12- Month PhD Report
December 2021

WILBR

Responsible Partner: Animal and Plant Health Agency, UK

Important Notes

- The full 9M report will be included in the Summary Progress Report due in September of each year.

- The full 12M report will be included in a new WP6 deliverable due in March of 2021/2022.

- A 'Summary' of each PhD project will be compiled from these 12M reports, and included in the Periodic report in February of each year.

    - The summary will be compiled from the content of the following sections :
        - Summary (approx. 500 words)
        - Overview of PhD progress
        - Deliverables and Milestones

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1. Summary

- For the 9-month report, summarise the key activities, results and achievements for the reporting period of January to September each year.

- For the 12-month report, summarise the key activities, results and achievements for the reporting period of January to December each year.

A literature review has been written 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.

Pig and presumptive gull faecal samples were collected over three time points in October 2017, 2018, and 2019 from a low antimicrobial usage pig farm as part of the APHA's work on the ARDIG project within the One Health European Joint Programme (https://onehealthejp.eu/jrp-ardig/). These were grown on antibiotic free and antibiotic selective agar, and 632 *E. coli* were purified from pig and gull faeces (n=342 and n=290 respectively) (see Annex 2, Table 1). 176 gull isolates (110 from T3, 66 from T5) that had not been subject to previous analyses were identified and underwent whole genome sequencing. WGS data for all isolates was run through APHA Seqfinder pipeline and Abricate to identify AMR determinants and plasmids. Seven allele MLST was carried out using SRST2 to identify the *E. coli* Sequence Type (ST) of isolates, which was used as an indicator of *E. coli* diversity in pig and gull populations. A total of 91 different sequence types were identified from the isolates included in this study, with 63 and 57 STs identified in gull and pig isolates, respectively. Considering the SNP differences is the next step to understand if the same "clone" or strain has been transferred rather than an AMR plasmid A core genome SNP alignment was generated from WGS data of all isolates using Snippy version 4.6.0 to compare the core genes that are present in all members of the same species, and a SNP distance matrix was generated using snp-dists version 0.7.0 to estimate the SNP differences between isolates to determine the levels of genetic variation in the ST744s, allowing any transmission and persistence of clones across host species and time points, on this farm, to be identified. Preliminary analysis allowed AMR profiles to be linked to ST types that are circulating in both wild bird and pig populations.

Another outdoor pig farm, known to have wild birds persistently present on farm, had been recruited for a longitudinal study and was visited in September 2021 where over 150 environmental swabs were collected. 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 2021 meeting, and an oral presentation on the PhD was presented virtually in the 3MT competition for the OHEJP ASM 2021, as part of communicating their research. The student won the prize for the best poster presentation at the OHEJP ASM 2021. The student has also attended two virtual plasmid workshops hosted by International Society for Plasmid Biology and Other Mobile Genetic Elements in order to further develop their skill set for tasks later on in the project.

2. Overview of the PhD project progress

- In both reports, please provide an update or key information with respect to any progress for all tasks in all work packages. If there has been no progress made, please explain the reasons why e.g. this task is not due to take place until July 2021.

Due to the Covid-19 pandemic in UK only desk and lab-based tasks have been recommended; currently farm visits are not permitted for research projects. A literature review has been completed 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.

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Over two hundred archived E. coli isolates from gull faeces that had not been subject to previous analysis 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 results are not complete.

A farm visit was carried out over 150 environmental swabs were collected. The student has used antibiotic free and antibiotic containing plates to isolate over 1500 isolates from these samples. The student is currently using biochemical tests and MALDI-ToF to determine the species of isolates in order to select a subset for sequencing.

An opportunity to examine the dissemination of AMR in wild bird populations across Great Britain for a 6 month period through the APHA's wild bird scanning surveillance programme is currently being pursued. It has been agreed that the wild bird scanning surveillance will provide 0.5-1.0g of caecal/faecal contents from every wild bird submitted as part of the APHA's wild bird scanning surveillance for a 6 month period. This study would allow a unique opportunity to establish the burden of AMR in the wild bird population of Great Britain. The study will provide context to our on farm studies by allowing wider comparisons to be made, highlighting any correlation between the AMR profiles identified in the farm environment and those found in wild birds across Great Britain.

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

- For the 9-month report please describe the key scientific results for the reporting period of January to September each year.
- For the 12-month report, please describe the key scientific results for the reporting period of January to December each year.
- Include a description of the project activities per task , plus detailed changes and any deviations compared to the annual work plan to which this 9 or 12 month report applies and the reason(s) for these differences.
- PI's may be requested to update the initial Annual Work Plans if the deviations reported are considered very significant.
- Please provide enough detail to enable the research to be understood, without the need for additional information. Wherever possible, link this to a specific task in the original PhD work plan describing the WP#, Task# and Task Name.

Deliverable 3 (9 month report) in AWP for Y4 has been completed and deliverable 4 (12 month report) is being undertaken.

Milestone 1 in AWP was delayed as due to further Covid-19 restrictions in the UK, all fieldwork at APHA had been stopped. An outdoor pig farm was visited in September 2021 and over 150 environmental swabs were collected, which resulted in the isolation of over 1500 bacterial isolates. Only one farm visit has been able to be carried out so far due to Covid-19 delays. Milestones 2 and 3 are delayed as a result of the initial delay of milestone 1.

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 in line with Milestone 2. This will result in a dataset including over 300 gull isolates and over 400 pig isolates from three time points that can be compared. AMR profiles and phylogeny are being generated from whole genome sequencing data to assess the diversity

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of *E. coli* strains and associated ARGs in gulls and pigs on the same farm, and assess the potential for transmission of AMR bacteria between wild birds and livestock. This work is still being carried out so the results are not yet complete.

An opportunity to examine the dissemination of AMR in wild bird populations across Great Britain for a 6 month period through the APHA's wild bird scanning surveillance programme is also being pursued. The wild bird scanning surveillance programme examines wild bird species for all disease and mortality investigations, including infectious and non-infectious disease. They receive submissions from all over Great Britain, and from many different species. It has been agreed that the wild bird scanning surveillance will provide 0.5-1.0g of caecal/intestinal contents from every wild bird submitted as part of the APHA's wild bird scanning surveillance for a 6 month period. Final logistics are being confirmed, taking in to account the risk from human and livestock pathogens that wild birds could be carrying.

4. Progress of the research project: milestones and deliverables

Deliverables

PhD Project Reference	Deliverable number	Deliverable name	Delivery date from Annual Work Plan	Actual Delivery Date	If not achieved: Forecast achievement date	Comments
WILBR	D-PhD10-1.3	Completion of 9 month review	M45			
WILBR	D-PhD10-1.4	Completion of 12 month review	M48			

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Milestones

PhD Project Reference	Milestone number	Milestone name	Delivery date from Annual Work Plan	Achieved (Yes / No)	If not achieved: Forecast achievement date	Comments
WILBR	1	Collection of microbiological samples for longitudinal study	M48			
WILBR	2	Molecular analysis of bacterial isolates and antimicrobial resistance	M48			
WILBR	3	Characterisation of mobile elements transmitting/proliferating AMR	M48			
WILBR	4	Completion of 9 month review	M45			
WILBR	5	Completion of 12 month review	M48			

5. Soft skills and Continuing Professional Development training

Please describe details of relevant soft skills or CPD training attended and undertaken by the PhD student.

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
EURL-AR Training Course	Working with bacterial sequence data in relation to the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria	26-29/04/2021	Technical University of Denmark

6. Publications and additional outputs

No publications or patents.

7. Remarkable outcomes

Please include 'Remarkable outcomes' i.e. deliverable, publication, folder, tool, etc. that are appropriate for communication purposes. You have the possibility to draw the attention to remarkable achievements that could be highlighted by the OHEJP communications team to illustrate the potential impact of your project.

The student won the prize for the best poster presentation at the OHEJP ASM 2021.

8. Impact & relevance

250 words - Describe how the PhD project contributes to and enhances the collaborative relationships between the partner institutes involved (both internal and external to the existing consortium).

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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.

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

The responses to previous ethical reviewers' comments have been accepted and this is closed.

10. Impact of COVID-19 crisis on the project

Please indicate the (sub)tasks and associated milestones and deliverables that are affected by the CoViD-19 crisis, propose new deadlines and indicate the reason: for instance issues in provision of equipment, materials or samples, shortage of staff (reallocated or sick), shortage of resources (reallocation of budget, etc.), or other. Also estimate the associated budget that will not be spent, or with delay.

If not a milestone of deliverable, don't forget costs associated with the organisation of meetings!

You can give more details in the 'Comments' field below the table.

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
			Milestone 2	M48	M54	COVID-19	not yet known	not yet known
			Milestone 3	M48	M57	COVID-19	Not yet known	Not yet known
			Milestone 4	M48	M60	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 visitied the farm recruited for longitudinal sampling four times. The original plan was to sample across four time points in one year, but that has not happened as a result of COVID-19. 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 wild birds.

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11. List of critical risks

Please indicate possible risk within your PhD project

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

Additional information:

12. 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.

Please describe any link (workshops, meetings etc.) with a Joint Research Project or Joint Integrative Project; with any external (EU, national and international) relevant project (name + website), and any OHEJP stakeholders.

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

13. List of dissemination and communication activities

Please fill in one table per event you attended/organised or for any publications which have no DOI reference (e.g. article in journal, publication in conference proceedings or workshop, book or monograph, chapter in a book, thesis/Dissertation, other).

Name of the activity:	*ISPB Plasmids Around the Globe*		
Date:	*06/05/2021*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	
Organisation of a Workshop		***Participation to a Workshop***	*Yes*
Press release		***Participation to an Event other than a***	
Non-scientific and non-peer-reviewed publication		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organized jointly***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication			
	Number		*Number*
Scientific Community (Higher Education,	***60***	***Media***	
Industry		***Investors***	

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Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*ISPB Plasmids Around the Globe*
Date:	*25/05/2021*
Place:	*Online*

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

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

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

	Number		*Number*
Scientific Community (Higher Education, Research)	***60***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

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Name of the activity:	*OHEJP ASM 2021 (poster and 3MT competition)*
Date:	*09-11/06/2021*
Place:	*Online*

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

Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	***550+***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

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