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Third periodic report on PhD Projects

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INTRODUCTION

To complement the One Health EJP's (OHEJP) research and integrative activities, between 2018 and 2019, 16 PhDs were co-funded by Work Package 6 (each funded 44% by the EU). The research focus of the individual PhD projects falls within at least one of the three research domains of the OHEJP: foodborne zoonoses, antimicrobial resistance and emerging threats, and all align with the Strategic Research Agenda. Each PhD project has at least two One Health EJP consortium partners collaborating on the project.

An additional PhD funded through Work Package 7 (WP7, Sustainability) also commenced in 2019. This PhD lies in the field of social sciences and public health. The progress of this PhD is monitored and assessed differently, and further details can be found in the report.

The PhD projects provide opportunities to explore and share skills, expertise and knowledge from the OHEJP consortium, therefore accelerating both the rate and quality of research in addition to developing the One Health scientific leaders of the future.

There is significant scope for inter-disciplinary networking among One Health EJP partners in addition to the interaction with the Joint Research Projects (JRPs) and Joint Integrative Projects (JIPs). The JRPs and JIPs have expertise that can support the PhD students, and provide opportunities to explore and share skills and knowledge, accelerating both the rate and quality of the research. These interactions help to bring the physical, biological, and social sciences together, and allow greater flexibility in the PhD projects to ensure innovative hypothesis driven research. The multi-country and inter-disciplinary approaches help inform decisions on market viability and EU policy relevance of project outputs.

The PhD projects provide excellent added value to the OHEJP, including improved integration (both geographical and interdisciplinary), and an opportunity to develop the next generation of scientists in One Health contributing to the sustainability of the One Health approach.

Each year, the PhD projects report on the research activities, progress, results, risks, ethics, and impact for the previous 12-month reporting period. A record of the dissemination activities and soft skills training that took place during this period is also reported. This deliverable report contains the 12-month reports for each PhD project for months 25 to 36 (Jan-Dec 2020) of the One Health EJP. The purpose of this deliverable is to monitor, report and disseminate the progress and results that can be shared publicly.

1. Summary of the PhD projects performance

In 2020, 15 of the 17 projects incurred delays caused by the COVID-19 global pandemic which impacted the feasibility of meeting the deadlines of the planned deliverables and milestones. The impact of the pandemic on the planned deliverables and milestones is described in further detail in each PhD report in this deliverable. Deliverables or milestones that had not been met on time were assigned new delivery dates within the timeline of the project.

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1.1. Overview of project deliverables and milestones

Deliverables in 2020	Total	Finalised	Delayed to 2021
Number	24	14	10
Percentage	100%	58%	42%

Deliverables in 2020	Due	Finalised	Delayed to 2021
ECO-HEN	3	2	1
LIN-RES	4	1	3
HME-AMR	0	0	0
KENTUCKY	2	1	1
METAPRO	0	0	0
PEMbO	2	2	0
MACE	3	0	3
DESIRE	0	0	0
UDoFRIC	1	1	0
WILBR	0	0	0
EnvDis	3	3	0
AptaTrich	1	1	0
VIMOGUT	1	0	1
ToxSauQMRA	0	0	0
TRACE	0	0	0
Codes4Strains	4	3	1
SUSTAIN (WP7 PhD)	Deliverables are not linked to specific tasks, and instead are defined and assessed by Roskilde University. See 12M report for further details.		

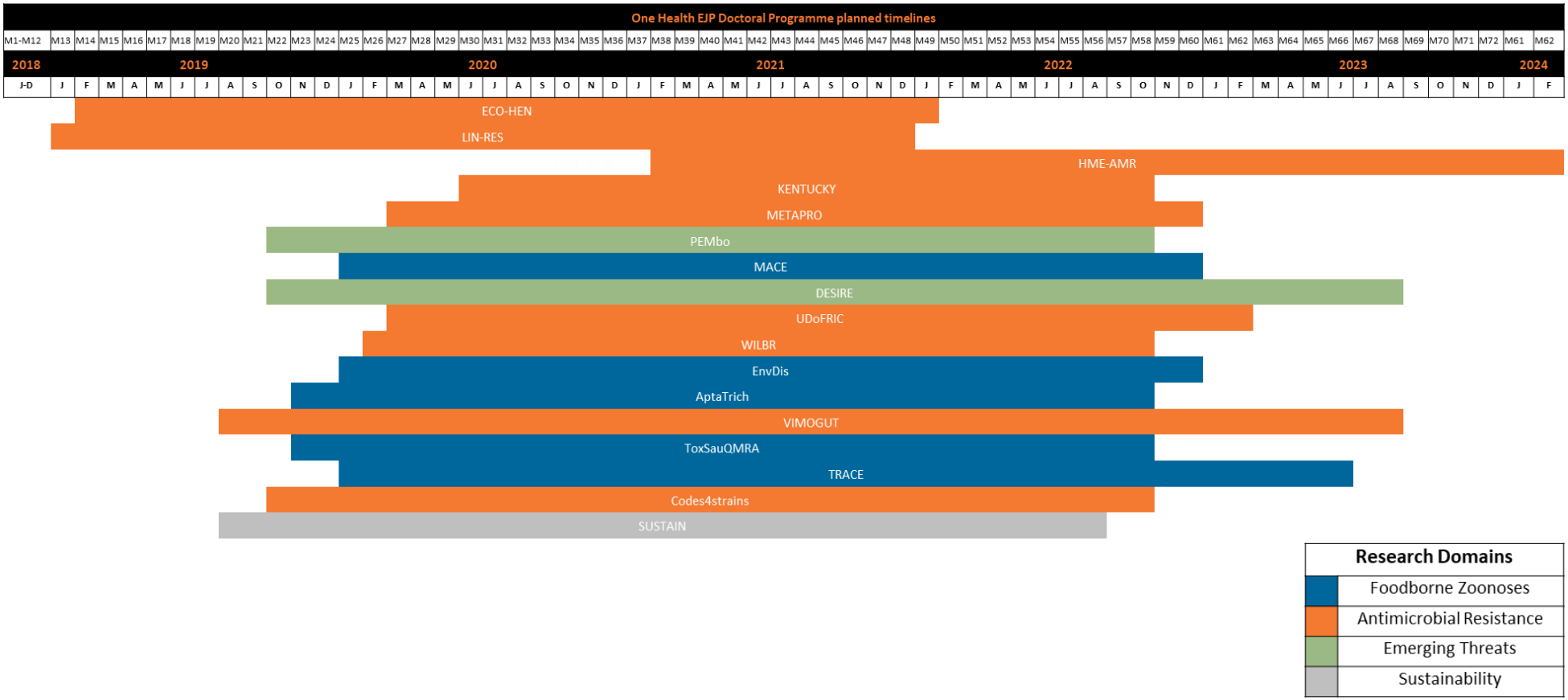
50% of planned milestones (18/36) were planned for months 25 to 36. As with the deliverables, many of these milestones were impacted by the COVID-19 pandemic. A new delivery date has been assigned within the project timeline.

The activities, deliverables and milestones reported in the 12M reports are those for the reporting period of Month 25- Month 36 of the One Health EJP (January – December 2020).

1.2. PhD project timelines and research domains – an overview

The planned timelines for the One Health EJP PhD projects is shown below.

Please note, the delays reported in this deliverable are associated with intermediate deliverables and milestones during the project. These delays have not had an impact on the expected end date, unless otherwise indicated. At this stage, it is premature to determine the impact on the expected end date, and a number of actions have been taken to alleviate the impact of these delays.



12M REPORTS OF PhD PROJECTS FOR MONTHS 25-36

2. PhD01 ECO-HEN

2.1. Summary

During the 12 months, from February 2020 to January 2021, the student carried out many activities.
At the beginning of the year the student participated in a research meetings programme called VISAVET JOURNAL CLUB, organized by the research institute itself. This activity consist of seminars in which a scientific article is presented and discussed among colleagues. It allows not only to learn to critically analyse an article, but also to expose your own opinion and argue with co-workers.
She also joined in May the annual scientific meeting that took place online. During this congress, the student had the opportunity both to share part of her work and to listen to the work of other colleagues. During the congress, a poster with the title “First report of trimethoprim resistance gene *dfrA36* on an *IncF*-plasmid in *Escherichia coli* isolated from day-old chicks” in which a work was presented that covered both laboratory analysis and computer one. During this congress, the student also participated in the thesis contest in 3 minutes, in which she was able to make a summary of her thesis and listen to the projects of her colleagues. Both when writing a poster and in the contest, the student was trained in writing scientific articles and in disseminating a work orally.
During this year, several training courses and activities have also been held.
In March the student participated in a course on epidemiological aspects and statistical studies of a research work, in which she learned what type of study to use for each type of data, as well as using software for statistical analysis.
In May, the 9-month online specialization diploma in bioinformatics analysis was completed. In these postgraduate studies, the student was able to train in the bioinformatic analysis of massive biological data, interpretation of the data from an omic experiment and plan their subsequent analysis by means of bioinformatics tools, etc.
In June, the student successfully completed a spreadsheet training course with EXCEL in which she was able to learn how to create attractive formatted spreadsheets, do automatic calculations using formulas and functions, and make graphs that allow you to visualize data effectively. In addition, another online course ended in December, the objective of which was to introduce the student to data visualization and the development of our own visualizations in Python In which the aspects to consider creating effective data visualizations and how to select the type of graph that best suits the data we want to show were discussed. Later it will be detailed how to create data visualizations in Python using different libraries.
In October, the student participated in a workshop organized by OHEJP "Communications and Media Workshop"
in which he had the opportunity to discuss with his colleagues how science achievements reach people and contribute to a better life, as well as listening to professionals talking about the importance of communication in science, among other things.
Finally, the student has attended both seminars and presentations of doctoral thesis of colleagues within the University, thus being able to listen to other ideas and other works that can enrich hers.

2.2. Overview of project progress

Tasks until WP4 were achieved on time.

WP5 and part of WP6 have been delayed for various reasons. In WP5 we wanted to carry out additional laboratory experiments because we found a newly described gene on the chromosome, in a large conjugative plasmid. The experiments we wanted to carry out required a laboratory, and since we were unable to use the laboratory for three months due to COVID-19, we had to delay those experiments. In addition, we have had technical problems regarding the extraction of plasmid DNA, since the concentration we obtained was not optimal. Now, the experiments that we want to carry out, and with which we believe that concentration problems will be solved, are already designed. Carrying out these

experiments is being delayed due to the snowstorm that makes it impossible for us to attend the center and the laboratories. WP6 (T6.4) has also been delayed by the COVID-19 quarantine. The isolates we wanted to sequence could not be sent until October, which delayed the manuscript. At this time, with the isolates already sequenced and analyzed, we are preparing this manuscript.

WP8, scheduled for 2021, has been able to go ahead. In 2021 the previous WPs that are unfinished will be finished, while the realization of WP8 takes place.

2.3. Progress of the research performed in the PhD project and key scientific results

During the reporting period (February 2020 to January 2021) the PhD work was focused on the in deep analysis of the surroundings of the trimethoprim resistance gene *dfrA36*, which was identified as the gene responsible of trimethoprim resistance in some isolates of *Escherichia coli* obtained from day-old chicks. In special, the big plasmid harboring this gene is under analysis.

In addition, the in deep characterization of the isolates obtained in selective media (with cefotaxime and with ciprofloxacin, respectively) is also in progress. The aim is to check if the absence of AMR use along the rearing and production periods affect their persistence on the farm throughout the production cycle, as well as the characterization of possible mobile genetic elements for these AMR determinants.

The key scientific results for the reporting period are:

WP5. Reconstruction of plasmids spreading AMR genes from animals' to egg shell's isolates

The objective of this WP is to reconstruct the plasmids responsible for dissemination of AMR genes across isolates from different sources. It is widely recognized that the epidemiology of certain AMR genes (e.g. those conferring resistance to critically important antimicrobials in human medicine such as third-generation cephalosporins and colistin) is linked mainly to AMR gene spread via plasmids rather than via bacterial clones and thus knowledge on the AMR plasmids is essential to describe the flow of AMR in different ecological niches. M26-M27 (feb / mar – 2020).

We have found a trimethoprim resistance gene in a large conjugative plasmid on, until now, four probably identical isolates belonging to the same sampling in day-old chicks. The gene, called *dfrA36,* was firstly described on the *Escherichia coli* chromosome isolated from calves in 2019. This information was presented in a poster at OHEJP ASM 2020.
This conjugative plasmid is being used as an example of a plasmid entering in the farm with day-old chicks. Details of the laboratory work are the following:
We are extracting the plasmids from the isolates with a commercial kit and performing an electrophoretic run for plasmids separation. Then, single bands are cut, and plasmids recovered and studied by PCRs to detect the *dfrA36* gene. When performing the PCR analysis, we could see that the different bands that we observed in the gel corresponded to the same plasmid, since we saw that the *dfrA36* gene was amplified from all the bands.
Furthermore, a plasmid conjugation experiment has been carried out to show that the plasmid in which the gene is found is conjugative what has been demonstrated after conducting the experiment.

From now on, we want to continue isolating the plasmids from these bacterial isolates and digesting them with the *NotI* enzyme. Once the plasmid is digested and linearized, we want to perform a long read sequence in order to reconstruct the entire plasmid and study other possible resistance genes that the mobile genetic element could carry which is now complicated since we have the sequences divided into contigs and it is usually not possible to reconstruct complete plasmid sequences from short read sequence data.

In addition, after sequencing other isolates from the farm with a similar resistance profile (simultaneous resistance to trimethoprim, sulphonamides and chloramphenicol), we found another isolate also with the same *dfrA36* gene that we had already detected by PCR.

- *Deviation: WP5 was planned to be done in February-March 2020. Due to both problems in plasmid DNA extraction, as well as the inability to access the laboratory due to the COVID-19 pandemic, the tasks of this WP was delayed to July 2020.*

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In addition to this delay, we wanted to carry out a transformation of the plasmid into electrocompetent cells, which has taken more time, so the long read sequencing has been delayed to 2021. In addition, with more additional experiments that we believe will inform the study, the WP5 will be lengthened and done at the same time as other WPs.

Further to experimental work, plasmids have been reconstructed in silico. Some plasmids that had resistance genes or structures of interest (such as integrons) have been identified, reconstructed, and tracked between the different isolates (Deliverable D-E14-5.1.).

WP6. Flow of AMR isolates between animals. The objective of this WP is to follow the dynamics of AMR, both, isolates and associated platforms, from day-old chicks to pullets and laying hens. M28-M33 (april / sept – 2020).

T6.1. Checking on the isolates data base looking for non-sequenced isolates putatively needed for this WP.

We are studying the resistance profiles of non-sequenced isolates, to identify relevant isolates for DNA sequencing to follow up the spreading of resistance genes and the putative platforms, especially plasmids Clonal dissemination of resistance genes will be also considered. In deliverable D-E14-6.1, we made a list of isolates to be sequenced that we have already sequenced in November 2020: L1M2-COL02, L5M5-02, L2M3-CTX08, L2M4-CTX01, L3M3-CIP03, L1M6-04, L1M3-CIP09, L3M3-CTX07, L1M1-CIP04, L1M3-CIP05, L3M3-CTX02, L4M4-CTX03, L5M5-08 and L2M2-CTX05.

T6.2. WGS of the isolates identified on T1.

After sequencing the isolates, the sequences in fastq format were converted through the SPAdes software to fasta format.

T6.3. Bioinformatic analysis of the WGS.

The sequences were analyzed using different software's to identify antimicrobial resistance genes, possible origins of plasmid replication, and MLST profile. They were also annotated with prokka software to identify integrons and other mobile genetic elements. These new sequences were compared with those that were already sequenced and previously analyzed, to study the same resistance profiles in isolates throughout the egg production cycle.

T6.4. Data analysis and scientific manuscript preparation.

The manuscript is under preparation.

- *Deviation: Due to the impossibility of performing laboratory tasks until during the COVID-19, the isolates were taken to sequence later, and the manuscript is delayed until January 2021.*

WP7. Flow of AMR isolates from animals to egg shells. WP7 takes place over the second and the third annual period of the OHEJP and is devoted to analysing relationships between AMR bacteria of laying hens and eggs. M34-M37 (oct 2020 / jan – 2021).

T7.1. Checking on the isolates data base looking for non-sequenced isolates putatively needed for this WP.

As was done in wp6 with animal isolates, possible egg isolates were evaluated to be sequenced, paying attention to the resistance profiles that could be associated with structures such as plasmids. (Deliverable D-E14-7.1)

T7.2. WGS of the isolates identified on T1.

Isolates from eggshells are being analysed and their resistance profile compared with those of other isolates, to study the dynamics of antimicrobial resistance genes.

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2.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD01-AMR2-ECO-HEN	D-E14-5.1.	A list of plasmids putatively present on *E. coli* isolates	M27	M31		Delay due to the COVID-19 pandemic.
	D-E14-6.1	A list of *E. coli* from animals isolates to be sequenced	M28	M33		Delay due to the COVID-19 pandemic
	D-E14-6.2.	Manuscript	M33	N/A	M37	Under preparation Delayed due to laboratory work.
	D-E14-7.1.	A list of *E. coli* from eggs to be sequenced	M37	M36		

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD01-AMR2-ECO-HEN	M-E14-3	New sequenced *E. coli* isolates from animals	M33	Yes		
	M-E14-4	New sequenced *E. coli* isolates from eggs	M37	Yes		Achieved M36

2.5. Soft Skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
VISAVET JOURNAL CLUB	Discussion of scientific publications	15/01/2020 05/02/2020 26/02/2020	VISAVET-UCM
Epidemiology workshop	Epidemiology and statics	03/03/2020 04/03/2020 02/06/2020 09/06/2020	UCM
OHEJP ASM 2020 Conference	Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats	27-29/05/2020	One Health EJP
CaM workshop	Communications and Media Workshop	05-06/10/2020	One Health EJP

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- VISAVET JOURNAL CLUB consists of sessions in which a scientific article is presented and discussed among colleagues. It allows not only to learn to critically analyse an article, but also to expose your own opinion and argue with co-workers.
- The epidemiology workshop is part of the student's CPD. In the workshop, you learned how to design a scientific study depending on the objectives you want to achieve, also being able to assess whether the studies presented in the published scientific articles followed the correct statistical model. The student also learned to use some very useful statistical software for future studies.
- The conference allowed the student to be in contact with professionals in the same field. Also, the student learned to make a poster for a communication, which will be very useful in her scientific career.
- CaM workshop allowed the student to learn methods to improve communication within a scientific project.

2.6. Publications and patents

- Irene Aldea, Alicia Gibello, and Miguel A. Moreno. First report of trimethoprim resistance gene *dfrA36* on an IncF-plasmid in *Escherichia coli* isolated from day-old chicks. Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 9.

2.7. Impact and Relevance

During this twelve-month period (January-December 2020) the PhD project has not been in direct collaboration with external institutes; nevertheless, the PhD student is in close contact with VISAVET researchers working in other ongoing OneHealthEJP projects like ADONIS, DISCOVER and MATRIX for improving their bioinformatics skills.

2.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
This project is focusing on laying hens. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the layers hens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so, please comment on any implications for the animal. Please state the 3Rs aspects of this work. Please describe how the animals' welfare are protected and considered (e.g. if the chicken is affected when taking samples, even if the work is dealing with faeces as these types of study can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals).	To the present moment, all the work has been performed with isolates stored in our laboratory and neither new farm visits nor new environmental sampling have been performed. Consequently there are no implications for animals regarding health or welfare.

2.9. Impact of COVID-19 crisis on project

Laboratory work and full access to computer tools have been stopped or diminished from March to June

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2020 due to COVID-19 pandemic, and in January 2021 , challenging weather conditions consequently a delay of all the milestones and deliverables scheduled from March 2020 for approximately four/five months must be applied. Indeed, now it is not possible to know if additional restrictions will be established during next months.

2.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	Yes*
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No**
Other risks (please describe)	N/A

Additional information:
*The DTU supervisor, Dr. Valeria Bortolaia is no longer working at DTU. A new DTU supervisor, Dr. Pimlapas Leekitcharoenphon, has been proposed.

** Not included those related to the COVID-19 pandemic

2.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Not applicable

2.12. List of Dissemination activities

Name of the activity:	One Health EJP ASM 2020 (presentation of a poster and participation in 3MT competition)		
Date:	May 27th - 29th, 2020,		
Place:	Online meeting		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*

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Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750+***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

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3. PhD02 LIN-RES

3.1. Summary

Between January 2019 and February 2020, 1325 faeces samples (from cattle, pigs or poultry) and 148 nasal swabs samples (pigs) collected in Belgium in 2019 for the official monitoring of antimicrobial resistance were analysed on blood agar supplemented with linezolid to select resistant strains (selective monitoring). One hundred forty-eight (148) (2 *Staphylococcus aureus* and 1 *Staphylococcus sciuri*, 143 enterococci (*Enterococcus faecium, faecalis, hirae, durans, gallinarum, asini, casseliflavus and saccharoliticus*), 1 *Pediococcus pentosaceus* and 1 *Lactobacillus johnsonii* (not in collection)) resistant to linezolid were isolated from these samples. Moreover, 3 *S. aureus* (2 from 2016 and 1 from 2013 from the Belgian monitoring of MRSA in pigs) were added to the collection of linezolid resistant bacteria. Five strains isolated from human samples (urine, blood culture or skin lesion), 1 *S. aureus* and 4 enterococci, were received from our Belgian partner (task 1.1). All the isolates (155) were completely sequenced (tasks 2.1 and 2.2). Three different resistance genes, *cfr, optrA* and *poxtA,* were found in this collection as well as mutations in the 23SrRNA gene conferring resistance to linezolid (task 2.1). A cgMLST analysis was conducted to study the relatedness of these isolates and compare with published sequenced of linezolid-resistant isolates.

3.2. Overview of project progress

The Annual Work Plan provided to do the sampling and the collection of bacteria (task 1.1) for the Y2 and this task was finished on time. The task 2.1, NGS resistance analysis, and the task 2.2, NGS subtyping of the strains and associated host specificity, was finished on time. The task 2.3 isn't started yet but will start in 2021 (postponed to ensure a faster submission of the first LIN-RES paper). The task 2.4 scheduled for the next period (M33-M48) is canceled because a very high number of isolates (~150) were found through selective monitoring performed for WP1 on samples gathered from official MRSA and Enterococci monitoring from food-producing animals in Belgium. Therefore, the project will focus on the NGS analysis of all these isolates and the investigation of putative risk factors associated with the numerous positive farms from which these isolates came from. The task 3.1 is completed (genetic organization of the contigs carrying LIN-RES genes as well as incompatibility groups were investigated) and the task 3.2 was already started: establishment and test of the protocol and a first conjugation experiment was performed to assess the transferability of linezolid resistance genes.

3.3. Progress of the research performed in the PhD project and key scientific results

During January 2020 and December 2020, 40 nasal swab samples and 392 faecal matter samples from 2019 were screened for linezolid resistant bacteria. Linezolid resistant bacteria were analysed by MALDI-TOF, broth micro-dilution. All the linezolid resistant isolates collected during the selective monitoring were sequenced by whole genome sequencing (WGS), assembled and analysed. A cgMLST analysis was conducted to study the relatedness of these isolates and compare with published sequenced of linezolid-resistant isolates. A paper is currently under writing.

The Annual Work Plan provided to do the sampling (1325 faeces samples (from cattle, pigs or poultry) and 148 nasal swabs samples (pigs) collected in Belgium in 2019 for the official monitoring of antimicrobial resistance) and the collection of bacteria resistant to linezolid (147 isolates from the official monitoring of 2019, 3 isolates from previous monitoring's and 5 human isolates from collaborators have been collected and stored for further analysis) (task 1.1) for the Y2 and this task was finished on time. The task 2.1, NGS resistance analysis of all isolates, and the task 2.2, NGS subtyping of the strains and associated host specificity, was finished on time. The task 2.3 (Research of genetic scars of horizontal transfer or recombination events) isn't started yet but will start in 2021 (postponed to ensure a faster submission of the first LIN-RES paper). The task 2.4 (investigation at the farm level) scheduled for the next period (M33-M48) is canceled because a very high number of isolates (~150) were found through selective monitoring performed for WP1 on samples gathered from official MRSA and *Enterococci*

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monitoring from food-producing animals in Belgium. Therefore, the project will focus on the NGS analysis of all isolates and the investigation of putative risk factors associated with the numerous positive farms from which these isolates came from. The task 3.1 is completed (genetic organization of the contigs carrying LIN-RES genes as well as incompatibility groups were investigated) and the task 3.2 (Laboratory experiments to demonstrate transferability of linezolid resistance genes and estimate transfer rates) was already started: establishment and test of the protocol and a first conjugation experiment was performed to assess the transferability of linezolid resistance genes.

3.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD02-AMR2/3/6-PhD LIN-RES	D-E27-2.3	Genetic resistance profiles and subtypes of strains sequenced until M36	M36	M39	N/A	Completed (confidential) will be submitted before M39 (accepted changed delivery date)
	D-E27-2.4	Results of in silico analysis of genetic scars of horizontal transfer or recombination events	M36	N/A	M48	Postponed to ensure a faster submission of the first LIN-RES paper; will be delivered together with in vivo transfer analysis results.
	D-E27-3.1	Results of in silico analysis of transferability	M36	M39	N/A	Completed (confidential) will be submitted before M39 (accepted changed delivery date)
	D-E27-3.2	Poster or oral presentation at an international conference of the results of the linezolid selective monitoring during 2019 in Belgium	M36	M36	N/A	These results were showed at the virtual OHEJP conference between the 26-29 May 2020 (confidential). This deliverable has already been send.

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PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD02-AMR2/3/6-PhD LIN-RES	M-E27-3	Synthesis of genetic resistance, subtyping and transferability markers analysis of the linezolid resistant bacteria collected.	M36	Yes	M36	The results are currently implemented in the first LIN-RES paper (writing in progress).
	M-E27-4	Poster or oral presentation at an international conference of the results of NGS analysis of linezolid resistant bacteria, including the in-silico analysis of transferability.	M36	Yes	M36	This milestone by replaced by another poster presentation (see D-E27-3.2) which was well achieved.

3.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Vulgariser sa recherche à l'écrit	Scientific vulgarisation	14 Decembre 2018	ULB
Vulgariser sa recherche à l'oral	Scientific vulgarisation	14 Decembre 2018	ULB
Galaxy live training 2019	NGS data analysis	4 April 2019	Sciensano
Formation sur l'encadrement d'équipe	Management	24-25-26 June 2019	ULB
Midi Cross-Experience - Infographie & data-visualisation	Data visualisation	13 Decembre 2019	ULB

3.6. Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 147.

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3.7. Impact and Relevance

This project allowed us to strengthen the links with the National Reference Center of *Staphylococcus aureus* in Belgium. It also, through the different presentations in international meetings, raised the awareness of the other countries and inside Belgium about the resistance to linezolid and gave the opportunity to share the scientific expertise between labs. The different conferences organized by the EJP gave the possibility to have a better visibility among the scientific community. The link with the collaborators (ANSES, Veterinair Microbiologisch Diagnostisch Centrum (VMDC) Utrecht University, Bundesinstitut für Risikobewertung (BfR) have been reinforced through this project and thanks to the Annual Scientific Meeting's.

3.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
Satisfactory ethics self-assessment Human biological samples The beneficiary must confirm that appropriate authorizations will be sought to collect the Human samples. Personal data processing The beneficiary must confirm that no personal data will be collected as part of the project; otherwise the GDPR (EU 2016/679) must be applied and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided. Animal This project is focusing on laying hens. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the layers hens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the chickens are affected when taking samples, even if the work is dealing with faeces as these types of study can, this can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals). Please provide a statement on the 3Rs aspects of this work If Ethical Approval is required, please state which Research Ethics Committee this will be sent to	Since we gather a lot of samples through official sampling campaigns, we will finally not need to sample by ourselves animal or human samples. Then, no more ethical issues are linked to the LIN-RES project.

3.9. Impact of COVID-19 crisis on project

Not Applicable

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3.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

3.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

We work with the FASFC (Federal Agency for the Safety of the Food Chain) and we work with samples of the nationwide antimicrobial resistance surveillance program.

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3.12. List of Dissemination activities

Name of the activity:	One Health EJP ASM 2020 (presentation of a poster and participation in 3MT competition)		
Date:	27-29 May 2020		
Place:	Digital conference		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*Yes*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	***Yes***	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750+***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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Name of the activity:	OneHealth@Sciensano online conference
Date:	27 November 2020
Place:	Virtual conference

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

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***+/-100***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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4. PhD03- HME-AMR

4.1. Summary

Project commencement delayed.

4.2. Overview of project progress

There has been a substantial delay to the commencement of this project due to recruitment issues and now the COVID-19 crisis. The first student who was recruited for the project in November 2019 changed his mind just after starting. This was followed by two further recruitment rounds. A suitable, highly qualified candidate, Mariel Aybar, was offered and accepted the position but Ms Aybar was unable to procure a study visa for Ireland due to travel restrictions, prioritisation of visa and the lack of an Irish Embassy in Peru. A revised start date of October 2020 was proposed with the final months of the project after the One Health EJP concluding to be funded by Teagasc. Unfortunately it was not possible to address the visa issue in a timely manner so the position was advertised once again. A candidate has accepted the position and is now in the processing of registering and should commence the February 2021. Furthermore, Dr Burgess and Dr Morris have spoken with Geological Survey Ireland (GSI) regarding the identification of suitable sampling sites to ensure the project sampling campaign can be started once the student takes up the position and are also collaborating with a nationally funded project to procure control samples in areas of high zinc application. The project has also been highlighted in internal workshops within Teagasc to encourage cross programme linkages.

4.3. Progress of the research performed in the PhD project and key scientific results

Project commencement delayed.

4.4. Progress of the research project: Deliverables and Milestones

- No deliverables due in months 25-36.

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD03-AMR2.1-HME-AMR	1	SOP in place for sampling culture based analysis	M27	No	M40	Delayed due to late recruitment of PhD student
	2	Sequences of sufficient quality obtained from metagenomic analysis	M35	No	M48	Delayed due to late recruitment of PhD student

4.5. Soft skills and Continuing Professional Development Training

Not Applicable. Student recruitment delayed.

4.6. Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 27.

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4.7. Impact and Relevance

Dr Burgess and Prof Morris currently collaborate on the Irish EPA funded project AREST which examines antimicrobial resistance in the environment and contribute to One Health orientated AMR research and policy making at a national and international level. This project is highly complementary to those activities and will facilitate an increasing collaborative relationship between the research groups. Dr Burgess has worked previously with Dr Johannessen of the Norwegian Veterinary Institute as part of the HUPLANT Control COST action focusing on microbial food safety issues and this project will further develop this relationship. This project has also led to Dr Burgess forging collaborative linkages with Geological Survey Ireland in relation to heavy metal mapping and with the SFI funded VISTA MILK research centre.

4.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
Biological samples The beneficiary must confirm that no Human and/or Animal samples will be collected for further analysis. Environmental and Health and Safety (H&S) Aspects Considering the area of work, the beneficiary must re-confirm that there are no environmental or H&S Aspects.	No human or animal samples will be collected as part of this project. There are no environmental or H&S aspects in relation to this project. All samples and lab work will be undertaken using SOPs in place in the partner institutes and all isolation work will take place in appropriate BSL2 labs.

4.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
1	Dec 2020	Feb 2022	D-PhD03-1.1	36	51	Recruitment delay	0	€53,700

COVID-19 has had a severe impact on recruitment for this project. Prior to the pandemic we reported issues with recruiting a suitable PhD candidate. A suitable candidate was identified in March 2020 and the position offered but failed to secure a visa by October 2020. Another advertising round commenced and another suitable candidate was selected and will start in February 2021. Tighter sampling schedules will be employed to address project delays to date.

4.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	Yes
Other risks (please describe)	Yes

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Additional information:
As outlined there has been a significant delay to the project start and associated delay in work plan execution and reporting. Nonetheless, Dr Burgess and Prof Morris believe the project remains achievable with the right candidate and slightly tighter timelines. This has been made possible through collaboration with other relevant projects as outlined in Section 11.

4.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

- An Initial meeting has taken place with Geological Survey Ireland and through collaboration with the Tellus survey (https://www.gsi.ie/en-ie/programmes-and-projects/tellus/Pages/default.aspx) this will enable suitable high and low metal content soils across Ireland for resistome analysis.
- This project is complementary to the EPA funded AREST project (https://www.nuigalway.ie/medicine-nursing-and-health-sciences/medicine/disciplines/bacteriology/research/arest/) which is examining antimicrobial resistance in the environment. Prof Morris is the coordinator of this project and Dr Burgess is a participant. The culture based methodologies being employed in AREST will be particularly relevant for HME-AMR. The student will have the opportunity to collaborate with these colleagues for methodologies and isolate characterisation.
- Dr Burgess and Prof Morris both contribute to Ireland's National Action Plan for Antimicrobial Resistance which is currently being updated and the results of this project will contribute to achieving the objectives of that plan.
- HME-AMR is complementary to an ongoing PhD project in Dr Burgess' group focusing on the impact of zinc oxide supplementation on the porcine resistome which will facilitate a smooth transition for the HME-AMR student in relation to methodologies and analysis.
- HME-AMR is complementary to a project led by Dr Orla O'Sullivan as part of the SFI funded VistaMilk project (https://vistamilk.ie/) which is examining the soil resistome in different sites across Ireland. Dr Burgess and Dr O'Sullivan will collaborate to ensure synergy of the projects and avoid duplication.

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4.12. List of Dissemination activities

Name of the activity:	One Health EJP ASM 2020 (presentation of a poster)		
Date:	27-29 May 2020		
Place:	Online		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes (poster)***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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5. PhD04- KENTUCKY

5.1. Summary

Salmonella enterica serovar Kentucky (*S. Kentucky*) is a common causative agent of gastroenteritis in humans. It is one of most notorious *Salmonella* serotypes, as it is strongly associated with antimicrobial resistance (AMR). Ciprofloxacin-resistant *S. Kentucky* (CIP^R *S. Kentucky*) belongs to a single sequence type (ST198), which acquired of a variant of the *Salmonella* genomic island 1 (SGI1) conferring resistance to first-line antimicrobials (β-lactams, aminoglycosides, sulphonamides, tetracyclines).
In addition to CIP^R, *S. Kentucky* is able to gain additional antibiotic resistance determinants through the acquisition of locally circulating plasmid-borne ESBL, AmpC and/or carbapenemase genes. Most recently, the situation has worsened, as ECDC launched an Urgent Inquiry (UI-464) on a CIP^R *S. Kentucky* ST198 strain carrying a chromosomally integrated *bla*$_{CTX\text{-}M\text{-}14b}$ gene encoding for cephalosporin resistance. The insertion event was traced back to Malta, but the strain has already spread to Belgium, UK, The Netherlands and five other EU countries. To date, this clone is only reported in humans, as opposed to (for example) the Cip^S *S. Kentucky* ST152 clone widely found in poultry in the USA but rarely reported in humans.[1] In this PhD project, we will investigate (i) what explains the evolutionary success of the multidrug resistant *S. Kentucky* ST198 clone, and (ii) what is the mechanisms of the integration (and potential further transfer) of the ESBL gene in its chromosome.

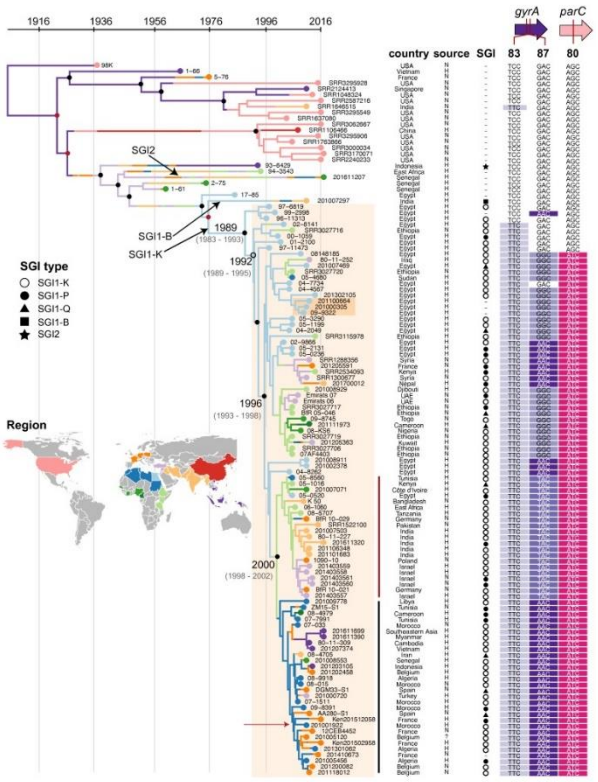

Figure 1. Phylogenetic analysis of *S. Kentucky* ST198[1].

In the first year of study, we focused on the genetic environment of the integrated *bla*$_{CTX\text{-}M\text{-}14b}$ gene. By hybrid sequencing, we reconstituted the entire genomes of four clinical *S. Kentucky* strains with this genotype. The ISEcp1B transposase, which is part of the IS1380 family, was detected in this region adjacent to the ESBL gene, and was hypothesized to catalyse the transfer. We expected on this finding by initiating an *in silico* database mining to correlate insertion sequences with AMR genes among clinical *S. enterica*, *Klebsiella pneumoniae* and *Escherichia coli* isolates. The results are expected by April 2021.

Meanwhile, we are investigating the transfer of the resistance gene from plasmid to chromosome using *in vivo* transposition dynamics via time-lapse microscopy. By cloning the gene cassette into the backbone of the IncHI R27 reference plasmid, and using the Datsenko Wanner method to knock-in 2 ParS sequences into pR27, we will be able to track and quantify the (inducers of) the genetic hopping using fluorescence microscopy. In 2021, we will explore the influence of serotype, species and antibiotics and other stressors on the chromosomal transfer, and therefore will be able to perform a risk assessment on this dangerous genotype.

5.2. Overview of project progress

The PhD is going according to schedule, although the project suffered from the COVID-19 crisis. Due to direct consequences of the pandemic, the original candidate (Jasper Van der Peet) had to return permanently to The Netherlands in M28. He was replaced by Alaa Albasiony, who picked up activities in M32. The transition between both candidates was smooth and Jasper is still involved in the project

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as advisor. During spring, the labs of Sciensano, INRA and KULeuven were closed for three months due to the pandemic, so no research could be done. Therefore, we hope that the project can be extended for (at least) three months, until April 2023. In 2021, Alaa will follow courses in statistics and soft skills, and supervise a master thesis in bioinformatics (Biancamaria Florenzi, KULeuven), who will perform the database mining described above.
In general, the KENTUCKY project is on track to be completed by the end of the OHEJP, if a three-month small extension is granted.

5.3. Progress of the research performed in the PhD project and key scientific results

Objectives.
Salmonella enterica serovar Kentucky (*S. Kentucky*) is a common causative agent of gastroenteritis in humans. It is one of most notorious *Salmonella* serotypes, as it is strongly associated with antimicrobial resistance (AMR). We hypothesize that the success of the MDR *S. Kentucky* ST198 is either due to (i) understudied accommodation of genetic elements encoded within mobile genetic elements (MGE) and their interaction with the core and accessory genome, or (ii) altered expression of a core genome-encoded virulence factor.

During the first nine months of the project, we selected four *S. Kentucky* strains for full genome sequencing using hybrid assemblies of short and long sequence reads. There were the following isolates from routine NRC practices in Sciensano: S16BD08730, S18BD00684, S18BD03994 and S18BD05011, either being *S. Kentucky* ST198*::bla*$_{CTX\text{-}M\text{-}14b}$ (i.e. chromosome-encoded), CIP^R, CTX^R or Kentucky ST198 p*bla*$_{CTX\text{-}M14\text{-}b}$ (i.e. plasmid-encoded), CIP^R CTX^R. These sequenced strains will form the basis for detailed investigation of these mobile genetic elements.

Methodology.
Short read sequencing libraries were prepared with an Illumina Nextera XT DNA Library Preparation Kit and sequenced on an Illumina MiSeq instrument with a 250-bp paired-end protocol (MiSeq v3 chemistry) according to the manufacturer's instructions. Trimming of the short reads was performed with Trimmomatic (version 0.32). First the Illuminaclip option was used to remove the Nextera adapter sequences. Then a sliding window approach of four bases and trimming when the Phred score dropped below 30 was employed. Lastly, the leading and trailing bases of a read were removed when the Phred score dropped below 3. Lastly, all reads that were smaller than 50 bp were removed.
The MinION long read sequencing library was prepared by using the 1D ligation sequencing kit (SQK-LSK108, Oxford Nanopore) according to the manufacturer's protocol for genomic DNA without barcoding. In total there were two MinION flowcells used, and libraries with 8 and 12 barcodes were loaded on them respectively (EXP-NBD103, Oxford Nanopore). From each isolate 1 µg of DNA was used at the start of the protocol. The optional steps of shearing the DNA to 8kb fragments with Covaris G tubes, while the DNA repair step was not performed. The sequencing was carried out on a R9.4 flowcell (Oxford Nanopore) and sequenced for 48 hours.
For the Flongle long read sequencing libraries, the adapted 1D ligation protocol for Flongles was used with the SQK-LSK109 sequencing kit. From each isolate 500 ng of DNA was used at the start of the protocol. DNA repair was no longer optional in SQK-LSK109 and therefore this was performed for the Flongle runs. In the SQK-LSK109 there are two washing buffers the SFB and LFB of which the latter enriches for DNA fragments >3,000 bp. Both these washing buffers and the inclusion or exclusion of the shearing step were used on separate Flongle flowcells. Moreover, on the Flongles no barcoding was performed.
Basecalling and demultiplexing of the Nanopore sequences was performed with Guppy (3.2.4). Then all Nanopore reads with a quality score lower than 7 or a length lower than 1000 were removed with NanoFilt. For the output of the sequencing runs and the theoretical coverage of each sample see table S14. The statistics of the Illumina reads was determined with FastQC and of the Nanopore reads was determined with NanoStat. Raw sequencing data and the de novo assemblies were submitted to NCBI Sequence Read Archive (SRA) and NCBI Genbank.

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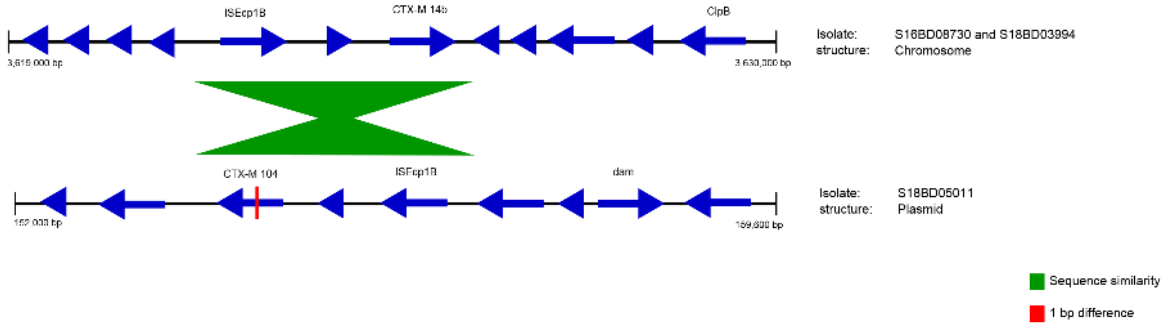
Results.

The chromosomes of each isolate were assembled in one contig and each isolate also showed one or multiple plasmids of which most were reconstructed in one contig (Fig 1). One plasmid from isolate S18BD00694 was split in multiple contigs, i.e. it was not possible to bridge a repetitive region in this plasmid due to the lower average read size in the MagCore DNA extracts. All isolates were determined to be of sequence type (ST) 198.

The chromosomes of isolates S16BD08730, S18BD03394 and S18BD05011 share the AMR genes *aac(3)-Id, aph(3'')-Ib, aph(3')-Ia, aph(6)-Id, sul1*, *tet(A) and aac(6')-Iaa.* All these AMR genes except for *aac(6')-Iaa* were localised very close to each other and by aligning this region to the NCBI nucleotide database it was determined that these genes were part of a *Salmonella* genetic island 1 K (SGI1-K). Two isolates (S16BD08730 and S18BD03394) carried the ESBL gene *bla*$_{\text{CTX-M-14b}}$ in the chromosome, while one isolate (S18BD00684) contained another ESBL gene, *bla*$_{\text{TEM-1B}}$, in the chromosome. Moreover, the latter isolate also contained the ESBL gene *bla*$_{\text{CMY-2}}$ on a plasmid. Isolate S18BD05011 contained no ESBL genes on its chromosome, but *bla*$_{\text{CTX-M-104}}$ and *bla*$_{\text{TEM-1B}}$ were localised on two different plasmids, contigs 2 and 3, respectively. Initially, ResFinder assigned *bla*$_{\text{CTX-M-14b}}$ to isolate S18BD05011 with an identity of 99.89%, but with the CARD database [2] it was determined that a point mutation at position 824 corresponds to the *bla*$_{\text{CTX-M-104}}$ gene. Upon further inspection, a **region of 2850 bp including the ESBL gene was found to be similar in the chromosome of S16BD08730 and S18BD03394 and in the plasmid (contig 2) of S18BD05011**. In these regions, there was only a 1 bp difference, resulting in either the *bla*$_{\text{CTX-M-14b}}$ (S16BD08730 and S18BD03394 on the chromosome) or *bla*$_{\text{CTX-M-104}}$ (S18BD05011, on a plasmid) variants. The ISEcp1B transposase, which is part of the IS1380 family, was detected in this region adjacent to the ESBL gene (Figure 2). In the NCBI database there were no exact matches, but with a literature search, a description of this 2850 bp fragment was found in Lei et al. 2020 in the chromosome of a *S. Kentucky* isolated from Chinese poultry.

Figure 2. Similarity between the chromosomes of S16BD08730 and S18BD03394 and the plasmid of S18BD05011 (contig 2). The location and orientation of the genes is indicated with blue arrows. There is a 1 bp difference between *bla*$_{\text{CTX-M14b}}$ and *bla*$_{\text{CTX-M104}}$.



We expected on this finding by initiating an *in silico* database mining to correlate insertion sequences with AMR genes among clinical *S. enterica*, *Klebsiella pneumoniae* and *Escherichia coli* isolates. The results are expected by April 2021.

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Meanwhile, we are investigating the transfer of the resistance gene from plasmid to chromosome using *in vivo* transposition dynamics via time-lapse microscopy, with the strategy summarized in Figure 3. By cloning the gene cassette into the backbone of the IncHI R27 reference plasmid, and using the Datsenko Wanner method to knock-in 2 ParS sequences into pR27, we will be able to track and quantify the (inducers of) the genetic hopping using fluorescence microscopy.

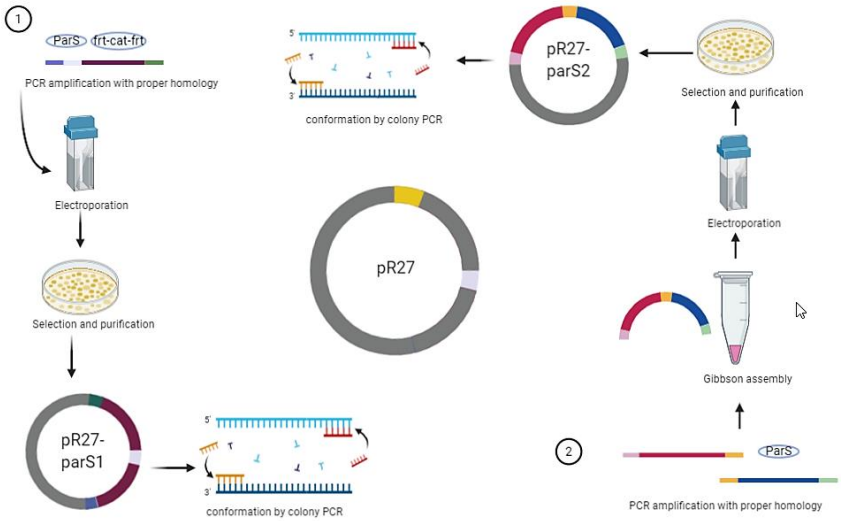

Figure 3. Cloning strategy followed to investigate the genomic transfer of the blaCTX-M gene into the *S. Kentucky* ST198 chromosome.

In 2021, we will explore the influence of serotype, species and antibiotics and other stressors on the chromosomal transfer, and therefore will be able to perform a risk assessment on this dangerous genotype.

5.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD04-AMR2.1-KENTUCKY	D-PhD04-1.1	Genomic Characterization of clinical Salmonella Kentucky strains	M32	M35		Small delay due to pandemic

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
PhD04-AMR2.1-KENTUCKY	M1	Quality training completed at Sciensano	M23	Yes		
	M2	*S. Kentucky* strain and genome collection completed	M30	Yes		
	M3	Completion of hybrid assemblies of four *S. Kentucky* strain	M36	Yes		

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5.5. Soft skills and Continuing Professional Development Training

CPD training events have been planned for 2021 and 2022

5.6. Publications and patents

No publication and patents thus far.

5.7. Impact and Relevance

The Kentucky project units **Sciensano, INRAE and** Laboratory of Food Microbiology of **KULEUVEN** (BEL), which clearly have complementary expertise. Sciensano holds the National Reference laboratory of Samonella, and has experience in short-and long read sequencing (see section 1 of this rapport). **Prof. Abram Aertsen**'s group uses analytical genetics and live (single-)cell biology approaches to study the spread, establishment and adaptive phenotypic impact of mobile genetic elements. **Benoît Doublet** and his team have long-term expertise in plasmid biology, and will study the transfer dynamics of these elements. It is clear these groups, which have never collaborated before, will greatly learn from each other and will exchange knowledge, strains and experiences along the way. The final goal is to improve our methodologies and understanding of transfer dynamics of these mobile elements, and its impact on antimicrobial resistance.

5.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
Environmental and Health and Safety (H&S) Aspects The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	I confirm that the PhD candidate has followed the necessary training in quality and biosafety to perform his work.

5.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Comments:

1. Due to the COVID-19 crisis, all laboratory activities were suspended for two months at Sciensano, KULeuven and INRAE. The PhD student shifted to bioinformatics (see section 1), so milestones M2 and M3 were already reached, while wet lab research was postponed. However, as we are still early in the project, the timing of deliverables and milestones still seems realistic.
2. Due to consequences of the COVID-19 crisis, the original candidate (Jasper Van der Peet) had to return permanently to The Netherlands in M28. He was replaced by Alaa Albasiony, who picked up the activities in M32.
3. The planned research visit to INRAE (M32) is very uncertain at this time, although crucial for buildup of expertise in conjugation and plasmid transfer methodology.
4. The Kentucky project only foresees budget for personnel cost, so the budgetary impact is limited. If the program will be extended with two months to compensate for the lab closures, we would need a budgetary injection of €6.000 to pay the salary of the PhD candidate.
5. A new candidate started in August 2020, and will build on the work of Jasper Van der Peet, who worked four months on the project.

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5.10. List of critical risk

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	Yes (see above)
Other risks (please describe)	No

Additional information:

1. Due to lab closure during the COVID-19 pandemic, research activities were suspended during two months (M27-28).
2. Also due to consequences of the COVID-19 crisis, the original candidate (Jasper Van der Peet) had to return permanently to The Netherlands in M28. He was replaced by Alaa Albasiony who will pick up activities in M30.

5.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Long-read sequencing was performed according to methodology derived from the Full Force project (JRP-14). No interactions with OHEJP stakeholders were made in this stage of the project.

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5.12. List of Dissemination activities

Name of the activity:	OneHealth@Sciensano online conference, "The evolutionary success of Salmonella Kentucky ST198"		
Date:	27 November 2020		
Place:	Virtual conference		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*Yes*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***+/-100***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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6. PhD05- METAPRO

6.1. Summary

The incorporation of the PhD candidate was in March 2020 and short after started the COVID-19 crisis, where our lab became part of a network to diagnose COVID-19 in elderly homes in Madrid. The candidate was actively involved in this activity and due to all the restrictions applied in our country no progress could be made in his PhD project until June. Also, in the period from June to September has been also quite difficult to make any advance, especially in the task of establishing the potential sampling points in Spain, because of the COVID-19 restrictions that we still have in our country. Therefore, we decided to make a small shift in our plans, and we resolved to start some analysis with samples that we collected from previous projects that our lab was involved in. Preliminary bioinformatic analyses have been made with metagenomic sequences belonging to different pig farms across Spain and several acquired 16S rRNA methyltransferases have been detected, including some of certainly low worldwide prevalence when using a read-based approach. The assembly-based analysis did not retrieve concluding results since the samples where only sequenced via Illumina (short reads). We still have faecal material frozen and DNA re-extraction and sequencing via both short and long-read technologies is foreseen in January 2021. In addition, in the period of November-December we established contact with a few potential sampling points in order to start sampling as soon as possible in the beginning of 2021. We are still missing several of the ecological niches that we intended to sample, but we hope that in the first months of 2021 the situation allow us to find suitable sampling spots.

6.2. Overview of project progress

- Spanish sampling design: We are having difficulties in finding sampling points, but we decided to make some modifications and start using already existing metagenomic data obtained in previous projects. In addition, we have established contact with a few potential sampling points in Spain and we still trying to find suitable spots for the rest of the ecological niches that we intend to study.

- Spanish sampling execution: for some of the samples it will take place, if possible due to COVID-19 restriction, in the first months of 2021.

- Metagenome sequencing of the Spanish sampling: this task is not due to take place until we perform the sampling.

- Enterobacteria isolation and WGS from the first sampling: this task is not due to take place until we perform the sampling.

- Analysis of the genomic and metagenomic data from the Spanish sampling: we started some of the analysis using metagenomic data obtained in previous projects from the lab. We tested part of our pipeline and we were able to detect already acquired 16S rRNA methyltransferases with our read-based approach.

- Sampling design in the United Kingdom: contact with our UK partners has been established in order to discuss sampling possibilities for the upcoming year.

- UK Sampling execution: this task is not due to take place until September 2021.

- Metagenome sequencing of the UK sampling: this task is not due to take place until September 2021.

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- Enterobacteria isolation and WGS from the UK sampling: this task is not due to take place until September 2021.

- Analysis of the genomic and metagenomic data from the UK sampling: this task is not due to take place until December 2021.

- Writing of a guideline for early detection of plazomicin resistance determinants to preserve plazomicin for human clinical use: this task is not due to take place until June 2022.

- Writing of the Thesis manuscript: this task is not due to take place until June 2022.

- Thesis defence: this task is not due to take place until February 2023.

6.3. Progress of the research performed in the PhD project and key scientific results

For the period of January to December of 2020 we had a few tasks proposed that sadly we had to delay due to the COVID-19 crisis several times. We were in lockdown for a good part of the year and even after, the restrictions imposed in our country limited our actions. We established contact with farms and sampling points and start designing our sampling strategy, but it has been really difficult to find suitable locations for all the ecological niches that we proposed and to start to execute the sampling as we planned. We expect to start sampling a pig farm and a veterinary hospital in the beginning of 2021 and find other optimal spots for sampling before May 2021. In addition, a sampling questionnaire has been prepared to obtain as much metadata as possible. It is intended to submit this deliverable by the end of January 2021.

Due the lack of sampling possibilities at the time, we have made a small modification in our strategy and we have started to work with samples that we already collected in the frame of other projects that our lab took part. We have started analysing metagenomic data from faeces of pig farms and did a preliminary test of our metagenomic pipeline. On our read-based approach we were able to find acquired 16S rRNA methyltransferases in our samples, including some that have been reported limitedly worldwide. We wanted to advance in the analysis of those samples and tested our assembly-based approach. Sadly, only short-read data is available for these samples and our pipeline rendered very little information. On the other hand, we still have faecal material from these samples frozen and we aim to re-extract DNA, test by PCR the presence of the genes encountered in the bioinformatic analyses and sequence it via both short-read (Illumina) and long-read (Nanopore) technologies.

Further, we had a meeting with our partners in the United Kingdom in order to discuss the sampling possibilities and to try to speed up the sampling in their country.

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6.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD05-AMR2/6.1/ET5-METAPRO	D-PhD05-4.1	Sampling questionnaire	M33	M37		

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD05-AMR2/6.1/ ET5-METAPRO	M1	Spanish sampling design	M33	No	M38	We are having difficulties in finding sampling points, but we decided to make some modifications and start using already existing metagenomic data obtained in previous projects (M9.2)
	M9.2	Analysis of metagenomic samples from previous projects	-	No	M48	The analysis has started, and we expect to include these analyses with the ones of our own Spanish metagenomic samples
	M2	Spanish sampling execution	M36	No	M41	We have found a few suitable sampling points, so we intend to start with these samples while trying to find spots for the rest ecological niches that we proposed
	M3	Metagenome sequencing	M39		M41	
	M4	Enterobacteria isolation and WGS	M39		M41	
	M9	Analysis of the Spanish genomic and metagenomic data	M48			

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	M5	United Kingdom sampling design	M42			Contacts with United Kingdom started

6.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Workshop “Aspectos epidemiológicos y estadísticos de un trabajo de investigación”	Epidemiology and Statistics	03-04/03/2020, 02/06/2020, 09/06/2020	Faculty of Veterinary Medicine, Universidad Complutense de Madrid
qPCR technician and analyst at Lab UCM COVID-19	COVID-19, qPCR	15/03/2020 – 01/06/2020	Universidad Complutense de Madrid
Course “Textos científicos con LaTeX”	LaTeX software	27/05/2020 – 16/09/2020	Universidad Complutense de Madrid
Biosafety seminar	Microbiology and Biosafety	14/07/2020	Faculty of Veterinary Medicine, Universidad Complutense de Madrid
One Health EJP Summer School 2020	One Health	17-28/08/2020	Wageningen University
Teaching of Practical classes in the Veterinary Medicine Degree	Microbiology and Immunology	Academic Course 2019/2020	Faculty of Veterinary Medicine, Universidad Complutense de Madrid
VISAVET Journal Club	Microbiology	Academic Course 2019/2020	VISAVET
Resistomap Webinar Series	Antibiotic Resistance and Environment	25/08/2020 – 24/11/2020	Resistomap
Course “Análisis de datos con Phyton”	Phyton	14/09/2020 – 11/12/2020	Universidad Complutense de Madrid
OHEJP FARMED Meeting	Metagenomics	29/10/2020	Sciensano
K-mer alignment training workshop	Metagenomics	6/11/2020	Technical University of Denmark
Webinar “Jornada Complutense sobre Resistencia a Antibióticos”	Antibiotic Resistance	23/11/2020	Universidad Complutense de Madrid

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6.6. Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 98.

6.7. Impact and Relevance

This PhD project connects three major laboratories focused on the fight of Antimicrobial Resistance, having all of them participated in other European Projects in the past. This offers a possibility to maintain the great relationship that has been always present between institutes. This fact is highlighted by the various exchanges of students that have had part and how successful they have been. In addition, since the PhD project supervisors study AMR from different perspectives, this new collaboration brings the opportunity to combine them all together to get the most out of it. But it is not only limited to that. Being part of the EJP networks allows to connect the partners and the student with different backgrounds and perspectives that are often needed.

6.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
Biological samples The beneficiary must confirm that no Human and/or Animal samples will be collected for further analysis. Environmental and Health and Safety (H&S) Aspects Considering the area of work, the beneficiary must re-confirm that there are no environmental or H&S Aspects.	Biological samples The intention is to use sewage from the different origins; therefore, the samples will not be collected directly from humans or animals. Environmental and Health and Safety (H&S) Aspects Our research will not cause any harm to humans, animals or the environment; and will not involve endangered fauna or protected areas.

6.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Spanish sampling design	M24	M38	Sampling questionnaire	M33	M38	Incompatibility to access potential sampling points		
Spanish sampling execution	M27	M39				Incompatibility to plan sampling		

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Metagenome sequencing	M30	M40				No sampling performed		
Enterobacteria isolation and WGS	M33	M40				No sampling performed		
Data Analysis	M36	M48				No sampling performed		
UK sampling design	M36	M42				No sampling performed		

Comments:
All the Annual Work Plans have been updated with the new estimated timeline of completion of the PhD project.

6.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

Additional information:
Due to a late incorporation of the candidate and the COVID-19 crisis the PhD project start date had to be postponed and the work plan has been also compressed. Any other special situation not predicted could cause a major delay in the work plan execution.

6.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

- FARMED: Fast Antimicrobial Resistance and Mobile-Element Detection using metagenomics for animal and human on-site tests (https://onehealthejp.eu/jrp-farmed/). Since the objectives and the techniques of both projects are similar, the candidate is taking part actively in the tasks performed at UCM associated to this project.

- EFFORT: Ecology from Farm to Fork Of microbial drug Resistance and Transmission (http://www.effort-against-amr.eu/). The group participated actively in the EFFORT project and its activities and resources are the foundation of the present research project.

- JPIAMR: Joint Programming Initiative on Antimicrobial Resistance. The lab is part of the NEAR-AMR (https://www.jpiamr.eu/near-amr/) and of the GAP-ONE (https://www.jpiamr.eu/gap-one/) projects. The candidate can benefit of these networks to extend the search of plazomicin

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resistance determinants in other parts of Europe and Africa or to estimate the economic burden of plazomicin resistance.

- AVANT: Alternatives to Veterinary Antimicrobials (https://avant-project.eu/). The student will also be involved in the development and test of alternatives to antimicrobials.

6.12. List of Dissemination activities

Name of the activity:	One Health EJP ASM 2020 (presentation of a poster and participation in 3MT competition)		
Date:	May 27th - 29th, 2020,		
Place:	Online meeting		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750+***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

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7. PhD06- PEMbO

7.1. Summary

WP1: Establishment of reference sequences from the main French clonal groups
Task-1.1: Culture, cloning and extraction of *M. bovis* strains (M21-M25)

We selected ten representative *Mycobacterium bovis* genotypes among the genetic diversity of the *M. bovis* strain genetic population in France. Strains were first cultured in liquid medium to recover them from the collection (stored at -20ºC) before a cloning step on solid medium. A single colony was picked, constituting the clone. Each clone was enriched in liquid medium prior to DNA extraction to obtain sufficient and concentrated DNA. This step was performed in the first 3 months of the PhD project (October-December 2019).
Given that genomic DNA purification performed in January 2020 with the NucleoBond® AXG kit (Macherey-Nagel), following the methodology of our previous studies (Branger et al 2020) did not provide correct DNA concentrations for long read sequencing, we shifted to an alternative phenol-chloroform DNA extraction protocol that allowed us to obtain a good quantity and quality of genomic DNA for each genotype.

Task-1.2: Sequencing and *de novo* assembly (M26-M37)

The validation of phenol-chloroform protocol was done by sending genomic DNAs belonging to two different genotypes to Genoscreen (Lille France), the chosen genomic sequencing platform performing the PacBio/Illumina sequencing steps of our project. The rest of the DNAs were sent to Genoscreen on the 22/12/2020 to control the DNA quality and sequencing our samples if they pass the QC.
Genomes of *M. bovis* strains will be sequenced by two complementary technologies, PacBio and Illumina (pe-250x2), in order to take advantage of the long readings and the low error rate obtained by each method respectively.

WP2: Genome sequence analysis
Task-2.2: Genomic markers analysis (M32-M43)

Genomic analyses on previously available genomes were performed. Ciriac participated to a trainee from the 19 to 21 November 2019, "Linux et script pour la bioinformatique", organized by the CNRS in Montpellier. The goal of this trainee was to get familiar with the Linux environment and to learn the fundamental requirements for Python coding. He is now able to write simple scripts to analyze WGS (Whole Genome Sequencing) data.
The first steps in bioinformatics focused on the presence and distribution of an insertion sequence, IS*6110*, in the genomes of *M. bovis*. IS*6110* is a very useful genetic marker of the *Mycobacterium tuberculosis* complex employed for TB direct detection by PCR and for genotyping. We adapted bioinformatics scripts to find the number and localization of IS*6110* in the bacterial genomes using contigs from reads of illumina sequencing.
We highlighted a wide diversity in the copy number and the localization of this sequence in the different genetic families of *M. bovis* in France. This study was presented as a poster at the OHEJPASM2020 (One Health European Joint Programme Annual Scientific Meeting 2020). These multicopy strains were grouped on specific nodes. These genotypes are mostly found in French bovine tuberculosis endemic regions and another study shows the strong stability of IS*6110* number and genomic location in these groups during time. At present, we are studying by *in silico* analyses, if these insertions can lead to phenotypic behaviors that could explain a better fitness or transmissibility and thus a potential epidemiological success.

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7.2. Overview of project progress

WP1: Establishment of reference sequences from the main French clonal groups

Task-1.1: Culture, cloning and extraction of *M. bovis* strains (M21-M26)
Ten genotypes were selected and cultured. This first step took between 3-6 months given that field *M. bovis* isolates are very slow growers.
The long read sequencing need a high DNA quantity and quality. After several tests and optimization, appropriate concentrations of DNA were obtained.
Two DNA genotypes were sent to Genoscreen on 23/11/2021 for quality checking and DNA purification protocol validation.
Genoscreen validated our methodology and we then sent the other 8 additional genomic DNAs the 22/12/2021. Quality control of these 8 samples is ongoing, if it is correct, sequencing of the genomes can start.
This task has been delayed because of the COVID-19 crisis, the lockdown and the above mentioned technical problems.

Task-1.2: Sequencing and *de novo* assembly (M27-M38)
This task has been delayed because of the COVID-19 crisis, the lockdown and the above mentioned technical problem as in WP1 task 1-1.
We hope that genomic sequencing will start soon (when DNA quality control will done). We have already started working on the assembly pipeline.

WP2: Genome sequence analysis

Task-2.1: Sequencing of supplementary strains (M38-M41)
This task, which depends on the genome sequencing of strains belonging to additional clonal complexes will start is pending and to be started in month M45.

Task-2.2: Genomic markers analysis (M33-M44)
We have already worked on IS*6110* to study the genetic diversity of *Mycobacterium bovis* French strains according to this element.
Another study used other sequencing data that obtained by other projects. This study focused on the IS*6110* evolution in the most prevalent French *M. bovis* genotype and showed a strong stability in the IS*6110* number and genomic location in each group during the time.
The task is ahead on this point.

WP3: Analysis of the antigenic variability: biochemical and lipidomic studies
This task can be started after the first genomic analyses planned in previous WP.

7.3. Progress of the research performed in the PhD project and key scientific results

WP1: Establishment of reference sequences from the main French clonal groups
Task-1.1: Culture, cloning and extraction of *M. bovis* strains (M21-M26)
Selection of ten representative *Mycobacterium bovis* genotypes among the genetic diversity of the *M. bovis* strain genetic population in France.
M. bovis strains cultures were done
M. bovis strains DNA extraction were carried out.

→ This task has been delayed because of COVID-19 crisis, lockdown and technical problem in DNA extraction protocol but currently done.

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Task-1.2: Sequencing and *de novo* assembly (M27-M38)
M. bovis strains sequencing at Genoscreen (Lille France).
De novo assembly of sequencing results.

→ This task is delay because of COVID-19 crisis, lockdown and technical problem in WP1 task 1-1.

WP2: Genome sequence analysis
Task-2.1: Sequencing of supplementary strains (M38-M41)
Sequence supplementary strains according to the future results.

Task-2.2: Genomic markers analysis (M33-M44)
Identification of genomic events (insertion / deletion or broad sequence polymorphism (LSP)) by comparison study of genetic variation between the new reference genomes sequenced.
Comparison between the new reference genomes and other genome already sequence with Illumina technology.
The analysis on the study of 182 virulence genes, genes involved in envelope biosynthesis and the study of excreted antigens will be done.
A study of IS*6110* number and localization in the *M. bovis* genome, which are representative of French diversity, was done.
The IS*6110* multicopy genotypes are mostly found in French bovine tuberculosis endemic regions (Côte d'Or, Dordogne-Haute Vienne and Pyrénées Atlantique). We focused on the IS*6110* evolution in these most prevalent French *M. bovis* genotype and showed a strong reccurence in the IS*6110* number and genomic location in each group during the time.

WP3: Analysis of the antigenic variability: biochemical and lipidomic studies
Task-3.1: Protein profiles determination (M40-M49)

A confirmation of first *in silico* result will be reinforced with *in vitro* studies.

Task-3.2: Lipidomic analyses (M40-M49)

A confirmation of first *in silico* result will be reinforced with *in vitro* studies.

WP4: Valorisation and dissemination of results
Task-4.1: Publication of results (M36-M56)

Two publications in peer review journals are planned. One of them will present the number and localization of IS*6110* in the diversity of *Mycobacterium bovis* French strains. This publication uses the ASMOHEJP2020 results and is currently being written.

The second publication will present the genomes sequence analysis, the genetic differences between these several strains and the possible consequences in their phenotype.

Task-4.2: Communication (M36-M56)
Ciriac presented his thesis subject at the speech competition in ASMOHEJP2020. Ciriac also presented a poster in the same event.
The *M. bovis* congress schedule on June 2020 has been postponed to June 2022 because of the COVID-19 crisis. We had been selected fora poster presentation on the 2020 planned session. According to our new results, we hope to be able to present a poster or oral communication on the 2022 session.
In 2021, the student will participate to ASMOHEAJP2021 (Annual Scientific Meeting OHEJP 2021 organized by OHEJP), EMBO tuberculosis 2021 (European Molecular Biology Organization tuberculosis 2021 organized by Pasteur institute) and SFM Microbes 2021 (organized by French Society of Microbiology).

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Ciriac presented his thesis subject during an intervention of “La bio au labo”. La bio au labo is a collaborative web site that allows scientists to present the daily life of researcher and this work. One scientist per week animates their Twitter, Instagram and Facebook pages. https://labioaulabo.tumblr.com/post/638210749663232001/ciriac-charles-doctorant-de-deuxi%C3%A8me-ann%C3%A9e-en

Task-4.3: PhD manuscript writing (M36-M56)
The student must write thesis manuscript.

7.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD06-ET5-PEMbo	D-PhD06-6.1	First steering committee report	M26	M33		Delay due to the COVID-19 crisis. Previously planned the 25 March 2020
	D-PhD06-6.3	Poster presentation at OHEJP ASM 2020 congress	M29	M35		Delay in submitting report, no delay in poster presentation.

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD6-ET5-PEMbo	M-E5-1	*PacBio + Illumina sequencing for obtaining reference genomes*	M30	No	M40	Delayed due to unexpected technical problems and COVID-19 crisis

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7.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Journée d'accueil des nouveaux doctorants	Introduction of the doctoral school; explanation of the good progress of the thesis during the next three years.	02/10/2019	ABIES
UZB training for working in the NSB3 lab	To learn how to work in a confined type 3 biosecurity lab	31/10/2019	ANSES
MOOC Bioinformatique: algorithmes et génomes	To develop skills in bioinformatics and genome data analysis.	1/11/2019→9/12/2019	MOOC Fun platform
Linux et script pour la bioinformatique	To develop skills in bioinformatics, especially Python.	19/11/2019→21/11/2019	CNRS Montpellier
Bases en épidémiologie des maladies animales et zoonotiques	To learn basic notions on epidemiology. To learn how to set up an epidemiology study. To learn how to use different statistics tests ...	01/12/2019→19/12/2019	MOOC Fun plateform
UZB training for working in the molecular biology lab	To understand UZB's molecular biology lab organisation and to evaluate my skills	19/12/2019	ANSES
ADOC - Construire et activer son réseau	To develop skills in professional presentation and elevator pitch. ADOC give tips to improve your networking.	20/01/2020	Paris Est university
Developing Fluency in English: Intermediate - Advanced level in English (B1-C1) session 2	To enhance the students' awareness appropriate vocabulary, pronunciation, intonation and improve their overall confidence in oral communications.	4/02/2020→21/04/2020	Paris Est university
Doc 'Avenir 2020	This was ABIES PhD candidates' annual day, organised by doctoral students to discuss about their future career, and to learn different techniques to help them applying and getting a suitable job. This year, the main subject was about networking and developing a good professional profile	12/02/2020	ABIES
Devenir acteur de la science ouverte : ouvrir ses publications	To understand concepts and practices in relationship to "open science".	08/07/2020	ABIES

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et les déposer dans HAL	To learn how to apply your rights for the deposit of publications in an open archives To learn how to use the HAL interface.		
research integrity in Scientific professions (EN and FR)	The objective of this training is to disseminate a culture of research integrity within institutions. Rather than passing on knowledge (this is not a learning process), it is a matter of raising awareness of the various issues associated with research integrity and encouraging a critical approach by proposing the basic elements necessary to understand and support the requirements of research integrity.	05/01/2021	MOOC Fun plateform

7.6. Publications and patents

- Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 76.

- C. Charles. (2020, December 4). C. Charles' first thesis project steering committee report. Zenodo. http://doi.org/10.5281/zenodo.4305724

7.7. Impact and Relevance

The aim of this thesis project, a collaborative study between ANSES, Animal Health Laboratory (Maisons-Alfort) and INRAE, Infectiology and Public Health laboratory (Nouzilly), two French EJP Partners, is to better understand the complex biology of *M. bovis* through the study of the complete genomes of a large panel of isolates of interest. The first two parts aimed at obtaining reference sequence (WP1) and identifying large genomic events (WP2). This part of the project will be carried out at ANSES. The third part of the project will consist on studying phenotypic traits that the genetic events disclosed by the genomic studies through analyses of antigenic variability, lipidomics and proteomics studies. This part of the project will be carried out at INRAE.

7.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
Biological samples The beneficiary must confirm that no Human and/or Animal samples will be collected for further analysis.	No human or animal samples will be collected for further analyses
Environmental and Health and Safety (H&S) Aspects The beneficiary must confirm that authorisations for relevant facilities (e.g. security classification of laboratory) have been obtained, and that safety procedures conforming to relevant local/national	Authorisations for relevant facilities have been obtained, and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.

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guidelines/legislation are followed for staff involved in this project. Non EU countries (*putative collaboration with Argentina)* The beneficiary must provide details on the material which will be imported to/exported from EU and confirm that the adequate authorisations have been obtained. Please provide more information on Nagoya Protocol Compliance	Whether any collaboration was established with one of the potential mycobacterial groups in Argentina, INTA-Castelar, the *M. bovis* French strains to be sent will be so only if a MTA between the two interested parties (Anses-INTA) was signed beforehand and the *ad hoc* import licence to send them to Argentina was appropriately established. Concerning the Nagoya Protocol in the French territory, within the Framework of the n° 2016-1087 law of the 8 August 2016 for reconquering biodiversity, nature and landscapes, which is the implementation of Nagoya Protocol, the decree n°2017-848 of the 9 May 2017 fixates the rules to have access to the genetic resources situated in the national territory and to share the advantages resulting in their use. Argentina complies with the Nagoya protocol by the law 2726 of the 26 November 2015.

7.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			**Milestones and Deliverables**				**Associated budget**	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Task-1.1	M25	M35	M-E5-2	M25	M35	Delayed due to unexpected technical problems and COVID-19 crisis		
Task-1.2	M37	M40	M-E5-3	M37	M40			

Comments:

We should have been in the last phase of lab manipulations (T-1.1) for starting genome analysis (T-1.2). We have encountered some technical difficulties (as you could see in the first part of this report) before the lab's lockdown; we have not been able to shift from T-1.1 to T-1.2 before the expected working month.Thus, the working program will be slightly delayed, although we are almost sure that we will be able to catch up time later on in PEMbo project.

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7.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

7.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Not Applicable

7.12. List of Dissemination activities

Name of the activity:	One Health EJP ASM 2020 (presentation of a poster and participation in 3MT competition)		
Date:	May 27th - 29th, 2020,		
Place:	Online meeting		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*

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Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750+***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

Name of the activity:	C. Charles' first thesis project steering committee report
Date:	04/12/2020
Place:	https://zenodo.org/record/4305724

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

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*

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Scientific Community (Higher Education, Research)	***19***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

Name of the activity:	La Bio au labo intervention		
Date:	04/01/2021→08/01/2021		
Place:	Twitter – Facebook – Instagram		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*No*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	***Yes***	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	***Yes***	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***0***	***Media***	*0*
Industry	***0***	***Investors***	*0*

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Civil Society	***0***	***Customers***	*0*
General Public	***1800 (Twitter), 600(Facebook) and 200 (Instagram)***	***Other***	*0*
Policy Makers	***0***		

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8. PhD07- MACE

8.1. Summary

An elicitation questionnaire was developed to capture the attitude to investment into disease surveillance sensitivity by stakeholders. This is in planning to be conducted in a representative sample audience of stakeholders.

A spatio-temporal model was developed to estimate the risk and prevalence of Cystic Echinococcosis in dogs across all regions of Uruguay (excluding Montevideo). This framework will be extended and adapted to support current planning of a screening programme across the country.

A review of Cystic Echinococcosis surveillance and control methods, as well as historical mathematical modelling approaches used, has been drafted in preparation for a report due 15 months after the start of the PhD (Confirmation report).

8.2. Overview of project progress

The PhD project has been progressing mostly as originally planed. The original intended start of the project was M21, however due to delay in finding a suitable candidate, the start date of the project was M24. This means the original timelines for the deliverables for Y2 and Y3 have been updated, delaying most by 3 months to account for the late start. Some tasks and deliverables have re-ordered, to take advantage of opportunities arising during the last year, as detailed in section 3. The student current focus country is Uruguay, as new initiatives are being planned where data is becoming available and where the project tasks can directly support policy. The project is still scheduled to finish before the end of WP6 of the OHEJP.

8.3. Progress of the research performed in the PhD project and key scientific results

The relevant literature has been flagged (MACE.Y2.A), with a draft being finalized for the student's confirmation report (due in M39), this output relates to deliverable (D-PhD07-6.1). The main focus of the student during the last year has been to conduct an elicitation with surveillance and One Health experts at an international conference. This exercise will allow to capture in the mathematical model developed in this project more realistic considerations regarding "willingness to pay" and "willingness to accept" of different control and surveillance strategies. This task relates to MACE.Y5.3; and was originally scheduled for later in the project (milestone MACE.Y5.A; M53), however a unique opportunity presented itself to conduct this task with the support of the organizers of the 4th International Conference on Animal Health Surveillance (ICAHS). This exercise has never been done for surveillance in a One Health context, and will have unique policy relevant impact, which will be shared with key stakeholders. Due to COVID-19 the ICAHS conference was delayed and eventually cancelled, the framework developed here has been adapted to support the COVID-19 response, by conducting the questionnaire within the WHO COVID-19 Stakeholder meeting in the context of investment in improving sensitivity for contact-tracing of cases.

Progress has been made towards deliverables D-PhD07-6.1 and D-PhD07-6.2. Coding of the model has started, but not finished (MACE.Y3.A). Simulations still need to be finished (MACE.Y3.B). with possible delays, as mentioned above, expected due to delayed start of the project. The timelines has been updated in the section below.

We are actively engaging with the Comisión Zoonosis in Uruguay and their Programa de Equinococosis quística (Programme of Cystic Echinococcosis). A spatial model was developed and fitted to historical data (D-PhD07-6.2), and the framework will be adapted and extended to support the screening programme that Uruguay is currently planning.

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An abstract was accepted in the BSP conference; however, the conference was canceled due to COVID-19. The conference will take place in the summer of 2021. A lightning talk was presented at the SVM research celebration conference at the University of Surrey.

8.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD7-ET2.1-MACE	D-PhD07-6.1	Draft of review of surveillance and control tools for CE	M24		M37	This deliverable has been delayed to focus on the elicitation.
	D-PhD07-6.4	Final draft of publication with the model and control scenarios	M32		M44	Progress has been made with the model, Delayed due to focus on elicitation
	D-PhD07-6.2	Spatial Temporal Model of CE validated in Uruguay	M32		M37	Progress has been made with the model, possible delay (due to delayed start of project). Focus to fitting data from Uruguay
	D-PhD07-6.3	Questionnaire to Elicit WTP/WTA of One Health Surveillance Activities	M53		M36	This deliverable was moved up during the first year of the PhD due to an opportunity presented at the time. Due to COVID-19 pandemic, the venue was cancelled and an alternante venue is being organised

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD7-ET2.1-MACE	MACE.Y2.A	Relevant literature on surveillance and control of CE identified	M23	Yes		Relevant literature identified. Draft document included in student's

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						Confirmation report (due M39)
PhD7-ET2.1-MACE	MACE.Y2.B	Training in Mathematical modelling	M24	Partial	M36	Partial training, some activities delayed due to COVID-19
	MACE.Y3.A	Fitted model of CE to data from Uruguay	M30	Partial	M36	First iteration of statistical model fitted to Uruguay historical data.
	MACE.Y3.B	Simulations of different control scenarios	M32	No	M44	Simulations to be run once transmission model is fitted
	MACE.Y5.A	Online polls with stakeholders completed	M53	Partial	M38	Poll finalised, conference cancelled. New sample audience being organised.

8.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
OHEJP Summer School 2020	One Health	17 August 2020 - 28 August 2020	OHEJP
Writing Coherently	Writing techniques	12 February 2020	University of Surrey Doctoral College
Driving your Doctorate	How to get the most out of your PhD	12 February 2020	University of Surrey Doctoral College
Engaging with you Literature: Finding Literature	Finding Literature for your projects	6 February 2020	University of Surrey Doctoral College
Welcome to your Doctorate	Introduction to your PhD	7 February 2020	University of Surrey Doctoral College
Python for Data Science and Machine Learning Bootcamp	Basic Python coding for Data analysis and visualisation	27 January 2020	Udemy
Judgement & Decision-making Lecture	Decision making from a Psychological view	9 March 2020	University of Surrey
Stage 3 Spanish	The Spanish Language	September 2020 – August 2021	Global Graduates Award
Confirmation process – virtual	Confirmation process	07 December 2020	University of Surrey Doctoral College

8.6. Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 47.

No other publications yet. Awaiting results of milestone MACE.Y5.A to finalise draft. Collaboration with stakeholders in Brazil (regarding mortality due to CE) is currently in the final draft stages.

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8.7. Impact and Relevance

This project has allowed collaboration with partners within the consortium (namely between UoS and ISS), as well as supporting stakeholders in South America. The PhD student has engaged in activities in Peru, Argentina, Brazil and Uruguay, supporting ministry of health officials through data analysis of current surveillance and control programmes. As this is the beginning of the project, the outcomes are fairly limited thus far, however the engagement with the stakeholders has been excellent. We are extending our network of collaborations and partnerships to maximize the impact of the work developed, currently engaging with partners in the East Mediterranean region.

8.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
Non EU countries **(Argentina and Peru)** -The beneficiary must provide details on the material which will be imported to/exported from EU and confirm that the adequate authorisations have been obtained. -As low middle income countries are participating in the study, the beneficiary must confirm that fair benefit-sharing arrangements with local stakeholders are ensured during the project (cf the Global code of conduct for research in resource-poor settings – www.globalcodeofconduct.org) This project states that the beneficiary will collect "data" from sheep and dogs. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. sheep and dogs). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the dogs or sheep are affected when collecting data. Are any animals restricting for data collection etc). Please confirm if there are any impacts on the animals. Please provide a statement on the 3Rs aspects of this work If Ethical Approval is required please state which Research Ethics Committee this will be sent to, in the EU and non-EU countries.	The project is using historical data that has already been collected through the ongoing control and surveillance programme in Rio Negro, Argentina and in Uruguay. The data is collected following standard protocols that have been approved in the countries (Argentina & Uruguay). Animal welfare is managed through the guidelines approved in the country. There are no physical materials transferred to the University of Surrey/EU, we only receive the data in silico (i.e. csv/excel files). The lead of the group in Argentina, Prof. Edmundo Larrieu, is registered as an external supervisor, which ensures fair benefit-sharing of all the outputs. Outcomes are also communicated with the local authorities (Echinococcosis surveillance and control programme in Rio Negro). A MoU is currently being drafted with the collaborators in Uruguay.

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8.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			MACE.Y2.B	M24	M36	Some training activities have been cancelled		
			D-PhD07-6.3	M53	M36	Conference for online poll was cancelled new sample audience has to be found		

8.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	No
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	

8.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

We are collaborating with Comisión Zoonosis in Uruguay and their Programa de Equinococosis quística (Programme of Cystic Echinococcosis). They are planning on initiating a national survey for cystic echinococcosis in dogs. We are preparing to assist with the planning of said programme.
In terms of OHEJP stakeholders, this project is a close collaboration between UoS and ISS, Rome. We are in frequent contact with Dr. Adriano Casulli from the Istituto Superiore di Sanitá in Italy, with further collaborative planned to analyse the economic burden of cystic echinococcosis in Bulgaria, Romania and Turkey. We are also planning on collaborating with Professor Majid Fasihi Harandi from the Kerman University of Medical Sciences in Iran with the studies they are conducting on Cystic Echinococcosis within the region's dog population.

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8.12. List of Dissemination activities

Name of the activity:	One Health EJP ASM 2020 (presentation of a poster and participation in 3MT competition)		
Date:	May 27th - 29th, 2020,		
Place:	Online meeting		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750+***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

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Name of the activity:	School of Veterinary Medicine, Research Celebration Event		
Date:	September 2020,		
Place:	Online meeting		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	***Yes***	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***50***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

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9. PhD08- DESIRE

9.1. Summary

The focus of the PhD candidate in the first year of her PhD study was on the field study. She started with interviews of various stakeholders, including municipal employees who are in charge of urban ecology and/or pest management. This resulted in a better problem definition and helped the design of the field study. The field study commenced in May and ran till October, leaving little time for other activities. It comprised two cities, of which one unfortunately did not result in many captured rats. Therefore, to ensure enough power to perform the proposed analyses, another city will be added to the project in 2021. After collection of the samples, necropsies were performed and the first set of diagnostic analyses were performed for zoonotic pathogens. However, this came to a halt due to lab restrictions at the RIVM due to COVID-19. The expected delay will be at least 3 months.

Furthermore, the PhD candidate has started NGS analysis of the bacterial pathobiome, using rat samples from the biobank. It was decided to extended this with the analysis of the viral pathobiome, which will be done in the first half of 2021.

Finally, the PhD candidate has participated in various courses and obtained an official degree to perform animal experiments (which also includes field work on the wild rats).

9.2. Overview of project progress

The main objective for 2020 was to design and start the field study in which the samples and data will be collected. This goal has been achieved, though the study needs to be continued in 2021 to ensure enough power for the planned analyses.

Furthermore, the objective was to gain experience with the laboratory analyses and develop new methods if necessary. The laboratory activities have started, but more emphasis on this part of the PhD will be in later years. This has also been limited by the laboratory restrictions due to COVID-19 in the second half of 2020.

9.3. Progress of the research performed in the PhD project and key scientific results

The following tasks had been planned for 2020 (Y3):

1. Collection of data through the mobile application.

The mobile application was launched in September 2019 and it was updated in September 2020. However, the number of participating pest controllers lacks behind expectations, and so does the number of reports. Promotion of the application has not yet led to increased numbers of participants so far. Though we still try to encourage participation and reporting through the application, the PhD candidate already initiated a plan B in case data collection with the mobile application will not result in sufficient coverage of the Netherlands. This plan B will be on assessing the home range of urban rats through genetic analysis.

2. Pathobiome analysis of brown and black rats

Laboratory analysis of the bacterial pathobiome of brown and black rats has been performed on samples available in the RIVM biobank. During 2020, it was decided to complement this with viral pathobiome analysis of the rats, of which the laboratory work is currently ongoing.

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3. Risk(-map) analysis

Relevant parameters for risk-mapping have been identified. As planned, the PhD designed and performed a field study, sampling rats in two cities in various urban environments that can be used for risk analysis and potentially risk mapping. In the field study, information on these parameters has been collected. Since we captured a smaller number of rats in one of the cities than anticipated, the PhD candidate will add another city to the field study in 2021, to ensure enough power for data analysis.

4. Assessment of measures for greening cities

The PhD candidate has held multiple interviews with professionals of municipalities. This led to the conclusion that comparing these measures on a small scale may be challenging. However, the insights gained from these interviews have been incorporated in the field study and will be analysed as variables in the risk analysis. This will be further worked in the coming years.

9.4. Progress of the research project: Deliverables and Milestones

No deliverables were due in months 25 to 36.

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
	M1	Pathobiome analysis: list of pathogens and protocols	M27	Yes (partly)	M48	An initial list and protocol was designed for analysis of the bacterial pathobiome early in 2020. However, we decided to complement this with additional analysis of the viral pathobiome. This will be finished in 2021.
	M2	Field study set-up	M30	Yes		The field study started already in May.

9.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Start to supervise BSc and MSc students	Learning basic tips on how to be a good supervisor	26-6-20	Wageningen University

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Presenting with impact	Train how to present your research with impact	2-3-20 & 9-3-20 & 16-3-20	Wageningen University
Brain friendly working and writing	Tips and tricks to work more brain-friendly and efficiently	18-3-20	Wageningen University
Laboratory Animal Sciences	Learn the context and regulations of setting up animal experiments. Also an article 9 (permission to design and perform animal experiments) is obtained.	30-11-20 t/m 11-12-20	Utrecht University

9.6. Publications and patents

No publications or patents yet.

9.7. Impact and Relevance

This PhD project involves three institutes (RIVM, WBVR/WUR, and FLI) and the collaboration between the involved partner institutes increased due to this PhD project. Regular meeting of the PhD supervisors of RIVM and WBVR offer the opportunity to discuss additional subjects and new collaborations as well. The PhD student is currently performing work at the WBVR. The intensified collaboration between the RIVM and FLI was exemplified by the PhD student joining a field project of the FLI in the third month of her PhD. This allowed the PhD student to gain insight already in the work practices of the FLI. She is planning to visit the FLI as part of her PhD project in autumn 2021, if the COVID-19 situation allows this.

While setting up the field study, meetings with partner institutes such as the Utrecht University and Wageningen University have been organized to discuss collaboration on smaller student projects that will benefit this PhD study. Furthermore, meetings with professionals of different Dutch cities have been held to collect their needs in the subject of urban wildlife/rats (related to urban greening).

9.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
<u>Environmental and Health and Safety (H&S) Aspects</u> Health and safety procedures conforming the relevant guidelines of the RIVM and the national legislation are followed. The work procedures have been discussed with our safety officer. The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project. <u>Animals</u> This project states that the beneficiary will capture and study rats. The beneficiary also indicated they may use / trap other rodent species or wild species	The appropriate health and safety procedures will be followed during this project by the PhD student and involved staff. RIVM has standard guidelines for working in the laboratory. For the fieldwork, the procedures have been discussed with a biosafety officer of the RIVM. In the Netherlands, wild rats are not included in the Nature Conservation Act, that lists wild animal species that are protected in the Netherlands. Thus, people are allowed to kill rats as long as it is done with tools that are approved for this.

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living in urban environments. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. rats). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the rats are affected when collecting data. Are any animals restricting / handled for data collection etc). Please confirm if there are any impacts on the animals. Please provide a statement on the 3Rs aspects of this work If Ethical Approval is required please state which Research Ethics Committee this will be sent to, in the EU and non-EU countries.	However, in this study rats are captured (with snap traps) for scientific purposes and are thus considered experimental animals. Permission was granted from the Central Authority for Scientific Procedures on Animals under the project number AVD3260020172104. The PhD student has been given permission to perform the necessary procedures. Furthermore, rats collected from regular pest control activities will be used instead of or complementary to rats that are specifically captured for the study, whenever possible.

9.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			**Milestones and Deliverables**				**Associated budget**	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Field study – laboratory analysis	Not specified in workplan, but it was planned to finish around April 2021	At least July 2021	D-PhD08-2.2	M54	M57	Laboratory restrictions (shortage of material, lockdown)		
			D-PhD08-3.1	M57	M60			

9.10. List of critical risks

Description of risk	**Yes/No**
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No (though delay due to COVID-19 possible)
Delay in work plan execution	Yes, due to COVID-19
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No

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Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	N/A

9.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

The PhD student and PI's are involved in a grant proposal about "Ticks and the city". It was not selected in the first round of applications, but it has been resubmitted. If this is granted, this would result in extensive collaboration between the projects. In the selection of the field sites, this has already been taken into account.

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9.12. List of Dissemination activities

Name of the activity:	One Health EJP ASM 2020 (Participation in 3MT competition)		
Date:	May 27th - 29th, 2020,		
Place:	Online meeting		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750+***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

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Name of the activity:	WIAS annual conference		
Date:	13&14 February 2020		
Place:	Lunteren (The Netherlands)		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***150***	***Media***	*0*
Industry	***5***	***Investors***	*0*
Civil Society	***0***	***Customers***	*0*
General Public	***0***	***Other***	*0*
Policy Makers	***5***		

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10. PhD09- UDoFRIC

10.1. Summary

The student has been making progress in the project's current objectives. The nine-month report has been submitted as the first deliverable. There has also been progress in the writing of a literature review focussing on the background and previous research conducted on Fluoroquinolone (FQ) resistance in *Campylobacter*. Data analysis has been carried out on a subset of the dataset available to this project, obtained by previous UK national research and surveillance studies in poultry. The project has access to six distinct datasets over a 24-year period. *Campylobacter* samples were obtained either by sampling wither caecal contents of broilers at slaughter or from broiler carcasses after processing. The student has been making progress analysing the trends of FQ resistance over time and production factors associated with FQ resistance (e.g. bird age, farming method, bird weight). Work has also been conducted identifying *Campylobacter* lineages and aims to determine if there is a link between MLST or whole genome sequence information and FQ resistance. Collection of historic isolates and information relating to them has also been collated. Using this collated information data gaps were identified. In order to create uniformity in the information available in each dataset for comparison, work has begun on historic isolates. These historic isolates have been recovered from the Campylobacter national reference laboratory archives, their species classification has been confirmed and the DNA of these isolates has been extracted and sequenced ready for further analysis.

The student has also been taking part in journal clubs, attending local presentations/ seminars at the APHA and meeting with staff members who have extensive experience and knowledge on the historic use of antibiotics (ABs). The student also attended the 2020 annual EJP scientific meeting where the student entered the 3MT competition and the medical school post graduate conference with Warwick University. They have also attended online presentations including the pubMLST new look showcase event and Resistomap's antibiotic resistance in the environment webinar. In December the student was approved for project upgrade from MPhil to PhD by Warwick University.

10.2. Overview of project progress

- Literature review

The first section of the literature review has focused on *Campylobacter* within broiler production systems, and its impact on industry and human health. Also, *Campylobacter* genetics, including virulence mechanisms, mutations and gene transfer.
The second section of the literature review has also been completed with a distinct focus on FQs. The first subsection focusses on FQ discovery, their chemical structure and the differences between different types of FQs, their uses, importance and mechanisms of action. Then, the review describes the historic trends in the use of these antibiotics and how this use has developed over time. It mainly focusses on when literature first discovered a link between the use of antibiotics both in medicine, the environment and in agriculture and how use in these sectors selected for antibiotic resistance. This link was then investigated by government bodies and the use of antibiotics as growth promoters was phased out by 2006 by EU law.
The third section, tying together FQs and their relationship with *Campylobacter* is currently being written. This section will focus on the specific relationship between FQs and *Campylobacter* and how this has developed over time. It will summarise research in the mechanisms of FQ resistance in *Campylobacter*, the spread of this resistance and previously identified factors associated with FQ resistance in *Campylobacter*.

- Data collection and identification of data gaps

There has been information gathered and reviewed of six datasets taken from research and surveillance of broiler production systems from 1994 to 2020. These datasets include information including phenotypic resistance to antimicrobials, genomic data (including some whole genome sequence data) and epidemiological meta-data. Data characterisation has been carried out on data from samples taken

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post-slaughter collected from the years 2008 and 2012-2015, making use of MLST and also whole genome sequence data pipelines that can predict presence of AMR. Using this data the student is working to characterise the *Campylobacter* lineages within our datasets and to identify trends and associated factors of FQ resistance Initial observations indicate that FQ resistance in *Campylobacter* has increased over time and that certain clonal complexes and sequence types may be associated with FQ resistance. Further work is needed to study the link between core genome MLST and FQR. Similar analysis will be carried out on wider dataset, allowing comparison of isolates over the full 24-year period.

- Training in WGS and bioinformatics

Training in Whole Genome Sequencing (WGS) and bioinformatics has been undertaken earlier than planned in lieu of microbiological practical techniques. This is due to COVID-19 preventing access to lab-based training. Bioinformatics training has involved the building of a database of known antimicrobial resistance (AMR) genes and mutations for Campylobacter, and initial training to assemble genomes, training to identify multi locus sequence types using Srst2 and detecting AMR genes and mutations using APHA SeqFinder. The first set of samples has been identified and will be used as a training set for the student to learn the basic bioinformatics process including downloading large WGS dataset.

- Expanding the datasets

Using the information gathered and data gaps identified, work was carried out on historic isolates in order to create uniform information across the 24-year period for comparison. The UDoFRiC team reviewed the collected data in order to discuss the identified data gaps. It was then decided the key areas in which to allocate financial resource available to this project for further DNA sequencing. It was decided that we would allocate resource to our oldest dataset (1990's?), and? our caeca dataset at the time closest to the 2006 ban of growth promoters in agriculture and our most recent dataset (totalling nearly 300 isolates, approximately 100 from each time point).
From November DNA extractions were carried out on the isolates from our oldest dataset and the beginning of our dataset closest to AB restriction in agriculture laws. A total of 125 isolates have since been grown from frozen stock, had their species identification confirmed, DNA also been extracted from these isolates and sequenced.

10.3. Progress of the research performed in the PhD project and key scientific results

- 9 Month Report

The student has submitted and completed the 9-month report containing updates on the project from March (project start month) to September 2020 in the deliverable template provided.

- Literature review of FQ in Campylobacter

The deadline for the literature review is at the end of Jan 2021. The student is currently on track to produce this on time with two of three sections completed.

- Description of the diversity of FQ resistance and acquisition of resistance variants over time

This work is currently on track to be completed by the appropriate deadline (May 2021). The current COVID-19 situation has delayed WGS due to the inability to access lab space with current social distancing measures. Work on previously characterised datasets has begun, mainly focussing on carcase data in the first instance with data obtained via caeca samples planned. Data analysis on carcase samples has been carried out in order to determine the levels of resistance over a temporal period, throughout a number of variables recorded at the time of sampling and extensive work has been done to identify key MLST groups (both clonal complexes and sequence types) that have an association with FQ resistance. Further work will be carried out to determine this lineage association at a higher level of resolution (core genome MLST). Likewise analysis will be carried out on the larger caeca datasets which will allow comparison of the two sampling methods and any variation in the data.

- Report on the relationship between WGS and phenotype

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This work has not begun as lab work (phenotypic resistance) has been delayed due to COVID-19. However, work has started on WGS with the earliest dataset available to this project and some of our 2008 caeca samples has been proportionally sampled, with these samples being DNA extracted and sent for WGS sequencing.

- GWAS studies and identification of strains for fitness trials

This work has not begun as it is not due until Sep 2021

- Selection and characterization of isogenic resistant strains

This work has not begun as it is due until Jan 2022

- In vitro fitness study: competition growth assays and growth kinetics

This work has not yet started as it is due until Mar 2022

- In vitro fitness study: comparison of survival on abiotic surfaces and on food matrices (e.g. Chicken skin model)

This work has not yet started as it is due until May 2022

- In vivo study: comparison of colonisation using chicken models

This work has not yet started as it is due until Sep 2022

10.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD09-FBZSH3/AMR2.1-UDOFRIC	D-PhD09-1.1	Completion of 9-month review	M33	M33		

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PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD09-FBZSH3/AMR2.1-UDOFRIC	1	Completion of literature review	M37	Yes		Layout and structure of the literature review has been established between the supervisor and student. The first section of the literature review is focused on *Campylobacter* and its impact, diversity and virulence. A second section is focusing on fluoroquinolones and their relationship with *Campylobacter*. The third and final section is currently being written up which focuses on the relationship between *Campylobacter* and fluoroquinolones over time
	2	Completion of data collation and identification of data gaps	M33	Yes		Analysis of an initial selection of the available datasets ranging from 2012-2016 is currently being carried out. Investigations into the variables of data collected with heightened levels of FQR is currently being completed. Initial findings indicate a relationship between FQR and *Campylobacter* species, clonal complex groups, abattoirs at which samples were taken and bird age.
	3	Completion of training in bacteriology and MIC	M36	Partially met		Due to current isolation guidelines set out within the UK because of COVID-19 the student has been unable to partake in lab-based training. For prolonged periods of time, therefore preventing MIC training.
	4	Completion of 9 month review	M33	Yes		n/a
	5	WGS and bioinformatics training	M36	No	M39	125 samples have been DNA extracted and awaiting WGS results. Initial bioinformatics training has started

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10.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
OHEJP Annual Scientific Meeting	Virtual scientific conference	27/05/2020 - 30/05/2020	One Health European Joint Project
Warwick University Medical school Post-graduate symposium	Virtual scientific conference	30/09/2020	Warwick University
pubMLST new look showcase event	An event showcasing the new layout and uses of the updated website	13/11/2020	pubMLST
AMR Plasmid Workshop	Antimicrobial resistance	22/09/2020	Animal and Plant health agency
AMR in the environment webinar	Antimicrobial resistance	24/11/2020	Resistomap

10.6. Publications and patents

No publications or patents yet.

10.7. Impact and Relevance

The UDoFRiC project combines the collaborative experience of the APHA, ANSES and Warwick University. It combines the expertise of microbiologists, epidemiologists, and bioinformatics throughout various institutions throughout the UK and France.

The project is supervised by Dr John Rodgers who leads the National reference lab for *Campylobacter* in animals, Professor Muna Anjum who leads bacterial characterisation workgroup and is the AMR research lead along with Dr Manal Abu Oun at the Animal and Plant Health agency (APHA).

Professor Noel McCarthy is the lead of Evidence in Communicable Disease Epidemiology and Control at Warwick University. The project also includes a year at the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) under the supervision of Dr Isabelle Kempf who is a researcher microbiologist specialized in AMR; Leading the Mycoplasmology Bacteriology Antimicrobial Resistance Unit in ANSES, Ploufragan and Dr Katell Rivoal , who works in the Hygiene and Quality of Poultry and Pork Products research unit and is leading scientific projects on zoonotic pathogens (*Salmonella, Listeria*, and *Campylobacter*) in poultry productions.

The study will interrogate archives of *Campylobacter* from UK research and surveillance activity in broilers from 1994 to 2020 (isolate, phenotype, MLST, WGS, production metadata), to determine the acquisition and diversity of resistance to FQ over time (temporal trends). In addition, data from French broilers and from other potential sources of *Campylobacter* exposure to people (livestock/environment) will be interrogated wherever possible (APHA/ANSES and Public access archives and databases).

Fluoroquinolone resistance in *Campylobacter* is a threat, with the World Health Organisation (WHO) naming it on their list of 12 priority pathogens. The information gathered during this project on drivers of resistance will help policy makers, the scientific community and the agricultural industry make decisions to prevent or hinder the continuous spread of FQ resistance in *Campylobacter*.

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10.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
Environmental and Health and Safety (H&S) Aspects The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project. Animals This project is focusing on broiler chickens. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. broiler chickens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the chickens are affected when taking samples, even if the work is dealing with faeces as these types of study can, this can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals). Please provide a statement on the 3Rs aspects of this work If Ethical Approval is required please state which Research Ethics Committee this will be sent to.	Environmental and Health and Safety (H&S) Aspects In the UK, the PhD will be fully trained to deliver all tasks safely and in compliance with the APHA General Health and Safety policy. This will ensure that all work is risk assessed and adequate training, supervision, facilities and equipment will be provided to protect the student and staff. As a minimum we will comply with the Health and Safety at Work etc. Act 1974 and the Management of Health and Safety at Work Regulations 1999. In France, the PhD student will be trained in hygiene and security (H&S) by the PhD supervisor, and by the agents in charge of H&S in the Anses Ploufragan Laboratory. The student will have all necessary protective equipment (laminar flow hood, masks, safety goggles, protective gloves…) and he will work in laboratory (L2 level) and animal facilities (A2 level), according to the French Labour Code, and conforming to relevant local/national guidelines/legislation. Animals All our animal facilities are under Directive on the protection of animals used for scientific purposes 2010/63/UE. When using conventional chickens, in case of breeding research, directives 98/58/CE, 1999/74/CE and 2007/43/CE must be applied. For experiments planned for the UDOFRIC thesis, we will proceed to obtain agreements from our ethic committees, and requirements of the national legislation or rules will be followed. Approval by the relevant ethics committees is required prior to the start of any animal experiment. Ethical committee on animal experiments is ANSES/ENVA/UPEC (Registered under the number 16 with the French ministry of research (Chairman: Luc Hettinger). Procedures are evaluated by this ethical committee ANSES/ENVA/UPEC and approved by the French ministry of research. Only approved experiments will be conducted. Where relevant planned experiments will also be reviewed by local Ethics Committees at APHA and the University of Warwick. Animal welfare laws: The study will be carried out in compliance with National Animal Welfare Regulations i.e. Ministerial Regulation of 1st February 2013 on the protection of animals used for scientific and educational purposes, which meets the 2010/63/EC Directive of the European Parliament and of the Council regarding 'the protection of animals used for experimental and other scientific purposes'. *Replacement* We are committed to use alternatives to animal studies wherever reasonable. The fitness of the susceptible and resistant strains will be studied *in vitro* (growth in culture medium and survival on different surfaces) but currently, the *in vivo* fitness (colonization, competition between colonizing strains) necessitates animal models. However, by regularly

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	consulting the Federation of Laboratory Animal Science Associations (FELASA) and the European Centre for the Validation of Alternative Methods (ECVAM), we will make sure that we can actively respond to the introduction of other validated alternatives and that we can actively contribute to improved protection and respect for the welfare of animals. *Reduction* The *in vitro* models as outlined above will help us to generate valuable information and limit animal experiments. Our experience of the *Campylobacter* models will also be used to reduce the numbers of included chickens, based on statistical study, to obtain scientific and statistically sound results. *Refinement* Animals will have appropriate enrichment. Campylobacter does not induce suffering. The animals will be observed daily to detect any sign of discomfort. They will be offered water and conventional feed ad libitum. In case of severe injury, birds will be euthanized. Sampling of faeces will be limited according to statistical studies (number of sampling per bird).

10.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			**Milestones and Deliverables**				**Associated budget**	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Completion of training in bacteriology and MIC	M33	M36	M2	M33	M40	COVID-19 isolation guidelines have prevented lab based training	n/a	n/a
WGS and bioinformatics training	M36	M39	M3	M36	M39	COVID-19 has prevented face-to-face interaction and therefore negatively effected teaching. The prevention of lab based training also presented the acquisition of WGS data for teaching exercises	n/a	n/a

Comments:

Lab based training was delayed due to COVID-19. As this report is being written (Jan 2021) we are entering a third lockdown in the UK for another unknown period of time.

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10.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

10.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Not Applicable

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10.12. List of Dissemination activities

Name of the activity:	One Health EJP ASM 2020 (Participation in 3MT competition)		
Date:	May 27th - 29th, 2020,		
Place:	Online meeting		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750+***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

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11. PhD10- WILBR

11.1. Summary

A PhD student (Olivia Turner) was successfully appointed to the WILBR project in February 2020. The student will be based at the APHA and primarily supervised by Prof. M. Anjum and Dr. M. AbuOun ; Prof. W. Gaze (University of Exeter) and Dr. S. Borjensson (SVA) are also part of the supervisory team. The PhD is registered at the University of Exeter.

Although, initial plans were made for desk, laboratory and farm based work to be performed in the first year of the PhD, due to the COVID-19 pandemic in the UK, only desk and lab-based tasks have commenced. During the past 12 months a literature review was undertaken on the role of wild birds in dissemination and persistence of antimicrobial resistance (AMR) in the farm environment. The review includes sections on identifying the current situation regarding AMR in different environments; drivers for AMR; the role of vectors and the environment in persistence and dissemination of AMR; the role of AMR surveillance; and evaluation of different methodologies for identifying AMR, both by phenotype and genotype, in bacteria.

Some historical *E. coli* isolated from gull faeces, collected from an outdoor pig farm, during a longitudinal study in the OH-EJP project ARDIG are being utilised in this PhD project as a result of the delays to farm based work, caused by COVID-19. Over two hundred previously unused isolates have undergone whole genome sequencing, and downstream bioinformatics is currently taking place.

Another outdoor pig farm, known to have wild birds persistently present on farm, had been recruited for a longitudinal study, but due to COVID-19 restrictions we were unable to undertake any farm visit to collect environmental samples, faecal samples from pigs, and caecal samples from corvids present on farm. This farm is now unlikely to be sampled during the course this project due to COVID-19 restrictions. An opportunity to examine the dissemination of AMR in wild bird populations across Great Britain for a 12 month period through the APHA's wild bird scanning surveillance programme is currently being pursued.
A poster was submitted to the OHEJP ASM 2020 meeting, and an oral presentation on the PhD was presented virtually in the 3MT competition for the OHEJP ASM 2020, as part of communicating their research. The student also took part in a workshop at University of Surrey on microbiomes in order to learn from other institutes taking part in metagenomic research, which may be included later on in the WILBR project. The student has also attended the One Health EJP Summer School on Global One Health, the EJP FULL FORCE Plasmid Analysis workshop, and two virtual plasmid workshops hosted by Christopher Thomas at University of Birmingham in order to further develop their skill set for tasks later on in the project.

11.2. Overview of project progress

Due to the COVID-19 pandemic in UK only desk and lab-based tasks have been recommended; currently all farm visits are not permitted for research projects. A literature review is being undertaken on the role of wild birds in dissemination and persistence of antimicrobial resistance (AMR) in the farm environment. The review includes sections on identifying the current situation regarding AMR in different environments; drivers for AMR; the role of vectors and the environment in persistence and dissemination of AMR; the role of AMR surveillance; and evaluation of different methodologies for identifying AMR, both by phenotype and genotype, in bacteria.

Over two hundred archived and previously unused *E. coli* isolates from gull faeces have undergone whole genome sequencing, and downstream bioinformatics is now currently taking place. These isolates were collected as part of the OH-EJP ARDIG project. Work is still being undertaken so there are no results as of yet.

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An opportunity to examine the dissemination of AMR in wild bird populations across Great Britain for a 12 month period through the APHA's wild bird scanning surveillance programme is currently being pursued.

11.3. Progress of the research performed in the PhD project and key scientific results

Deliverable/Milestone 1 in AWP is currently being undertaken. The purpose of the literature review is to identify the gaps in current knowledge and provide ideas for study design. A thorough review of the scientific literature on AMR and the role wild birds might play in helping dissemination and persistence of AMR in the farm environment. Review will focus on identifying the current situation regarding AMR, what is currently known about wild birds in relation to AMR, and evaluation of different methodologies for identifying AMR.

Deliverable 2/Milestone 4 in AWP was the 9 month report and completed and submitted to EJP.

Milestone 2 in AWP was adapted, in response to COVID-19, as originally the plan was to recruit three outdoor pig farms to participate in a longitudinal study. Sampling from the same farms over several time points would allow us to see if there are any effects of bird movement and migration through the seasons on levels of AMR in the farm environment. This was changed to the recruitment of a single farm, to adapt to the COVID situation, which was achieved successfully, with fieldwork delayed and expected to start in February 2021. Due to further COVID-19 restrictions in the UK, all fieldwork at APHA has been stopped. For the same reason Milestone 3 in the AWP is unable to be completed. The original plan was that the farms successfully recruited for the longitudinal study would be visited once in Y1 and an as of yet undecided number of pig faecal samples will be collected. In collaboration with the wildlife team based at Sand Hutton (York) gull faeces will be identified from the environment and collected. The project requires further re-evaluation to determine what work will be done in place of this.

Some historical *E. coli* isolated from gull faeces, collected from an outdoor pig farm, during a longitudinal study in the OH-EJP project ARDIG are being included in this PhD project as a result of the delays caused by COVID-19. Over two hundred isolates archived from this farm have undergone whole genome sequencing, and downstream bioinformatic analysis is now being carried out. This will result in a dataset including over 300 gull isolates and over 400 pig isolates from three time points that can be compared. This work is still being carried out so there are no results as of yet.

An opportunity to examine the dissemination of AMR in wild bird populations across Great Britain for a 12 month period through the APHA's wild bird scanning surveillance programme is currently being pursued.

11.4. Progress of the research project: Deliverables and Milestones

No deliverables due in months 25 to 36.

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD10-FBZSH3/AMR2.1-WILBR	2	Recruitment of farms for longitudinal study	M33	Yes		
	4	Completion of 9 month review	M33	Yes		

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11.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Scientific Writing Skills Workshop	Development of skills in scientific writing	04-05/02/2020	Animal and Plant Health Agency
OHEJP Summer School	Global One Health	17-28/08/2020	Wageningen University
Animal Microbiome Workshop	Animal Microbiome	28/02/2020	University of Surrey
OHEJP FULL FORCE Plasmid Analysis Workshop	Plasmid Analysis	07-08/09/2020	One Health EJP
Virtual Plasmid Workshop	Plasmid Analysis	08/09/2020 and 22/09/2020	University of Birmingham
Resistomap Webinar Series	Antibiotic Resistance and Environment	25/08/2020 – 24/11/2020	Resistomap

11.6. Publications and patents

No publication or patents.

11.7. Impact and Relevance

The supervisory team brings together leading experts in veterinary, wildlife and environmental AMR, with expertise spanning veterinary and molecular microbiology, bioinformatics, microbial ecology and evolution, as well as wildlife disease.

William Gaze is working with the United Nations Environment Project on AMR in the environment, having recently authored the UNEP Frontiers report on AMR and the environment. He is currently located within two interdisciplinary units, Exeter's Centre for Environment and Human health, and the Environmental and Sustainability Institute, which is also part of the University of Exeter

Beside his work as a researcher within SVA, Stefan Börjesson is involved in the Swedish AMR monitoring program and is also a senior lecturer in clinical microbiology with an emphasis on AMR in a One-health perspective at Linköping University at the Department of Clinical and Experimental Medicine.

Muna Anjum leads the Bacterial Characterisation Workgroup and is the AMR Research Lead at the APHA working at the interface of molecular and veterinary microbiology, within the One Health remit. As lead for the AMR research, she is also involved in supporting national AMR surveillance activities and APHA's response to national outbreaks, and identifying new and emerging threats. She is a member of the DEFRA Antimicrobial Resistance Coordination Group, which advises and reviews the DEFRA activities on antimicrobial usage in animals and AMR in microorganisms from feedstuffs, animals and food, the APHA lead for the Defra AMR in the Environment group, and works closely with colleagues in Public Health England in various research projects and national activities.

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11.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	The student will receive extensive training in microbiological procedures that will be carried out, and is required to read and work to the standards stipulated within APHA risk assessments when going on farm surveys and carrying out microbiological testing. Farm surveys will be undertaken alongside other scientists/vets.
Please comment on any implications for the animal. Please state the 3Rs aspects of this work. Please describe how the animals' welfare are protected and considered	Faecal samples will be collected directly from the ground and farm environment; therefore no direct animal handling is required and is outside of the Animal (Scientific Procedures) Act 1986. Animal welfare on farm is covered by the animal welfare act 2006, and additional controls are required by other farm assurance schemes, which pig farms also adhere to.

11.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			2	M33	?	COVID-19	not yet known	not yet known

Comments:

The project has been significantly affected by COVID-19 as the student by this point should have been visiting the farm recruited for longitudinal sampling. The sampling has been delayed and if these restrictions persist it may have a major impact on the proposed PhD plans. The original plan was to sample across four time points in one year, but this is unlikely to happen as a result of COVID-19. A new estimated completion date is hard to predict, as it is reliant on external factors, such as lockdown restrictions being lifted to allow access to the farms. To help mitigate risks posed by COVID-19 on visiting and sampling on farms, we have already included characterization of archived isolates and are also planning to explore bird caecal samples collected from national surveillance of avian influenza.

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11.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	None

11.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

This PhD overlaps with the ARDIG project, where wild birds on farm have already been sampled.

There will be possible collaboration with FULL FORCE OHEJP project as some samples collected during the PhD will be used for this project. The student attended the FULL FORCE OHEJP plasmid workshop.

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11.12. List of Dissemination activities

Name of the activity:	One Health EJP ASM 2020 (Participation in 3MT competition)		
Date:	May 27th - 29th, 2020,		
Place:	Online meeting		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750+***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

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12. PhD11- EnvDis

12.1. Summary

As a result of the literature review, salmonellosis continues to be the second most commonly reported foodborne disease in humans in Europe, with a seasonal pattern of incidence focused on the warmest weeks of the year. Amongst the food sources of infection, eggs and chicken meat were identified as relevant sources of dissemination.

As a first step in the project, we developed an equation linking the growth of *Salmonella* on eggs and chicken meat dependent of temperature. Assuming that the probability for a human getting infected is proportional to the temperature-dependent number of bacteria, we created a simplistic predictive model using the high-resolution meteorological records for the past 20 years kindly provided by the MetOffice. To validate our model we compared our predictions to the incidence of salmonellosis in England and Wales provided by PHE for the same period of time. Both curves have the same pattern albeit some minor differences, suggesting that the three parameters considered (eggs, chicken and temperature) play a big role on the seasonality of salmonellosis. The preliminary results were presented in some virtual conferences (6th World One Health Conference) and workshops (6th Cogwheel Workshop, OHEJP Communication Workshop).

For this work, substantial progress on programming skills were developed being able now not only to write functions and commands with ease, but also to create complex charts and troubleshoot and fix bugs quickly. Different exploratory charts were created to find further trends and causality, such as a geographical effect by comparing different areas of England and Wales; hypothesise different contribution in the risk factor associated with eggs versus chicken, as well as the modelisation of the effect of an average increasing temperatures (simulating one of the effects of climate change) in the incidence of the disease. These are templates that will be used for improved future versions.

The preliminary results lead to future planning steps, namely including other climate variables (e.g. relative humidity) and perform a conditional incidence of salmonellosis based on the combination effect of both, and extend it to more variables, like UV light in an attempt to fit our simulation curves as closer as possible to the historic incidence data. The effect of environment on animal disease will be considered, as well as other foodstuff involved in infection.

12.2. Overview of project progress

Under the WP1 with the objective of *developing a general tool to assess the risk of infectious diseases (in particular zoonosis) when we have information of relevant environmental factors*:

- *Task 1 « Test and discuss findings of the model for Salmonellosis »* has been partially completed, but it is due to be finalised in December 2022 (M60). Preliminary results suggests that the three parameters considered so far (eggs, chicken and temperature) play a big role on the seasonality of salmonellosis. More variables will be taken into consideration.
    - *Subtask 1 « Test and discuss findings of the model for Salmonellosis »* idem for this task, where temperature has been seen to be a determinant seasonality factor, but more variables (e.g. humidity) will be taken into consideration applying a conditional incidence analysis to the model. This task is due in April 2022 (M52).
    - *Subtask 2 « Complete the same tasks for Leptospirosis »* not due until August 2022 (M56).
    - *Subtask 3 « Write up thesis »* not due until December 2022 (M60).

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12.3. Progress of the research performed in the PhD project and key scientific results

Task 1: Test and discuss findings of the model for Salmonellosis; Complete the same tasks for Leptospirosis; write up thesis.
Activities :

- **Literature review** on (which will be part of thesis and 1st year report):
  - Importance of *Salmonella*
  - Empirical/experimental impact of environment on *Salmonella*
  - Theoretical works to understand and predict the impact of environment on *Salmonella*
- **As above on leptospirosis**. This part of the project was paused as we may explore another disease with a link to food sources of infection instead.
- **Become familiar with infectious disease modelling concepts**. Some courses were pursued for this purpose.
- **Familiarise with R and git. Learn how to write codes that will be published.** Several codes for different purposes (e.g. different plots, a preliminary model linking eggs, chicken and temperature) were done and will be used as templates for future adjustments and improvements of the model. Basic training on Git was done.
- **Familiarise with the data:** the format of data provided by PHE and MetOffice was explored:
  - Some delay in the data coming from PHE was experienced given the extraordinary circumstances. Partial data has now been received (weekly salmonella cases in humans from 1989 to 2012), but we are still waiting for more updated and smaller scale data.
  - The high-resolution MetOffice data was gathered (daily averages for all the post codes in England and Wales from 1978 to 2019) and selected the weather parameters of interest for exploring its impact on the project: global radiation (kJ/m2), mean air temperature (ºC), mean dewpoint (ºC), precipitation (mm).
  - Re-adapting the method from Lo Iacono et al., 2020. for campylobacteriosis to salmonellosis with additional weather variables.
- **Do all the trainings, seminars, conference, external courses etc.** (see section 5.)
- **Formalize detailed research questions and decision on what approach to use next:**
  - Perform a Wavelet analysis as a base for demonstrating seasonality of the weather parameters selected over time. It will be used to compare different models and choose the most adequate.
  - Perform different length duration aggregation averages (7, 14, 30 days) of weather parameters to reduce the effect of incubation delay from the weather variation to its perception in human cases detection.
  - Perform a conditional incidence analysis combining more than one weather factors (e.g. temperature + humidity).
  - Explore the further food parameters that are influenced by weather by means of R plots.
  - Adjust the model to fit historic prevalence data by incorporating more variables (weather, and other relevant food sources, if found)
  - Explore the effect of environment on animal salmonellosis.

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12.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD11-FBZ4/5-EnvDis	D-PhD11-1.1	Mandatory and Basic Trainings	M30	M30		Attendance to following trainings: • Welcome to your Doctorate RDP 12/02/2020 • Workshop on Git with Dean Roe 04/02/2020 • Infectious disease modelling with Giovanni Lo Iacono 18/02/2020 • Introduction to HPC with Pritesh Tailor 24/02/2020 • Programming with R. Online resources • News and updates meeting with the NTD group. University of Surrey Biweekly • News and updates meeting with the vHive group. University of Surrey Weekly
	D- PhD11-1.2	Presentation of findings (e.g. conferences, internal school seminars)	M36	M29		During the first 6 months Laura has reviewed about 56 papers, taking notes and compiling the relevant information as well as populating a table with quantitative values related to the growth and survival rates of *Salmonella* in the main modes of transmission that will be used in the final thesis.
	D- PhD11-1.4	Attending Relevant Training courses (this is optional, and it might happen in year 2 depending on the student's needs.)	M36	M30		Laura has also completed a few relevant trainings, including: - Introduction to Infectious Disease Modelling and its Applications (SIDM) at the London School of Hygiene & Tropical Medicine, UK (15-26/06/2020). - Infectious Disease Modelling Specialization from Imperial College, UK (01-12/06/2020).

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PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD11-FBZ4/5-EnvDis	M-PhD11-1.1	Review of the literature on *Salmonella, Leptospirosis*, environmental epidemiology and modelling approaches;	M30	Yes		During the first 6 months Laura has reviewed about 56 papers, taking notes and compiling the relevant information as well as populating a table with quantitative values related to the growth and survival rates of *Salmonella* in the main modes of transmission that will be used in the final thesis.
	M-PhD11-1.2	Collection of the available data from different sources, MEDMI, APHA, CEH etc. (Objective 1);	M36	Partially	M41	Data from MEDMI are available to collaborators. It would be easier to access the data from their physical hard-drive (remote transfer is difficult due to stringent security measures). Following Covid-19 lockdown this has not been possible. Laura is however working on other aspects of the PhD, so this has not been a big problem. We are aware that our collaborators in PHE are working on sharing the data in the upcoming months.
	M-PhD11-1.3	Generate specific hypotheses about the underlying mechanisms involved (Objective 2-3);	M36	Partially	M48	Completed, but Laura will keep on working on this by reviewing the literature during the second year.
	M-PhD11-1.4	Interact with colleagues at PHE and Gianni Lo Iacono who have estimated the probability of *Salmonella* cases, knowing recent environmental parameters at certain locations (Objective 2-3)	M36	Partially	M45	Laura is in contact with Prof Nichols from PHE. She is also in contact with Dr Emma Gillingham who was working on the estimation of the probability of *Salmonella* cases, knowing recent environmental parameters. Because of COVID-19, however, Dr Gillingham has not been able to work on this. After discussing with our

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						collaborators, Laura will take over this task as soon as she has access to the data.

12.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Postgraduate Research Showcase	Self-Management	23/01/2020	UoS (Doctoral College/IoD)
Demonstrating in Laboratories	Teaching	29/01/2020	UoS (RDP)
Assessment and Feedback	Teaching	03/02/2020	UoS (DHE)
Introduction to Teaching in Higher Education	Teaching	07/02/2020	UoS (DHE)
Presentation skills with James Green	Communication	12/02/2020	UoS (ELSP)
Presentation skills with James Green	Communication	19/02/2020	UoS (ELPS)
Statistics and R (online course)	Programming	01-29/11/20	Harvard edX
Public speaking and presenting	Communication	13/11/20	Teach First
Presentation skills in Practice	Communication	30/11/20	UoS (DC)
Intermediate R	Programming	22/05/2020	DataCamp
Developing the SIR model	Modelling	01-08/06/20	Imperial College/Coursera
Intro. to Infectious Disease Modelling and its Applications	Modelling	15-26/06/20	LSHTM
French weekly classes	Languages	07/11/20- 28/04/21	UoS (GGA)

12.6. Publications and patents

- Laura Cristina Gonzalez Villeta. (2020, December 20). EnvDis deliverables Y1. Zenodo. http://doi.org/10.5281/zenodo.4516634.

12.7. Impact and Relevance

The project involves the University of Surrey, Public Health England (PHE) and Zoetis. Laura's project builds on current work being done at PHE by Dr Gillingham and Prof Nichols and myself (Lo Iacono). Essentially PHE will focus on a phenomenology of *Salmonella* in England and Wales and Laura's project will complement this by using mechanistic models. The project fits also very well with research interests of Dr Kanellos in Zoetis and the activities led by Prof Cook in vHive (https://vhive.buzz/). Following initial meetings with partners we are now planning to extend the collaboration by applying for extra funding focusing on the use of big data (e.g. weather data) to understand and measure the impact of the environment on infectious diseases.

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12.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
So far there appear to be no ethical issues since data is already in the public domain or granted permission to be shared. This might change as we are going to have access to PHE data.	N/A

12.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Task 1	NA	NA	M- PhD11-1.2	December 2020	May 2021	Delayed data on origin	NA	NA
Subtask1	NA	NA	M- PhD11-1.4	December 2020	October 2021	Collaborator unavailable	NA	NA

Comments:

M- PhD11-1.2 Laura is waiting for PHE to provide some data and this action keeps suffering delays due to the COVID-19 urgent management undertaken by PHE. Despite this delay, Laura still can work without the data, focusing on preliminary work that needed to be done anyways: e.g. literature review, designing the modelling approach (i.e. selecting route of transmission and identify suitable environmental factors based on the literature rather than data), simulating the dependence of *Salmonella* on these factors.

M- PhD11-61.4 Dr Emma Gillingham who was working on the estimation of the probability of *Salmonella* cases, knowing recent environmental parameters. Because of COVID-19, Dr Gillingham has not been able to work on this. After discussing with our collaborators, Laura will take over this task as soon as she has access to the data. She will re-orientated the approach to go around it by adapting an existing method in a similar paper applied to *Campylobacter*.

12.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	NO
Loss of technical training staff delaying progress of the work	NO
Delay in work plan execution	NO
Conflicts between the collaborative partners that support the PhD	NO
Lack of commitment between the collaborative partners that support the PhD	NO
Delay in duties, tasks or reporting	NO
Poor working relationships within the PhD project team	NO
Change in PhD student circumstances requiring temporary leave	NO
Other risks (please describe)	Yes*

Additional Comments: The major difficulty that Laura is experiencing is a lack of concentration due to the lack of a daily routine since working from home.

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12.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Not applicable now although we are exploring a few initiatives.

12.12. List of Dissemination activities

Name of the activity:	2nd One Health European Joint Project (OHEJP) Annual Scientific Meeting 2020. Presentation of poster and participation in 3-minute Thesis competition.
Date:	27th – 29th May 2020
Place:	virtual

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

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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Name of the activity:	Vet School Research Celebration Event. University of Surrey
Date:	9th September 2020
Place:	Virtual

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

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***120***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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Name of the activity:	Communication and Media Workshop (OHEJP)		
Date:	5th – 6th June 2020		
Place:	virtual		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*No*
Organisation of a Workshop	*No*	***Participation to a Workshop***	***Yes***
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***35***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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Name of the activity:	6th World One Health Congress		
Date:	30 Oct -3 Nov 2020		
Place:	virtual		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	***Yes***	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***+1500***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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Name of the activity:	6th Cogwheel Workshop (SafeConsume/OHEJP)		
Date:	25 Nov 2020		
Place:	virtual		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***40***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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Name of the activity:	EnvDis Deliverables Y1
Date:	20/12/2020
Place:	https://zenodo.org/record/4516634#.YD0qWU5xeUk

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*No*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	***Yes***	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	***Yes***	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	*0*	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

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13. PhD12- AptaTrich

13.1. Summary

Two protocols for *Trichinella spiralis*-specific aptamers selection were planned. The first one is against whole Muscle Larvae (ML), the infective stage of this nematode. The second one is against protein (s) considered as potential biomarker (s) from the excretory/secretory ML products. Firstly, the PhD student has been trained at McGill institute (extra EJP-partner) for the SELEX (Systematic Evolution of Ligands by Enrichment) method (cell-SELEX, protein-SELEX,…). For both protocols, in the past few months, much work has been accomplished in producing single-stranded DNA (ssDNA) sequences from double-stranded DNA (dsDNA) PCR products, an absolutely essential step in the successful isolation of target specific aptamers. Then the protocols to adapt the SELEX method to *Trichinella spiralis* whole Muscle Larvae (ML) have been established and optimized at Anses.

Regarding the whole larvae-SELEX, *T. spiralis* ML has been recovered from infected mice and fixed in ethanol. They have been used and are currently in use for the whole larvae-SELEX method defined. Selection of single stranded DNA-based aptamers specific for *T. spiralis* whole muscle larvae (ML) has begun using a highly variable and randomized ssDNA library by SELEX method (Systematic Evolution of Ligands by Enrichment). Intact *T. spiralis* whole ML are incubated with the DNA library for multiple rounds (up to now 5 successful cycles have been performed) with a progressive decrease in ML number and interaction time at each round. In addition, larvae washing volume and frequency with a buffer solution are gradually increased to remove aptamer sequences that are less specific for the target. Then ssDNA was prepared by asymmetric PCR incorporating a biotin and recovered using streptavidin beads.

For the protein-SELEX, a first step of optimal biomarker identification was necessary. Currently, *Trichinella spiralis* muscle larvae have been recovered from infected mice. Excretory/Secretory proteins, containing potential biomarkers of infection, have been produced and collected for further top-down Mass Spectrometry (MS) analysis at the McGill Institute. Upon identification, specific biomarker proteins can be used as targets in the protein-SELEX experiments to generate aptamers to be used in diagnostic applications.

13.2. Overview of project progress

- The project has begun and the student has been well trained in the whole-larvae SELEX methodologies along with other key laboratory protocols. Furthermore, many *Trichinella spiralis* muscle larvae have been produced and fixed in ethanol for future experimentations.
- As shown in the gel (image #1, below), following 5 cycles of whole-larvae SELEX, DNA sequences have been successfully isolated from the surface of the muscle larvae. However, with the technique used at the time, it is not possible to see whether any single-stranded DNA is present.
- The gel (image #2, below) illustrates the successful production and identification of single stranded DNA. This new method will be implemented shortly in a SELEX context to yield specific single stranded aptamers. These results are very promising.
- Recently, *T. spiralis* excretory and secretory (ES) antigens have been produced. These proteins are being kept at -80C for further identification by Liquid Chromatography Mass Spectrometry (LC-MS/MS). This work is to be conducted at the Canadian partner institution. Upon identification of potential biomarkers, work on producing recombinant proteins will begin to initiate protein SELEX.

13.3. Progress of the research performed in the PhD project and key scientific results

Key Scientific Findings

Round 5 of aptamer selection against Whole *T. spiralis*

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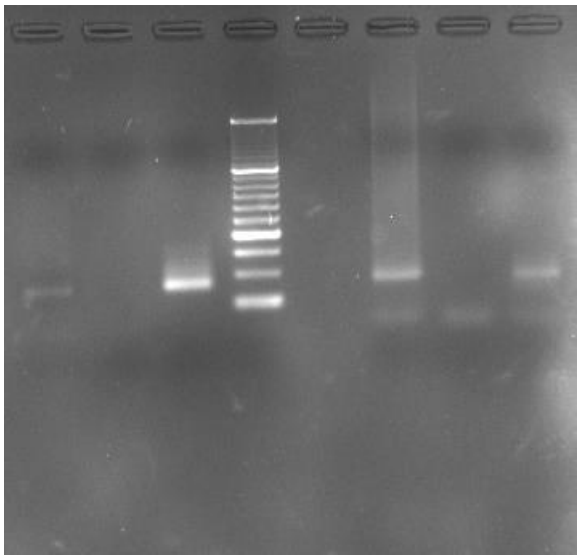
- 5 rounds of aptamer selection have been conducted successfully with the retrieval of *T. spiralis* specific sequences and the following PCR amplification.
- Upon heating the larvae, sequences are recovered and subjected to amplification by PCR for the next round of selection.
- The image below illustrates that indeed our desired products of 80bp are present in lane 6.
- This means that aptamers specific for *T. spiralis* muscle larvae have been selected.

Gel Image #1

R5 purified = 22.5ng/ul

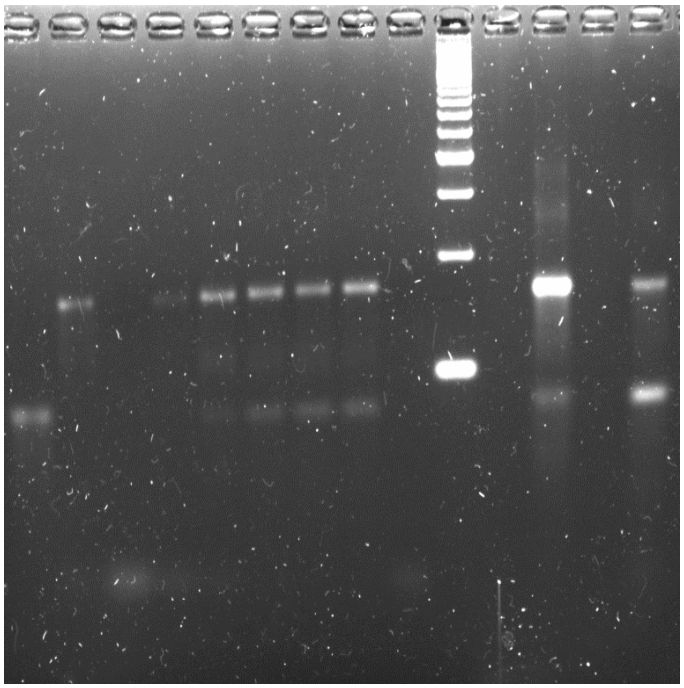


Gel Loading Sequence

1 – R5 symmetric PCR (12 cycles)
2 – Symmetric neg control
3 – Symmetric pos control
4 – 50bp ladder
6 – R5 pur (10 cycles asymmetric PCR)
7 – asymmetric neg control
8 – symmetric pos. control

Isolation of single stranded DNA

Gel Image #2



Gel Loading Sequence

1 – 1uM 20N ssDNA
2 – 20N ssDNA amplified 8 cycles symmetric PCR
3 – Symmetric PCR negative control
4 – Symmetric PCR products amplified 0 cycles asymmetric PCR
5 - Symmetric PCR products amplified 10 cycles asymmetric PCR
6 - Symmetric PCR products amplified 20 cycles asymmetric PCR
7 - Symmetric PCR products amplified 30 cycles asymmetric PCR
8 - Symmetric PCR products amplified 40 cycles asymmetric PCR
9 – Asymmetric negative control amplified 40 cycles asymmetric PCR
10 – 50bp ladder
14 – Asymmetric PCR product treated with streptavidin sepharose beads

Important to note here are the products present in lane 14 of the gel. Below the 50bp mark we can see a bright band at approximately the 40bp mark. These products correspond to ssDNA at 80 nucleotides

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in length. On the gel, these 80 nucleotide sequences correspond to a weight of 40bp. These are our sequences, or aptamers, of interest. While these sequences were directly derived from the amplified ssDNA library, and were at no point introduced to the *T. spiralis* muscle marvae, it is a proof of concept which will be implemented shortly in the generation of *T. spiralis* specific aptamers.

13.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD12-FBZSH9-AptaTrich	D-PhD12-1	*T. spiralis* ML are now fixed in ethanol	M24	M27		*T. spiralis* Muscle larvae have successfully been fixed in ethanol
	D-PhD12-2	Aptamers set on whole larvae is selected	M29	M29		Pools of aptamers specific for *T. spiralis* muscle larvae have been produced
	D-PhD12-3	Aptamers set on stage specific proteins is selected	M36	N/A	M42	

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD12-FBZSH9-AptaTrich	M-3	The aptamers bind to Trichinella spiralis ML	M36	Yes		

13.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organizing Institute
Module général de la Formation en expérimentation animale - Niveau conception et réalisation des procédures.	Training in Animal Experimentation	Start : 07/09/2020 End : 11/09/2020	Université de Paris Est (UPEC) école Nationale Vétérinaire d' Alfort (ENVA)

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13.6. Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 24.

13.7. Impact and Relevance

The partners involved in the project offer various elements of expertise to optimally support a PhD student in the development of a new method. The partners come from institutes with a wide interdisciplinary background.
ANSES laboratories conduct their activities in three major areas: animal health and well-being, food safety (chemical and biological) and plant health. The French NRL is also an OIE Collaborating Centre, it is part of the Animal Health Laboratory of Anses, which is internationally renowned and carries out critical missions for France, Europe and World in the field of animal health and public health, food safety, epidemiology. Researchers of the NRL work in close collaboration with medical doctors, veterinarians and give their expertise when outbreaks occur.
The Federal Institute for Risk Assessment (BfR) is the German national scientific institution in the area of consumer health protection, food safety, authenticity and risk assessment/ risk communication. The NRL for Trichinella mainly operates in the field of diagnostics, food safety and risk assessment, but also provide consultant support in human diagnostics.
The Canadian National Reference Centre for Parasitology (NRCP) is located within the Research Institute of the McGill University Health Centre (RI-MUHC) and provides reference serological and molecular diagnostics for parasitic diseases. Investigators at the Centre are active in several areas, including clinical parasitology, parasite diagnostics, parasite epidemiology, vaccine immunology, as well as cold-climate parasitoses and circumpolar health.
The existing close collaboration between the partners, which operate between the medical, veterinary and food science fields, will facilitate the success of the proposed PhD project under a One Health approach.

13.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
<u>Human biological samples</u> The beneficiary must confirm that appropriate authorizations will be sought to collect the Human samples. <u>Non EU countries (Canada)</u> The beneficiary must provide details on the material which will be imported to/exported from EU and confirm that the adequate authorisations have been obtained. This project is focusing on laying hens. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the layers hens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the chickens are affected when taking samples, even if the work is dealing with faeces as these types of study can, this	<u>Human biological samples</u> All serums have already been sampled with the authorizations that must be obtained in accordance with Canadian laws, and within the framework of the NRC in place at the McGill Institute. <u>Non EU countries (Canada)</u> Both the French and German NRLs have defined *Trichinella* positive and negative pig sera already available in their respective repository and the Canadian reference center has defined some *Trichinella* positive Human sera in its repository. Each project participant will test the effectiveness of the test on its own samples. Therefore, no shipment is planned. If, however, the evolution of the project leads to shipments, the Nagoya protocol will be respected. This project will not focus on laying hens. On the other hand, we will have to use mice to obtain larvae.

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can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals). Please provide a statement on the 3Rs aspects of this work If Ethical Approval is required please state which Research Ethics Committee this will be sent to.	Their number will be very limited since each mouse contains thousands of larvae. The experiments on mice were approved by an ethical review committee (C2EA-16 Comité d'éthique ComEth ANSES/ENVA/UPEC, under the approval number: saisine 12-0048, ComEth 13/11/12-4).

13.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			**Milestones and Deliverables**				**Associated budget**	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
Aptamers selection on *T. spiralis* whole larvae	M29	M32	D-2 & M-3	M36	M39	Lab closed during the crisis	€11250	€11250
Production of stage specific proteins and apply protein-SELEX	M36	M42	D-3 & M-3	M36	M42	Lab closed during the crisis		

Comments: For now, as It Is still the beginning of the thesis, we estimate that the work was delayed for 3 months. The ongoing confinement and curfew measures have added some difficulty in accessing the laboratory.

13.10. List of critical risks

Description of risk	**Yes/No**
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

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13.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Not Applicable

13.12. List of Dissemination activities

Name of the activity:	2nd OHEJP Annual Scientific Meeting 2020. Presentation of poster and participation in 3-minute Thesis competition.		
Date:	27-29 May 2020		
Place:	Virtual		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	***No***		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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14. PhD13- VIMOGUT

14.1. Summary

Over the past year the PhD student has made progress in both work packages of the VIMOGUT project. During the first lockdown of the COVID-19 crisis in the Netherlands, she has participated in several online courses and home-training in community ecology, multivariate analysis and analysis of microbiome data. For Work Package 1 a dataset was generated in year 2 of the microbiome of broiler chickens on a single farm that complements a preliminary dataset from the same flock to generate additional statistical power to the analysis. During the lockdown she has merged the datasets and analysed these together. While the data analysis has uncovered some unforeseen issues in the generated data, the outcome of the analysis is currently prepared in a manuscript for submission to a peer-reviewed scientific journal. Preparations to commence sampling of new broiler chicken farms in the Netherlands will commence shortly and, provided that this is possible during the COVID-19 pandemic, farms will be visited in 2021 to generate datasets from additional broiler flocks on additional farms in order to confirm if the differences in microbiome composition that were measured in the first dataset can be found in additional flocks.

The second work package of the PhD project relates to the set up of an *in vitro* model of the broiler caecum. A continuous culture fermenter system was designed in year 2 and delivered in year 3. The model is seeded with caecal content of broiler chickens in order to create a stable microbial community. This community can be used to test the effects on the composition of the community from feed interventions and to study the effects of substances on the transfer of AMR encoding plasmids between bacteria in the community. Courses to control the processes and perform the culturing in the fermentors were provided online by the supplier of the system. Workflows for the process were created and several test runs were performed. Based on these test runs, challenges were identified regarding the pH and oxygen maintenance in the system. Adjustments to the protocols and workflows have mostly solved these challenges. Initial runs have been performed using to test the effects of two phytochemicals for which microbiome analysis is currently carried out.

14.2. Overview of project progress

For Work Package 1 the student has made much progress in key theoretical concepts of microbial ecology and the research of animal microbiomes. She has attended several courses in order to understand the background of the field and the major challenges. Furthermore, she attended courses to gain the necessary skills in bioinformatics to perform 16s microbiome analysis independently. As 16s microbiome analysis is a key component for both Work Package 1 and 2, it is vital that the student is comfortable using this technique. The work for Milestones 5 and 7 have been delayed somewhat due to a technical difficulty in combining the preliminary dataset and the dataset that was generated in Y2.

For Work Package 2 the progress has suffered more from the COVID-19 crisis and the following lockdown as laboratory facilities were temporarily closed for non-essential work. The student has been able to gain a lot of technical experience on how to design workflows and perform experiments using the fermentor system from online courses on these topics from the supplier of the system, Applikon biotechnologies. As such the technical testing of the system has been somewhat delayed. While a technical challenge of the pH control of the system has been resolved, another challenge in the detection of (unwanted) oxygen and its removal is currently faced by several technical solutions. These challenges have given the student much technical insight into the operation of the system and will benefit her for certain further challenges that will be identified later.

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14.3. Progress of the research performed in the PhD project and key scientific results

Since the start of the PhD project, the student has followed several courses and seminars to learn about the background of microbiome analysis and microbial ecology. These included KNVM: Microbial Ecology, Baseclear: Animal Microbiome Expert, WUR-VLAG Graduate School: Intestinal Microbiome of humans and animals, Utrecht University: Analysis and interpretation of Microbiome and Metagenomics data and online courses on 'The introduction to multivariate analysis' and 'Advanced community ecological data analysis' from the University of Regina. Furthermore, she has received in-house training on the use of 16s barcoding and further molecular biology techniques, completing Milestones 1 and 2. Samples that were collected before the start of the project have now been sequenced using 16s barcoding as part of Milestone 4. Analysis of this data was performed successfully and reconfirm the findings of a preliminary dataset. The maturation of the chicken gut occurs in three successional stages in which the richness of the composition of the community greatly increases over the first 21 days. While all animals were eventually colonised by ESBL producing *E. coli*, those with a greater diversity in the second stage were colonised later indicating a possible window of opportunity for intervention strategies. There were technical difficulties in the analysis when the preliminary and novel datasets were merged. The student has addressed these difficulties with a collaborator from iDiv Germany. Analysis on the merged dataset confirmed that broilers that were colonised by ESBL early in the production round have statistically less diverse microbiomes. This concludes the analysis for Milestone 5 and currently a manuscript is being prepared describing the relationship of the chicken gut microbiota and the colonisation of the gut by ESBL E. coli, as described in Milestone 7/Deliverable 1.

For Milestone 3, a visit was planned to APHA for training on the in vitro chicken gut model that is operational there. However, due to difficulties to obtain a UK visum and the fact that Applikon Biotechnolgies, the supplier of the Bio-reactor system that was obtained by WBVR, provides a basic cultivation course and a course ti design and implement workflows to control the system, this was considered sufficient training. The design and implementation of the system was discussed with APHA via Skype and APHA will be included in the progress of the set-up of the system at WBVR.

The test runs of the Bio-reactor system revealed challenges regarding the control of the pH and the exclusion of oxygen. While control of the pH worked in the presence of water, in test runs with culture media the values would oscillate due to addition of large volumes of alkali and acid. Recalibration of the pumps to smaller volumes have solved this problem. Currently, the amount of oxygen that is detected in the system is still too high. It is uncertain if this is a leak in the system, a sensor problem or a side-effect from components in the culture media that are used. An adaptation to the delivery of nitrogen gas in the system should help to determine the optimised state of the system. After this installation, experiments will be carried out to determine the CFU of ESBL E. coli to deliver an efficient colonisation of the system, finalising milestone 6.

To start running the model seeded with a caecal community, a large batch of fresh chicken caeca was acquired from a slaughterhouse and caecal content was mixed and stored aliquoted in glycerol at -80 °C in order to allow identical start cultures for all experiments that are run for VIMOGUT. Initial experiments with these communities have been performed to determine the effects of the phytochemicals berberin and quercetin, two feed additives that are currently used in broiler feed. 16s microbiome sequencing of these experiments is currently carried out and will give insight into the stability of the communities over time in the model. Furthermore, these results can contribute to the work described for milestone 10 where intervention strategies against the colonisation by ESBL E. coli are tested.

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14.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD13-FBZ8/AMR2-VIMOGUT-AMR	D-PhD13-1.1	Manuscript on preliminary findings for the relationship between chicken gut microbiome maturation and ESBL colonisation.	M36	-	M39	Unforeseen challenges with the data analysis described for Milestone 5 are currently being addressed. This is the final work for this manuscript.

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD13-FBZ8/AMR2-VIMOGUT-AMR	M4	Perform 16S barcode sequencing on currently collected samples.	M27	Yes		
	M5	Perform analysis of initial 16S experiment	M30	Yes		Initial analysis was presented in OH-EJP ASM poster.
	M6	Perform initial test runs on *in vitro* gut model to determine CFU for reliable ESBL colonisation.	M24	No	M42	The work on the *in vitro* chicken gut model has been delayed considerably due to the COVID-19 pandemic. Some standardisation and initial test runs in the *in vitro system* have been performed but the model is not completely in use yet.
	M7	Write manuscript on initial results relationship	M36	No	M39	See deliverable 1: Unforeseen challenges with

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		between chicken gut microbiome maturation and ESBL gut colonisation.				the data analysis are currently being addressed. This is the final work for this manuscript.

14.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
PhD competence assessments	Various essential skills for PhD students	26/11/19	Wageningen Graduate School
Resilience during change	Dealing with unexpected events	28/04/20	YoungWUR
Lucullus	Training on software controls of fermentor systems	May-December 20	Applikon Biotechnology
Introduction to multivariate analysis	Data analysis	07/07/20	University of Regina
Advanced community ecological data analysis Vegan	Data analysis	09/07/20	University of Regina
PhD writing course	Effective academic writing	20/09/20	University of Leipzig
Webinar Animal microbiomes		25/09/20	Baseclear NL
Basic cultivation course	Hands-on lab course for culturing in fermentors.	12-15/10/20	Applikon Biotechnology
Scientific integrity		27/10/20	Wageningen Graduate School

14.6. Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 31.

14.7. Impact and Relevance

The work that is carried out during VIMOGUT enhances the existing collaboration between WBVR and the APHA. The APHA has contributed tremendously through sharing their experience with their own *in vitro* gut model which has been the foundation for the development of the current model at WBVR.

Within Wageningen University and Research, the VIMOGUT project has initiated a new collaboration between WBVR and the Plant Science Group of which Prof. Arjan Stegeman is part. His involvement into the project as promotor and his experience in microbial ecology are essential for the analysis of the data that is generated in the project.

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The work that is carried out has produced a new collaboration with iDiv in Leipzig where Dr. Stephanie Jurburg has come to aid when we proposed the difficulties of merging our datasets from the two *in vivo* datasets described above.

Outside of the consortium there has been much attention to the development of the *in vitro* model. Social media posting of the installation of the fermentors was well received and various groups and commercial parties are interested in receiving updates of the development of the model. The initial experiments described above using berberin and quercetin as the first feed additives to detect changes in the composition of the microbiome were performed as part of a PhD-student research visit where Mr. Will Hutton from the group of Dr. Adam Roberts at the Liverpool School of Tropical Medicine (LSTM) visited WBVR for a month.

14.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
This project is using broiler chickens at conventional farms. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the broiler chickens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the the broiler chickens from sampling etc. Please describe how the animals' welfare are protected and considered (e.g. if the chickens are affected when taking samples, even if the work is dealing with faeces as these types of study can, this can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals). Please provide a statement on the 3Rs aspects of this work If Ethical Approval is required please state which Research Ethics Committee this will be sent to.	During the VIMOGUT project, sampling at conventional broiler farms and possibly slaughter houses will be carried out. If samples are taken at slaughter houses, the intestinal tract of animals will be collected after slaughter and evisceration are carried out. No changes are made to the standard procedures of the site and no additional animals are slaughtered for the benefit of the research. For the sampling at farms, only conventional broilers will be used with no restrictions or manipulation of the animals' diets. Animals will not be sacrificed for the research carried out for VIMOGUT unless approval has been given by the local animal welfare committee (IvD) at WUR and the national board on animal experiments (CCD), as per local guidelines. Sampling at conventional broiler farms will include the collection of fresh droppings for which animals may be briefly isolated to relieve themselves. Handling of the animals will only be performed after careful instruction and under the constant supervision of a trained veterinarian. As per local law in the Netherlands, the 3Rs are always considered when animal experiments or on-site sampling is performed. The in vitro model that is set up during VIMOGUT is part of the strategy to replace the need for animal experiments in microbiota research. To refine the experiments and reduce the number of animals that are sacrificed, fresh droppings will be used for this study instead of caecal content of the animals, unless there is there is a clear need for the use of caecal content. As mentioned above, permission will be sought from the local animal welfare committee IvD and the national board on animal experiments CCD.

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14.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
			M6	M24	M42	Lab closure		

Comments:

The COVID-19 crises has had effects on the feasibility of meeting deadlines of the milestones as described above. While the analysis work on *in vivo* microbiome has mostly been able to be continued, the work on the *in vitro* model has had significant delays due to the temporary closure of the laboratory facilities and the postponement of an essential course to properly commence this part of the work. An arrangement was made with the supplier and this course has now been followed and experiments with the *in vitro* systems have commenced.
While the situation in the Netherlands is progressing well, it is currently still unclear if the crisis will have an impact on the feasibility to collect.

14.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

Additional information:
As described above, the COVID-19 crisis has had effects on the planning of the work in this PHD project.

14.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Data generated in VIMOGUT is shared with the National Monitoring program for AMR in Livestock in the Netherlands.
Analysis of samples from the VIMOGUT *in vitro* chicken gut model will be tested with the Long read metagenomic approach that is developed during OH-EJP FARMED.

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14.12. List of Dissemination activities

Name of the activity:	OHEJP Annual Scientific Meeting 2020. Presentation of poster and participation in 3-minute Thesis competition. *WINNER of 3MT competition*		
Date:	27-29 May 2020		
Place:	virtual		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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15. PhD14- ToxSauQMRA

15.1. Summary

The present research project aims to answer to the scientific question: "What is the attribution of the traditional raw pork products in the human Toxoplasma infection? " based on three main areas of study consisting of (i) a thorough investigation of *T.gondii* predilection sites in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst) (WP1); (ii) evaluate the impact of the manufacturing process (including different incorporation rates of nitrites and NaCl) and the conservation of dry sausage on the viability of *T. gondii* (WP2); (iii) a quantitative microbiological risk assessment analysis to be conducted for the various raw pork products (dry sausage, dry ham, etc) (WP3). The 3-year project spans over four Annual Periods (Y2-Y5). The key activities, results and achievements for the reporting period of January to December of this year are the following:

Key scientific results:

1. Successful experimental infection of pigs with both parasitic forms (oocysts and tissue cysts)
2. Successful collection of meat samples in spite of Covid19 shut-down
3. Complete artificial digestion of meat samples from "oocysts" pig group
4. Manufacturing of 168 dry sausages and their analysis by bioassays
5. Manufacturing of long-salting dry ham and the analysis by bioassays

Challenges:

1. Failure in oral infection with tissue cysts form of the parasite
2. Covid19 shut-down twice (March-May and November)
3. Strike in harbour of Marseille

Adaptations:

1. Repeating the oral infection with tissue cysts form of the parasite for 3 times
2. In house production of tissue cysts
3. The Covid19 shut-down that forced us to adapt our sampling schemes and planning
4.

15.2. Overview of project progress

Concerning the project progress in various tasks and work packages for the last 12 months, we are clearly within our objectives and goals since for:

<u>WP1 - Investigation of *T.gondii* predilection sites in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst)</u>

WP1-T1: Experimental infection of pigs: **done**

WP1-T2: Predilection sites of *T.gondii* in pigs: **partially done, due to 1st and 2nd confinement**

<u>WP2 - Assessment of the persistence of *T. gondii* during the production and storage of dry sausages and dry ham</u>

WP2-T1: Manufacture of dry sausages and dry ham: **done**

WP2-T2: Assessment of the persistence of viable *T. gondii* in pork delicatessen: **in progress, due to 1st and 2nd confinement, anyhow the task is due for M42**

<u>WP3 - Quantitative microbiological risk assessment</u>

WP3-T1 Review of prevalence of *T. gondii* in pigs and pork products: **this task is not due to take place before M37**

WP3-T2 QMRA modelling for human *T. gondii* infections: **this task is not due to take place before M47**

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15.3. Progress of the research performed in the PhD project and key scientific results

Within the WP1 (Investigation of *T.gondii* predilection sites in experimentally infested pig carcasses with two different stages (tissue-cyst versus oocyst)), we have worked on two different tasks: WP1-T1: Experimental infection of pigs and WP1-T2: Predilection sites of *T.gondii* in pigs with the help of external partners: Institut du Porc (IFIP) and Université de Champagne-Ardennes (URCA).
For WP1-T1, the main focus was to be able to collect meat massively contaminated with *Toxoplasma gondii*. Therefore an infection with a high dose (1000) of parasites was carried out (strain ME49) in pigs. These tests were carried out with 2 parasitic forms that may be at the origin of contamination in pigs: the oocyst (ingestion from the environment) and the tissue cyst (ingestion from infested meat). Three pigs were inoculated with oocysts and 3 others with tissue cysts. Serological monitoring of the serum of these pigs was carried out by Modified Agglutination Test (MAT) on a weekly basis until D30, then every 15 days until the end of the protocol (3-4 months/80-90 kg).
However due to several logistic problems (late delivery/lack of viability of parasitic forms, last-minute change of the experimental facilities), the inoculation of the 3 pigs with the tissue cysts form of the parasite has failed twice. Four-weeks post-infection, they revealed negative by MAT, while the 3 pigs inoculated with oocysts revealed positive. Therefore an experimental infection of mice with viable oocysts of *T. gondii* has been set up, in order to produce our own tissue-cysts for experimentally infection of the remaining 3 pigs. Eight weeks later, the mice were culled, the brain has been collected, homogenized, the tissue cysts of *T.gondii* identified and counted. A dose of 1000 tissue cysts /pig has been inoculated orally. Serological monitoring of the serum of these pigs was carried out by Modified Agglutination Test (MAT) on D2, 5, 7, 9 p.i. and at the end of the protocol, since the Covid19 shut-down was set up. At the end of the protocol the 3 pigs were confirmed by MAT to be positive for *T.gondii* infection.

After euthanasia of the MAT positive pigs, 4 tissues /pig were collected for analysis: heart, breast, shoulder and ham as tissues used in the manufacture of dry sausage. For each of the anatomical regions studied, the different muscles (4 for breast, 7 for shoulder and 13 for ham) were pooled. Some 40 supplementary muscles per carcass were individually collected, representing the most important anatomical regions. One hind leg was collected for each pig for dry ham production.

Concerning the WP1-T2, the main focus was to identify the predilection sites of *T.gondii* presence in pig tissues. Therefore, mouse bioassays and quantitative PCR was performed on a part (200g) of the pooled sample per region. The remaining parts of the pools were used for industrial (salting, smoking, etc) processing (WP2).
The analysis of tissue samples (by qPCR and mouse bioassays) is still under way, delayed by the 1st and the 2nd confinement due to COVID19 sanitary crisis in France.

Within the WP2 (Assessment of the persistence of *T. gondii* during the production and storage of dry sausages and dry ham), we have worked on two different tasks: WP2-T1: Manufacture of dry sausages and dry ham and WP2-T2: Assessment of the persistence of viable *T. gondii* in pork delicatessen with the help of external partners: Institut du Porc (IFIP), Université de Champagne-Ardennes (URCA) and INRAe Corte (Corsica).

For WP2-T1 the manufacture of dry sausages was carried out on a pilot scale by IFIP, according to a protocol representative of those used by a commercial factory. Briefly, after mincing, the muscle pool was divided into seven portions (corresponding to the 7 combinations of nitrites (as sodium NaNO2 nitrites) and NaCl concentrations that was to be compared: 120 (maximum dose of nitrites mentioned in the Code of Practice), 60 and 0 ppm of nitrites combined with the usual dose of 26 g/kg NaCl or a reduced dose of 20 g/kg NaCl or 0 g/kg NaCl. For each of the tested formulations, 3 dry sausages was collected at different dates (D0, D2, D10, D20, D30 and D50). On each analysis date, IFIP has carried out a physico-chemical monitoring (pH, Aw, weight loss) and a count of the lactic flora from a dry sausage per formulation, in particular to check that the process is running properly. A total of 168 dry sausages was required for this study (3 dry sausages × 7 recipes × 6 analysis dates for *T. gondii*

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monitoring as well as 1 dry sausage × 7 recipes × 6 analysis dates for physico-chemical and bacteriological analyses).
The dry hams were meant to be sent and manufactured by INRAe Corte (Corsica), using two traditional salting techniques (a long one : 2.5 days/kg and a short one: 1 day/kg). However due to a local strike in the harbour of Marseille, the hams arrived with 10 days of delay, causing the sanitary quality of meat to be questionable in terms of manufacturing. Therefore, a long salting technique has been applied only, with 300g of product that was taken at D30 and D90 due to Covid19 shut-down.

Concerning the WP2-T2 the main focus was the analysis of the dry sausages for the presence of viable *T. gondii* by bioassay in mice that has been performed in the animal facility of URCA. The presence of *T. gondii* DNA in the inocula was quantified by URCA using a qPCR and is under analysis by ENVA by MC-qPCR.

The analysis of dry sausages is still under way, delayed by the 1st and the 2nd confinement due to COVID19 sanitary crisis in France.

Key scientific results:

1. Successful experimental infection of pigs with both parasitic forms (oocysts and tissue cysts)
2. Successful collection of meat samples in spite of Covid19 shut-down
3. Complete artificial digestion of meat samples from “oocysts” pig group
4. Manufacturing of 168 dry sausages and their analysis by bioassays
5. Manufacturing of long-salting dry ham and the analysis by bioassays

Challenges:

1. Failure in oral infection with tissue cysts form of the parasite
2. Covid19 shut-down twice (March-May and November)
3. Strike in harbour of Marseille

Adaptations:

1. Repeating the oral infection with tissue cysts form of the parasite for 3 times
2. In house production of tissue cysts
3. The Covid19 shut-down that forced us to adapt our sampling schemes and planning

15.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD14-FBZ4-ToxSauQMRA	D-PhD14-1	Final report on the experimental infection of the pigs (serological monitoring, rectal temperature, weight) and predilection sites of *T.gondii*	M36		M39	Due to Covid19 sanitary shut down a 3 months delay has to be taken into account (for the moment)

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PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD14-FBZ4-ToxSauQMRA	M-PhD14-01	Pig infection experiment is terminated. Weekly/monthly weight and rectal temperature are collated and presented in a graph. Samples are collected and prepared for testing and dry sausage and ham processing.	M27	Yes		Due to Covid19 sanitary shut down and the need to repeat 3 times the tissue-cyst oral infection, 6 months delay has to be taken into account. The milestone was achieved during M33
	M-PhD14-02	Serological testing of pigs is finalised and weekly levels of Igs are collated in a graph.	M30	Yes		
	M-PhD14-03	Testing of tissue samples prior to processing is finalised and quantitative PCR, Magnetic-capture PCR and mouse bioassay data are collected in tables.	M36	No	M39	Due to Covid19 sanitary shut down a 3 months delay has to be taken into account (for the moment)
	M-PhD14-05	Testing of dry sausage samples is (almost) finalised and quantitative PCR and mouse bioassay data are starting to be collected in tables	M36	No	M39	Due to Covid19 sanitary shut down a 3 months delay has to be taken into account (for the moment)

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15.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Communication and Media Workshop	Communication of science	05/10/20 - 06/10/20	Bulgarian Food Safety Agency
Cours Francais Langue Etrangère niveau débutant	Language	22/09/20 - 14/12/20	Université Paris-Est Créteil

15.6. Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 40.
Moreover, during this period, Filip DAMEK published the article: "Detection of *Toxoplasma gondii* in retail meat samples in Scotland" Jacqueline Plaza, Filip Dámek, Isabelle Villena, Elisabeth A.Innes, Frank Katzer, Clare M.Hamilton Food and Waterborne Parasitology (2020) (https://doi.org/10.1016/j.fawpar.2020.e00086) in relation with his previous work but still in the field of Toxoplasma.

15.7. Impact and Relevance

From the beginning, the PhD project was built as an interdisciplinary project involving partners from the Vet (Anses, RIVM), Med (URCA, RIVM) and industrial (IFIP) area. The topic itself touches all three domains, fitting perfectly into the OneHealth approach. Therefore the PhD student is working in an inter-disciplinary environment, gathering together vets, researchers, doctors, pharmacists, engineers, technicians with a broad spectrum of activities such as parasitology, food-product manufacture, risk assessment analysis, statistics, epidemiology. Precisely, the experimental infection of pigs has been performed by JRU BIPAR, the sausages were manufactured by Institut du Porc (IFIP), and tested both by JRU BIPAR and URCA. Later on the results will be interpreted with the help of statisticians (ANSES) and the QMRA model will be run and performed in RIVM. The PhD project and the PhD student are playing perfectly the role of a pivot within this interdisciplinary environment. Without them this part of the research would not have been possible.

15.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
The beneficiary must confirm that appropriate health and safety procedures conforming to relevant local/national guidelines/legislation are followed for staff involved in this project.	All new personnel working in the ANSES laboratories has to undergo a ½ day training/visit of the laboratories with the local Health and Safety correspondent. During this visit, the personnel are highlighted the specific dangers and critical points for each section of the lab and are informed about the relevant local and national guidelines that needs to be followed. The visit ends with the signature of a training sheet summarizing all this points. One copy being kept by the personnel.
This project is focusing on pigs. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the pigs). If these are not	The entire experimental infection has been already approved by the local Ethical Committee (ANSES – ENVA –UPEC) and

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experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the animals. Please describe how the animals' welfare are protected and considered (e.g. if the pigs are affected when taking samples, even if the work is dealing with faeces as these types of study can, this can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals). Please provide a statement on the 3Rs aspects of this work If Ethical Approval is required please state which Research Ethics Committee this will be sent to.	the Ministry of Research under the number 18-035 (n° APAFIS: 2018032908554996) where all these aspects (3R, welfare, etc) has been detailed.

15.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			**Milestones and Deliverables**				**Associated budget**	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
WP1-T1:Experimental infection of pigs	M25	M31	M-PhD14-01	M27	M33	See 1 below		
WP1-T2: Predilection sites of *T.gondii* in pigs	M36	M39	M-PhD14-03 D-PhD14-1	M36	M39	See 2 below		
WP2-T2: Assessment of the persistence of viable *T. gondii* in pork delicatessen	M42	M45	M-PhD14-05	M45	M48	See 2 below		

Comments:

1. Due to Covid19 sanitary shut down and the need to repeat 3 times the tissue-cyst oral infection, 6 months delay has to be taken into account. The milestone was achieved during M33.
2. Due to Covid19 sanitary shut down (twice) a 3 months delay has to be taken into account (for the moment), the time needed for the collection and processing of all samples by various techniques. During the shut-down no lab work was permitted.
3. Due to the Covid19 crisis, a delay of 3 months has to be taken into account, so the estimated completion date is December 2022

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15.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	Yes (see below)

Additional information:

Filip DAMEK is an excellent PhD student, dynamic and pro-active From the beginning of the PhD project we had to deal with several unexpected problems, but this has not discouraged him, demonstrating he can adapt and is well suited to finalise this project successfully. The challenges included:

1. A last-minute change of the animal facilities: the experimental infection facilities of INRAe Tours cancelled our reservation 2 days before the beginning of the project with very few explanations. We had to find an emergency solution in order to stick to the scheduled time-plan. The ENVA/Anses animal facilities were luckily available to host our animals.
2. A lack of viable parasites: when checking the positivity of our animals at day 30 p.i. we were surprised to realise that all animals in the tissue-cysts group were negative, while the animals from the oocysts group were positive, forcing us to repeat the oral infection. A second infection failed again, most likely to a lack of viable parasites provided by our external partner. A third infection was finally successful, when we fed orally the pigs with mice-brain homogenate following an oral infection performed in ENVA/Anses animal facilities.
3. Strike in Marseille harbour: after collecting the hams from the experimentally infected animals and sent to INRAe Corte in Corsica for short/long time salting, a strike has been declared in Marseille harbour (https://www.leparisien.fr/economie/la-corsica-linea-annule-toutes-ses-traversees-mercredi-jeudi-et-vendredi-14-01-2020-8236179.php), blocking our shipment for several days. In fact the hams arrived 12 days after leaving our lab, in a degraded sanitary condition. Therefore only a long salting procedure has been performed.
4. Covid19 shut-down: from 17/03 to 11/05 no lab activities were available postponing all sample collection and treatment. Beginning with 11/05 Filip had a limited access to the lab, but the research activities were restarting very slowly with multiple difficulties due to the sanitary constrained situation (no more than 1 person/room, social distancing, etc). During the second confinement (01.11-15.12), a general reduced activity has been noticed, with administrative people or lab technicians been absent, involving a certain delay for the project due to a delays in acquiring reagents or in helping with the lab work.

15.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

Filip DAMEK has been involved in the OHEJP JRP Toxosources, as part of the Anses team, participating at the kick-off meeting (3-4.02, Copenhague, Denmark) and since then participating at all the videoconferences of the various WPs, where Anses is involved in. Filip visited the RIVM (24.02.20 to 28.02.20) to get acquainted and discuss plans with the QMRA team.

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Similarly, part of the Anses team, Filip DAMEK is involved in the national research project n° 0917003490 financed by the French Ministry of Agriculture through the France Agri Mer agency with the title: Study of the tropism and persistence of *Toxoplasma gondii*: from pork carcass to sausage, gathering the above mentioned partners (Ifip, URCA, Inrae Corte), representing in the same time the fundament/basis of his PhD programme.

15.12. List of Dissemination activities

Name of the activity:	2nd OHEJP Annual Scientific Meeting 2020. Presentation of poster and participation in 3-minute Thesis competition.		
Date:	27-29 May 2020		
Place:	virtual		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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16. PhD15- TRACE

16.1. Summary

Due to the COVID19 crisis (which started just a few months after the start this PhD project) the PhD project is delayed by approx.. 6 months). The PhD candidate worked on COVID19 in minks and published a paper on this topic. Regarding TRACE, this year some progress was made on establishing optimisation of sample (pre)processing (enrichment) to make whole genome sequencing possible (WP1). The work of WP2 (phylodynamics) will start February 2021.

16.2. Overview of project progress

WP1: During the first 9 months of the project work was done to establish a sample (pre)processing (enrichment) to make whole genome sequencing possible. Different methods were used and DNA depletion treatments were explored. mRNA of 18S (host) and 16S (bacterial) origin was successfully removed. HEV whole genome sequences were successfully generated using above enrichment methods and specific primers. Currently work is done to analyse sensitivity. HEV culture preceding sequencing may be considered to increase the amount of HEV virus (work on a culture method is part of BIOPIGEE project).

WP2: Using HEV whole genome sequences data generated in WP1, we will construct a timed-phylogeny of HEV based on core-genome SNP analysis. From this analysis we can infer time-points of evolutionary divergence of different HEV types. Coupled to geographic location data of the isolates we will also include a spatial dimension to the phylogeny which will inform us on the geographical origin and spread of HEV types over time. Comparison of sequences over time will also be used to identify sequences that differ between time periods in which we observed a different epidemiology of HEV, giving indications of probable increased colonization success in pigs, increased environmental survival and/or increased virulence. This work is delayed due to COVID-19, but will start next month.

WP3: Not planned to start yet.

WP4: Not planned to start yet.

16.3. Progress of the research performed in the PhD project and key scientific results

Work Package 1 Whole genome sequencing.
During the first 9 months of the project work was done to establish a sample (pre)processing (enrichment) to make whole genome sequencing possible. Different methods were used and DNA depletion treatments were explored. mRNA of 18S (host) and 16S (bacterial) origin was successfully removed. HEV whole genome sequences were successfully generated using above enrichment methods and specific primers. Currently work is done to analyse sensitivity. HEV culture preceding sequencing may be considered to increase the amount of HEV virus (work on a culture method is part of BIOPIGEE project).

Work Package 2 HEV phylodynamics.
Using HEV whole genome sequences data generated in WP1, we will construct a timed-phylogeny of HEV based on core-genome SNP analysis. From this analysis we can infer time-points of evolutionary divergence of different HEV types. Coupled to geographic location data of the isolates we will also include a spatial dimension to the phylogeny which will inform us on the geographical origin and spread of HEV types over time. Comparison of sequences over time will also be used to identify sequences that differ between time periods in which we observed a different epidemiology of HEV, giving indications of probable increased colonization success in pigs, increased environmental survival and/or increased virulence. This work will start next month.

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Work Package 3 Identification of HEV virulence genes and HEV quasispecies.
Work on this work package is planned to start in month 42

Work Package 4 Data analyses and data evaluation
Work on this work package is planned to start in month 42

16.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
	D-PhD15-1.1	Sample processing protocol for HEV RNA positive target samples of different origin and associated deep sequencing procedure for HEV from such samples	M36		M48	Due to the COVID-19 crisis there is a delay
	D-PhD15-2.2	Report/publication on HEV dynamics including information about the geographical origin of predominant virulent strains and identification of genetic traits changed over time.	M36		M48	Due to the COVID-19 crisis there is a delay

No milestones planned for Y3.

16.5. Soft skills and Continuing Professional Development Training

Not Applicable

16.6. Publications and patents

No publications have been produced yet.

16.7. Impact and Relevance

OHEJP Strategic Research Agenda (SRA) Updated List and Descriptions of Priority Research and Integrative topics: “risk factors and infection dynamics”.
Development and harmonisation of deep sequencing-based methods for detection and tracing of foodborne zoonotic agents and emerging threats have been identified as a main research item within the updated EJP One Health SRA. Data from this project will lead to improved surveillance and more

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harmonized data analyses on the foodborne zoonosis HEV. This will contribute to broader and flexible actions to detect actual hazards, main reservoirs, trends and routes of transmission as well as common approach and timely analysis and data sharing which will be needed more and more with ongoing globalization.

16.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
The beneficiary must confirm that appropriate authorizations will be sought to collect the Human samples. Please reconfirm that this work is not using or interacting with animals directly, please reconfirm this.	In this case the appropriate authorization regarding the collection of human HEV sequences is RIVM itself. In addition, we confirm that this research is not using animals directly.

16.9. Impact of COVID-19 crisis on project

Tasks or Subtasks			Milestones and Deliverables				Associated budget	
Name of Task or Subtask	End date according to AWP 2020	Expected end date due to crisis	Associated Milestone or Deliverable	Deadline according to AWP 2020	New proposed deadline	Reason for delay	Budget that will not be spent	Budget that will be spent with delay
HEV Whole genome sequencing	M48	M54	D-PhD15-1.1	M48	M54	COVID-19	NA	€2500
HEV Phylodynamics	M48	M54	D-PhD15-2.2	M48	M54	COVID-19	NA	€2500
Identification of HEV quasispecies	M60	M64	D-PhD15-3.3	M60	M64	COVID-19	NA	€2000
Analyses HEV bioinformatics data	M60	M64	D-PhD15-4.4	M60	M64	COVID-19	NA	€1000

16.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	No

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16.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

This PhD project relates to the OHEJP project BIOPIGEE: HEV viability screening method developed In BIOPIGEE may be used In TRACE as applicable.

16.12. List of Dissemination activities

No dissemination activities to report.

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17. PhD16- Codes4Strains

17.1. Summary

Aim

Whole genome sequencing allows the tracking of pathogenic strains and informs infection control, diagnostic and sometimes treatment strategies. To track strains globally, and as they spread between the environment, food, animals and humans, universal strain nomenclatures are necessary. The core genome Multilocus Sequence Typing (cgMLST) approach is an accurate, reproducible and portable strain genotyping method that underlies widely used strain nomenclatures, in which groups are generally determined by single-linkage clustering. However, cgMLST groups are unstable due to the possibility of group fusion upon subsequent sampling. Recently, a new coding approach named LIN (Life Identification Number) was introduced by Marakeby et al. (1). It provides a numerical code for each genome based on its similarity (estimated using the Average Nucleotide Identity, ANI) to the closest genome already encoded. As LIN codes are attributed to genome rather than groups, they are stable. A common feature of both approaches is that single linkage groups and LINcodes can be defined using several similarity cutoffs, in which case they inherently convey phylogenetic proximity information. cgMLST additionally provides, through the number of allelic mismatches among cgMLST profiles, intuitive human-understandable metrics of differences among strains involved in epidemiological events (*e.g.*, '5 cgMLST allele differences').

The aim of the PhD project is to develop a novel genome-based genotyping approach taking the best of the two above classification approaches, *i.e.,* combining the advantages of cgMLST (discrimination, standardization) with those of the LIN code approach (complete stability). That is, we aim to develop and explore the strain classification utility of cgMLST-based LIN code (cgLINcodes) systems, where the pairwise distance is based on the number of allelic mismatches, rather than ANI. We also aim to compare the cgLINcodes approach with other existing classification approaches: the SNP address and multi-level single-linkage classifications (hierarchical clustering, HierCC). We use *Klebsiella pneumoniae* (Kp) and *Escherichia coli* (Ec) as two important pathogens to develop and evaluate our approach.

Report on the period of January to December 2020

Before the reporting period, we had selected our pilot genome dataset for the pathogen Kp (December 2019, **D-PhD16-2.1**).

During this reporting period, we developed procedures (bioinformatics, algorithmic) to follow our goal of a cgMLST-based LIN code systems. First, we generated cgMLST profiles from genomes, using defined schemes. To this aim, the choice and the evaluation of the cgMLST schemes was finalized. For Kp, we defined 629 gene loci for the cgMLST scheme from a previously published scheme. For Ec, we chose to use the cgMLST scheme from EnteroBase, which includes 2513 gene loci (**D-PhD16-3.1**).

As a second task, the development of the cgMLST-based LIN code algorithm has been defined and a bioinformatics implementation was developed in Python (**D-PhD16-3.2**). To apply the algorithm, an important point is the definition of the set of thresholds. We have used a set of bioinformatics tools and metrics to define thresholds, considering the phylogenetic structure of the species, and with the view to maximize its usefulness in population biology and epidemiology. For Kp, we have used a large dataset of 7060 high-quality genomes to define the overall structure and diversity of the population, and finalized a cgLINcode system for Kp comprising 11 thresholds. Subsequently, we generated cgLINcodes for the dataset of 7060 genomes based on the 11 defined thresholds. Regarding the foodborne pathogen Ec, we used a genome dataset that was previously published (2) by Public Health England (PHE). In this article, 7-gene sequence typing (MLST), cgMLST profiles and the SNP addresses were provided. For cgLINcodes system set-up, the selection of the set of thresholds was based on those used in EnteroBase for HierCC, to facilitate comparison with this widely used approach. We thus leveraged preexisting data and typing systems for Ec, which allowed us to generate a database of cgLINcodes

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aligned with other approaches. Finally, a comparison between the different classification systems (HierCC, cgLINcodes and SNP address) was carried ou for Ec. For Kp, we only compared cgLINcodes and multi-level Single Linkage clustering (equivalent to HierCC), as the SNP database required for the SNPaddress approach remains to be constructed.

17.2. Overview of project progress

The initial objectives proposed for the PhD project are the following:

- To define a collection of pilot dataset of genomes including published outbreak sets
- To develop the cgLINcode approach and compare with HierCC and SNPaddress approaches on large genomic datasets
- To simulate genomic evolution and use the three approaches to encode resulting evolved genomes; compare resulting codes with expectations
- To write publications on the cgLINcodes concept and implementation with comparison with HierCC and SNPaddress approaches
- To disseminate the cgLINcodes approach towards collaborating partner institutes and to evaluate this approach in one Health and Global Health contexts
- To write-up the PhD dissertation

The first two objectives have been mostly achieved, according to schedule. The second objective was divided into 4 subtasks: define the cgMLST schemes to be used for the two pathogens; collect a full dataset of public genomic sequences and scan them to define their cgMLST allelic profiles; development of the cgLINcodes algorithm; and create and/or analyze the SNPaddress database and compare cgLINcodes with HierCC and SNPaddress approaches.

The scheduled work that is not complete yet is the development and analysis of the SNPaddress approach for Kp. We are first finalizing the manuscript on cgLINcodes for Kp, as this is the most innovative development and the manuscript is already complex and long. We thus reserve the comparative objective for Kp, for the next period.

The planned objectives for the next period are (i) the study of simulated evolved genomes to test the ability of the three coding methods to recover the right phylogenetic relationships; and (ii) to complete the writing of the publication on the cgLINcodes approach.

17.3. Progress of the research performed in the PhD project and key scientific results

Background

One of the main objectives of the PhD project is to develop a novel nomenclature system, potentially applicable to all bacterial pathogens. Here we first focus is on a highly antibiotic resistant bacterial pathogen, *K. pneumoniae* (Kp), and on a foodborne bacterial pathogen, *Escherichia coli* (Ec). The aim is to combine two main microbial strain nomenclatures approaches that are currently being used separately.

First, core genome Multilocus Sequence Typing (cgMLST) is widely applied, especially in the context of bacterial pathogen surveillance. It relies on sets of predefined gene loci, the sequence variants of which are given unique identifiers (allelic numbers). Resulting allelic profiles are given unique identifiers (cgST) or are grouped based on their similarity, generally using the single-linkage clustering method. An alternative approach known as the SNPaddress was developed at Public Health England (PHE). Different from MLST, it is based on single nucleotide polymorphisms (SNP) compared to a reference genome. As for cgMLST, single-linkage clustering is performed based on the resulting SNP distance between isolates. An original contribution of the SNP address as implemented in SNapperDB (a database for SNPs and corresponding SNPadresses developed by one of us at PHE) is to apply several thresholds of SNP differences to defined classification groups. The resulting ‘address’ is a multi-positions code, where each position corresponds to the cluster membership at descending thresholds of genetic (SNP) distance among strains. The resulting multi-level nomenclature provides a good approximation of the phylogenetic relatedness among isolates. Likewise, several cgMLST thresholds can be used to provide phylogenetic information on top of classification purposes, as was implemented

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by the HierCC (Hierarchical Clustering of CgMLST) tool from EnteroBase. However, one major limitation of current SNP address or multi-level cgMLST classifications is that they suffer from instability: in case of discovery of 'intermediate' genotypes, the fusion of pre-existing groups can happen, due to the inherent mathematical property of single linkage. This introduces nomenclatural confusion and requires manual management of group delineation and updates.

Second, a strain naming approach that avoids the genotype identifier instability problem was introduced by Marakeby, Vinatzer and colleagues (see above), and is known as the Life Identification Number (LIN) code. This approach provides a multi-level numerical code for each genome based on its similarity (Average Nucleotide Identity, ANI) to the closest genome already encoded. As it does not rely on single-linkage clustering, LINcodes provide a stable, definitive identifying code to each genome. However, ANI is not an accurate metric and is not reproducible enough for strains that are very closely related, as is typically the case in epidemiological surveillance or outbreak investigation studies. Until today, cgMLST and LIN codes have been implemented separately.

Progress of the research performed in the PhD project

Before this study period, we had defined a pilot dataset of *Klebsiella pneumoniae* genomes (**D-PhD16-2.1**). We used the publicly accessible database of *Klebsiella* genomes (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html), which comprises MLST data submitted by more than 400 labs worldwide, some of which being submitted with complete genomes. A set of genomes were removed after quality filtering and for lack of public availability. The pilot dataset of genomes was defined to comprise 751 isolates. This dataset represents different levels of diversity of *Klebsiella pneumoniae*, from different phylogroups to isolates groups derived from single outbreaks.

During the period of January to December 2020, our activities were subdivided into 4 tasks/deliverables and are detailed below.

D-PhD16-3.1. cgMLST schemes for K. pneumoniae and E. coli

One of the main objectives of the PhD project is to develop a new system of nomenclature, based on a highly reproducible metric to estimate the genetic distance between genomes. Our intention was to use pairwise distances between cgMLST allele profiles. We first had to make a choice of which cgMLST scheme (i.e., set of gene loci and their allelic templates) to use.

For Klebsiella pneumoniae, we based our work on a previous scheme: In 2014, Bialek-Davenet et al (3) defined a cgMLST scheme (scgMLSTv1) of Kp that included 634 highly conserved and syntenic genes. Here, we updated the scgMLST scheme based on a reassessment of scgMLSTv1 loci, with the following improvements. First, two loci (KP1_2104 and aceB=KP1_0253) were removed because they were absent or truncated in multiple strains, based on our pilot genomic dataset (**D-PhD16-2.1**, project id 11 at https://bigsdb.web.pasteur.fr/cgi-bin/bigsdb/bigsdb.pl?db=pubmlst_klebsiella_isolates&page=projects). Second, the remaining 632 loci templates were modified so that they would include the start and stop codons of the corresponding coding sequence (CDS); This was not the case for all CDSs in scgMLSTv1, as some loci corresponded to internal portions of CDSs. These templates redefinitions were done in order to harmonize locus definitions across the scheme, and because defining loci as complete CDSs facilitates genotyping by allowing to precisely identify the extremities of novel alleles through the search of the corresponding start and stop codons. As a result of these locus template extensions, the 629 scgMLSTv2 genes have a summed length of 512,856 nt (9.8% of the genome of reference strain NTUH-K2044), as compared to 507,512 nt (9.7% of the genome of reference strain NTUH-K2044) for the corresponding loci in scgMLSTv1.

To define the allelic profiles, the 629 loci were scanned for presence and allelic identity in the 751 genomes. Pairwise cgMLST distances were then computed as the number of distinct alleles, ignoring missing data in pairwise comparisons, *i.e.,* the number of mismatches was divided by the proportion of loci called in both strains.

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For Escherichia coli, we have chosen the cgMLST scheme from EnteroBase (https://enterobase.warwick.ac.uk/ species/ecoli/download_data), which includes 2513 loci. This choice was based on an assessment of existing schemes and their usage. The one from EnteroBase was published, and used for more than 153,043 genomes (present in EnteroBase), so that it is a *de facto* reference.

We did not have to define a pilot dataset for this pathogen, because the cgMLST scheme had been evaluated and published. Besides, the cgMLST profiles used for our analyses were extracted from the EnteroBase.

D-PhD16-3.2. cgLINcodes algorithm defined and implemented on full dataset

1. *Algorithm*

We have implemented the cgLINcode attribution algorithm using the Python programming language and made the script available publicly (**M-PhD16-3.2,** https://gitlab.pasteur.fr/BEBP/LINcoding).

The developed cgLINcoding software tool takes as input a file of allelic profiles and a set of thresholds, defining a cgLINcode scheme. Then, the tool returns two files:

- cgLIN DataBase: a file containing the cgLINcodes associated with each allelic profile, allowing to create new cgLINcodes from future profiles;
- Genome cgLINcode list (genome names and their cgLINcode)

These cgLINcodes are composed of a set of p-positions, each corresponding to a threshold of similarity between genomes. These similarity thresholds are sorted in ascending order (*i.e.*, $s_p < s_{p+1}$), the first positions of the code (on the left side) thus corresponding to low levels of similarity. Following the initial proposal, the codes are assigned as follows: (step 1) The code is initialized with the first strain being assigned the value "0" at all positions; (step 2) The encoding rule for a new genome i is based on the closest genome j already encoded as follows, from the similarity $s_{ij} \in]s_{p-1}\,;\,s_p\,]$:

i) identical to code j up to and including position $p-1$.
ii) for the position p : maximum value observed at this position (among the subset of codes sharing the same prefix at the position $p-1$) incremented by 1.
iii) "0" to all downstream positions, from $p+1$ included.

For each genome to be encoded, step 2 is repeated. This encoding system conveys phylogenetic information, as two genomes with identical prefixes in their respective cgLINcodes can be understood as being similar, to an extent determined by the length of their common prefix. Isolates having cgMLST profiles with 100% identity (no mismatch at loci called in both genomes) will have exactly the same cgLINcode.

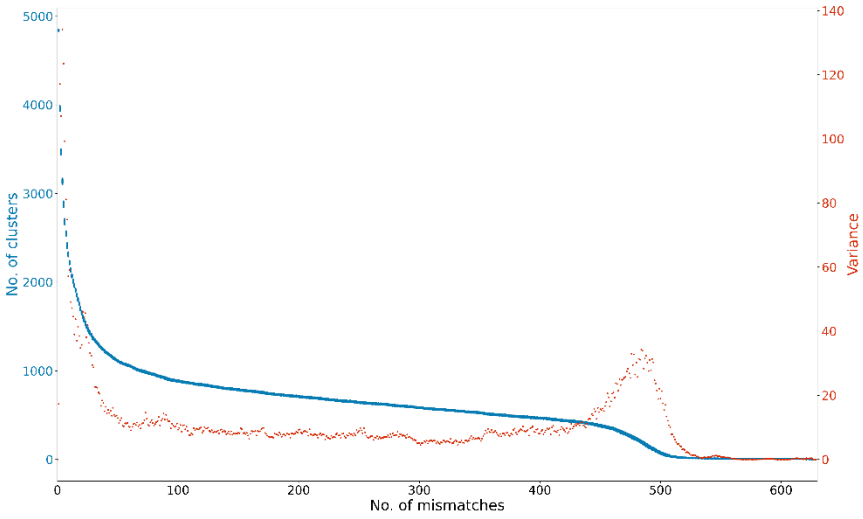

Figure 1: Example of the evaluation of the impact of input order for the Kp dataset.

In the course of our experiments and literature searches, we noticed that this method has a significant problem of dependency on the order of entry of isolates. In other words, according to their encoding order, the same set of allelic profiles can lead to LIN codes resulting in categorizations by different prefixes. We evaluated the impact of input order on the number of inferred values, as

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defined by the number of distinct prefixes: for each threshold varying from 1% to 99%, a LIN encoding was defined using this threshold, and the 7060 high-quality, non-hybrid cgMLST profiles were encoded 500 times with random input orders. This experiment made it possible to determine (i) the threshold values associated with a stronger variability in the final number of prefixes; and (ii) the magnitude of this variability. In the example illustrated in ***Figure 1***, we observed that the number of distinct prefixes was affected by the order of encoding, especially in the range of 450 to 530 mismatches. Knowing these results, we therefore sought to minimize this problem by defining an optimal input order. The one that answered our expectations is the input order guided by a Prim's algorithm (4).

2. Application to K. pneumoniae

An important choice to make is the definition of the set of thresholds. We aimed to define thresholds taking into account the phylogenetic structure of the species, and to maximize its usefulness in population biology and epidemiology. For this, we analyzed the properties of cgMLST groups for all possible threshold values (ranging from 1 to 629 cgMLST mismatches). We first estimated for each cut-off value, the quality of single linkage clustering of the population by using the parameter Silhouette S_t, which reflects both the homogeneity within groups and the distinctness among groups (5). Second, we estimated the stability of cgMLST clusters using a sub-sampling procedure and quantifying concordance across samples with the Wallace Index W_t (6), which led to identify threshold values of nearly complete stability. Combined with the distribution of pairwise distances, these analyses led to define four levels of intra-phylogroup classifications. The two first thresholds, 610 and 585 allele mismatches, corresponded to species and subspecies delineations, respectively. Next, we defined a threshold at the level of 190 cgMLST mismatches, corresponding to the optimum of combination of stability W_t and silhouette analysis S_t. Finally, a threshold of 43 cgMLST mismatches was defined, for it matches the existing ST classification optimally. We also wanted to define shallow-level classifications, which might be useful for epidemiological tracking purposes. To provide flexible case clusters definitions, we classified Kp genomes using six thresholds: 0, 1, 2, 4, 7 and 10 cgMLST mismatches. These choices are based on the study of epidemic strains in the full dataset.

To summarize, we have defined a set of 10 thresholds based on our current understanding of the phylogenetic structure of the Kp species. We believe these short 10-number codes will be intuitive enough to be widely adopted by epidemiologists. Based on the above choices, cgLINcodes were generated on our full dataset of 7060 Kp strains, resulting in 4889 distinct cgLINcodes (**M-PhD16-3.2 for *K. pneumoniae***).

Figure 2 illustrates the representation of a set of cgLINcodes in the form of a prefix tree, showing that cgLINcode prefixes reflect phylogeny. As cgLINcode prefixes define classification categories or phylogenetic lineages at given similarity thresholds, cgLINcodes represent a nomenclature tool that is both simple and phylogenetically informative. For example, ***Figure 2*** shows that cgLINcodes can provide a taxonomy, which is predictive of phylogeny at the phylogroup level, as a single cgLINcode prefix defines each phylogroup with no exception: phylogroup Kp1 has as prefix 0_1; Kp2 has as prefix 2_0; Kp3 has as prefix 1_0; Kp4 has as prefix 2_1; Kp5 has as prefix 1_1; Kp6 has as prefix 3_0 and Kp7 has as prefix 4_0.

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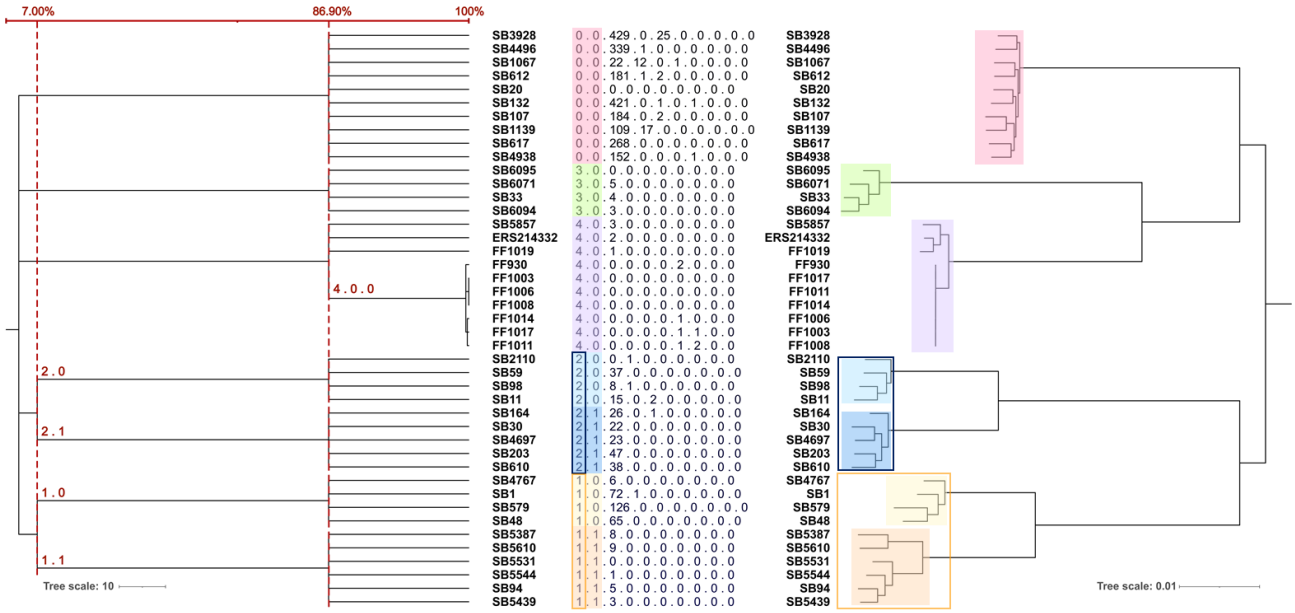

Figure 2: Representation in tree form of the 43 reference strains. *On the left, the prefix tree generated from cgLINcodes and on the right an IQ-tree from a cgMSA. The cgLINcodes are also shown in this figure, between the trees.*

3. *Application to E. coli*

With regards to the generation of the cgLINcodes of the *Escherichia coli* species, our choice of the set of thresholds is based on those defined by EnteroBase, in the approach Hierarchical Clustering (HierCC). These 13 thresholds are defined by a number of mismatches between the profiles (https://enterobase.readthedocs.io/en/latest/features/clustering.html), and are: HC0, HC2, HC5, HC10, HC20, HC50, HC100, HC200, HC400, HC1100, HC1500, HC2000, HC2350. In the HierCC notice (https://enterobase.readthedocs.io/en/latest/HierCC_lookup.html#hiercc-equivalents), it is mentioned that HC2350 corresponds to the separation of species and clades in *Escherichia* genus. Moreover, in the article of Zhou et al. (7), the authors write that *Escherichia* HC1100 corresponds to ST Complexes and the HC5-10 is for detecting local transmission chains.

Next, we used for Ec the dataset published by Holmes et al. in 2018 (2), for which the cgMLST profiles had been generated in EnteroBase. Besides, other information such as address SNPs and HierCCs were provided. Of the 150 published strains, 149 had a cgMLST profile. We processed these with our cgLINcode algorithm, resulting in 128 distinct cgLINcodes (**M-PhD16-3.2 for *E. coli***).

D-PhD16-3.3 SNapperDB for E. coli implemented for full dataset

To achieve our comparative objective, we analyzed SNPaddresses from the *E. coli* strain dataset. As *E. coli* SnapperDB is already implemented by Public Health England, we used available SNPaddresses. We have chosen the dataset published by Holmes et al. (2). The fastq files have been processed in the SnapperDB. This approach uses *E. coli* O157 as the reference, and pairwise SNP comparison and single-linkage clustering at 7 different levels of increasing similarity (SNP thresholds, 250, 100, 50, 25, 10, 5, and 0) were performed to produce a SNPaddress. From the publication (2), we extracted 109 distinct SNPaddresses from the 152 strains.

We next compared SNPaddresses with cgLINcodes. This analysis allowed us to establish the correspondence between the SNPaddresses thresholds and the different mismatch levels used for by the cgLINcodes, as given in the table below:

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THRESHOLD OF SNPADDRESSES	NO. OF MISMATCHES OF BEST CGMLST THRESHOLD	RAND INDEX
SNP0	0	0.841792
SNP5	2	0.924894
SNP10	5	0.927842
SNP25	20	0.707476
SNP50	50	0.755378
SNP100	100	0.977602
SNP250	100 (this threshold was better that the next ones: 200 and 400 and up)	0.987471

This table shows that overall, the partitions created by the two approaches are similar at shallow levels of intra-specific diversity. We also compared the topologies of the trees created from the SNPaddresses and cgLINcodes and found that they are identical (***Figure 3)***. This shows that the two approaches are highly concordant in their classification of strains.

Figure 3: Representation in tree form of the 149 strains*. On the left, the prefix tree generated from LINcodes and on the right the prefix tree generated from SNPadresses. This figure is exported from Dendroscope after running the Tanglegram algorithm between two trees.*

D-PhD16-3.4 SNapperDB for K. pneumoniae implemented for full dataset

We did not create a SNapperDB database for the pathogen Kp yet. This implies that SNPaddresses are not generated yet for this pathogen. We are first finalizing the manuscript on cgLINcodes for Kp, as this is the most innovative development and the manuscript is already complex and long. We thus reserve this objective for the next period.

References:

(1) Marakeby, H.; Badr, E.; Torkey, H.; Song, Y.; Leman, S.; Monteil, C. L.; Heath, L. S.; Vinatzer, B. A. A System to Automatically Classify and Name Any Individual Genome-Sequenced Organism Independently of Current Biological Classification and Nomenclature. *PLOS ONE* **2014**, *9* (2), e89142. https://doi.org/10.1371/journal.pone.0089142.

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(2) Holmes, A.; Dallman, T. J.; Shabaan, S.; Hanson, M.; Allison, L. Validation of Whole-Genome Sequencing for Identification and Characterization of Shiga Toxin-Producing Escherichia Coli To Produce Standardized Data To Enable Data Sharing. *J. Clin. Microbiol.* **2018**, *56* (3), e01388-17. https://doi.org/10.1128/JCM.01388-17.

(3) Bialek, S.; Lavigne, J. P.; Chevalier, J.; Marcon, E.; Leflon-Guibout, V.; Davin, A.; Moreau, R.; Pages, J. M.; Nicolas-Chanoine, M. H. Membrane Efflux and Influx Modulate Both Multidrug Resistance and Virulence of Klebsiella Pneumoniae in a Caenorhabditis Elegans Model. *Antimicrobial agents and chemotherapy* **2010**, *54* (10), 4373–4378. https://doi.org/10.1128/AAC.01607-09.

(4) Prim, R. C. Shortest Connection Networks And Some Generalizations. *Bell System Technical Journal* **1957**, *36* (6), 1389–1401. https://doi.org/10.1002/j.1538-7305.1957.tb01515.x.

(5) Rousseeuw, P. J. Silhouettes: A Graphical Aid to the Interpretation and Validation of Cluster Analysis. *Journal of Computational and Applied Mathematics* **1987**, *20*, 53–65. https://doi.org/10.1016/0377-0427(87)90125-7.

(6) Wallace, D. L. A Method for Comparing Two Hierarchical Clusterings: Comment. *Journal of the American Statistical Association* **1983**, *78* (383), 569–576. https://doi.org/10.2307/2288118.

(7) Zhou, Z.; Alikhan, N.-F.; Mohamed, K.; Fan, Y.; Achtman, M. The EnteroBase User's Guide, with Case Studies on Salmonella Transmissions, Yersinia Pestis Phylogeny, and Escherichia Core Genomic Diversity. *Genome Res* **2020**, *30* (1), 138–152. https://doi.org/10.1101/gr.251678.119.

17.4. Progress of the research project: Deliverables and Milestones

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
PhD16-FBZ2/AMR6.1-Codes4strains	D-PhD16-3.1	cgMLST schemes for Kp and Ec	M36	M36		None
	D-PhD16-3.2	LINcodes algorithm defined and implemented on full dataset	M30	M30		None
	D-PhD16-3.3	SNapperDB Ec implemented for full dataset	M33	M33		None
	D-PhD16-3.4	SNapperDB Kp implemented for full dataset	M36		M48	We did not create SNapperDB for the pathogen Kp yet. We are finalizing the manuscript on cgLINcodes for Kp first.

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PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
PhD16-FBZ2/AMR6.1-Codes4strains	M-PhD16-3.1	cgMLST schemes defined for Kp and Ec	M36	Yes		
	M-PhD16-3.2	LINcodes algorithm defined	M30	Yes		
	M-PhD16-3.3	SNapperDB databases set-up for both pathogens	M36	Yes/no		Yes for Ec; not yet for Kp

17.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
RESEARCH ETHICS AND SCIENTIFIC INTEGRITY	• Open to all students and the whole scientific community of Sorbonne University. • Each conference focuses on a specific theme, and is given by an internationally recognized speaker. Speakers are selected from the recognized resources (US Office of Research integrity, French OFIS, EUA-CDE, LERU). The lectures are captured in video for live broadcast on the other campuses of Sorbonne	19/12/2019	Sorbonne Université
ETHICS OF SCIENTIFIC RESEARCH	3h Workshop, based on the Dilemma Game of the Erasmus University of Rotterdam. • A maximum of 5 groups of 5 people are trained at each workshop. Each group must answer a dozen questions on scientific integrity, chosen according to the origin of the participants. When the group does not agree on a response, it must discuss it to seek consensus. • Pooling of group results, using the same consensus-finding and discussion process where this is not possible.	21/02/2020	Sorbonne Université

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OPEN SCIENCE, BY AND FOR THE BENEFIT OF RESEARCHERS	• Definition of open science • Institutional context • The need for openness in science	25/02/2020	Sorbonne Université
OPEN ACCESS: GENERAL ASPECTS	• Development of arguments and editorial solutions for Open access • Presentation of institutional and disciplinary open archives • Learning to use the portal HAL Sorbonne University	05/03/2020	Sorbonne Université
SEMINAR BIBLIO@PHD	• The program is focused on points of view from different actors of scientific publishing and varies depending on the years. • The programs and slides of prior seminars are available online: https://paris-sorbonne. libguides.com/bibliodoctorat	31/01/2020	Sorbonne Université
DISCOVER THE MAIN PRINCIPLES OF MANAGEMENT	• History of management • Management styles and team types • Management and teamwork • The skills of the manager in situation	12/02/2020	Sorbonne Université
DISCOVER THE PRINCIPLES OF EFFECTIVE WRITTEN AND ORAL COMMUNICATION.	• Decrypt the act of communication • Principles of effective written communication • Principles of effective oral communication	22/01/2020	Sorbonne Université
CONDUCT YOUR INTERVIEWS AND MEETINGS EFFICIENTLY	• Preparing and structuring interviews and meetings Anticipate and anticipate objections or questions • Learn how to manage the types of interlocutors • Improve verbal and gestural expression techniques • Build your argumentation and develop your strength of conviction and persuasion • Use the educational tools in the right situation • Practice dialogue, participation and facilitation skills Learn how to regulate and manage voltages face-to-face or in a group setting	09/03/2020 10/03/2020	Sorbonne Université

17.6. Publications and patents

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 71.

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Badell E, Hennart M, Rodrigues C, Passet V, Dazas M, Panunzi L, Bouchez V, Carmi-Leroy A, Toubiana J, Brisse S. Corynebacterium rouxii sp. nov., a novel member of the diphtheriae species complex. Res Microbiol. 2020 Apr-Jun;171(3-4):122-127. doi: 10.1016/j.resmic.2020.02.003. Epub 2020 Feb 28. PMID: 32119905

Hennart M, Panunzi LG, Rodrigues C, Gaday Q, Baines SL, Barros-Pinkelnig M, Carmi-Leroy A, Dazas M, Wehenkel AM, Didelot X, Toubiana J, Badell E, Brisse S. Population genomics and antimicrobial resistance in Corynebacterium diphtheriae. Genome Med. 2020 Nov 27;12(1):107. doic: 10.1186/s13073-020-00805-7. PMID: 33246485; PMCID: PMC7694903.

Hennart Melanie, & Brisse Sylvain. (2021). Deliverables of Codes4strains project (Part1) (Version 1). Zenodo, https://zenodo.org/record/4471354#.YD4b9E5xc2w

17.7. Impact and Relevance

The project will define, implement and evaluate a novel bioinformatics strategy to classify and name strains within pathogenic bacteria, from the level of deep subspecific lineages down to shallower levels of diversity that differentiate epidemiological related strains from non-related ones. It will be first tested on Ec and Kp, two important ubiquitous 'One Health' pathogens. However, the general applicability of the approach means that in the future, the classification and nomenclature of strains of other pathogens could benefit from the PhD project outcomes.
By facilitating in the future, communication on bacterial strains across sectors and countries, the project is highly relevant to multiple topics and objectives of One Health EJP: antibiotic resistance clonal dissemination, emerging pathogens, cross-sector transmission, public health and basic microbiology integration.
The project will deliver a novel nomenclature system of bacterial pathogens genomes that will be stable by design, unlike existing systems based on SNPs and cgMLST single linkage groupings. This has a far-reaching impact on possibilities to integrate efforts of agencies (*e.g.*, at the international levels, ECDC, EFSA, PulseNet international) to detect, monitor, understand and control the spread of pathogens.

17.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

No requirements from Ethics Advisors

17.9. Impact of COVID-19 crisis on project

No impact, as our bioinformatics work was not affected.

17.10. List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	No
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	No
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	No
Other risks (please describe)	None

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17.11. Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

The novel method developed in the PhD project will find natural dissemination ways via the existing networks of collaborations in which the main investigators are involved: MedVetKlebs (just finished JRP), KlebNET, SpARK, kleb-GAP and Nor-Kleb-Net for Kp (see MedVetKlebs final report); and Ec surveillance networks and national and international levels (e.g., French NRC & NRL @Pasteur and ANSES; ECDC; PulseNet international).

The novel nomenclature system will be compared with existing nomenclatures (dictionaries of nomenclature correspondence between LIN codes and current SNP/cgMLST nomenclatures for wider communication and backwards-compatibility) and will need in the future to be integrated in existing platforms that serve nomenclatures of bacterial strains (e.g., SnapperDB, EnteroBase, BIGSdb-Oxford and PulseNet international for *E. coli*; BIGSdb-Pasteur for *K. pneumoniae*). Future interactions with 'dev-op' specialists (application developers) will be established with free software such as EnteroBase, BIGSdb and Innuendo; or commercial software such as BioNumerics or SeqSphere.

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17.12. List of Dissemination activities

Name of the activity:	2nd One Health European Joint Project (OHEJP) Annual Scientific Meeting 2020. Presentation of poster and participation in 3-minute Thesis competition.		
Date:	27-29 May 2020		
Place:	Virtual		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***750***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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Name of the activity:	Deliverables of Codes4Strains (part 1)
Date:	20/12/2020
Place:	https://zenodo.org/record/4471354#.YEtSVrBxc2w

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*No*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	***Yes***	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	***Yes***	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		*No*

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	*0*	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		*0*

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18. PhD17- SUSTAIN

This PhD is funded and managed by WP7 (Sustainability). The progress of this PhD project is measured differently to the PhDs funded by Work Package 6. Instead of defined tasks, deliverables and milestones with the One Health EJP, this PhD has a number of challenging targets and deliverables to meet each year defined by Roskilde University to measure student progress. Therefore, please be advised the format of this report will differ in some areas.

18.1. Summary

Fieldwork:
A three month fieldwork stay in Sweden at the Swedish Veterinary Agency was planned for 2020 in spring. However, due to the COVID-19 pandemic, I remained in Denmark and conducted interviews online via Microsoft Teams and Skype instead of conducting face-to-face interviews. Another three month fieldwork stay in Italy was shortened to one month in October. It is planned to conduct remaining fieldwork in Italy in 2021. In October, I stayed at the Istituto Superiore di Sanità (Italian Public Health Institute) in Rome, where I was integrated into the research environment and was able to conduct interviews.

Research work:
In the WS2020, the main focus was to transcribe and analyse interviews. This was done with exchange with the supervisors as well as research groups at the university. Additionally, I supported the NOVA project, where I conducted and analysed interviews.

Scientific outputs:
In 2020, two articles were published and one is submitted and under review.

- "The state of One Health in academic research – a bibliometric analysis". In the Elsevier journal "One Health". https://doi.org/10.1016/j.onehlt.2020.100146

One co-authored article:

- "Attention to the Tripartite's one health measures in national action plans on antimicrobial resistance". In the "Journal of Public Health Policy". https://doi.org/10.1057/s41271-021-00277-y

Teaching:
In spring 2020, I engaged in teaching activities at the Roskilde University, where I supervised students in their semester projects and taught a bachelor and master class in Global Health Governance.

Conferences:
I participated at the Second Annual Scientific Meeting by engaging in the three minute competition and presenting a poster. At the World One Health Congress I participated, presenting another poster.
At the 6th Cogwheel Workshop, I presented my research to the OHEJP and SafeConsume.

18.2. Overview of project progress

The SUSTAIN PhD project is connected to WP7 of the OHEJP. However, it is not connected to specific tasks, deliverables or milestones.
Nevertheless, there has been progress. In 2020, data gathering has started as planned for the PhD project. This included conducting interviews with experts from public health, veterinary, environment and food agencies in Sweden and Italy. Data gathering for the interviews has been slowed down due to the COVID-19 pandemic and the following travel restrictions. Most data is gathered, but few interviews are left to be conducted with Italian experts.
Further, the preparation of a survey started, which will be conducted in spring 2021 and addressed to ministries, EU institutions and high level members of staff from public health, veterinary, environment and food agencies.

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Within the PhD project, a number of articles must be produced. In 2020, two articles were published (see Publications), and one was submitted in December 2020.
Lastly, all teaching obligations were completed after the spring semester 2020.

18.3. Progress of the research performed in the PhD project and key scientific results

In 2020, data gathering has started as planned for the PhD project. This included conducting interviews with experts from National Public Health, Veterinary, Food and Environment agencies in Sweden. Initially, it was planned to travel to Sweden to conduct the interviews in person. Due to the COVID-19 pandemic, the interviews were conducted via Microsoft Teams or Skype for Business in the period from March to June 2020. A fieldwork trip to Italy was planned for autumn 2020, but was shortened. In October, I stayed one month at the Istituto Superiore di Sanità (ISS), where I was integrated into the everyday work of the institute and was able to conduct interviews. The interviews were conducted with experts working in different departments (public health, veterinary health, environment, nutrition and food) at ISS. Data gathering for the interviews has been slowed down due to the COVID -19 pandemic and the following travel restrictions. Most data is gathered, but few interviews are left to be conducted with Italian experts, especially working in the veterinary (Istituto Zooprofilattico Sperimentale) and environmental institutes (Istituto Superiore per la Protezione e la Ricerca Ambientale).

Data from the interviews in Sweden was analysed in NVivo. An article was prepared, discussed with the supervisor and in research groups, and submitted in December 2020. The data analysis from the Italian interviews initiated in November 2020 and is ongoing into 2021. The process will be completed when all interviews, the remaining ones, will be conducted and analysed. Thereafter, an article will be prepared.

Further, the preparation of a survey started. The survey will be addressed to ministries, EU institutions and high level members of staff from public health, veterinary, environment and food agencies. It will be about the understanding of One Health and how One Health is put into practice. The online survey will be launched in spring 2021

Within the PhD project, a number of articles must be produced. In 2020, two articles were published (see publications) and one was submitted in December 2020.

Lastly, all teaching obligations were completed after the spring semester 2020. The teaching included supervision of two bachelor groups (group size five and two) in the programme of international studies, and the supervision of two master groups (group size 3 and two) in the programme of global studies. For the supervision, the students need to complete a written semester project. The supervisor meets four times with the group to guide them through the process. At the end of the semester, the written projects were evaluated and oral exams were taken by the supervisor as well as a censor. Additionally to the supervision, two classes were taught, one at bachelor, the other at master level called International Organisations and Global Governance.

Dissemination of the research was done via presenting at two conferences in 2020. At the OHEJP ASM in May, my research was presented at the 3MT and as a poster presentation. At the World One Health Congress in October-November, I presented my research again as a poster. Additionally, I participated at the Cogwheel Workshop in November, where I was invited to present my PhD project.

18.4. Progress of the research project: Deliverables and Milestones

As mentioned at the start of this report, this PhD is funded by WP7. Therefore the structure of deliverables and milestones differ to the PhD projects funded by Work Package 6.

The deliverables of this PhD are determined by Roskilde University's PhD school.

Deliverables of Roskilde University's PhD School:

Every 6 months, the status of the PhD progress is assessed by the half-yearly evaluation, which entails detailed description of the past 6 months in terms of status, changes in the time schedule or budget

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plan, the nature of supervision, research stays at other institutes, the assessment of all attended events (courses, conferences) as well as teaching hours.

Total of 30 ECTS for whole period of PhD

In 2020:

- Attendance and presentation at conferences
  - OHEJP ASM & World One Health Congress
- Taking courses
  - NVivo course
  - Data management
  - Qualitative research interviews
  - Qualitative research methods
  - Research design
  - OHEJP Summer school
- Remaining ECTS will be mainly spent on presentations at conferences in 2021 and 2022

Fieldwork and change of research environment stay, 3- 6 months

- 1 month stay in Rome, Italy in October 2020
- Previous stay: 2 week stay in Uppsala, Sweden in October 2019
- Remaining time of fieldwork will be spent in 2021

Publications (Mandatory: three articles of which at least two are self-authored articles)

- Two co-authored articles published
- One single-authored article submitted
- Remaining articles to be done in 2021

18.5. Soft skills and Continuing Professional Development Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
OHEJP Summer School 2020	Global One Health- From Research to Practice	17.-28.08.20	Wageningen Bioveterinary Research
NVivo course	NVivo beginners and advanced	07. & 10.01.20	Roskilde University
Data management	Research data management and the FAIR principles in Open Science	05.11.20	Roskilde University
Qualitative research interviews	Hands-on, intensive interview training course	10.-12.06.20	Roskilde University
Qualitative research methods	Contemporary discussions on case studies, document analyses, observations, interviews, and focus groups. Tools for choosing qualitative research methods and applying them.	25.-29.04.20	Roskilde University
Research design	Interrelationships between research question, research strategy, literature review, theory, data collection and mixed methods, and interactive research.	04.-13-04.20	Roskilde University
Supervision of bachelor group (5 students) + examination	International studies	Spring semester 2020	Roskilde University

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Supervision of bachelor group (2 students) + examination	International studies	Spring semester 2020	Roskilde University
Supervision of master group (3 students) + examination	Global studies	Spring semester 2020	Roskilde University
Supervision of master group (2 students) + examination	Global studies	Spring semester 2020	Roskilde University
Teaching at bachelor level: International Institutions and Global Governance	Global governance Policy Area IV: Health	15.04.20	Roskilde University
Teaching at master level: Advanced Study Course: International Organisations and Global Politics	From International Health Policy to Global Health Governance	21.04.20	Roskilde University

18.6. Publications and patents

Sarah Humboldt-Dachroeden, S., Olivier, R., Frid-Nielsen, SS. (2020). The state of One Health research across disciplines and sectors – a bibliometric analysis. *One Health,* 10, pp. 100146. DOI: 10.1016/j.onehlt.2020.100146

Munkholm, L., Rubin, O., Bækkeskov, E. , . Attention to the Tripartite's one health measures in national action plans on antimicrobial resistance. *J Public Health Pol* (2021). https://doi.org/10.1057/s41271-021-00277-y

Proceedings of the 2nd Annual Scientific Meeting of the One Health European Joint Programme on Foodborne Zoonoses, Antimicrobial Resistance and Emerging Threats. Online meeting, May 27th - 29th, 2020, page 74

18.7. Impact and Relevance

The PhD project has a unique angle on One Health. It investigates the whole project in terms of One Health integration, implementation and how One Health is put into practice within public health, veterinary, food and environment Institutes. The results of this research can be used by all partners to evaluate and consider their coordination and collaboration efforts. It can be used to enhance disease surveillance nationally and internationally. It produces useful knowledge and examples for institutes on challenges for collaboration and how to overcome those. On a political level, it will provide insight into sharing and translating knowledge between scientists and politicians.

18.8. Follow-up of recommendations and comments in previous reviews by Ethics Advisors

Requirement (from ethical reviewers)	Measures and actions taken
The beneficiary must confirm that no personal data will be collected as part of the project; otherwise the GDPR (EU 2016/679) must be applied and the contact address of the Data Protector Officer of the institution in charge of processing the data obtained must be provided.	No personal data will be collected as part of the project.

18.9. Impact of COVID-19 crisis on project

The COVID-19 pandemic has affected my PhD project, as there were changes in terms of fieldwork, conferences and courses. Conferences were postponed or rescheduled to online events, which impedes

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the ability to network.

My fieldwork was postponed or cancelled and cut short. In spring 2020, a research stay in Sweden at the Swedish Veterinary Agency was planned to conduct my fieldwork. However, due to the COVID-19 pandemic, I remained in Denmark and conducted my interviews online via Microsoft Teams and Skype instead of conducting face-to-face interviews.

A three month fieldwork stay in Italy at the Istituto Superiore di Sanità in the Autumn semester 2020 was shortened to a one month stay. It is planned to finish the fieldwork in Italy in 2021.

18.10 List of critical risks

Description of risk	Yes/No
Loss of PhD supervisor(s)	No
Loss of technical training staff delaying progress of the work	No
Delay in work plan execution	Yes
Conflicts between the collaborative partners that support the PhD	No
Lack of commitment between the collaborative partners that support the PhD	No
Delay in duties, tasks or reporting	Yes
Poor working relationships within the PhD project team	No
Change in PhD student circumstances requiring temporary leave	Yes
Other risks (please describe)	Yes

18.11 Interactions with on-going JRPs/JIPs or with external (EU or national) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), OHEJP stakeholders, national and international surveillance programmes.

I am collaborating in the JPR: NOVA (WP1) on the project of *Surveillance barriers and opportunities as perceived by med-vet-food experts*. Here, I have assisted with conducting and analysing Interviews of Swedish and Norwegian experts.

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18.12 List of Dissemination activities

Name of the activity:	*6th Cogwheel Workshop*		
Date:	*25.11.2020*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*No*
Organisation of a Workshop	*No*	***Participation to a Workshop***	***Yes***
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	***Yes***
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***21***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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Name of the activity:	2nd One Health European Joint Project (OHEJP) Annual Scientific Meeting 2020. Presentation of poster and participation in 3-minute Thesis competition.
Date:	27-29 May 2020
Place:	Online

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories

	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	***Yes***
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories

	Number		*Number*
Scientific Community (Higher Education, Research)	***750***	***Media***	*0*
Industry	*0*	***Investors***	*0*
Civil Society	*0*	***Customers***	*0*
General Public	*0*	***Other***	*0*
Policy Makers	*0*		

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Name of the activity:	*World One Health Congress – Poster presentation*		
Date:	*30.10.-03.11.2020*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference	*No*	***Participation to a Conference***	*Yes*
Organisation of a Workshop	*No*	***Participation to a Workshop***	*No*
Press release	*No*	***Participation to an Event other than a Conference or a Workshop***	*No*
Non-scientific and non-peer-reviewed publication (popularised publication)	*No*	***Video/Film***	*No*
Exhibition	*No*	***Brokerage Event***	*No*
Flyer	*No*	***Pitch Event***	*No*
Training	*No*	***Trade Fair***	*No*
Social Media	*No*	***Participation in activities organized jointly with other H2020 projects***	*No*
Website	*No*	***Other***	*No*
Communication Campaign (e.g. Radio, TV)	*No*		
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***500***	***Media***	*20*
Industry	***0***	***Investors***	*0*
Civil Society	***0***	***Customers***	*0*
General Public	***0***	***Other***	*0*
Policy Makers	***0***		

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