Oral presentation of the project results by Gro Johannssen (Norway) at the OneHelath EJP first annual scientific meeting in Dublin. Oral presentation of the project by Jeffrey Hoorfar at the 70th annual meeting of the European Association of Animal Production (EAAP), Ghent, Belgium.

4.1.2.9.2 Project-specific milestones and deliverables

4.1.2.9.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
AIR-SAMPLE	N/A	N/A				

4.1.2.9.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
AIR SAMPLE	M-JRP9-3	Local field studies completed.	M18	Yes		All partners have conducted local field sampling in chicken farms, as described the project plan.

4.1.2.9.3 Description of the project activities per task

4.1.2.9.3.1 WP1. Method DevelopmenT

4.1.2.9.3.1.1 *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

4.1.2.9.3.1.2 *JRP9-WP1-T2: Development of a protocol for non-complex DNA extraction for diagnostic qPCR and metagenomics analysis from gelatine-filter samples (M3-M13)*

Based on intensive in-house studies at several participating labs, the consensus protocol was intensively discussed and agreed upon. It consists of two steps: Pre-treatment of gelatine filters taken by AirPort8 device, and DNA extraction using QIAgen DNeasy Blood&Tissue Kit, according to manufacturer's instruction with several modifications. The main step in filter preparation is the addition of the Protex protease to completely dissolving the gelatine. The modifications that we found to be important to performance of the commercially available DNA extraction kit were: 1) Addition of RNAse (due to our intended later use of the DNA product for downstream metagenomics analysis); 2) The replacement of kit buffer with TE-EDTA buffer; 3) The addition of AL buffer.

4.1.2.9.3.2 WP2: Validation and Standardization

4.1.2.9.3.2.1 *JRP9-WP2-T1: Validation of air sampling and DNA extraction methods (M13-M21)*

Three harmonized protocols were prepared for 1) field airsampling, 2) DNA extraction, 3) PCR testing. The protocols were validated on the field airsamples from chicken farms around Europe collected during the summer 2019. A sample bank was established for later use for metagenomics diagnostics. The protocols will make the basis for draft standards to be submitted to CEN and EFSA for consideration as part of the continued standardization work for Campylobacter detection in chicken farms. This part of the work is planned for Spring 2020. A second manuscript is planned for preparation based on the outcome of the summer sampling in 2019.

List of planned tele- or video conferences, face to face meetings in the next yearWe will continue to use the well-received newsletter style through email as the main internal communication tool, while Skype meetings will be used in the case of emergencies, or where there is lack of progress. Besides the second annual meeting in Brno on Oct. 15-16, 2019, a third annual (and final) face-to-face meeting will be held in May/June 2020. A dissemination, wet-lab workshop will be planned to take place at DTU during spring 2020.

4.1.2.10 *MoMIR-PPC*

4.1.2.10.1 Summary

The **MoMIR project** aims to develop new approaches to predict, identify and prevent the appearance of animal and human Super-shedders based on immune response and gut microbiota composition. In order to achieve this aim the project will focus on four objectives.

1.   Defining **Predictive markers** that will signal the risk of both animals and Salmonella isolates becoming a super-shedder of *Salmonella*.

2.   Immune and microbiota **biomarkers of excretion** to detect animal super- shedders and/ or human prolonged carriers.

3.   **Preventive measures and /or control measures** of this zoonotic problem by the characterisation of pre- biotics, pro-biotics and nutraceutical products, for use in both animals and humans

4.   Development of **mathematical models to provide new risk management tools**. These tools will lead to creation of a **pool of biosecurity measures** at the farm levels, each with a **cost effectiveness** consideration.

In order to meet these objectives a consortia of partners from across Europe are working together. However, initiation of the project was delayed due to unavoidable waits for ethical approval of animal studies and the replacement of two partners.

To date the project team has undertaken *in vivo Salmonella* infection studies in both chickens and pigs. Immunological and serological analysis of these studies has now also been completed, with microbiome analysis currently ongoing. These studies allowed us to identify predictive biomarkers based on gut microbiota composition. The *Salmonella* isolates recovered from high and low shedding animals (pigs) are currently undergoing characterisation, with various *Salmonella* associated virulence factors being considered during this analysis. Recruitment of participants in the human aspect of the project is also now ongoing, with analysis being carried out on a rolling basis. Concerning the development of preventive measures, the comparison of the gut microbiota compositions of the *E. faecium*-inoculated and control groups revealed an overall impact of the inoculated *E. faecium* candidate strain. Besides, one of the assays performed noticed that when *Bacteroides* are absent from piglet microbiota during first week of life, *Clostridium perfringens* is abundant. However, when *Bacteroides* spp. reach around 10 % abundance in piglet gut microbiota, *C. perfringens* decreases below 1 % of total microbiota. Nutraceuticals (alperujo) are currently tested in broiler chicks.

The partners met for their midterm meeting in February 2019, hosted by VISAVET, Madrid. The majority of partners represented and able to give updates on the progress of the aspect of the MOMIR project. During the meeting it was agreed that the project should be extended by 12 months, due to the unexpected delays encountered at the start of the project. The extension has been accepted by the EJP board. It was also agreed that at the end of the project Partner 23 (University of Surrey) will host a final project meeting, with the date of this meeting to be agreed at a later date.

4.1.2.10.2 Project-specific milestones and deliverables

4.1.2.10.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
MoMIR-PPC	D-JRP10-1.02	Identification of risk factors for shedding of Salmonella in pigs and poultry farms	12	24	24	The task has been delayed due to difficulties in obtaining all the necessary permissions to use personal and health related data.
MoMIR-PPC	D-JRP10-2.01	In vitro effect of already characterized probiotics on Salmonella growth and cell invasion	10	24	24	Some experiments have been delayed due to the modification of the participating partners. The recovery of *Salmonella* strains required for this task has thus been delayed.
MoMIR-PPC	D-JRP10-2.02	Description of the microbiome and resistome in farms	12	36	36	NDRVMI has supplant Vet-DTU at month 6 of the project.
MoMIR-PPC	D-JRP10-4.2	Data management policy and strategies	4	18	18	Completed

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
MoMIR-PPC	D-JRP10-0.4	Minutes of project meetings (mid term meeting)	13	18	18	Completed
MoMIR-PPC	D-JRP10-0.6	Financial and scientific reports for the EJP Coordinator (Intermediated report)	13	13		Completed
MoMIR-PPC	D-JRP10-1.11	Recovery of all human samples	15	32	32	Sampling start delayed by 12 months due to delayed ethical clearance that resulted in extension of sampling period until August 2020 Update September 2019: work now in progress/
MoMIR-PPC	D-JRP10-2.03	Characterization of protective commensal bacteria able to inhibit Salmonella colonization (two rounds)	14	34		Work now in progress Protective activity of *Enterococcus faecium and L. reuteri* isolates strains has been tested in pigs or chickens

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
MoMIR-PPC	D-JRP10-1.12	Antimicrobial susceptibility testing, serotyping and whole genome sequencing of all *Salmonella* isolates from participants submitted to the Norwegian Reference laboratory	17	34		Will be finished approx. two months after the last *Salmonella* sample has been received.
MoMIR-PPC	D-JRP10-1.08	Characterization, evolution and comparison of the microbiome of pig and poultry with different shedding status of Salmonella	18	24		Completed for chicken experiments and in progress for pigs. The majority of samplings have been done. Faecal samples have been received by UOS from the *in vivo* pig infection study at ANSES and DNA extraction is underway. Shipping of samples from the ISS *pig in vivo* study will be carried out in the near future

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
MoMIR-PPC	D-JRP10-1.04	In vitro virulence levels of different *Salmonella* strains recovered from high and low shedders in animals and humans (first round)	20	24		Work in progress. Some animal experiments are still ongoing. (UoS) will also analyse the attachment and biofilm formation. This work will begin 17/06/19.
MoMIR-PPC	D-JRP10-1.06	Definition of predictive immunological markers associated to the high and low shedders in chickens and in pigs.	20	34		Deliverable delayed Analyses are ongoing
MoMIR-PPC	D-JRP10-1.07	Definition of immunological markers associated to the high and low shedders in chickens and in pigs.	20	34		Deliverable delayed Analyses are ongoing
MoMIR-PPC	D-JRP10-1.09	Definition of predictive microbiota markers associated to the high and low shedders in chickens and in pigs.	20	32		Completed in chicken and new experiments are now in progress (UoS) samples have been received and 16S analysis is currently underway (see D-JRP10-1.04).

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
MoMIR-PPC	D-JRP10-1.10	Definition of microbiota markers associated to the high and low shedders in chickens and in pigs.	20	32		Completed in chicken and new experiments are now in progress (UoS) samples have been received and 16S analysis is currently underway (see D-JRP10-1.04).
MoMIR-PPC	D-JRP10-1.14	Identification, from in vitro studies, of immune parameters related to high and low shedders	20	24		Work in progress.
MoMIR-PPC	D-JRP10-2.11	Evolution of the immune-signature in pig, chicken and/ or human according to the context (infection, treatment…)	20	34		Partly completed. Work in progress.
MoMIR-PPC	D-JRP10-3.03	Intervention measures inventory	20	24		First version (Milestone) is completed; systematic inventory due in December 2019

4.1.2.10.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MoMIR-PPC	M-JRP10-7	Recovery of samples from the first round of experimentally infected animals	8	In part	24	Some experiments have been delayed due to the modification of the participating partners. (UoS) faecal samples have been received from the *in vivo* pig infection study conducted at ANSES and DNA extraction is underway. Shipping of samples from the ISS *pig in vivo* study will be carried out in the near future (June 2019).
MoMIR-PPC	M-JRP10-8	Four sets of NGS derived 105 mimotope sequences – positively and negatively enriched in IgM and IgA	8	yes		Resulting from the left of SAIM (A. Pashov), this task has been deleted in the new version of the project. Part of this task has been performed by partner 18 (INRA).

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MoMIR-PPC	M-JRP10-9	Recovery of samples from experimentally infected animals and from farms, pretreated with pre-biotics or neutraceuticals	8	In part	24	Pig experiments have been performed. Chicken experiments have started on May 2019. Work in progress
MoMIR-PPC	M-JRP10-10	In vitro infection of cell lines and organoids with the *Salmonella strains recovered from high and low shedders in animals and humans (from the first experiments)*	10	In part	24	Some experiments have been delayed due to the modification of the partner participants. The recovery of *Salmonella* strains required for this task has thus been delayed.
MoMIR-PPC	M-JRP10-12	Comparison of immune response of high and low shedders in chickens and pigs	11	No	14	Animal samples have been recovered. Work in progress

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MoMIR-PPC	M-JRP10-13	Comparison of microbiota composition of high and low shedders in chickens and pigs.	11	In part	32	Completed for chickens. Three experimental pig infection studies have been performed (in ISS, IZLER and ANSES). All fecal samples from these studies will be shipped to UoS for 16S community analysis. (UoS) faecal samples have been received from the *in vivo* pig infection study at ANSES and DNA extraction is underway. Shipping of samples from the ISS *pig in vivo* study will be carried out in the near future (June 2019).
MoMIR-PPC	M-JRP10-15	Comparison of the predictive markers obtained in pigs with those obtained in chickens.	12	No	32	Pig experiments have been delayed.

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MoMIR-PPC	M-JRP10-16	Comparison of the transcriptomic immune response induced *in vitro between different strains to identify immunological markers*	12	No	32	Some experiments have been delayed due to the modification of the participating partners. The recovery of *Salmonella* strains required for this task has thus been delayed
MoMIR-PPC	M-JRP10-17	Recruitment of the first three sets of human participants including stool sampling and Salmonella culture	12	In part	32	Recruitment and sampling of the human subjects started in 2019. This milestone is not applicable any more as the sampling protocol has been changed to continuous recruitment and sampling of subjects. All samples are expected to be collected by Aug 2020. See also comments to D-JRP10-1.11

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MoMIR-PPC	M-JRP10-20	First inventory of intervention measures completed	12	yes	16	Completed with delay due to MSc Student project timing
MoMIR-PPC	M-JRP10-21	First version of economic analysis tools completed	12	No	24	New planning due to better availability of research capacity in 2019
MoMIR-PPC	M-JRP10-22	Organization of consortium meetings (intermediate and closure)	13	Yes	34	Mid-term meeting held in Feb 2019. The final meeting will be held at UoS, but a date has not been set yet.
MoMIR-PPC	M-JRP10-23	Recruitment of the fourth set of human participants including stool sampling and Salmonella culture	15	In part	36	The milestone is no longer applicable. See comments to D-JRP10-11.11 and M-JRP10-17.
MoMIR-PPC	M-JRP10-24	Recovery of samples from experimentally infected animals and from farms, pretreated with probiotics	16	In part	32	This work has been delayed. Suitable pre- and probiotics have now been identified by the UoS and will be shipped when required.

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MoMIR-PPC	M-JRP10-25	Comparison of immune response of high and low shedders in chickens and pigs	18	No	32	This work has been delayed.
MoMIR-PPC	M-JRP10-26	MALDI TOF Imaging processing and workflow on the paraffin wax slides of intestine wall in high and low shedder from chickens and pigs	18	No	-	This milestone has been cancelled
MoMIR-PPC	M-JRP10-27	Comparison of microbiota composition between high and low shedders in chickens and pigs and in humans between prolonged and short *Salmonella* excretion.	18	In part	Humans: 36	Human microbiota composition will be analysed after all samples have been collected (month 32).
MoMIR-PPC	M-JRP10-28	In vitro infection of several cell lines and organoids with the different Salmonella strains recovered from high and low shedders in animals and humans (second round)	18	In part	24	This work is in progress
MoMIR-PPC	M-JRP10-29	Comparison of the immune signature of high and low shedders in chicken and pig	18	In part	32	This work is in progress
MoMIR-PPC	M-JRP10-30	Comparison of the predictive markers obtained in pigs with those obtained in chickens.	19	In part	32	This work is in progress

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MoMIR-PPC	M-JRP10-31	Comparison of the transcriptomic immune response induced in vitro between different strains to identify immunological markers	20	No	32	This work is in progress
MoMIR-PPC	M-JRP10-32	Final inventory of intervention measures completed	20	No	34	

4.1.2.10.3 Description of the project activities per task

4.1.2.10.3.1.1 JRP10-WP0-T1: Draft and agree Consortium Agreement (M1-M6)

This task has been completed, see annual report 2018

4.1.2.10.3.1.2 JRP10-WP0-T2: Produce project-planning, control documentation and Data Management Plan (M1-M24)

This task has been completed with the help of the EJP Board.

4.1.2.10.3.1.3 JRP10-WP0-T3: Control and manage activity progresses, the timely delivery of project tasks and outputs (M1-M24)

This task is ongoing. An exceptional extension of 12 months of the project at no extra expense has been obtained by the EJP board. This extension was motivated by:

- The withdrawal of SAIM from the EJP consortium after the approval of our project.

- Astrid-Louise Webster has left the FHI, just before the beginning of the project, so that we have adapted the project with Anke-Stüken. These modifications have delayed the ethics committee approvals, resulting of one year delay in the human experiments.

- The fact that after the beginning of the project, Vet-DTU has been closed by its Government. To compensate for this loss, we looked for new partners in the EJP consortium and in June (6 months after the beginning of the project), H. Daskalov (NDRVMI) has joined our consortium. Consequently, part of the work related to WP2 has been delayed by one year.

- Finally, several partners had had difficulties to obtain the ethics committee approval for animal experiments, which has also delayed some experiments.

4.1.2.10.3.2 WP1. Risk prediction for Super-shedder animals and human asymptomatic carriers through the use of gut microbiota and immune status analyses.

4.1.2.10.3.2.1 JRP10-WP1-T1: Predictive immunological markers associated to the high and low shedders in chickens and pigs (M1-M20)

Partner 27 carried out experimental studies on 40 and 20 CD1 mice, respectively. During two different experiments, in which mice were inoculated at day 1, by intraperitoneal route, with 2×10^2 CFU of a 14028 *Salmonella* Typhimurium wild type strain, which allowed us to produce a sub-lethal infection in susceptible mouse model with a good response to the infection. At day 0 and 7 the blood was collected from orbital sinus. At day 7 after the infection, the animals were euthanized and spleens were collected. The number of viable *S*. Typhimurium 14028 strain was determined by plating serial dilutions on LB agar plates. We obtained a wide distribution of the colonies, from a low to a high, confirming a different ability of *Salmonella* Typhimurium to colonize the spleens. These allowed us to select two groups of mice, with low and high *S*. Typhimurium 14028 spleen colonization, for further studies on the involvement of the immune system specific for the two levels of infection spread. Results are still ongoing, to analyze the immune system:

First results showed that IFN-gamma was significantly higher in the high shedders compared to the low-shedders in both spleen and serum. Other cytokines analyses are still on-going. The spleens were also used for the transcriptome studies to obtain a specific transcriptome databases for the identification of genes that are differentially expressed in the two distinct shedding populations. The

NGS analysis identified a clusterization pattern between the low and high super-shedders, comparable with the microbiological shedding state. We are then analysing deeply several genes and patterns to understand similarities and differences between these two groups.

Only recently, **Partner 29** from IZSLER, obtained the authorization in accordance with directive of the Service for Biotechnology and Animal Welfare of the Istituto Superiore di Sanità and authorized by the Italian Ministry of Health. To determine what an infected/uninfected baseline microbiota look like and how colonisation of the gut by *Salmonella* might change microbiota, a total of 30 pigs, post weaning at the start of the experiment and sourced from the same location, were split into 2 groups (control and infection). Pigs were housed with other group mates (but, remote from the other group to maintain non-*Salmonella* status of control group). Food, housing, care and animals age matched between the groups to ensure as little variation as possible in gut microbiota. Faeces were collected from the animals before inoculation with *S.* Typhimurium and following inoculation faeces were collected three times weekly for the duration of the experiment. The animals were followed-up for 14 days (in order to observe disruption and recovery of the microbiota). Gut microbiota analyses will be performed in collaboration with the UNIVERSITY OF SURREY, School of Veterinary Medicine**, (Partner 23)**.

Samples were also collected for *S.* Typhimurium culture on LB agar plates by plating serial dilutions, to allow quantification of the *S.* Typhimurium shedding status of each animal. We obtained a wide distribution of the colonies, from low to high, passing through an intermediate population, confirming a different ability of *S.* Typhimurium to colonize the animals. These allowed us to select two groups of pigs, with low and high *S.* Typhimurium colonization. Animals were bled before infection. Blood was stimulated in vitro with inactivated *S.* Typhimurium in order to assess the capability of blood leukocytes to produce cytokines after stimulation. In addition, 5 days after infection and before killing animals were bled and serum was collected and stored to quantify cytokines. Analyses are in progress. At the moment, we tested TNF-alfa on blood and serum collected. We found no correlation between TNF-alfa in serum of infected animals and spleen colonization (fig.1), but a negative correlation between the capability of animals to constitutively produce TNF-alfa and spleen colonization. The statistical analysis of this negative correlation, however, only approached the statistical significance (r= - 0.39; p=0.07) (fig.2).
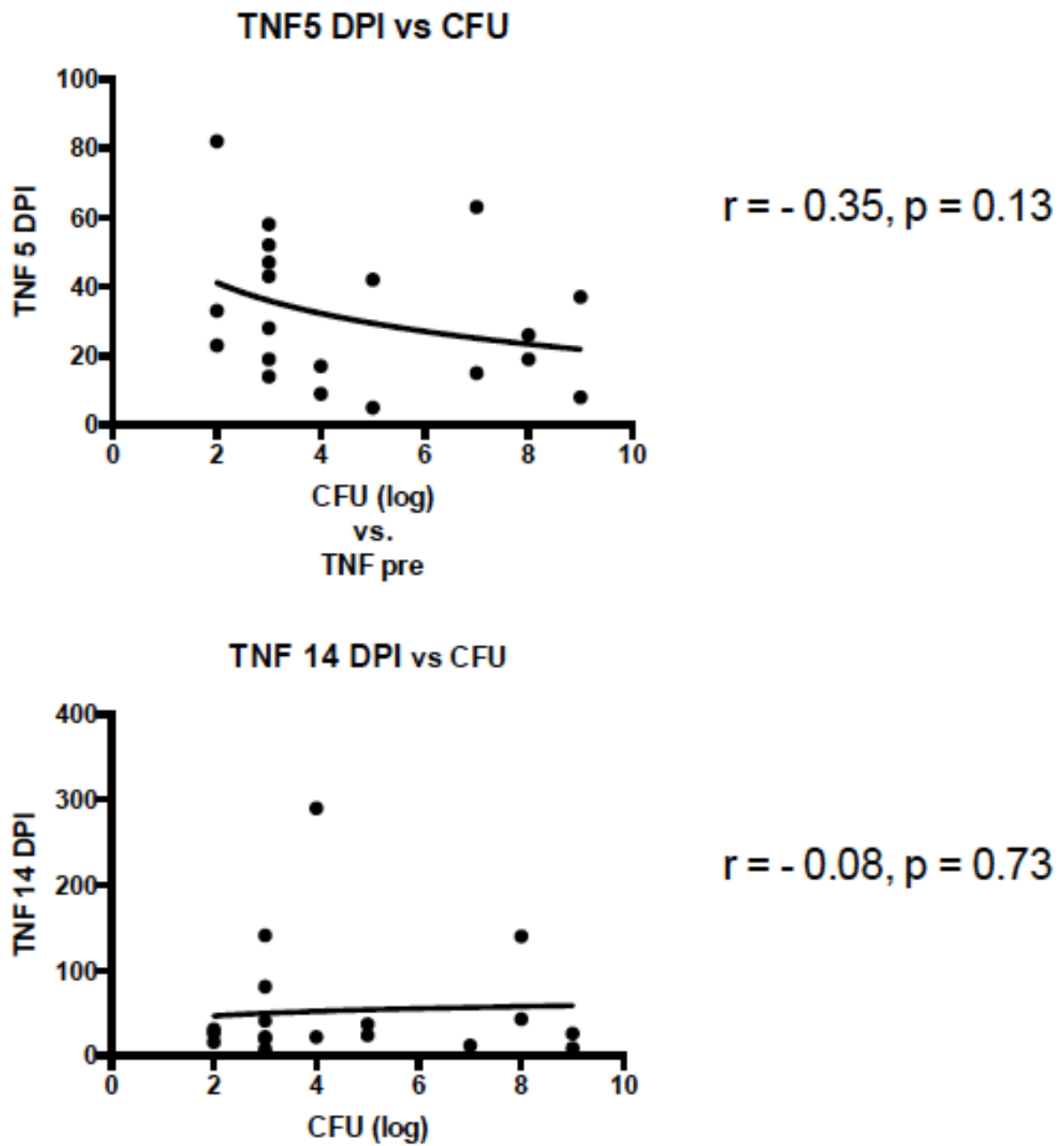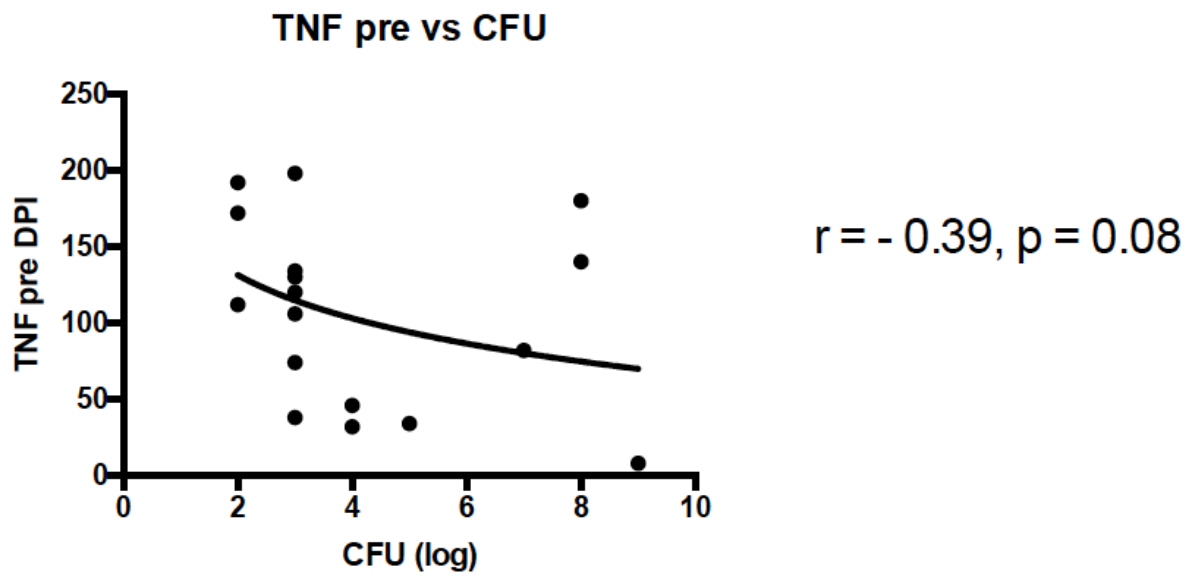
Figure.1

TNF5 DPI vs CFU

r = - 0.35, p = 0.13

TNF 14 DPI vs CFU

r = - 0.08, p = 0.73

Figure 2.

TNF pre vs CFU

r = - 0.39, p = 0.08

Partner 1, from ANSES, has performed a trial with a total of 45 piglets from 9 sows, which were divided into 5 random groups: one group with five piglets as control and 4 groups each with 10 piglets (n=40) as inoculated pigs. The 40 inoculated piglets at 7 weeks of age received orally 10 ml of suspension of 10^8 UFC/ml of a monophasic variant of *S.* Typhimurium strain. Following challenge the pigs were monitored for 3 weeks then necropsied. Twice a week, faeces were sampled in order to quantify *Salmonella* and follow the excretion of *Salmonella* from the pigs during the trial, which has been determined by calculating the LAUC (Log area under the curve). The LAUC allow them to classify the 40 pigs in three classes of shedding, which gathered 13, 16 and 11, high, intermediate and low shedders pigs respectively (Fig 1).
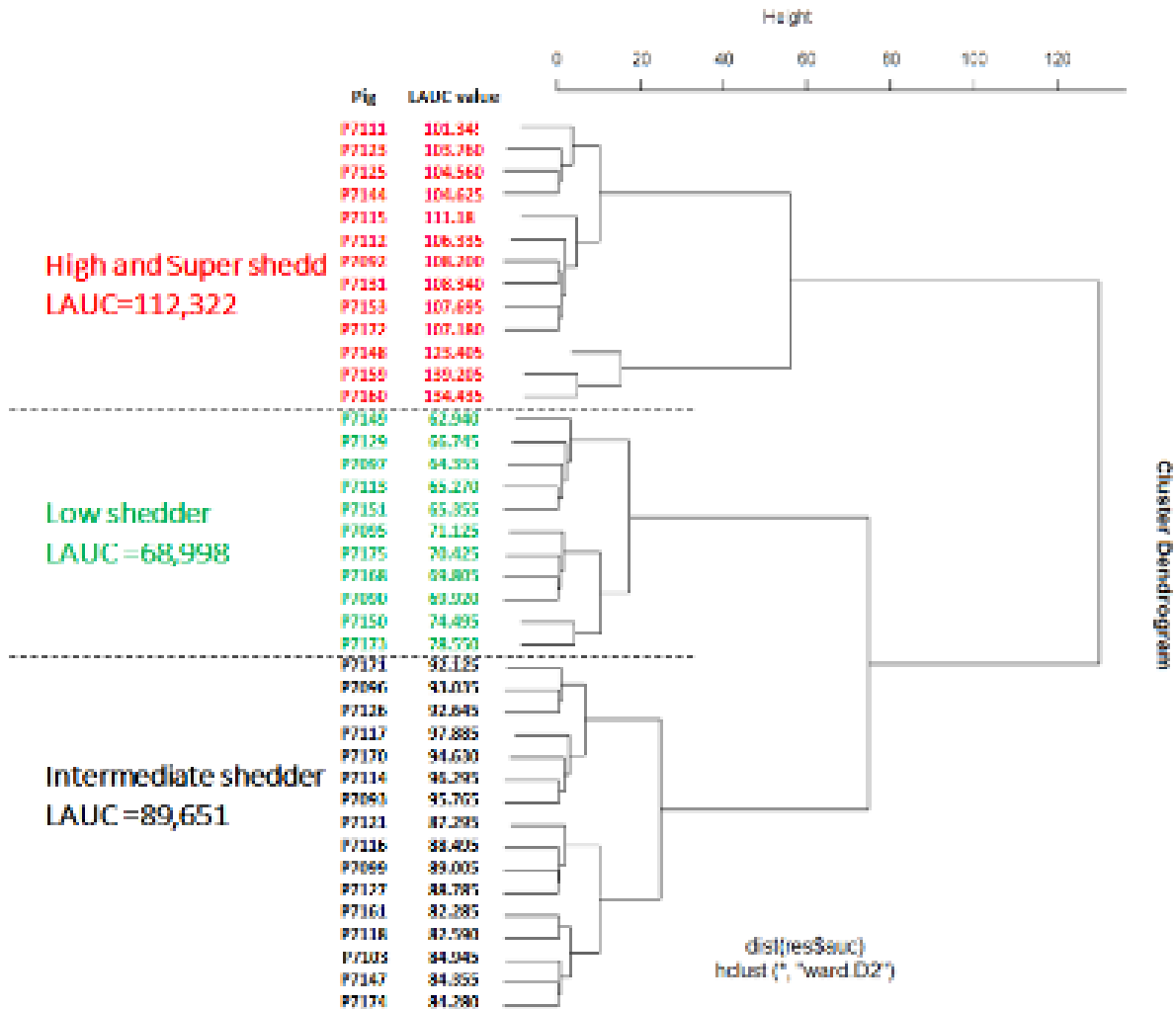

Fig 1 : Hierarchical classification of pigs according the LAUC calculated from numeration values.

After necropsies, tonsils, caecum and ileum contents were highly contaminated (in mean, 5.6, 3.7 and 3.5 Log_{10}CFU/g, respectively) unlike MLN (in mean, 0.85 Log_{10}CFU). We observed that for the group of high shedders, levels of contamination was significantly higher for MLN, ileum and caecum contents than for the group of low shedders (p<0.01). During the experimental trial, blood samples were taken twice a week and total blood cells (TBC) were enumerated. ELISA tests have been performed on serum (*Salmonella*, Cytokines). Statistical analyses are in progress, as well as for TBC results. Other blood samples will be sent very soon to **partner 18** for transcriptomic analysis. **Partner 23** (University of Surrey – Participant 22) has now received faecal samples from **partner 1** and DNA extraction for 16S community analysis is currently underway.

Two experiments were performed in chickens by **Partner 18** (INRA). Blood samples were recovered before and after infection. Hierarchical clustering performed on the level of *Salmonella* in feacal and caecal samples has identified super and low shedder phenotypes. All RNA from super and low shedders have been extracted. The analysis of gene expression is in progress with the Biomark.

4.1.2.10.3.2.2 JRP10-WP1-T2: Predictive microbiota markers associated to the high and low shedders in chickens and pigs (M1-M20)

A collaboration between the ISS-IZSLER (**partners 27-29**) and the UNIVERSITY OF SURREY, School of Veterinary Medicine, (**Partner 23**) partners is taking place to analyze the predictive microbiota markers associated to the high and low shedders pigs (see JRP10-WP1-T1). Currently **Partner 23** is working with **partner 29** in order to arrange the shipping of faecal samples from their *in vivo* pig study. These samples will be subjected to DNA extraction and 16S community analysis.

As described in the first report, **partner 18** (INRA) performed a *Salmonella* infection experiment of chickens reared in isolator. The analysis of gut microbiota from feacal samples showed that nine bacterial taxa presented significant differential abundances among the 3 shedding categories, for at least one sampling time. Interestingly, this included two genera presenting high relative abundance (> 5%) before infection in the low-shedder chicks: *Enterococcus* spp. (at 7 days age, *i.e.* just before infection) and *Streptococcus* spp. (at 7 and 11 days (before and after infection)). This result suggested that high level of *Enterococcus* and/or *Streptococcus* before infection correlated to the low shedder phenotype. The correlation that high *Enterococcus* level before infection are observed in the low *Salmonella* group before infection has been confirmed in an independent experiment. These results suggested that presence of *Enterococcus* (probably faecium) could be a predictive markers of low shedder phenotype and therefore flocks including "low risk *Salmonella* infection" chickens. It should be noted that these results were obtained mainly using faecal samples. This kind of biological material could enable the use of a non-lethal, cost-effective detection method to predict the outcome of *Salmonella* infection. Such fast, robust and early diagnostic methods could be applied to control strategies of chicken salmonellosis by aiming to remove future super-shedder individuals before infection, or to intervene at the level of gut microbiota in order to prevent super-shedding. An article has been submitted for publication. In WP2, we tested the protective activity against *Salmonella* colonization of one *Enterococcus faecium* strain isolated from chicken.

4.1.2.10.3.2.3 JRP10-WP1-T3: Risk factors associated with prolonged convalescent Salmonella shedding in humans (M1-M24)

Partner 32 Participant recruitment started 01.01.2019. 297 people have been invited to participate in the study, of these 137 gave their consent to participate and 112 faecal samples have been received at NIPH (status June 6th, 2019). *Salmonella* culture of these samples is in progress. WGS data from all human *Salmonella* isolates has been sent to the Norwegian reference laboratory and is ongoing, and so is the WGS of *Salmonella* strains isolated from faecal samples.

4.1.2.10.3.2.4 JRP10-WP1-T4: Virulence of Salmonella strains originated from high and low shedders (M7-M20)

Salmonella strains isolated from high and low shedders pigs during animal experiment performed in Anses Ploufragan **(partner 1)** have been sent to INRA **(partner 18)** in March. Partner 18 is analyzing the adhesion and invasion capabilities of *Salmonella* strains recovered from pigs and chickens on pig and chicken cell lines.

Partner 23 (University of Surrey) will receive the samples currently held by INRA (**partner 18)** from Partner 1. These isolates will be screened for their ability to attach to abiotic surfaces and for biofilms. Comparisons will be made between the abilities of high and low shedders. This work will commence on the 17/06/19.

4.1.2.10.3.3 WP2. Prevention of the appearance of Super-shedder animals and asymptomatic carriage in humans and animals by modifying feed and/or microbiota

4.1.2.10.3.3.1 JRP10-WP2-T1: Use of probiotics in chickens and pigs (M7-M24)

The main objective of this task will be to improve immune response and barrier effect procured by gut microbiota by introducing already identified probiotics.

In WP1 **Partner 18** (INRA) has identified *Eneterococcus faecium* as a predictive biomarker for the most resistant phenotype. To test the protective activity of this species, isolated from chicken, against *Salmonella* colonization, a candidate *E. faecium* strain was isolated, purified and orally inoculated to a group of 30 chicks reared in a large isolator (CFU=1×10^9 per chick) at 1 and 6 days of age (i.e. before *Salmonella* infection). A control group was inoculated using the bacterial medium alone. At 7 days of age, all the chicks received an oral inoculation of *S.* Enteritidis. Levels of *Salmonella* were determined in faecal samples collected on day 4, 6, 11, 14 and 20 and in the caecal samples collected on day 21. Under these conditions, they did not observe any significant differences between the *Salmonella* shedding levels of the *E. faecium*-inoculated and control groups (at any time point; $0.11<p<0.61$). To determine whether the *E. faecium* strain had colonized the gut of chicks and whether this inoculation had modified the gut microbiota composition, a subset of 13 individuals was analysed, including *E. faecium* inoculated chicks (N=8) and control chicks (N=5). The comparison of the gut microbiota compositions of the *E. faecium*-inoculated and control groups revealed an overall impact of the inoculated *E. faecium* candidate strain. Interestingly, in these experiments, OTU corresponding to *Salmonella* were detected among the gut microbiota. A significant relationship was found between *Salmonella enterica* abundance and the binary variable differentiating the *E. faecium*-inoculated and control chicks. The negative correlation showed that *Salmonella enterica* abundances were lower in the samples recovered from the *E. faecium*-inoculated chicks (p=0.013). The contradiction between the two estimations of *Salmonella* abundance may be explained by the fact that these two measures are in part intrinsically independent. Bacteriological analyses aim to determine the number of 'colony forming units' which reflect the number of total viable and culturable *Salmonella*. However, that quantity does not take into account the viable but non-culturable bacteria, and may lead to an under-estimation of the actual number of bacteria. Works are in progress to better understand this discrepancy.

During this period, **Partner 8** (VRI) have performed some assays regarding the use of probiotics. *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Bacteroides fragilis* and *Bacteroides xylanislovens* are common colonizers of intestinal tract of piglets during lactation. However, these species nearly disappear from pig gut microbiota within a week post weaning and sows are only lowly positive for *Bacteroides* spp. In addition, they noticed that when Bacteroides are absent from piglet microbiota during first week of life, *Clostridium perfringens* is abundant. However, when *Bacteroides* spp. reach around 10 % abundance in piglet gut microbiota, *C. perfringens* decreases below 1 % of total microbiota. They used this observation and in a herd with continuous fatalities due to *C. perfringens* they orally administered 10 and 16 piglets of two independent litters with a mixture of above mentioned *Bacteroides* species. Half of the piglets in each litter were given intramuscularly amoxicillin for 3 days. They found out that intramuscular amoxicillin administration did not interfere with gut colonisation. However, there were no fatalities also among the piglets which were administered only the probiotic mixture, i.e. without antibiotics which are used by the farmer to keep the epidemiological

situation in his herd under control. This experiment is now (in June 2019, first litter was given probiotic *Bacteroides* spp. on June 12 and two more litters are expected on June 13 and 14) repeated and data from the current experiment will be presented in the next report.

4.1.2.10.3.3.2 JRP10-WP2-T2: Use of pre-biotics and nutraceutical already defined by the consortium partners in chicken and pig (M1-M24)

The goal of this Task is to measure the effect of several pre-biotics and nutraceuticals on gut microbiota composition and protection against pathogens in farms and in experimental conditions.

Partner 8 (VRI) has used short pieces of *Sutterella*, *Bacteroides*, *Phacolarctobacterium*, *Dialister* or *Prevotella* genome obtained by metagenomic sequencing of bacterial mass washed from anaerobically cultured nutrient agars specific primers design for *Bacteroides barnesiae*, *Bacteroides massiliensis*, *Bacteroides mediterraneensis*, *Paraprevotella clara*, *Phascolarctobacterium*, *Succinatimonas hippei*, *Prevotella lascolaii*, *Sutterella wadsworthensis*, *Lactobacillus mucosae* and *Dialister succinatiphilus*. Caecal contents from a donor hand were serially diluted and individual growing colonies were tested by a set of designed PCRs. This selection protocol resulted in successful isolation of *Bacteroides barnesiae*, *Bacteroides mediterraneensis*, *Paraprevotella clara*, *Sutterella wadsworthensis*, *Phascolarctobacterium* and *Lactobacillus mucosae*. These isolates are currently tested in chickens for their probiotic potential. The experience obtained from the culture of chicken gut anaerobes was also applied to select some *Bacteroides* spp. originating from pigs. They have obtained and sequenced *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Bacteroides fragilis* and *Bacteroides xylanislovens*. These isolates were tested by PCR for the absence of the most frequent horizontally transferrable antibiotic resistance genes *tetQ* and *linA*. Following these controls, the strains were used for inoculation of piglets in a herd subjected to natural *Clostridium perfringens* infection in task 2.1.

Partner 16 (VISAVET-UCM) have just started the animal experiments that were delayed due to BSL3 boxers availability and Community of Madrid authorization. This study has started on the 27th of May using broiler chicks of 1 day of life. The animals were divided in two groups: one that was fed with conventional feed and a second one that was fed with "alperujo" since their arrival. All the chicks were confirmed as negative to *Salmonella* spp. during the first week after the analysis of the transportation cages. After a week of adaptation, animals were challenged with a *Salmonella* strain resistant to colistin. Besides, a subgroup of animals will be challenge after 21 days consuming the "alperujo". The objective of these approaches will be to analyze the modification of the microbiota **and intestinal mucosa changes** before and after the challenging, comparing treated and control groups. The results obtained from the current experiment will be presented in the next report.

Partner 23 (University of Surrey) has characterised novel *Lactobacillus* probiotics for use in pigs. *In vitro* testing of the isolates is nearing completion and expected to be complete in July 2019. To date 85 potentially probiotic isolates have been isolated from pig faeces and 120 from chicken faeces. All isolates have been confirmed as belonging to the *Lactobacillus* genus by PCR and determined to be genetically unique by RAPD. All isolates have also been tested for survival within low pH conditions. In addition, pig isolates have been tested to ensure that they display inhibitory activity towards the growth of both *S.* Typhimurium and *E. coli* type strains, survival in 0.3% and 1% bile and have been speciated by 16s sequencing. Following this screening 20 pig isolates were whole genome sequenced and analysis of this data is currently ongoing. Following completion of this characterisation two probiotic isolates, each from pig and chicken will be selected and used in *in vivo* on-farm intervention studies. This work will be carried out in collaboration with NDRVMI (**partner 6**). Samples collected during the study will be shipped to Partner 23 for genomic analysis (16S metagenomic analysis in the first instance). Ethical approvals, MTA's and import licences are in place for these studies.

4.1.2.10.3.3.3 JRP10-WP2-T3: Use of pre-biotics in human travelers to high-risk areas for contracting salmonellosis and AMR (M1-M24)

This task has been cancelled in the last version of the project due to A.L. Wester (FHI) withdrawing.

4.1.2.10.3.4 WP3. Modelling the transmission of zoonotic agents to improveintervention strategies on livestock farms

4.1.2.10.3.4.1 JRP10-WP3-T1: Transmission modelling at within-host and between-host scales (M1-M24)

4.1.2.10.3.4.1.1JRP10-WP3-T1-ST1: Within-host scale: modelling individual responses and shedding (M1-M24)

Partner 18-Jouy (INRA) analyzed a first set of time series of gut microbiota composition in chicken (with **partner 18-Tours** and **partner 8**) in order to infer interactions between the pathogen (here *Salmonella*) and the resident microbiota. However, the results are not satisfactory, we need to adapt our method to handle time series of species frequency instead of absolute abundances. We presented our method for data analysis to the other project partners in the mid-term project meeting in February in Madrid.

A paper on our 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 submitted in March. Our current work is to improve the model with a better representation of the immune response.

4.1.2.10.3.4.1.2JRP10-WP3-T1-ST2: Between-host scale: modelling transmission, linked to within-host results (M15-M21)

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 designed, studying the indirect transmission of both *Campylobacter* and *Salmonella* between broilers, and is to be carried out in October-December 2019. Presently applications to the ethical committees are being prepared.

4.1.2.10.3.4.2 JRP10-WP3-T2: Interventions strategies: Identification and evaluation tools (M1-M24)

4.1.2.10.3.4.2.1JRP10-WP3-T2-ST1: Systematic inventory of relevant intervention measures (M17-M19)

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 will be worked out to a systematic inventory in M20-M24.

4.1.2.10.3.4.2.2JRP10-WP3-T2-ST2: Inclusion of potential interventions into the modelling (M10-M24)

This task will be carried out later (M25-M36).

4.1.2.10.3.4.2.3JRP10-WP3-T2-ST3: Development of economic analysis tools (M1-M24)

The first twelve months have been used to make a plan about the scope and approach of the tools: to evaluate the cost effectiveness (utility) of intervention strategies using probiotics to reduce *Campylobacter* prevalence in broilers. The tools determine the cost per averted disease burden (in €/Disability-Adjusted Life Year, DALY), by taking into account the attribution of DALY's to broiler sector, and correcting for import/export. The cost-utility is obtained by calculating the cost per averted disease burden as a function of the on-farm efficacy. The model will be parameterized for four or five different European countries spanning a range of differently structured poultry sectors: taking into account differences in technical and economic farm performance, and scale of poultry production. Since then the work on the tools is under way and a draft scientific paper is being written. The task is still planned to be completed in M24.

4.1.2.10.3.5 WP4: Communication and Dissemination for Impact

4.1.2.10.3.5.1 JRP10-WP4-T1: Dissemination of data within the project and management of data (M1-M24)

All partners shared their results in the mid-term project meeting in February in Madrid.

Exchanges of methods and data have been performed during face-to-face meetings but also via skype meetings and email contacts.

4.1.2.10.3.5.2 JRP10-WP4-T2: Dissemination of data outside the project and management of data (M1-M24)

Some results have been already presented in several congresses (oral communications and poster sessions).

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

The final meeting will be held at University of Surrey, but a date has not been set yet.

4.1.2.11 *MedVetKlebs*

4.1.2.11.1 Summary

The first goal of MedVetKlebs project was the development, optimization and harmonization of detection and isolation methods for *Klebsiella pneumoniae* (Kp) from different sources, using culture

and molecular detection approaches. In order to achieve this purpose, we have first updated the taxonomy of the Kp complex, including now 7 phylogroups corresponding to 5 different species (Rodrigues et al. 2019 Res Microbiol. PMID: 30817987). Although not initially planned, we decided to evaluate the potential of MALDI-TOF mass spectrometry, a fast and cost-effective technique that is well established in routine laboratories for microbial identification, to identify all taxa of the *Kp* complex (Rodrigues *et al.,* 2018: https://doi.org/10.3389/fmicb.2018.03000).

For detection and isolation of the members of the Kp complex, we have established SCAI (Simmons Citrate with Inositol) as the most suitable medium based on the productivity and specificity results, and showed it allows selective growth across the breadth of Kp taxonomic diversity. Besides, to adapt the Kp culture strategy to food microbiology processes, the Kp isolation protocols were optimized by testing several routinely used enrichment conditions and incubation temperatures for selected types of matrix/sources: food, soil and water (task 1.1).

Regarding the molecular approach (task 1.2), we have developed a real-time PCR (called the ZKIR PCR) for the identification of the entire *Kp* complex (including its seven different phylogroups or species) directly in the samples in order to allow a more efficient broad sampling. Two partners were involved in this task (IP, INRA). The ZKIR qPCR consists in the amplification of a specific intergenic region of 78 bp between *zur* (zinc uptake regulation protein) and *khe* (annotated as coding for a haemolysin) using a SYBR Green chemistry. The sensitivity of this qPCR was tested on spiked soil. The protocol was already distributed to all the partners and all Institutions validated the method (task 1.3). To render the protocol usable by the community, it was made publicly available through protocols.io: https://www.protocols.io/view/detection-of-klebsiella-pneumoniae-and-closely-rel-6gvhbw6. Finally, we are also developing a qPCR method for the identification of Kp phylogroups (or species) directly in the samples (task 1.2). For this a pan-genome strategy was used to define the target genes specific for phylogroups. qPCR primers/probes were designed and validated on a reference panel of strains. The protocol is being finalized at INRA before being distributed to the partners.

Based on sampling of multiple sources (mainly food and environmental samples, task 2.1), we have identified chicken meat and salads as important potential sources of Kp for humans. A multicentric study with 6 partners (INRA, ANSES, AGES, NUIG, SSI, ISZAM) is ongoing, sampling chicken meat (~25 in each centre) and ready-to-eat salads (~25) in each center (task 2.2). In this work, qPCR and culture methods are being compared, further validating the novel qPCR (task 1.2).

Whole genome sequencing (task 3.1) was started for isolates identified by the qPCR and/or MALDI-TOF MS as belonging to the Kp complex from a diversity of environmental sources as well as chicken meat and salads. Modelling (task 3.2) will start in Nov 2019, awaiting the recruitment of a post-doctoral researcher.

Regarding project management (task 4.1), on 10-11th January 2019, we have organized at Institut Pasteur (Paris, France) our 1-year meeting with all the MedVetKlebs partners, who were invited to present the progress of the work carried during the last year. We have also invited researchers who are coordinating other Kp-dedicated networks (Davide Sassera and collaborators from SpARK project, Orjan Samuelsen from NOR-KLEB, Damien Thiry and Francois Gravey from Caen hospital Normandy-Kleb) and Arnaud Callegari (Coordinator's Representative of the H2020 One Health EJP). Our final meeting is scheduled to take place in Paris, April 16-17th, 2020.

Regarding exploitation of results (task 4.3): the main results of these 18 months of the MedVetKlebs project were presented at the OneHealth AMS2019 conference (6 posters and 1 oral presentation) and at the Nor-Kleb-Net workshop (Sommaroy, Norway, 21st Aug 2019). In addition to the publications cited above, three publications related to the project are being prepared.

4.1.2.11.2 Progress of the research project: milestones and deliverables

4.1.2.11.2.1 Deliverables

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
MedVetKlebs	D-JRP11-2.1	List of high-Kp occurrence sources	12		24	Optimization of isolation protocols was done; qPCR detection protocols were defined and implemented among partners. Multiple (several hundreds) samples were screened by the consortium and this is still going on. This task will gain impetus in the coming months due to availability of the qPCR detection method. Some sources where Kp is prevalent were already identified including waste water, chicken meat and some ready to eat salads.
MedVetKlebs	D-JRP11-4.6	Communication strategy plan	12		21	A first draft has been produced and distributed to partners for review.

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
MedVetKlebs	D-JRP11-2.2	Prevalence in selected sources	18		24	Chicken food and salads were selected as important potential Kp transmission sources; 170 samples are nearly finished to be screened using the harmonized protocol within the consortium
MedVetKlebs	D-JRP11-2.4	Quantification of Kp in selected sources	18			This task will probably not be achievable during the project due to its complexity. However the developed qPCR will allow this to be done in future dedicated studies. An approximation might be attained within the MedVetKlebs project using Ct values obtained with the qPCR results from salads and chicken screening and the calibration curve that we have derived from extracted DNA.
MedVetKlebs	D-JRP11-2.6	Genome sequence data	18		28	Genome sequencing has started for soil and some food isolates.
MedVetKlebs	D-JRP11-3.1	Source distribution of clonal groups, plasmids and genes	18		28	Depends on above deliverable

JRP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
MedVetKlebs	D-JRP11-3.4	Computer program for dynamic models simulation	18		28	Hiring of postdoc is in process; scheduled to start this fall
MedVetKlebs	D-JRP11-4.2	Project Periodic Reports	18		24	As scheduled (9, 12, 18 months reports were delivered)
MedVetKlebs	D-JRP11-4.7	Plan for the dissemination and exploitation of results	18		24	A first draft has been produced and distributed to partners for review.

4.1.2.11.2.2 Milestones

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MedVetKlebs	M-JRP11-2	Preparation of draft of the strategic communication plan	10	No	September 2019	A first draft has been produced and distributed to partners for review.
MedVetKlebs	M-JRP11-6	Broad survey of Kp in multiple sources complete	12	No; but has started	December 2019	This task has started recently in a large-scale, once optimization of isolation and detection protocols were defined.

JRP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
MedVetKlebs	M-JRP11-7	Development of model frameworks for dynamic modelling and source attribution	12	No	March/April 2020	Not started yet, although the strategy is defined; we are hiring a postdoc this fall (modelling PI was on maternity leave)
MedVetKlebs	M-JRP11-8	Initial prevalence, quantification and genomic data for model refining	18	No	December 2019	In process, except for quantification.
MedVetKlebs	M-JRP11-9	1st batch of clonal groups, plasmids and genes defined, for refinement of models	18	No	December 2019	Sequencing in process.
MedVetKlebs	M-JRP11-10	Compilation and integration of the data produced in WP1 and WP2 to be used in the dynamic and source attribution models	18	No	March/April 2020	To be discussed with to-be-hired modelling postdoc.
MedVetKlebs	M-JRP11-11	Identification of a list of scenarios for control measures to be assessed through model simulations	18	No	March/April 2020	To be discussed with to-be-hired modelling postdoc

4.1.2.11.3 Description of the project activities per task

4.1.2.11.3.1 WP1. Methods for Kp detection and isolation

4.1.2.11.3.1.1 JRP11-WP1-T1: Evaluation and optimization of culture-based approaches (M1-M12)

This task has been completed, see annual report 2018.

4.1.2.11.3.1.2 JRP11-WP1-T2: Detection and quantification (M1-M12)

This task is finalized for the methodological development component: we have developed a SYBR-green based qPCR (ZKIR qPCR) for Kp detection, which was validated in six labs/institutes; and we are now using it to screen chicken and salads. We are also developing a multiplex qPCR for the identification of Kp phylogroups.

4.1.2.11.3.1.3 JRP11-WP1-T3: Harmonization and alignment (M1-M24)

Culture methods have been optimized collectively and harmonized across partners, to detect Kp from food, water, soil and fecal material (humans, animals). qPCR method was disseminated, implemented and validated in six partners' labs. Taxonomic updates and MALDI-TOF identification method of Kp was disseminated.

4.1.2.11.3.2 WP2. Sampling

4.1.2.11.3.2.1 JRP11-WP2-T1: Broad sampling of potential reservoirs and sources of Kp (M1-M12)

This task was voluntarily delayed once we realized our developed qPCR would be much more efficient to screen large numbers of samples. Sampling/screening was started in some partner's labs, but the coordinated effort to sample with harmonized protocols must now begin based on qPCR.

4.1.2.11.3.3 WP3. Genomics and Modeling

4.1.2.11.3.3.1 JRP11-WP3-T1: Analyses of genomic sequences (M13-M24)

Genomic sequencing was started in Austria, Ireland and Denmark for food samples (n=61 isolates; 54 from chicken meat and 7 from ready-to-eat salads) and in Paris for soil isolates (214 isolates) collected at INRA Dijon (France). More sequencing will follow.

4.1.2.11.3.3.2 JRP11-WP3-T2: Modeling and source attribution (M1-M24)

This work package was delayed because the PI (L Opatowski) was on maternity leave for five months (see 6-months extension request). As she was back a few weeks ago, we met and defined the strategy for modelling and have already identified a post-doc candidate to work on this topic; she is due to start October or November 2019 (pending administrative validation).

4.1.2.11.3.4 WP4: Management, dissemination, exploitation

4.1.2.11.3.4.1 JRP11-WP4-T1: Implementation of the project management structure (M1-M24)

Nothing specific to report here except that we have asked for the 6-months extension.

4.1.2.11.3.4.2 JRP11-WP4-T2: Administrative, legal, financial and ethical support to the consortium (M1-M24)

Nothing specific to report here. Financial reports have been submitted as scheduled.

4.1.2.11.3.4.3 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 [1–3]:

1 Wisgrill L, Lepuschitz S, Blaschitz M, Rittenschober-Böhm J, Diab-El Schahawi M, Schubert S, *et al.* Outbreak of Yersiniabactin-producing Klebsiella pneumoniae in a Neonatal Intensive Care Unit. *Pediatr Infect Dis J* 2019;**38**:638–42. https://doi.org/10.1097/INF.0000000000002258.

2 Rodrigues C, Passet V, Rakotondrasoa A, Diallo TA, Criscuolo A, Brisse S. Description of Klebsiella africanensis sp. nov., Klebsiella variicola subsp. tropicalensis subsp. nov. and Klebsiella variicola subsp. variicola subsp. nov. *Res Microbiol* 2019. https://doi.org/10.1016/j.resmic.2019.02.003.

3 Rodrigues C, Passet V, Rakotondrasoa A, Brisse S. Identification of Klebsiella pneumoniae, Klebsiella quasipneumoniae, Klebsiella variicola and Related Phylogroups by MALDI-TOF Mass Spectrometry. *Front Microbiol* 2018;**9**:3000. https://doi.org/10.3389/fmicb.2018.03000.

Three more publications are being prepared on (1) the definition of two new species of the

Klebsiella oxytoca complex (submitted), (2) on the ZKIR qPCR method and (3) on the prevalence, antimicrobial susceptibility phenotypes and genomic characterization of Kp in chicken meat and salads. The latter paper will include culture media strategies comparison.

Several posters and one oral presentation from the MedVetKlebs consortium were presented at Dublin One Health EJP ASM:

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

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

3. 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).

4. 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).

5. 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).

6. 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).

7. Violeta Di Marzio, Gabriella Centorotola, Cristina Marfoglia, Alessandra Cornacchia, Maria Antonietta Saletti, Carla Rodrigues, Sylvain Brisse, Francesco Pomilio. A Comparative Study of Productivity, Selectivity and Specificity of Three Selective Culture Media for *Klebsiella* spp. Detection (poster).

Sylvain BRISSE has presented the MedVetKlebs project optimized culture methods and qPCR protocol at

the Nor-Kleb-Net meeting in Sommaroy, Norway, August 21st, 2019.

The ZKIR qPCR protocol has been made publicly available on protocols.io for all scientists to be able to

use it. This has been twitted by S Brisse. Please see: https://www.protocols.io/view/detection-of

klebsiella-pneumoniae-and-closely-rel-6gvhbw6

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

We do TC meetings as felt necessary, with no particular planning but with regularity (every three months

approx.)

We are planning our final face to face meeting in Paris Pasteur on April 16th, 2020.

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

In October 2018 the second call for internal projects (JRP and JIP) was launched. As planned, the Letters of intent were received before Christmas 2018 and were put in the private space on the OneHealth EJP website, accessible to the major stakeholders ECDC and EFSA, to PMT members and to REA. We received feedback on most of the documents and informed each of the candidate Project Leaders (cPL) individually of these remarks in a mail on 18 January 2019. All cPL were invited to submit their full proposal in which the various comments had to be addressed. Therefore, cPL did not have to submit a revised Letter of Intent.

The following proposals were submitted:

- Foodborne zoonoses (one application for each topic was received):
  - Source attribution of bacterial foodborne zoonoses and antimicrobial resistance considering also the environment and non-livestock reservoirs (e.g. pets and wildlife) as sources: DiSCoVeR, Discovering the sources of *Salmonella*, *Campylobacter*, VTEC and antimicrobial Resistance
  - Benchmarking biosecurity practices for pig farming across Europe using national surveillance data and management standards for identifying best practice to prevent biological hazards, particularly *Salmonella* and hepatitis E virus, from entering the food supply chain: BIOPIGEE, Biosecurity practices for pig farming across Europe
  - Source attribution and transmission routes of foodborne pathogens other than bacteria, with emphasis on *Toxoplasma gondii*: TOXOSOURCES, *Toxoplasma gondii* sources quantified
  - Determinants of the reversal of the decreasing trend in *Salmonella* incidence in humans and poultry in the EU: ADONIS, Assessing Determinants Of the Non-decreasing Incidence of *Salmonella*
  - Better tools for detection and investigation of foodborne outbreaks, including antimicrobial resistant pathogens: BeONE, Building integrative tools for OneHealth Surveillance
- Antimicrobial Resistance (two topics for which two full-proposals each were received)
  - New tools for early (real-time) detection & new diagnostic tools, on-site tests
    - FARMED, Fast Antimicrobial Resistance and Mobile-Element Detection using metagenomics for animal and human on-site tests
    - WORLDCOM, Development of new tools for real-time detection of zoonotic bacteria and antimicrobial resistance in veterinary, human and environmental sources
  - Dynamics of AMR selection, clonal spread and horizontal gene transfer
    - FED-AMR, The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain
    - FULL_FORCE, Full-length sequencing for an enhanced EFFORT to map and understand drivers and reservoirs of antimicrobial resistance
- Emerging Threats (two topics for which two full-proposals each were received)
  - Toolkit to characterize ET by combining genomic and phenotypic information

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

- TELE-Vir, Point-of-incidence toolbox for emerging virus threats

o NGS and non-NGS methods for the detection of foodborne parasites

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

- PARADISE, PARAsite Detection, ISolation and Evaluation

Initially, based on the Letters of Intent, four more proposals were expected but some candidate Project Leaders encountered difficulties and withdrew their proposal: three on AMR (DetectNOHERE, PORE and STRETCH) and one ET-related proposal (InVitroRiZk). Some of the issues mentioned were that the comments on the Letter of Intent were perceived as discouraging, the consortium found that their proposal was not mature enough or the consortium encountered problems with the management and considered them too difficult to overcome.

The external experts that registered last year through the online tool, and thus agreed to assess proposals, were contacted in February 2019 to confirm their willingness to participate in the evaluation process. On 17 April, we sent the full proposals to the experts, who were assigned into three JRP panels, i.e. on FBZ, AMR and ET. All members of each of the panels received the same proposal documents, in order to guarantee an optimal quality of the assessment. After this individual evaluation, a consensus teleconference was organised per panel (on 4, 7 and 17 June, for FBZ, ET and AMR, respectively) in order to agree on a common score and to draft recommendations, useful for PMT, SSB and the candidate Project Leaders as well. The reports of these teleconferences were drafted by the rapporteur of each of the panels and were submitted soon after the meetings.

In their assessment, experts validated only four out of the five <u>Foodborne Zoonoses proposals</u> (that all belonged to different topics), judging that one proposal didn't meet the scientific standards required. The experts considered that in project BIOPIGEE two pathogens are studied with totally different epidemiology, i.e. *Salmonella* and Hepatitis E, and that therefore the biosecurity measures for both should be considered separately. Also, the possible recurrent re-introduction of the pathogens via feed (*Salmonella*) or breeding stock was not sufficiently taken into consideration. In addition, some specific remarks dealing with biofilm and climate change were formulated.

As for <u>Antimicrobial Resistance</u>, although WORLDCOM has obtained a lower score than the competing project FARMED (18.00 vs 19.25) the former is preferred by the reviewers as the on-site test proposed seems easier for implementation and use than the MinION technique and therefore will obtain a significant larger impact. For the second AMR topic, for which both scores are equal, i.e. 19.25, FULL-Force has the advantage that it is more in line with the objectives of the call text, it links with other EU projects SOLIDNESS and EFFORT, and it aims to harmonise protocols over Europe.

Regarding the <u>Emerging Threats projects</u> IDEMBRU and TELE-Vir, the latter scored somewhat higher (15.7 vs 15.3) but most of all it is more challenging than IDEMBRU, which has a more limited scope. Both proposals in the second Emerging Threats topic were considered excellent (20.0 for MeME and 19.3 for PARADISE). Since MeME has a better network and the pathogens are more challenging than Giardia and Cryptosporidiosis, this one is preferred by the experts.

The outcomes of the expert evaluations was discussed with the PMT in a teleconference on 25 June 2019. Despite the low scores of one JRP (i.e. BIOPIGEE) and one JIP project (MATRIX, see further), the PMT considered that both proposals are valuable because they meet the expectations and needs of ECDC and EFSA (cfr high ranking in the list of topics), they are large consortia (15 partners in BIOPIGEE

and 19 in MATRIX) and comply with the objectives of the OneHealth EJP as outlined in the DoA. Also, the budget is available and it is cumbersome to reallocate this to similar research and integrative activities in a timely matter. However, the recommendations of the reviewers are considered essential to improve the initial proposals. For these reasons, the PMT adjusted the comments and recommendations for the candidate Project Leader in order to clarify the weaknesses of the BIOPIGEE proposal and to encourage the submission of an amended version. In their TC meeting on 28 August 2019, PMT came to the conclusion that the amended version of the proposal has significantly improved and that it can be hand over to the SSB. Thus, the high-ranked topic concerning biosecurity on pig farms can still be valorised.

The Scientific Steering Board in its meeting in Madrid on 19th September 2019 exanimated all proposals and decided to select all submitted proposals (a total of 16 new projects).

4.1.4 Task 3.4: Organisation of annual scientific meetings (ASM) where results from JRP are presented.

Several teleconferences have been set up for the further preparation of the ASM in Dublin in 22-24 May 2019. Due to the efficient organisation at Teagasc and the University of Galway, no relevant difficulties have been noticed. The conference welcomed 288 participants from 25 different countries. All member states who are part of the OneHealth EJP were represented with the exception of Romania and Estonia. On the scientific side, 93 posters were displayed in the conference halls (41 for foodborne zoonoses, 33 for Antimicrobial Resistance, 12 for emerging threats and 7 for Integrative activities) and 90 oral presentations were given. Out of them, 55 (±60%) were given by scientist from OneHealth EJP partner institutes. Furthermore, a number of satellite meetings took place adjacent to the ASM, i.e. before (the WP6 meeting on Digital Innovation and Data Management and several annual meetings of JRP and JIP consortia) and during the conference, i.e. the first meeting with the External Scientific Steering Board. All details of the scientific programme can be found on the conference website https://www.ohejp2019.com. There was a general appreciation by the participants that the scientific level was very good (both in oral and poster presentations), that the possibility to make new contacts and to reinforce older ones was appreciated, and that the social events were excellent.

In July 2019, Pikka Jokelainen (SSI) started as WP3 Deputy Leader, with focus on coordinating the organising of ASMs. The timeline of the organisation of the ASM2020 meeting in Prague, Czech Republic was updated for optimal synergy with other One Health events in 2020.The planning and preparing processed as planned.

4.2 Deliverables and Milestones

4.2.1 Deliverables

Del. Ref.	Deliverable title	Submission
D3.6	Updated guidelines for Evaluation of proposal + Selection of projects (JRP&JIP)	31 Jan 2019
D3.7	1st periodic report on ongoing JRP	11 Feb 2019
D3.8	Abstract book for 1st Annual Scientific Meeting (ASM)	06 Jun 2019

Del. Ref.	Deliverable title	Submission
D3.9	Guidelines for WP3/WP4 on the evaluation of final reports	29 June 2019
D3.10	Second call for projects: Evaluation reports of full proposals	31 July 2019

4.2.2   Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS30	External experts contacted for proposal evaluation, 2nd call	M13	The external experts were invited to participate to the assessment process on January 21st, 2019. The external evaluation was achieved on May 17, 2019.
MS31	First Annual Scientific Meeting	M16	1st Annual Scientific Meeting (ASM) took place in Dublin, Ireland from 22nd to 24th May 2019. The abstract book of the ASM was delivered as D3.8 on 4th June 2019.
MS32	External experts contacted for final report evaluation (#1)	M21	In progress

5   WP4 - Joint integrative projects

5.1   Work carried out to date

5.1.1   Task 4.1: Development of procedures and guidelines for submission and selection of JIP proposals, and for reporting and evaluation

Together with WP3, this task has prepared guidelines for evaluation of project proposals (D3.6), as well as a procedure for conducting teleconference consensus meetings with the experts reviewing the proposals. Guidelines directed to the project leaders, on final reporting on Joint Integrative Projects, have also been produced (D4.12). (These guidelines will not be needed until Y3, since all JIPs are 3-year projects). Input has also been given on the WP3 deliverable Guidelines for evaluation of final reports (D3.9).

Procedures for extension and enlargement of project consortia have also be jointly developed with WP3 (no deliverable linked to this activity).

5.1.2 Task 4.2: Supervision of JIPs

In M13, WP4 submitted the 1st periodic report about the ongoing JIPs, ORION and COHESIVE (D4.10). A series of actions are being taken as a result of the follow-up:

- The expectations on the continuous reporting of milestones and deliverables, including where to place them, is being more explicitly communicated in an updated version of the reporting guidelines (D3.1 and D4.1). This is linked to an overarching discussion in the PMT, about the project's dissemination strategy.

- The difference between milestones and deliverables has been better communicated for 2nd call proposals, to allow for a cleaner precision in the follow-up.

- The WP4 team has also identified a need to define whether deliverables are to be regarded as public (PU) or confidential (CO). While this is implicit for projects such as the OHEJP itself, where this information is specified in conjunction with the submission of project proposals, this was not the case for the projects funded in the first call of the OHEJP (JRP:s as well as JIP:s). This has been taken care of in the 2nd call to ensure, as far as possible, a timely and open dissemination of deliverables.

- The JIPs flagged that it may be a challenge to get partners external to the project involved in the integrative missions, since the funding from WP4 has been mainly for mobility). WP4 has therefore suggested a reallocation of funding from the 2nd call for JIPs, where one of four topics did not attract a proposal (see Task 4.4), to be used to allow a greater involvement (with national co-funding) in the JIPs. This proposal has been accepted by the PMT.

Throughout the year, WP4 has had informal contacts with JIP project leaders, addressing various practical questions regarding the implementation. WP4 also attended the ORION prioritisation workshop (in January) and the annual meeting of COHESIVE (in April) to get further insight into the progress and to present the overarching direction of the OHEJP.

The progress of the two JIPs is described in more detail below:

5.1.2.1 ORION

5.1.2.1.1 Summary of the work carried out in the EJP

The ORION project progressed successfully from the "Inventories and requirement analysis" phase into the "Improvements and new resources" phase as described in the PERT chart of the project proposal. This second phase includes the preparation of dedicated WP-specific/ country-specific One Health (OH) pilots as well as the development of new resources identified as urgently needed during the requirement analyses carried out in year 1.

In WP1 the conceptual design phase of the overarching ORION "OH Surveillance Codex" (OHS Codex) has been completed. The OHS Codex complies with and extends the "Tripartite Guide to Addressing Zoonotic Diseases in Countries" as it evolved into a guidance document that provides practical recommendations, solutions and resources from all ORION WPs. WP1 further developed / extended the OHEJP Glossary (previously referred to as ORION glossary) based on extensive curation and review work from OH experts of each sector (animal health, public health and food safety). In WP2-Epi the general structure of the OHS Knowledge Base Epi was drafted and first technical implementations were accomplished. The work on improving the content of the Knowledge Base Epi started including the research on advanced methods (Rasch model) for analysis of questionnaire data. WP2-NGS focused on building the basic conceptual and technical framework for the OH NGS handbook and on organizing a

round of request for comments. WP2-Integration continued to provide integration opportunities between WPs specifically supporting the designs and preparation phase of the pilots carried out by WP1-WP3. WP3 focused on developing new harmonisation infrastructure to support data interoperability among OH surveillance agencies. This covered the areas of understanding the use of surveillance data for decision making in surveillance practice, promoting collaboration between sectors, the development of a knowledge model to annotate surveillance data which enables semantic interoperability, and the development of tools to support adoption of semantic resources in the process of report generation.

Finally all ORION WPs started discussions on the selection of their WP-specific / country-specific OH pilot projects. The aim of these pilots is to illustrate and validate the usefulness and added value of various ORION results in year 2 and 3 of the project.

The project coordination established shared project management resources including a shared space for documents, a shared calendar, an online mailing list and several other features. The project holds trimonthly web meetings for the whole ORION consortium (including stakeholders and interested EJP members) and a monthly call for the WP leaders & deputy leaders. The project organized and performed joint web meetings with EFSA and ECDC, contributed to the EJP DMP and initiated collaborations and information exchange with other EJP projects. Members of the project presented ORION and its work at several international conferences.

5.1.2.1.2 Progress of the integrative project: milestones and deliverables

5.1.2.1.2.1 Deliverables

JIP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
ORION	D-JIP1-1.1	Report on requirement analysis for "OH Surveillance Codex"	12	31-12-2018	Delivered on time	
ORION	D-JIP1-2.1	Report on requirement analysis for an "OH Knowledge Base – Epi"	12	14-01-2019	14-01-2019	
ORION	D-JIP1-2.2	Report on requirement analysis for an "OH Knowledge Base - NGS"	12	28-02-2019	28-02-2019	This report was delayed due to lack of manpower. That was fixed, and the report was handed in on the new forecasted delivery date.
ORION	D-JIP1-2.3	Report on requirement analysis for an "OH Knowledge Base – Integration"	12	28-02-2019	28-02-2019	The DTU ORION post doc is leaving his post before completing the work. The work was delivered a little later than planned.

JIP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
ORION	D-JIP1-3.1	Report on requirement analysis for an "OH Harmonisation Infrastructure Hub"	12	29-12-2018	Delivered on time	The deliverable detailed a literature review on interoperability in health surveillance, the results of an interview of partners regarding data interoperability and publishing among health surveillance agencies, and a requirement analysis on the technical architecture for adoption of ontologies in practice.
ORION	D-JIP1-4.1	Two internal training workshops for ORION partners	12	09-2018	Delivered on time	
ORION	D-JIP1-4.2	Draft on Sustainability Roadmap	18	30.06.2019	Delivered on time	

JIP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
ORION	D-JIP1-1.2	Draft on OH Surveillance Codex	24	31-12-2019	Expected to be delivered on time	The document will give an introduction to the OHS Codex framework (i.e. background, target audience, purpose, scope). It will further describe the purpose, scope and proposed method of each of the four postulates principles. The tools/ technical solutions for each principle and examples of their use will be given in the appendix.
ORION	D-JIP1-2.4	Status report on OH Knowledge Base – Epi	24	31-12-2019	Expected to be delivered on time	
ORION	D-JIP1-2.5	Status report on OH Knowledge Base – NGS	24	31-12-2019	Expected to be delivered on time	
ORION	D-JIP1-2.6	Status report on OH Knowledge Base – Integration	24	31-12-2019	Expected to be delivered on time	

JIP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
ORION	D-JIP1-3.2	Status report on OH Harmonisation Infrastructure Hub	24	31-12-2019	Expected to be delivered on time	The deliverable will provide an update on the progress of the 3 WP tracks: a practice track which carried our workshops for inter-agency communication and data sharing; am ontology track that developed an ontology of health surveillance; and a tech track that developed an Excel plug-in to allow ontology use in practice without changing current workflows.
ORION	D-JIP1-4.3	Two training workshops for other EJP	24	31-12-2019	Expected to be delivered on time	

5.1.2.1.2.2 Milestones

JIP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
ORION	M-JIP1-1	Requirement analysis synchronization workshop	4	Yes		
ORION	M-JIP1-2	Prioritization workshop	15	Yes		Workshop held in Uppsala on 16th- 18th January 2019

5.1.2.1.3 Progress of the integrative project

5.1.2.1.3.1 WP1: "OH Surveillance Codex"

5.1.2.1.3.1.1 JIP1-WP1-T1: Inventories and requirement analysis for "OH Surveillance Codex" (M1-M12)

This task has been completed; please see annual report 2018, or deliverable D-JIP1-1.1.
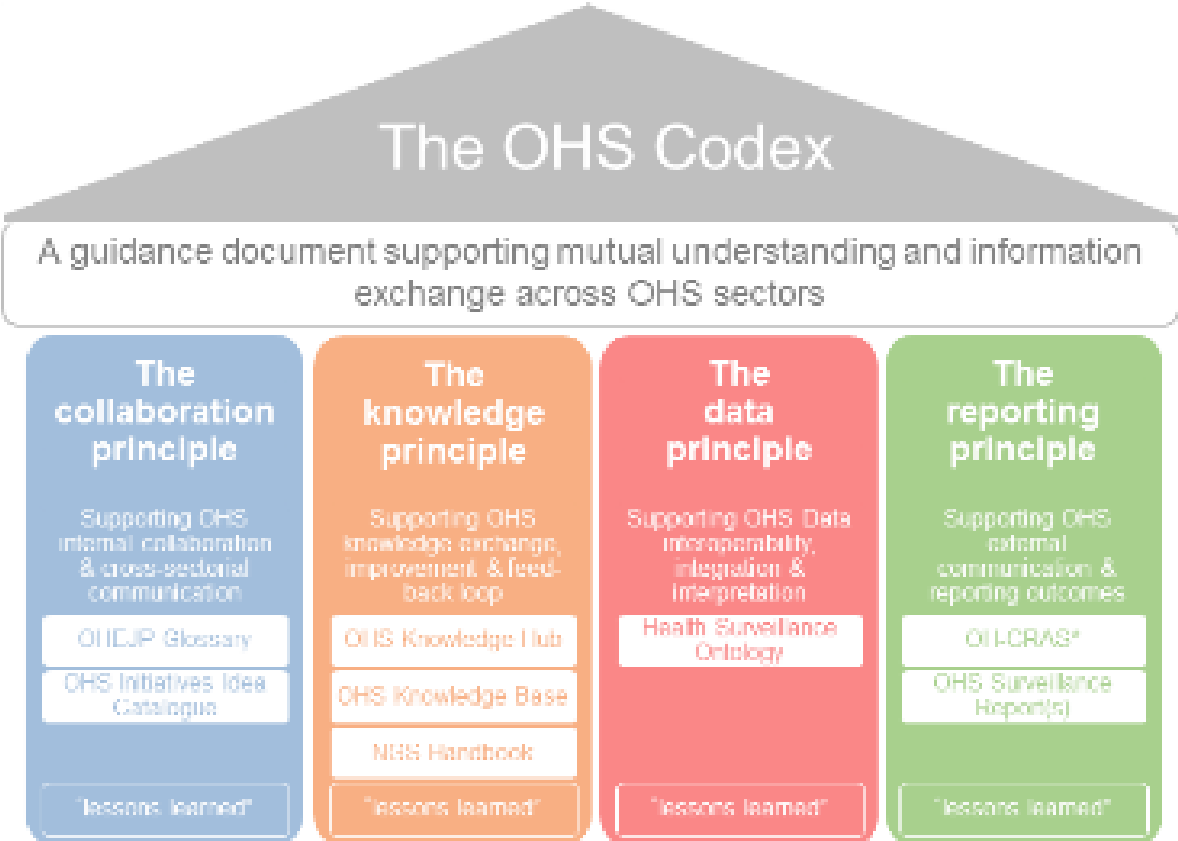
5.1.2.1.3.1.2 JIP1-WP1-T2: Development of "OH Surveillance Codex" (M13-M24)

The conceptual design phase of the "OH Surveillance Codex" (OHS Codex) has been completed (Subtask 1.2.1). The OHS Codex aims at establishing a high-level framework that supports mutual understanding and information exchange between OHS sectors, which are perquisites for integrated OHS data analyses. To bring this framework into "action" the OHS Codex postulates a set of four high-level principles as well as a description of resources (e.g. tools, technical solutions, guidance documents) and example implementations supporting the adoption of each OHS Codex principles. The resources outlined in the OHS Codex include developments from WP1, WP2, WP3 and WP4. A detailed description of each resource will be given in the Annex of the OHS Codex, together with practical examples and "lessons learned" from the pilot studies.

The design of the OHS Codex follows the ambitions of the "Tripartite Guide to Addressing Zoonotic Diseases in Countries" (specifically Chapter 5.2), which highlights the importance of multi-sectorial OHS information sharing. With the OHS Codex document the ORION project provides specific resources / solutions that support the practical implementation of actions proposed by the "Tripartite Guide" for a multi-sectoral One Health approach.

Figure 1 : The conceptual design of the « OH Surveillance Codex », postulating four high-level principles / pillars together with practical tools/technical solutions for improved OHS information sharing.

5.1.2.1.3.1.3   JIP1-WP1-T3: One Health pilot (M7-M30)

5.1.2.1.3.1.3.1 **JIP1-WP1-T3-ST1: Selection of the OH pilot study topic (M7-M12)**
This task has been completed; please see annual report 2018, or deliverable D-JIP1-1.1

5.1.2.1.3.1.3.2 **JIP1-WP1-T3-ST2: Planning and performing work necessary to prepare the execution of the OH pilot (M13-M18)**

The OHEJP Glossary and OH-CRAS are essential WP1 resources developed within the framework of the OHS Codex. Both resources will be used for the execution of the OH pilot in WP1 (JIP1-WP1-T3).

The content and technical infrastructure of the OHEJP Glossary (previously referred to as ORION Glossary) were further developed/ extended in year two of the ORION project. An essential part of the work was extensive curation and review of the terms and definitions by OH experts from each sector (animal health, public health and food safety). For the technical implementation of the OHEJP Glossary,

we developed a Google Sheet macro[1] that enabled more harmonized provision of terms, definitions and references. Moreover, a publicly available interface for the OHEJP Glossary was developed that provides a citable Uniform Resource Identifier (URI) for each definition. The OHEJP Glossary was made publicly available in M17 at http://orion.onehealthejp.eu/ and https://foodrisklabs.bfr.bund.de/orion-one-health-ejp/ .

To support OHS report harmonization we are proposing the establishment and adoption of a so called "One Health Consensus Report Annotation Schema" (OH-CRAS) in future surveillance reports. The aim of OH-CRAS is to support the systematic collection of OHS metadata across all sectors and to allow for structured reporting of these metadata. The development of OH-CRAS is performed in close collaboration of WP3. The schema of CRAS is based on the Generic Statistical Business Process model (GSBPM) and a first version for the pilot execution is expected in M19.

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[1] a single instruction given to a computer that produces a set of instructions for the computer to perform a particular piece of work (https://dictionary.cambridge.org/dictionary/english/macro, accessed on 21.08.2019)

GSBPM	Surveillance pathway	Data and metadata to be collected	Reporting
1) Specify needs	Set context for entire system	hazard/disease	Introduction / Background
		country	
		susceptible species	
		historical presence	
		surveillance objective	
		year started	
	Start listing specific surveillance components (activities)	year	Methods
		surveillance purpose	
	legal background	institutions involved	
		legal classification	
		legal basis	
2) Design	Sampling design	surveillance context	
		Target population (Species and production type for animals; population groups for humans)	
		sampling strategy	
		sampling unit	
	Reporting obligations	reporting obligations and process, for instance reporting trigger (suspicion, confirmation, etc)	
		case classification	
		case definition	
		reporting actors	
		date used for statistics	
3) Build	Sample collection	timeline	
		surveillance sampler type	
		sampling stage	
		sample type/specimen (matrix)	
4) Build	Laboratory analysis (collect raw data)	assay (laboratory test)	
		pooling	
5) Process	data aggregation, epi data collation	population coverage	Results
		sampling area	
		sample size calculated	
		Number of samples tested	
		Number of samples positive	
		Sample weight	
		cases	
		domestic cases	
		prevalence	
		incidence	
		results per group (per age, gender, sector, etc)	
6) analyse	epidemiological analysis	seasonal trends	Discussion
		historical trends	
		geospatial analysis	
		outbreak detection	
		source attribution	
		typing and cluster analysis	
7) disseminate	reporting and feedback loops	information relevant for consumers/patientes/other target audience of the report	
8) evaluate	surveillance evaluation and actions triggered	any evaluation of performance attributes	
		any review of surveillance practices recommended/decided	
		actions taken in case of outbreaks	

Figure 2 : A draft of OH-CRAS as of 26th August 2019

5.1.2.1.3.1.3.3 JIP1-WP1-T3-ST3: Execution of the OH pilot - applying the "OH Surveillance Codex" guide (M19-M30)

The execution of the OH pilot in collaboration with ARDIG and GOHI (for details see annual report 2018, or deliverable D-JIP1-1.1) is in preparation. A detailed strategy for the execution and evaluation of the OH pilot has been created together with the description of the pilots of the other WPs in M19.

5.1.2.1.3.2 WP2: Epi

5.1.2.1.3.2.1 *JIP1-WP2-T1: Inventories and requirement analysis for OH Knowledge Base Epi (M1-M12)*

5.1.2.1.3.2.1.1 JIP1-WP2-T1-ST1 – literature review

This task has been completed, see annual report 2018.

5.1.2.1.3.2.1.2 JIP1-WP2-T1-ST2 – requirement analysis workshop

This task has been completed, see annual report 2018.

5.1.2.1.3.2.1.3 JIP1-WP2-T1-ST3 - Survey and/or interviews with internal / external experts

In the fall of 2018, a survey was sent to all EJP members. The response rate was very low. Considering the amount of parallel activities and input gathering going on within the project at the same time, rather than push on this EJP survey, we decided to take the time to consolidate all input from project members, and then review results with key stakeholders (EFSA and ECDC). This will lead to a more mature version of the tables for inventory of surveillance systems, and we will conduct another round of questions when sending out those inventory tables.

5.1.2.1.3.2.1.4 JIP1-WP2-T1-ST4 – first version of the OH knowledge base-epi

A general structure for the knowledge base was drafted for presentation in the prioritization workshop planned for month 13. It was decided that the structure will be a collection of 3 inventories:

- an inventory of surveillance data sources available among partners,

- an inventory of surveillance activities related to One-Health and

- an inventory of methods and tools used for surveillance.

5.1.2.1.3.2.2 *JIP1-WP2-T2: Improving OH Knowledge Base – Epi (M13-M24)*

After discussions initiated in the prioritization workshop (M13), it was decided to work in small groups, to fine-tune the three inventory tables, within each health sector. Each sector needs to define the concept lists (which will be drop-down lists of options in the inventory forms) for the respective fields and complete those according to existing EFSA and ECDC lists and guidelines. We also created a handbook file for each table with descriptions on what information is needed in the individual fields.

Data base – statistical tools and methods:

Statistical tools and methods of specific importance in surveillance projects were collected in a data base. The database entries were collected in a comma separated file (*.csv) and translated into a browser readable format by using R Shiny. This format increases clarity and readability of the data base and adds user comfortable ways to search and sort the data base. A first draft of the data base was published at https://www.shinyapps.io/

We collaborated with representatives of EFSA and ECDC on the details and sustainability questions of the inventories. A specific discussion meeting was carried out at EFSA in M19.

The technical specifications and options to publish the inventories with the given platforms have been evaluated. Publishing in the EJP website proved more challenging than anticipated, and therefore an alternative solution will be developed.

Based on the experiences with the first draft of the questionnaires (JIP1-WP2-T1-ST3 - Survey and/or interviews with internal / external experts) we further developed the scoring method provided by the Rasch model to optimise the information gain. To obtain more detailed information about the survey projects the overall quality score was split into several quality scores reflecting specific topics like: planning and analysing standardisation, reporting standardisation and focus of institutions. The coding of the Rasch model was finalised by using the statistical programming languages R and JAGS.

The coding of latent class analysis and structural equation models for a more detailed analysis of the questionnaires in terms of detection of structural dependencies and characteristics that can be combined into classes with specific shared properties, was continued and adjusted at the suggested questions.

5.1.2.1.3.2.2.1 JIP1-WP2-T2-ST1 - Data collection and integration

A preliminary data collection was performed in M4, and a more thorough data collection will be started after M19, when we consolidated all internal input, plus input from ECDC and EFSA.

5.1.2.1.3.2.2.2 JIP1-WP2-T2-ST2 - Data analysis and validation

This task will be particularly active between M20 and M24, after data collection.

5.1.2.1.3.2.2.3 JIP1-WP2-T2-ST3 - Knowledge integration and Decision support

This task will be particularly active between M20 and M24, after data collection.

5.1.2.1.3.2.3   JIP1-WP2-T3: Epi - OH pilot studies (M7-M30)

5.1.2.1.3.2.3.1 JIP1-WP2-T3-ST1: One Health Pilot 1: Toxoplasma gondii (carried out by FLI and BfR, Germany) (M7-M30)

The pilot is in the second phase, where the literature search has been completed and data is in the process of being extracted.

The obtained results on surveillance activities in AH and the food sector on Toxoplasmosis will be described according to the defined outline of the inventory tables. This will be used to make a gap analysis on Toxoplasmosis surveillance activities, analysing mainly the data collection and formatting discrepancies between the sectors.

5.1.2.1.3.2.3.2 JIP1-WP2-T3-ST2: One Health Pilot 2: Salmonella (M7-M30)

Currently in the planning and early implementation phase with three PHE/APHA meetings held to discuss requirements for data confidentiality, metadata requirements, methodology for WGS data sharing. Draft standard operating procedures document and Memorandum of Understanding in development.

5.1.2.1.3.2.3.3 JIP1-WP2-T3-ST3 One Health Pilot 3: Hepatitis E (carried out by WBVR and RIVM, the Netherlands) (M7-M30)

Currently, the pilot study is in the phase of planning with objectives and expected outcomes being defined. Data about hepatitis E gathered in PH and AH are hardly combined at the moment. One of the goals is to set up a collaboration between several parties/institutions. Aims to be considered are

the needs for a good (hepatitis E) surveillance and collaboration, and perform epidemiological and NGS data analyses in a concerted action.

5.1.2.1.3.3 WP2: NGS

5.1.2.1.3.3.1 *JIP1-WP2-T4: Inventories and requirement analysis for OH Knowledge Base – NGS (M1-M12)*

This task has been completed, please see annual report 2018, or deliverable D-JIP1-2.2.

5.1.2.1.3.3.2 *JIP1-WP2-T5: Improving OH Knowledge Base – NGS (M13-M24)*

The focus for this task this year has been on building the basic framework for the OH NGS handbook. The framework draws on the information collected during the first year, and is described in the 2018 annual report. We have found a technical platform to host the handbook (Github), and are in the process of organizing a round of "Request for Comments" for the framework for the handbook. The first round is internally in the project, the second involves domain experts and stakeholders.

For the pilot project we are to set up and do analysis on two specific pathogens. We have as our first pathogen chosen *Listeria monocytogenes*, and are in the process of collecting information regarding current practices on sequencing for surveillance for that pathogen. This includes information on analysis pipelines, information that will both be collected for the handbook and also used as a basis for pipeline implementation in the pilot project. We are in close collaboration with the ListAdapt project in this regard.

We will in the pilot be setting up a collaborative analysis platform between the NVI and the NIPH. Within this platform, we will have a need for storing metadata about the samples. For this purpose, we are collaborating both with WP3 and with WP4.1 of the COHESIVE project to get a good strategy for metadata handling. This work will also be documented in the handbook.

In collaboration with WP3, we have identified initiatives that provide tools for WGS-based surveillance, and we are organizing a Cogwheel workshop which will take place on Month 21 of the ORION project. Initiatives with a strong leadership in both WGS data analysis, and data interoperability tools have confirmed presence: IRIDA, INNUENDO and COMPARE. EFSA and ECDC have also confirmed presence.

WP2-NGS is also organizing a [Nextflow workshop](#) on M22 for the entire EJP consortium, with a total of 30 spots and with priority for ORION and COHESIVE member institutions. Nextflow is a tool that enables the creation of portable, reproducible and scalable genomics analysis.

5.1.2.1.3.3.3 *JIP1-WP2-T6: NGS OH pilot studies (M7-M30)*

For the pilot project, we are currently exploring the compute infrastructure that will be needed to host a collaborative data analysis platform that both the NIPH and the NVI can use. We are also exploring data management and analysis platforms, with an emphasis on platforms that can be managed nationally (examples are [IRIDA](#) and the [INNUENDO](#) projects). Once that evaluation is complete, we will install the chosen platform in the national compute infrastructure, and start implementing the pipelines and the metadata storage as detailed in Task JIP1-WP2-T5. Once there is a working joint analysis framework up and running for *L. monocytogenes*, we will choose a second pathogen and repeat the process. The lessons learned from this project will feed back into the handbook.

5.1.2.1.3.4 WP2: Integration

5.1.2.1.3.4.1 JIP1-WP2-T7: Inventories and requirement analysis for OH Knowledge Base – Integration (M1-M12)

The inventory report was completed and delivered to the new deadline of 28/02/2019. Furthermore, the interviews were published in a report: "One Health integration in Surveillance – ideas and inspiration" as inspiration to member states http://www.food.dtu.dk/english/news/nyhed?id=545ac065-3134-4572-bf2e-40834d21ac83. The link to the report was circulated via EFSAs Focal Point network and through ECDCs FWD-Net.

The milestone report contained analysis of OH surveillance initiatives in the EU. The interviews highlighted many interesting initiatives, but also challenges and requirements along the surveillance pathway. Among others, no initiatives were identified at the sampling level stage, highlighting that in the EU, OH approaches may not be relevant in all surveillance steps. Furthermore, it was expressed by the interviewees that at the step of data analysis and interpretation, true collaboration was rare. Often each sector prepared their data and they collated it into a joint surveillance output. This was often due to tradition, lack of either willingness or legislative or technical barriers in regards to data sharing.

5.1.2.1.3.4.2 JIP1-WP2-T8: Improving OH Knowledge Base – Integration (M13-M24)

We continued to seek integration opportunities between work packages and provide fora and opportunity to do so. The pilot studies designs and preparation have been the focus of the last 9 months. We supported identification of opportunities for collaboration and integrating the knowledge from experts within ORION.

In June 2019, we held a workshop to identify expected outcomes and performance indicators for all pilots and share objectives and sub-objectives. This highlighted further opportunities for collaboration and integration within the OHS Codex. A plan for sharing pilot designs internally and externally was made and an evaluation matrix was agreed and initiated. The knowledge sharing and added value from the enhanced collaborations between institutes will contribute to improve the OH Knowledge Hub as well as support MS with implementation of further OH approaches in surveillance.

WP2 integration also participated in a COHESIVE workshop to discuss the application of the "A Tripartite Guide to Addressing Zoonotic Diseases in Countries » for a European setting and will continue involvement in this work.

5.1.2.1.3.4.3 JIP1-WP2-T9: Integration OH pilot studies (M7-M30)

Planning of the Danish pilot study. The focus will be on integrating surveillance data from different sectors to improve interpretation and enhance collaboration between sectors. Three main tasks have been identified:

1) Improving the interpretation of surveillance data on AMR in zoonotic bacteria, by integrating surveillance data from all four branches of DANMAP: Use of antimicrobials in people, Usage in animals, AMR in people and AMR in animals. The work has started this year and new version of the DANMAP chapter on AMR in Campylobacter is under development. The task will continue by using similar approaches to transform the chapter of Salmonella resistance throughout 2019 with the aim to have a template ready for the report in 2020. (PH, FS and AH).

2) Description of the campylobacter surveillance system from farm to patient to enhance understanding of on-going surveillance between sectors. The work is progressing and will continue throughout 2019. Collaboration with pilot projects in NL and BE will be sought (PH, FS, AH).

3) Improving analysis and understanding of Campylobacter surveillance data by integrating data from across food and animal sectors, both public and private. The data is in house and analysis are about to start. The data interpretation will be done in collaboration with the poultry industry to enhance collaboration and impact. We are also investigating the possibility of integrating WGS data into the analysis of traditional surveillance data (FS, AH).

5.1.2.1.3.5 WP3: OH Surveillance Harmonisation Infrastructure

5.1.2.1.3.5.1 JIP1-WP3-T1: Inventories and requirement analysis for OH Harmonisation Infrastructure (M1-M12)

This task has been completed, please see annual report 2018, or deliverable D-JIP1-3.1.

5.1.2.1.3.5.2 JIP1-WP3-T2: Improving OH Surveillance Harmonisation Infrastructure (M13-M24)

After the requirement analysis performed in Year 1, in Year 2 this WP is focusing on a harmonisation infrastructure to support data interoperability among health surveillance agencies through a structure of 3 tracks: a practice track, focused on understanding the use of surveillance data for decision in surveillance practice, and promoting collaboration among PH, AH and FS; an ontology track, developing a knowledge model to annotate surveillance data which enables sematic interoperability; and a technology track, developing tools to adopt the ontology while keeping current workflows. These 3 tracks are supporting other ORION WPs as detailed in the subtasks below.

5.1.2.1.3.5.2.1 JIP1-WP3-T2-ST1: Systematic compilation and further development of infrastructural harmonisation resources - supporting high priority needs identified in WP2 (M13-M24)

We have reviewed and aligned terminology from the ECDC, EFSA and previous key projects in animal health surveillance design (RISKSUR and AHSURED), as well as current EFSA projects on surveillance reporting (SIGMA). This work will support the inventories to be carried out in WP2-epi. During the course of Year 2, all these concepts will be added to the ontology developed. During Month 21, the ontology becomes already publicly available, and webinars are planned to get further community feedback.

An Excel plug-in is being developed, so that surveillance data – as well as the data from the WP2-epi inventories – can be annotated with the ontology using simple Excel templates. To decide which data should be shared among health surveillance agencies, and which data should be made public, the surveillance practice track has promoted collaboration workshops among AH, PH and FS agencies. This work is closely tied to the work done in WP2-integration. To support WP2-NGS, we have identified initiatives that provide tools for WGS-based surveillance, and we are organizing a Cogwheel workshop which will take place on Month 21. Initiatives with a strong leadership in both WGS data analysis, and data interoperability tools have confirmed presence: IRIDA, INNUENDO and COMPARE. EFSA and ECDC have also confirmed presence.

5.1.2.1.3.5.2.2 JIP1-WP3-T2-ST2: Systematic compilation and further development of infrastructural resources - supporting high priority needs identified in WP1 (M13-M24)

The work of all WP3-tracks has been highly tied to the work within WP1. Besides taking active part in the development of the OHS Codex and OH-CRAS, WP3 has supported WP1 needs by making sure that the developed tools are a means to adopt WP1 tools. That is, once these WP1 resources exist to guide

the reporting of OHS data and inter-agency communication, the harmonisation infrastructure provided by WP3 will serve as the tools to annotate data following these guidelines. The ontology, in particular, is being structured following the top level structure set by OH-CRAS.

5.1.2.1.3.5.3 JIP1-WP3-T3: One Health pilot (M7-M30)

In Year 1, active discussions have led to the decision of focusing the OH-pilot in three main chapters of the Swedish surveillance report: Campylobacter, Salmonella, and VTEC/EHEC. During months 13-18, the surveillance practice followed closely the production of the surveillance report for these three zoonotic agents, conducting workshops that gathered AH, PH and FS surveillance officials to discuss opportunities for data sharing, joint data analysis, and publication of main findings that highlighted the OH aspects of the activities and findings of the previous year. The results will be compiled during the second semester of Year 2 (months 19-24) to elaborate an Excel template for data gathering and sharing; and guide the content needs for the ontology. This work will ensure that on Year 3 (months 25-30) we have a cycle of surveillance reporting fully supported by the harmonisation and data interoperability framework set forward by this WP.

5.1.2.1.3.6 WP4: Coordination, Communication, Training and Sustainability

5.1.2.1.3.6.1 JIP1-WP4-T1: Internal project coordination (M1-M36)

In month 13 the ORION Pilot Prioritization workshop took place in Uppsala. This workshop also served as a physical full consortium meeting for ORION. 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 stakeholders and interested EJP members) and a monthly call for the WP leaders & deputy leaders. EFSA & ECDC representatives as well as the leads of EJP WP 4 and 5 and the coordinator of the COHESIVE project are invited to join the full consortium calls. ORION compiled the Data Management Plan (DMP) and supported the overarching EJP project in all requested activities (e.g. presentations at PMC meeting, offering short term missions, sharing of information, etc.). For all physical and web-meetings the ORION coordination created meeting minutes that were shared via email and the ORION VRE.

5.1.2.1.3.6.2 JIP1-WP4-T2: External project integration (synchronized with EJP WP5) (M1-M36)

The project coordination contributed to the EJP DMP, to all overarching EJP activities and continued to extend collaboration and information exchange between EJP projects. EJP stakeholders were actively informed on project results via e.g. invitation to project web meetings, WP specific web-meetings, newsletters (WP3) or via invitation to physical meetings. ORION also supported the EJP WP5 in their efforts to establish a "capacity map".

5.1.2.1.3.6.3 JIP1-WP4-T3: Sustainability roadmap (M7-M36)

As described in the ORION project plan the draft of the ORION Sustainability Roadmap has been created and released as deliverable D-JIP1-4.2 by the end of June 2019. In agreement with the project description this roadmap will be continuously updated over the course of the ORION project.

5.1.2.1.3.6.4   JIP1-WP4-T4: Training and Dissemination (M1-M36)

5.1.2.1.3.6.4.1   JIP1-WP4-T4-ST1: Internal training (sharing knowledge on currently available national solutions) (M1-M12)

In 2019 additional ORION internal training activities were carried out:

- WP2int: Two cross-institutional meetings to discuss pilot project design, pilot execution and to share.

- WP3: Three workshops, focusing on 3 specific zoonoses (Salmonella, Campylobacter and VTEC/EHEC) were conducted as part of the OH pilot in Sweden (WP3). These workshops gathered surveillance officials from the AH, PH and FS agencies. The flow and tools for surveillance data analysis and reporting was reviewed and shared among agencies.

- A Cog-wheel infrastructure workshop, has been organized on 16/17th September 2019 in Dubrovnik, Croatia by WP2-NGs and WP3 where initiatives with a strong leadership in both WGS data analysis, and data interoperability tools have confirmed presence: IRIDA, INNUENDO and COMPARE. EFSA and ECDC have also confirmed presence.

5.1.2.1.3.6.4.2   JIP1-WP4-T4-ST2: Knowledge integration (web portal, Wiki, curricula, tutorials, videos, sample data) (M7-M36)

The ORION project explored with the support from the EU-funded project AGINFRA+ if so called Virtual Research Environments (VRE) could be used as knowledge integration platform. So far the ORION VRE has been mainly used by ORION project members for sharing project documents and project internal information. As similar features are now available via the official EJP website (within the "ORION Group") it was further explored with the EJP WP1 if this EJP infrastructure could serve as OHS Knowledge Hub as well. Unfortunately, the EJP platform does not provide sufficient flexibility to e.g. create or maintain a project specific website that could serve as OHS Knowledge Hub. After further analysis of the specific needs of all WPs and the available functionalities and dependencies for the different technological solutions (VRE, EJP website, other) the ORION project decided to develop an independent solution on the basis of BfR's "FoodRisk-Labs" web-page https://foodrisklabs.bfr.bund.de/. Via the ORION One Health EJP section on this platform it is now possible for ORION project partners / WPs to integrate and link to their specific platforms and resources:

- WP3: for the "health surveillance ontology": http://bioportal.bioontology.org/ontologies/HSO or as machine readable permanent link: http://w3id.org/hso

- WP2Epi: The technical basis for the OH KnowledgeBase including the inventories for surveillance systems, data sources and tools and methods will be developed as R Shiny app.

- WP2Int: First results are collected in the publication "OH integration in surveillance – inspiration and ideas" https://www.food.dtu.dk/english/news/Nyhed?id={545AC065-3134-4572-BF2E-40834D21AC83}

- WP2NGS: a OH Surveillance NGS Handbook will be developed jointly and evolves continuously

- WP1: the OH EJP Glossary and the OHS Codex will be available as dedicated web-services using the infrastructure of BfR's KNIME Server as well as the ORION VRE.

5.1.2.1.3.6.4.3 JIP1-WP4-T4-ST3: Training and support for other EJP projects & partners (M7-M36)

The ORION project contributed actively to all dissemination event organized by the overarching EJP project, e.g. with four presentations during the ASM Scientific conference in Dublin (https://www.ohejp2019.com/). Further there are dedicated collaborations by several work packages with other EJP projects, e.g. COHESIVE, RADAR, NOVA, ListAdapt etc. A specific mean to facilitate training and support will be the ORION pilots where planning has been finalized by June 2019. The synopses of all ORION pilots will be made available for EJP members by the end of September 2019 via the EJP portal.

WP2-NGS is also organizing a Nextflow workshop on M22 for the entire EJP consortium, with a total of 30 spots and with priority for ORION and COHESIVE member institutions. Nextflow is a tool that enables the creation of portable, reproducible and scalable genomics analysis.

An overview on all ORION training activities – including the webinars hold and planned in Year2 - is available within the continuously evolving ORION Stakeholder Involvement and Dissemination Plan:

https://docs.google.com/document/d/1nDCx7KVxdi2RJSoa8uf-zOfcm34cX9rWCr-CoiKaLB8/edit?usp=sharing

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

The ORION project pursued its annual physical full consortium meeting in January 2019. Monthly WP leader calls are performed to monitor progress of project work and make decisions on project related issues. Every three months there is a full consortium conference calls that is open for stakeholders, COHESIVE and the EJP WP3, 4 and 5. In addition each WP organizes further conference calls on their specific schedule and needs. For WP2Epi there are bi-monthly web-meetings. Furthermore there is a face-to-face WP2-Epi satellite meeting planned at the annual meetings.

A general overview on all overarching ORION activities is available within the continuously evolving ORION Stakeholder Involvement and Dissemination Plan:

https://docs.google.com/document/d/1nDCx7KVxdi2RJSoa8uf-zOfcm34cX9rWCr-CoiKaLB8/edit?usp=sharing

5.1.2.2 *COHESIVE*

5.1.2.2.1 Summary of the work carried out in the JIP

A main event in 2019 was the annual meeting that was held on April 10-12 at SVA. For an integrative project as COHESIVE, coming together and extensively discuss issues is very important. One element, in addition to work on the content of the project, is getting to know each other and build trust and respect. Since the whole project is built around strengthening collaboration between med-vet-food in the area of zoonotic diseases, realizing the value of this collaboration in an international setting is also key nationally. All tasks were discussed and several workshops took place. Also time was reserved to inform stakeholders via a videoconference.

WP2: For WP2.1 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 to zoonoses. Since in March 2019 an extended, update version of the Tripartite Zoonoses Guide (TZG) was published, the focus of the workshop was shifted towards getting insight in to what extend the new TZG was useful in setting up/strengthening such collaborations in European countries. Although the TZG is useful, added value is seen in a dedicated, simple European guideline with focus on implementation (more practical). During a two-day meeting in Brussel on July 1-2 first steps were made drafting the implementation guideline.

For WP2.2 the goal is to develop a tool to help decide which tool/model best to use for risk assessment in a specific situation. An early prototype decision tree has been developed in excel, using a limited number of publicly available quantitative risk assessment tools and also includes disease prioritization tools. To make the application easily accessible and available (avoiding proprietary software) a version in R-Shiny is being made. A webinar was held on 6th September for partners in COHESIVE to introduce the tool ahead of the 'show and tell' session planned for the annual meeting.

WP3: A workshop was held on WP3.1, focusing on how information around an event/outbreak is shared. It was concluded that detailed descriptions of systems may not be very helpful for other countries trying to build up new systems/ways to share signals. Rather, it may be more useful to identify different factors that are believed to contribute to well-functioning systems/ways to share signals, trying to answer "<u>why</u> does signal sharing work well in this context". An interview guideline has been made and in-depth interviews are in being held.

For WP3.2 the first task was to make an inventory and analysis of horizon scanning tools. Evaluation of a questionnaire has been conducted, literature inventory and workshops have been performed within the task to identify horizon scanning tools. An important tool is the formation of an expert team. After formation of a team with all the needed expertise, a horizon scanning exercise will take place as a satellite meeting before the general COHESIVE meeting in November 2019.

For WP3.3 systematic mapping of zoonoses detection systems within the UK has been started. By using a reversal process we are starting with identifying the formal outputs of systems that are based around, or contain, zoonoses information.

WP4: For WP4.1 a survey has been conducted in order to gather detailed information on existing databases and information systems for WGS data management and analysis adopted or available among countries. A demo version of the COHESIVE prototype information system (CIS) is made and available for Italy, The Netherlands and Norway to perform a feasibility study.

In WP4.2, a list of available tracing tools was compiled, evaluated and published as a web service that can be updated by interested partners in the future. Secondly, the physical setup of the tracing web portal with initial features was realised and several prototype modules for data collection, cleaning, visualisation and reporting were implemented within the portal or are going to be implemented in the upcoming months. Data formats to collect sample and case data were developed. Their visualisation was tested in case studies and in a current outbreak.

For WP4.3 the goal is to make a platform-independent risk modeling framework. A prototype of the risk modeling framework in R shiny for quantitative microbiological risk assessment has been developed. To make the web application easily available an online version is under development.

5.1.2.2.2 Project-specific milestones and deliverables

5.1.2.2.2.1 Deliverables

JIP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
COHESIVE	D-JIP2-3.3	Pathway analysis of exchanging signals	10		24	Interview-guideline is developed and interviews will take place in summer and autumn, analysis in autumn. Due to having to prioritize working on an outbreak, finalization of the task had to be further postponed.
COHESIVE	D-JIP2-4.8	Report section about user requirements, relevant modelling modules and final specification for a modelling tool	10		18	Contact with external cooperation partners has been established, with a delay in feedback
COHESIVE	D-JIP2-4.5	Report of available tools and algorithms and ranking of most valuable features	12	30-06-2019		The report was finished and uploaded to the EJP platform end of June 2019.
COHESIVE	D-JIP2-1.3	Annual meeting	14	12-04-2019		
COHESIVE	D-JIP2-1.4	Annual report	16	18-01-2019		

JIP name	Project deliverable number	Deliverable name	Delivery date from AWP	Actual delivery date	If deliverable not submitted on time: Forecast delivery date	Comments
COHESIVE	D-JIP2-4.1	Implemented database	17	30-06-2019		The workload of this subtask was underestimated, in particular because it depended by the first workshop which was postponed to Month 11.
COHESIVE	D-JIP2-2.3	Development of tool for systematic risk-assessment	18		24	Development of prototype is on track, extra time for further user testing and refinement. Webinar held to introduce the prototype ahead of user testing
COHESIVE	D-JIP2-3.4	Inventory and analysis of tools for horizon scanning	18	30-06-2019		In addition, an extra deliverable will be a pilot horizon scanning team exercise that is the planned in the autumn of 2019.
COHESIVE	D-JIP2-2.4	Thematic workshops	20	02-07-2019		Two workshops have been done
COHESIVE	D-JIP2-3.2	Thematic workshops	20	30-06-2019		One workshop has been done and given input for the interview guidelines

5.1.2.2.2.2 Milestones

JIP name	Milestone number	Milestone name	Delivery date from AWP	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
COHESIVE	M-JIP2-5	Prioritization of requirements for risk modeling framework	12	Yes		Typical components have been identified that support quantitative microbiological risk assessment
COHESIVE	M-JIP2-5	Annual report	16	Yes		
COHESIVE	M-JIP2-6	Databases for WGS data consistent with COMPARE standards, for the metadata of the samples included in the WGS database, and for the data collected by classical epidemiology investigations (case-control studies)	17	Yes		The workload of this subtask was underestimated, in particular because it depended by the first workshop which was postponed to Month 11
COHESIVE	M-JIP2-7	Development of tool for systematic risk-assessment	18	No	24	Development of prototype is on track, extra time for further user testing and refinement.
COHESIVE	M-JIP2-8	Fully operating and linked databases	21	Not due		

5.1.2.2.3 Progress of the integrative project

5.1.2.2.3.1 WP1: Coordination, communication and sustainability

5.1.2.2.3.1.1 *JIP2-WP1-T1: Coordination (M1-M36)*

In 2019, two institutes were welcomed as partners. From Portugal INIAV became a partner, as well as VRI from the Czech Republic. Several other institutes have shown interest in becoming a partner and we are in the process of making them partners.

COHESIVE has produced a DMP (data-management plan) which will be kept updated through-out the project. There have been several contacts with EFSA and ECDC, mainly concerning WP4. Also regular video-conferences will be organized between the contact persons of EFSA/ECDC and the coordinator of COHESIVE. ORION and NOVA were identified as other OH EJP projects to which COHESIVE could relate. The coordinator of COHESIVE was present at (part of) the ORION annual meeting. ORION, NOVA and COHESIVE are working together at a glossary including terms used within the different projects.

5.1.2.2.3.1.2 *JIP2-WP1-T2: Communication/dissemination (M1-M36)*

An annual meeting was organized in April 2019 at SVA in Sweden. During this meeting there were several workshops organized as well as a stakeholder meeting, in which the progression of the COHESIVE project was presented via a videoconference.

The COHESIVE project delivered text for two newsletters, for information on the OH-EJP website and used twitter and linkedin. The coordinator presented the project at the PMC meeting and also at the POC meeting the project was presented. Also during the ASM in Dublin COHESIVE was presented.

5.1.2.2.3.2 WP2. Integrated risk-analysis at the national level

5.1.2.2.3.2.1 *JIP2-WP1-T1: Coordination (M1-M36)*

In 2019, two institutes were welcomed as partners. From Portugal INIAV became a partner, as well as VRI from the Czech Republic. Several other institutes have shown interest in becoming a partner and we are in the process of making them partners.

COHESIVE has produced a DMP (data-management plan) which will be kept updated through-out the project. There have been several contacts with EFSA and ECDC, mainly concerning WP4. Also regular video-conferences will be organized between the contact persons of EFSA/ECDC and the coordinator of COHESIVE. ORION and NOVA were identified as other OH EJP projects to which COHESIVE could relate. The coordinator of COHESIVE was present at (part of) the ORION annual meeting. ORION, NOVA and COHESIVE are working together at a glossary including terms used within the different projects.

5.1.2.2.3.2.2 *JIP2-WP1-T2: Communication/dissemination (M1-M36)*

An annual meeting was organized in April 2019 at SVA in Sweden. During this meeting there were several workshops organized as well as a stakeholder meeting, in which the progression of the COHESIVE project was presented via a videoconference.

The COHESIVE project delivered text for two newsletters, for information on the OH-EJP website and used twitter and linkedin. The coordinator presented the project at the PMC meeting and also at the POC meeting the project was presented. Also during the ASM in Dublin COHESIVE was presented.

5.1.2.2.3.3 WP3 Towards an EU zoonoses structure

5.1.2.2.3.3.1 JIP2-WP3-T1: "Explore current ways for exchanging signals between countries and cross disciplines – pathway analysis" (M1-M24)

During the workshop in the annual meeting in Uppsala in April 2019, it was concluded that the information retrieved for this task so far (previous workshop and a questionnaire) was quite general and that it would be beneficial to go more in depth. One of the overall purposes of the project is to find good examples of ways to exchange signals cross disciplines/cross borders and learn from each other, but it was further concluded that detailed descriptions of systems may not very helpful for other countries trying to build up new systems/ways to share signals. Rather, it may be more useful to identify different factors that are believed to contribute to well-functioning systems/ways to share signals, trying to answer "why does signal sharing work well in this context". In parallel, in the discussions it became apparent that there is also a need to try to identify factors that prevent signal sharing. To identify these factors, persons working at different levels in the involved organisations need to be approached and encouraged to share their experience. One more text-based questionnaire was not believed to be successful, and it was concluded that in-depth interviews would be the best approach. An interview guideline has been developed and participants have been recruited to perform interviews and thematic analysis. The interviews will be performed during summer and autumn, each country will do a separate thematic analysis and there will be a joint analysis in the autumn.

5.1.2.2.3.3.2 JIP2-WP3-T2: Select tools for Horizon scanning and signal detection (M1-M24)

Horizon scanning is a specific foresight methodology that utilizes various steps to identify issues at the edge of current thinking that may have significant impact in the medium to long-term future. Consequently horizon scanning requires structured information gathering. A one health horizon scanning tool is based on assembly of information sources and assembly of analysis teams with assigned topics. Various one health team members examine signals in a structured way. Evaluation of a COHESIVE questionnaire has been conducted, literature inventory and workshops have been performed within the task to identify horizon scanning tools. An important tool is the formation of a expert team. The formation of a horizon scanning team composed of experts within public health, animal health, food safety and environment is under progress. With this team a horizon scanning exercise will be planned in Autumn 2019, which can be seen as an extra deliverable.

5.1.2.2.3.3.3 JIP2-WP3-T3: Retrospective systems analysis of detection of outbreaks (M6-M30)

Systematic mapping of zoonoses detection systems within the UK has been started. Using a reversal process we are starting with identifying the formal outputs of systems that are based around, or contain, zoonoses information. These include formal publicly available publications at the highest level through to less formal, restricted access, reports and documents. Having identified the outputs and the bodies responsible for producing them the next stage will cover identifying the personnel, data and information sources that are used in their production and how they interact.

5.1.2.2.3.4 WP4: Data platform to facilitate risk-analysis and outbreak control

5.1.2.2.3.4.1 JIP2-WP4-T1: Molecular typing data and metadata – database creation

A survey has been conducted in order to gather detailed information on existing databases and information systems for Whole Genome Sequencing (WGS) data management and analysis adopted or available among the Member States. The survey represented a first step for the evaluation of the technical feasibility of the inter-connection of genetic data produced by the laboratories and metadata

archives available in each country and to provide the project with a reasoned list of the available Information Systems for WGS data and associated metadata. Eight countries answered to the questionnaire: Sweden, Norway, Belgium, Portugal, The Netherlands, Austria, Czech Republic and Germany. The 75% of countries reported that no OH surveillance system is in place at the moment, but where it exists WGS data are shared.

Proposals as a result from the discussions during the WP4 workshop (held at APHA-Weybridge November 2018) and the questionnaire results have been analysed.

A clearer description of the task 4.1 phases has been presented to EFSA representatives on March 2019 and during the stakeholder meeting at the COHESIVE Annual Meeting (held at Uppsala, April 2019). EFSA declared that Task 4.1 purpose is both clear and interesting to them. Two Member States decided to perform the evaluation and feasibility study of the project: The Netherland and Norway.

5.1.2.2.3.4.1.1 JIP2-WP4-T1-ST1: Workshop on data and DBs (M1-M6)

This task has been completed, see annual report 2018

5.1.2.2.3.4.1.2 JIP2-WP4-T1-ST2: Design and implementation of DBs (M6-M17)

- The system is operative as a demo version. One more month has been used to refine documentation and realize the virtual machines to be used by the Member States participating in the feasibility study

5.1.2.2.3.4.1.3 JIP2-WP4-T1-ST3: Interconnection of the three DBs (M13-M21)

The activity is ongoing on the COHESIVE prototype information system (CIS) for the Italian scenario.

5.1.2.2.3.4.1.4 JIP2-WP4-T1-ST4: Analysis of the systems in involved countries (M11-M22)

- The study of the systems for Italy is in progress. Data structures for data of NRL for *Listeria* and *Campylobacter* is ready. Integration with National Animal Identification and Registration System is ready. Integration and harmonization with the human part is ongoing.

- The study of the systems for Norway is in progress. Preliminary documents have been sent.

- The study of the systems for The Netherlands has just started. A first physical meeting took place in The Netherlands in July.

5.1.2.2.3.4.1.5 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". The activity is ongoing on Italian scenario.

5.1.2.2.3.4.2 JIP2-WP4-T2: Development of a platform-independent tracing framework (M1-M32)

5.1.2.2.3.4.2.1 JIP2-WP4-T2-ST1: Evaluation of all available approaches, algorithms and tools for tracing, epidemiological analysis and visualization combined with WGS data (M1-M12)

- In addition to the work performed in 2018, in 2019 we were able to integrate even more available tools. The result is a web-based interactive table-like compilation that compares the

functionalities of the software tools found. This table was published on the EJP platform together with a report end of June 2019.

5.1.2.2.3.4.2.2 JIP2-WP4-T2-ST2: Programming a software and developing an algorithm (M1-M32)

- 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

- The overall status and progress of the whole FoodChain-Lab project can be inspected at https://foodrisklabs.bfr.bund.de/foodchain-lab.

- The specific status and new software versions of the FoodChain-Lab (FCL) tracing web portal are deployed automatically to a test server accessible at https://fcl-portal-dev.bfr.berlin where new features of the tool can be seen live.

- A first demonstrator of a reporting module – the Rapid Outbreak Assessment (ROA) style – was implemented and integrated in the tracing web portal. The ROA style visualises tracing, sample and case information in a format which is suitable for publishing the results of tracing analyses in outbreak reports such as the EFSA-ECDC Joint Rapid Outbreak Assessments just in one click. For this, a JSON-based data exchange module was developed to import delivery data obtained from EFSA.

- A prototype of a data cleaning module was developed as a KNIME server workflow. This module will be implemented in the FCL tracing web portal soon.

- A prototype of an online data collection mask for tracing data was developed but is not yet integrated in the tracing web portal. The mask is available in multiple languages: German, English, Italian and Norwegian. Currently, a task and user management feature is being developed.

- During summer 2019, a web-security audit of the tracing web portal will be conducted to ensure secure handling of sensitive information.

5.1.2.2.3.4.2.3 JIP2-WP4-T2-ST3: Integration of surveillance and outbreak data into the software platform for analysis (M13-M32)

- In several presentations on FoodChain-Lab the audience highlighted the importance for integration of WGS data into tracing network visualisations.

- Meetings with public and veterinary health institutes were conducted to clarify data needs e.g. in terms of case, sample and animal movement data.

- A case study was developed in which WGS sample data i.e. the phylogenetic distance was implemented within the weight of the stations in the tracing network and visualized via different colors. The functionality was implemented in the FCL desktop application and still needs to be implemented in the tracing web portal.

- In a current outbreak investigation the sample status of companies and cases

(confirmed/probable/not outbreak strain) was implemented and displayed within the tracing network in the FCL desktop application.

- Sample data can be assessed via the data collection mask. Furthermore a data format for sample and case data was developed in the JSON format. Both can be displayed within the ROA style mentioned above. Visualisation was tested in the framework of case studies.

5.1.2.2.3.4.3 JIP2-WP4-T3: Development of a platform-independent risk modeling framework (M1-M33)

5.1.2.2.3.4.3.1 JIP2-WP4-T3-ST1: Requirement analysis (M1-M9)

Data suitable as case study is ongoing and further models will be tested. In particular, contacts were established with the Veterinary Faculty of Berlin and the Friedrich Loeffler Institute. The project partners were also asked to provide examples of quantitative risk assessments.

5.1.2.2.3.4.3.2 JIP2-WP4-T3-ST2 Implementation (M10-M30)

We have developed a prototype of the risk modelling framework in R shiny for quantitative microbiological risk assessment. The risk assessment tool is online for testing purposes. The selected server does not yet meet the requirements for continuous operation. Further discussions with the project coordinator are necessary to arrive at a final solution that also meets the sustainability requirements.. At the next project meeting in November 2019 we will demonstrate our preliminary web application.

5.1.2.2.3.4.3.3 JIP2-WP4-T3-ST3: Validation of the risk modelling framework (M19-M33)

We are currently working out a detailed validation plan and will start next month.

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

- WGS-based surveillance: a cog-wheel workshop to detect links and promote collaboration among OH-EJP projects and external initiatives, 16-17 September 2019. Dubrovnik, Croatia

- WebEx seminar to demonstrate the WP3.3 progress of a systems analysis for signal detection, Autumn 2019

- Meeting on WP2.1 at APHA, October, London

- Horizon scanning exercise, Rome, November 2019

- General project meeting, including workshops, Rome, 27-29 November 2019

- Monthly VC steering group and task leaders

- Regular VC per WP

- Regular VC with EFSA and ECDC will be planned

5.1.3 Task 4.3: Integrative support

5.1.3.1 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,

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joint priorities and opportunities for collaboration with external actors/initiatives. In September, a thematic/cogwheel workshop (#3) will be held in conjunction with the 12th International Meeting on Microbial Epidemiological Markers in Croatia. The timing of cogwheel #3 (originally scheduled for M16) was postposed to accommodate this opportunity. The theme is on institutional implementation of next generation sequencing, and the consortium is cogging with INNUENDO, IRIDA and COMPARE, who are all external initiatives that are offering cross-sectoral platforms for the integration of genomics in the surveillance of food-borne pathogens. This initiative has been very much welcomed by the stakeholders (EFSA, ECDC) who will also be attending the workshop. Planning for cogwheel #4 is underway.

A meeting has been held with the management of COMPARE, to explore how output from COMPARE can be taken on-board by the EJP. As a first idea, a COMPARE user group will be set up to allow COMPARE to get relevant input on their data hubs, and OHEJP partners to explore this resource in more detail.

5.1.3.2 *Subtask 4.3.2: Support function for integration of additional partners in ongoing JIPs*

This subtask is responsible for so called integrative missions, aimed at helping OHEJP partners that are not originally partners of our JIPs ORION and COHESIVE to join in. The first call for integrative missions was launched in April 2019. In this call, the format of the integrative missions has been redefined. In the Details of Work in the Grant Agreement, two types of mobility instruments were described – Short Term Integrative Missions (STIM), and Integrative Mentoring (IM). Consequently, there are milestones linked to the execution of such specific activities, for example MS54 and MS55. However, it has become clear that the JIP's themselves are better placed to decide on the format and target for these integrative missions, within certain boundaries. In other words, other formats and target groups are also possible, as long as it conforms with the overarching goal of integration. The funding available through WP4 (funding rate 100%) is mainly for mobility (travel grants), and has been applied for by OHEJP partners attending COHESIVE's annual meeting as well as a consortium workshop arranged at APHA. This has opened doors for further engaging with COHESIVE, both through COHESIVE's own budget but also through funding reallocated from the 2nd call for JIPs (see task 4.4), all to be co-funded by the new partners. So far, four additional countries (CZ, PT, FR, PL) have decided to formalise partnership with COHESIVE, with PT (INIAV, INSA) and CZ (VRI) having come the furthest. Discussions are also underway with IE. ORION has not yet involved additional partners but have defined a series of integrative missions where they see that such interaction would be feasible. The pickup of these activities is likely to increase as the project progresses.

5.1.4 Task 4.4: Organisation of a second round of Joint and Integrative projects and their supervision.

Together with WP3, WP4 has managed the 2nd call for projects that was launched by the end of Y1, engaging external experts in the review of three proposals for joint integrative projects. Their input has been consolidated and reported in D4.14. The output was presented at the SSB meeting in September, where the decision about projects to fund was taken. Proposals were only received for three of the four call topics under WP4, and therefore alternative means of using the allocated budget has been explored by WP4 and proposed to the PMT. This includes more funding for integration of partners into ongoing projects, support to DMP implementation in 1st call projects, arrangement of a simulation exercise in Y4 and setting up a COMPARE user group.

Furthermore, funding will be reallocated to a so-called data sharing initiative. WP4 has identified issues around national Med-Vet data sharing as an area of joint interest to partners. This is a generic issue

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but has been further flagged by stakeholders and partners in relation to uncertainties introduced when the new regulation on data protection (GDPR) was launched in May 2018. The initiative will be operationalised as a specific project that will be launched during Y2 (to be funded in Y3). The aim is to bring together the relevant legal expertise/practitioners from partners to explore the possibilities to have more harmonised approached to the interpretation of our joint legislation. The initiative has been well received both by the stakeholders and by the Programme Managers.

5.1.5 Task 4.5: Open data management

Guidance on the development of project-specific data management plans (DMP) has been produced and made available on the open science platform Zenodo, both in the form of a webinar (given in Dec 2018) and a report (D4.7). These resources can be accessed via the Open Data access point on the website (D4.9). In addition, a thematic workshop on data management was given in conjunction with the Annual Scientific meeting, jointly with WP6 (D4.8). This interactive workshop attracted approximately 40 delegates. WP4 continues to provide support to partners with respect to data management planning. A network of institutional contact points has been set up, with a dedicated group on the OHEJP website (private area). This group enables to share relevant DMP guidelines and the DMPs from all JRPs and JIPs.

WP4 has also taken the lead in the development of the OHEJP publication policy, which is one of the documents supporting the overall dissemination strategy.

5.2 Deliverables and Milestones

5.2.1 Deliverables

Del. Rel. No	Deliverable title	Submission
D4.7	Guidelines for DMP implementation	13 Mar 2019 NB webinar with partners given in M12
D4.8	Report from thematic meeting I ((Digital Innovation and Data Management)	28 June 2019
D4.9	Open data access point on the website	28 Feb 2019
D4.10	1st periodic report on ongoing JIPs	11 Feb 2019
D4.11	Report from 3rd cogwheel workshop	Postponed to 31 Oct 2019
D4.12	Instructions for final reporting of JIPs	18 July 2019
D4.13	Reports on evaluation of JIP proposals, 2nd call	31 July 2019

5.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS51	External reviewers for JIP proposals recruited	13	The external experts were invited to participate to the assessment process on January 21st, 2019. The external evaluation was achieved on May 17, 2019.
MS52	Draft instructions for final reporting of JIPs presented to SSB	14	The drafting of the Instructions for final reporting of JIPs (D4.13) is in progress. Following modification of the validation procedure, the SSB will not be requested for validation, to be carried out by the PMT in accordance with the common procedure.
MS53	All projects from 1st call have started implementing the DMP	17	Achieved. All projects have their DMP on DMPonline.be, and they will also be uploaded in the joint DMP group on the website.
MS54	Eight IM visits conducted	18	See subtask 4.3.2
MS55	Fifteen STIMs conducted	20	See subtask 4.3.2

6 WP5 - Science to Policy translation to stakeholders

6.1 Work carried out to date

The progress of WP5 activities followed the planned timeline despite a change in staff member (Donna Dietz was replaced by Ludovico Sepe as workpackage collaborator). The objectives were met.

6.1.1 Task 5.1: Identification of the stakeholders and establishment of communication links

During the second reporting year (M13-21), WP5 focused on consolidating interaction with EU stakeholders. This included also the broadening of interaction with the JIPs and JRPs of highest interest for them. Moreover, WP5 worked to establish contacts with the international stakeholders FAO, OIE and WHO, as well as with the European agencies EEA and EMA.

Communication with stakeholders was active by email, on the OHEJP website, and in several occasion by direct contact. One of the major instruments are the Stakeholders Committee Meetings. During

year 2, in proximity to the Annual Scientific meeting, the third SC meeting with ECDC and EFSA representatives was organised. During this meeting the communication strategy, the procedures to meet various stakeholders' needs, approaches to work towards the sustainability of the activities, and the state of selecting projects of the 2nd call were discussed. The feedback was overall positive. The fourth SC meeting is scheduled for October/November 2019.

Additionally, stakeholders' representatives were invited to join meetings of selected JIPs and JRPs as well as the Annual Scientific Meeting. In order to support interaction and improve understanding between consortium's projects and stakeholders' nominated contacts, WP5 representatives took part in stakeholders' meetings of the JIPs COHESIVE and ORION.

As regards communication links, during the second year one of the priorities is to establish links with international stakeholders, e.g. FAO, OIE and WHO. To achieve this, the added value of the OHEJP for them is described in a targeted document relating their policy and research needs with ongoing work of OHEJP. Furthermore, FAO representatives are invited to join a specific meeting together with COHESIVE to support exchange on ongoing activities and to identify common fields of interest.

Moreover, WP5 took advantage of the virtual "groups" on the OHEJP website to communicate with the stakeholders. The groups permit to easily share information and files. Separate groups were established to address the specific needs of national, EU and international stakeholders.

Additional targeted support to the EU stakeholders is provided through new procedures established in year 2. Management and ad hoc communication on this will be organised through a "helpdesk" (Task 5.4).

6.1.2 Task 5.2: Identification of the research needs of EU stakeholders

Using the communication links established in Y1 and consolidated in the first half of Y2 (Task 5.1), WP5 together with other OHEJP structures engaged the EU stakeholders in active dialogue concerning their research and integrative needs in the area of foodborne zoonoses, antimicrobial resistance and emerging threats.

The research and integrative needs of ECDC and EFSA were initially identified in Y1 (Deliverable 5.3) and actively kept up to date during the second reporting year (M13-M21) in the platform established for this. During the second year, ECDC and EFSA were updated on the progress made in selecting projects through the call of the 2nd round of JIPs and JRPs. To further match the stakeholders' needs with the activities of the consortium, feedback on the letters of intent was given directly by ECDC and EFSA. The candidate project leaders then incorporated the recommendations into the full proposals, and the compliance with the recommendations was assessed as part of the selection process. This procedure was positively evaluated by the EU stakeholders, and was seen as a major improvement compared to the application and selection of the first round of projects, when no consultation with key EU stakeholders was possible.

In parallel to this active communication, additional procedures were developed to increase awareness on and understanding of research and integrative needs of EU stakeholders as well as their activities and aiming to ensure complementary / to avoid duplication of work of ongoing and future activities within the OHEJP. WP5 screened stakeholders' documents in order to identify potential needs, synergies and potential overlaps with OHEJP research, and to address complemental activities. This screening was also taken advantage of to link the identified potential needs with the OHEJP research and integrative activities, and the result of this process is being incorporated in the Capacity Map (Task 5.3).

The procedure in which the policy needs and support requests are identified and communicated was discussed with ECDC and EFSA during the 3rd Stakeholders Committee Meeting. In order to improve

the flow of information, for example, it was decided to update the matrix of needs not at regular intervals, as in the original plan, but as needs emerge, to keep the procedure more flexible.

In contrast to the original plan, EU stakeholders will not regularly validate the list of needs identified for the integration into the Strategic Research Agenda. A new process is currently established where WP2, WP5 and WP7 will collaborate to consolidate the input for the SRIA.

To update more efficiently the list of their ad hoc needs, the stakeholders welcomed the proposition of the establishment of a "helpdesk" (Task 5.4). For topics transferred through this tool, potential for action will be analysed and negotiated.

6.1.3   Task 5.3: Linking of the scientific capacity available in the EJP with the stakeholders' identified needs: closure of knowledge gaps

The capacity map is an important tool linking the stakeholders' needs with the scientific and integrative results of the consortium. It highlights expertise and selected technical infrastructure within the consortium, 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 OHEJP. The capacity map targets not just the key EU stakeholders, but also national stakeholders, and supports internal collaboration and dissemination as well with a focus on the interaction on One Health topics.

During the second reporting year (M13-M21), following feedback from the stakeholders, the scope and coverage of the capacity map was narrowed in order to make it more focused. Moreover it was split into a capacity map, to depict the potential and scientific results of the consortium, and an assessment map, to illustrate the progress of the different areas covered in the capacity map in a timely manner.

The backbone of the capacity map is being implemented in the form of a relational database, for which an entity relationship diagram was drafted. To ensure timely identification of potential overlaps and synergies of OHEJP activities with similar activities of stakeholders, WP5 screens stakeholders' documents (Task 5.2) and accordingly incorporates relevant information in the capacity map.

During the 3rd Stakeholders Committee Meeting, the functionality and usage of the capacity map was illustrated and its potentiality as a tool for dissemination was recognized, especially concerning internal dissemination, and communication with national stakeholders. For key EU stakeholders, additional support through the help desk was preferred.

Importantly, the capacity map 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 the sustainability of the consortium. It thus 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).

After consultation within the PMT, the Communication Team and the OHEJP website developers, it was decided to implement the capacity map as an electronic tool built in the OHEJP website, in a way that it will complement existing databases and inventories of EU and international stakeholders. All projects within the OHEJP are encouraged to use this platform to describe their approaches, skills, tools etc. and to include links to their specific activities and outcomes.

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.

Targeted support to the EU stakeholders is provided through new procedures established in year 2. This covers the offer to provide input to scientific meetings, e.g. on outcomes from the OHEJP or through experts within the OHEJP network. Furthermore specific support might be given through commissioning specific activities or the identification of relevant expertise or platforms to fill identified needs. Management and ad hoc communication on this will be organised through a "helpdesk" (Task 5.4).

6.1.4 Task 5.4: Dissemination of new knowledge, tools and materials

WP5 implemented a variety of tailored dissemination strategies in order to meet the needs of national, European and international stakeholders, and to maximise the impact of the consortium's output. For example, EU stakeholders were updated about the scientific output of the consortium through a Targeted Report, which consisted of a brief description of results, ongoing work and publications of JIPs and JRPs, focusing on projects of highest interest for the stakeholders.

The overall dissemination strategy was discussed with the Key EU stakeholders and subjected to revision. A tool in the form of a "helpdesk" is being designed to enhance interaction with stakeholders. This tool will allow to easily retrieve focused information, as well as communicate their emerging needs. Internal dissemination and dissemination to national stakeholders will be strengthened by applying the aforementioned Capacity Map (Task 5.3). Furthermore, new activities will be started to encourage establishing national mirror groups as this is a well-recognized way to ensure impact on the national level.

Dissemination to international stakeholders (mainly FAO, OIE and WHO) is being also implemented. Awareness of OHEJP is being raised through a targeted document depicting the consortium's activities with potential to contribute to global health issues.

WP5 also supported consortium members to give input to information collection activities, namely from the FAO/OIE/WHO Tripartite Collaboration, and from JPI-AMR during the second year. With the former we are contributing to the FAO/OIE/WHO Tripartite Zoonoses Guide (TZG) by sharing information on tools and resources to establish a coordinated One Health surveillance system for zoonotic diseases in the frame of the Tripartite Surveillance and Information Sharing Operational Toolkit. The latter is a survey on resources (research infrastructure, collections of biological material and databases) that are relevant for antimicrobial resistance research, promoted by ZonMw (the Netherlands Organisation for Health Research and Development) , on behalf of JPI-AMR and the consortium VALUE-Dx.

6.2 Deliverables and Milestones

6.2.1 Deliverables

Del. Ref.	Deliverable title	Submission
D5.4	Report on a capacity map and indicators developed	04 Feb 2019
D5.5	Report on the dissemination strategy developed	04 Feb 2019
D5.6	First annual report on dissemination activities to international stakeholders	04 Feb 2019

6.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS66	Production of a capacity map with an associated indicator to measure the impact of integration activities developed	12	Concept delivered and presented to stakeholder committee

7 WP6 - Education and training

7.1 Work carried out to date

The progress of the WP6 funded PhD projects and scientific missions combined with the success of the WP6 training events will help to create a generation of scientists to build a One Health community.

7.1.1 Task 6.1: Short-Term Missions

The Short Term Mission (STM) 2019 call was launched in M13, and a total of 5 applications were received. Each application was reviewed by three independent expert reviewers selected by the Scientific Steering Board. In total, 4 Short Term Missions were selected for co-funding.

The Short Term Mission 2020 call was launched in M17 and was closed in M20. To increase the success of the 2020 call, not only was the co-funding available for the missions increased, but so was the flexibility of when these missions can take place in 2020. The call closed in M20 and 6 applications were received and will be sent off to reviewers. The funding arrangements will be reviewed and adapted with the aim to increase the number of STM applications received.

7.1.2 Task 6.2: Workshop programme (satellite to Annual Scientific Meetings)

The ASM Satellite Workshop 2019 call was closed in M14 and the validated procedure was followed to select the organisers.

The event was co-organised by SVA, Sciensano, and University of Surrey, and the workshop theme was 'Digital Innovation and Data Management'. The workshop was hosted by Teagasc at the ASM in Dublin on 21st May 2019, the day before the Annual Scientific Meeting (ASM). The workshop hosted 40 delegates from across our consortium, speakers in the field of Artificial Intelligence and Machine Learning from the University of Surrey, and speakers in the field of Data Management, from Open Aire, Sciensano and the University of Surrey.

The workshop aimed to provide a platform to discuss digital innovation within the One Health EJP consortium. In addition to developing an understanding and interest in data management plans, the event provided an excellent opportunity for delegates to understand how data management plans can be applied to their own research.

The ASM Satellite Workshop 2020 call was launched in M16 and the deadline closed in M18. The validated procedure was followed to select the organisers. The event will be organised by RIVM (the Netherlands) and the workshop theme will be 'New and Emerging diseases'

7.1.3 Task 6.3: 'One health' Summer School for medical and veterinary science

The Summer School 2019 call was closed in M13 and the validated procedure was followed to select the organisers. The 'One Health' Summer School 2019 was selected to be organised by the University of Surrey (UoS) in collaboration with Public Health England (PHE) and Wageningen Institute.

This event was an open event, and proved to be very popular with over 130 applications for the 20 available places.

12/20 delegates belonged to participating OHEJP institutes from across the EU e.g. UK, Italy, France, Spain, Poland, Hungary; but also extended to outside our consortium hosting delegates from Russia, India, Switzerland and Thailand.

The Summer School 2019 theme was 'Approaches towards One Health Operationalisation' and was hosted by UoS and Chatham House from 18th August to 30th August 2019 (M20), in addition to pre-online learning material and follow up tasks and projects.

The Summer School boasted an excellent teaching programme consisting of a large selection of international experts who have a wealth of experience in academic institutions, renowned research centres, European one health networks, government public health departments and programmes targeting a One Health approach across the European Union in areas of human health, animal health and environmental health.

The summer school aimed to provide:

(1) an understanding of the complexity of health challenges and how to use a One Health approach to address these.

(2) ways to identify, characterise, and quantify the drivers of value from One Health approaches across programmatic areas (e.g. prediction, detection) and stakeholders.

(3) an ability to recognise the methods and operational approaches for effective preparedness and response to One Health threats.

(4) an understanding of the application of robust monitoring and evaluation tools to implement One Health interventions.

Students learned a number of technical and organisational skills that they practiced on a number of case studies. The programme delivered an introduction to One Health basics, prediction approaches, analyses of integrated disease surveillance, outcomes research, risk management, and decision quality.

The Summer School 2020 call was launched in M16 and the deadline was at the end of M17, and the validated procedure was followed to select the organisers. The event will be organised by Wageningen BioVeterinary Research (the Netherlands) which will build on the curriculum from the first summer school.

7.1.4 Task 6.4: Doctoral Training Programme

The second call of the doctoral programme was launched. In total, 24 applications were received, and these were processed following the validated procedure to select the projects to be funded. Each application was reviewed by three independent expert reviewers selected by the Scientific Steering Board. In this second call, a further 12 projects were selected.

In the first call, 4 PhD grants were successfully selected. Students were successfully recruited for two of these PhD grants (VISAVET-UCM and Sciensano). These commenced in M13 and M14, respectively.

In total, the One Health EJP has successfully co-funded 16 PhD grants, which is 4 more than originally anticipated. All PhD grants will commence before M25 of the One Health EJP.

7.1.5 Task 6.5: One-Health Continuing Professional Development (CPD) Module

The CPD 2019 call was closed in M16, and the validated procedure was followed to select the organisers. The organisers were selected in M18. The first CPD module will be a two-day event in early 2020 organised by RIVM, with the theme 'outbreak preparedness', with a further longer CPD event planned later in 2020.

The CPD 2020 call was launched in M17 and the deadline to apply is in M21.

7.1.6 Task 6.6: Communications workshop and media training

The Communication and Media workshop call was launched in M14 and the deadline was in M18. The validated procedure was followed to select the organisers. The Bulgarian Food Safety Agency will host and organise this two-day event in 2020, collaborating with the University of Surrey.

7.2 Deliverables and Milestones

7.2.1 Deliverables

N/A

7.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS75	Launching of annual call for Short Term Missions n°1	M16	Call for Short Term Missions was launched on 31st January 2019

8 WP7 – Sustainability

8.1 Work carried out to date

8.1.1 Task 7.1: Gathering Stakeholders´ Needs and Expectations

This task received the input from WP5 which identified the stakeholders which are relevant for the EJP and for the SRA (report 5.1). A SWOT analysis has been conducted. A questionnaire was developed and sent to a pool of relevant stakeholders (ca. 200 valid e-mail addresses: programme owners, programme managers, project management team, EFSA, ECDC, scientists involved in the project…). The deadline of the survey was scheduled on May 10th 2019. Ca. 40% of the stakeholders answered to the survey. Based on these answers, a sound SWOT analysis has been developed showing the strengths, weaknesses, opportunities and threats of the EJP. The SWOT's outputs will help WP7's team

to find out the most appropriate scenario to make the EJP sustainable which match with the stakeholders' needs.

8.1.2 Task 7.2: Strategic Research and Innovation Agenda (SRIA) 2021-2030 (SRIA 2021-2030).

Members of the WP7 took part in some activities related to the definition of the SRA, as facilitator in the expert meeting. The updated research topics defined will be one of the inputs for the task 7.2.

8.1.3 Task 7.3: Making the EJP sustainable through other funding and/or legal basis

The WP7 team has monitored the calls for funding which could correspond to the scientific scope of the OHEJP. The calls are mostly European and come from Horizon 2020 (societal challenges, Marie Sklodowska Curie actions), JPI AMR, JPI Healthy diet for a healthy life, IMI2.

The negotiations on the next framework programme, Horizon Europe have been monitored.

Indeed, Horizon Europe could be a financial mean to ensure the sustainability of the EJP. The negotiations regarding Horizon Europe's partnerships have been particularly monitored in close collaboration with programmes owners.

8.1.4 ask 7.4: Making the bridges between EJP's beneficiaries and stakeholders sustainable

This task has been initiated during Y2, by setting up a collaboration with the Department of Social Sciences and Business, Roskilde University who will cofund a PhD in Global studies. The PhD will carry out the tasks under 7.4 with an aim to analyse One Health implementation across the EU-member states involved in the OHEJP, and produce a roadmap for institutionalisation of One Health. The PhD will commence in September 2019 and the exact work plan for Y3 will then be drafted.

8.2 Deliverables and Milestones

8.2.1 Deliverables

N/A

8.2.2 Milestones

Mil. Ref.	Milestone title	Expected Delivery/ Achievement Month	Notification
MS97	Analysis of the Stakeholders´ Needs and Expectations inputs collected	M12	SWOT analysis has been conducted and a summary of the preliminary results established

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