SHORT-TERM MISSION SUMMARY REPORTS 2018

Eight summary reports from completed missions funded during the 2018-2019 call have been received from Maria Cabral, Clazien de Vos, Jonathan Betts, Janneke Duijster, Victor Lorente Leal, Pilar Pozo Piñol, Frits Franssen and Kaya Stollberg.

2018_STM_01_Cabral

Modelling disease incursion risks using the quantitative risk assessment model COMPARE

Maria Cabral *from Wageningen Bioveterinary Research (WBVR), Lelystad, the Netherlands visited Dr. Rachel Taylor from the Animal and Plant Health Agency (APHA), UK from 24th February – 8th March 2019.*

OBJECTIVE: To provide additional training to Maria in quantitative risk assessment and to assist Maria in expanding her network in the area of risk assessment. More specifically, the STM was aimed to introduce Maria to a quantitative model (COMPARE) developed at APHA to estimate disease incursion risks, to help her understand its input and algorithms, to adapt the model to a specific disease, and to run the model independently.

REPORT: Maria visited the Department of Epidemiological Sciences at APHA to work with the Biomathematics and Risk Research workgroup. During this STM, she was introduced to a quantitative risk assessment model for disease incursion (COMPARE) that was developed by APHA. The aim of Maria's visit was to understand the model's framework, algorithms and data requirements, and to practice running the model.

In the first week, Maria had an introductory meeting with Dr Rachel Taylor explaining the model's inputs and outputs, its algorithms and the pathways considered. Dr Taylor organised exercises for Maria to practise performing spatial import risk assessment in R. In addition, Maria did a DataCamp course to consolidate her knowledge of spatial modelling in R.

In the second week, Maria started using the COMPARE model on a previous case study to practise running the model and understanding each step of the model algorithms. She also began parameterisation of the model for foot and mouth disease incursion into the UK and the Netherlands for a new case study. Therefore, by the end of the STM, Maria was able to run the model independently and was ready to begin the new case study of Foot and Mouth Disease.

The STM also enabled Maria to expand her professional network in the area of risk assessment. She was introduced to both the Biomathematics and Risk Research workgroup and the Epidemiology workgroup in the Department of Epidemiological Sciences, attended department meetings and had the opportunity to participate in a meeting with the UK's Chief Veterinary Officer.

2018_STM_02_Vos

Import risk assessment for exotic livestock diseases

Clazien de Vos *van Wageningen Bioveterinary Research, Lelystad, the Netherlands visited Prof. Krzysztof Smietanka and Anna Gierak at the National Veterinary Research Institute, Pulawy in Poland from 28 January 2019 to 1 February 2019.*

OBJECTIVE: To train scientists at the National Veterinary Research Institute in Pulawy in import risk assessment by (a) supervising one of Prof. Smietanka's PhD-students, Anna Gierak, who is modelling the incursion risk of avian influenza to Poland, and (b) giving an introductory course on risk assessment to the Department of Epidemiology and Risk Assessment.

REPORT: In January 2019, Clazien de Vos has visited the National Veterinary Research Institute in Pulawy to train scientists in import risk assessment with a focus on exotic livestock diseases. She has provided a 2-day introductory course on risk assessment to the Department of Epidemiology and Risk Assessment, covering the topics of (a) qualitative and quantitative risk assessments, (b) model pathways, (c) deterministic and stochastic risk models, (d) risk assessment for vector-borne diseases, and (e) risk communication. Furthermore, she has reviewed a quantitative risk model that has been developed by a PhD student of the Department of Epidemiology and Risk Assessment to evaluate the probability of introducing low pathogenic avian influenza into Poland by trade in poultry. Model assumptions and input parameters were evaluated and discussed and suggestions for improvement were given. Furthermore, Clazien has helped the PhD student in setting up a detailed sensitivity analysis to validate this risk model.

Clazien has also used the opportunity of visiting the National Veterinary Research Institute in Pulawy to get an update on the epidemiology of African swine fever in Poland.

2018_STM_04_Betts

Novel compounds for the treatment of *Streptococcus pneumoniae*: an unexplored species in zoonotic disease

Jonathan Betts *of the School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford UK, visited Dr Apostolos Liakoupoulos and Dr Daniel Rozen at the Institute of Biology, Leiden, Leiden University, the Netherlands from 20th to 28th October 2018.*

OBJECTIVE: the key aim of this mission was to determine the antibacterial activity of the manganese complex [$Mn(CO)_3$(tpa-k^3*N*)]Br against multidrug-resistant isolates of *Streptococcus pneumoniae.* Introductory training on whole genome sequencing (WGS) and library preps for future analysis of *S. pneumoniae.*

REPORT: The rise of antibiotic resistance (AMR) in humans has been a significant topic of interest over the past decade. However, many also consider animals as a reservoir of resistance mechanisms, due to previous overuse of antibiotics. The zoonotic potential of many bacterial pathogens enables their transfer to humans, carrying any resistance genes with them. This can lead to limited therapeutic options for clinicians in veterinary and human medicine. *S. pneumoniae* is a problematic human pathogen, which is often resistant to multiple classes of antibiotic. However, it has been isolated in several animal species, both wild and domestic. A group of novel antibacterial agents with potential against *S. pneumoniae,* are Mn carbonyl complexes. During the visit to the University of Leiden, the efficacy of one of these complexes, [$Mn(CO)_3$(tpa-K^3*N*)]Br, was evaluated against multidrug-resistant (MDR), clinically important strains of *S. pneumoniae*. [$Mn(CO)_3$(tpa-K^3*N*)]Br was found to be 8- to 16-fold more active against these strains, than previously observed against MDR avian pathogenic *Escherichia coli*. This activity was also confirmed *in vivo,* using the *Galleria mellonella* model of infection. Data from these experiments have been submitted for presentation at the European Congress for Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, 2019.

Training received on whole genome sequencing (WGS), confirmed *S. pneumonia* as a reservoir of resistance genes and will allow the future analysis and comparison of the molecular characteristics of *S. pneumoniae* strains, isolated from humans and animals. Work is underway, to determine if *S. pneumoniae* in animals are not only a reservoir of resistance genes but also potential zoonotic pathogens.

2018_STM_05_Duijster

***Salmonella* infection and colon cancer**

Janneke Duijster *from the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands visited Dr. S. Ethelberg and Prof. K.A. Krogfelt from the Statens Serum Institut (SSI), Copenhagen, Denmark from 14-18 January 2019.*

OBJECTIVE: The mission to SSI in Copenhagen had two main objectives, which are both part of a project on the association between *Salmonella* infection and colon cancer. The project is carried out in collaboration with the SSI.

1) To provide methodological input and assistance in the epidemiological analysis of salmonellosis and colon cancer data and to collaborate on the interpretation of results;

2) To gain knowledge in the application of an indirect ELISA method used at the SSI to determine the serum IgG, IgM, and IgA levels against *S*. Enteritidis and *S*. Typhimurium and to assist in the analysis of the samples.

REPORT: Over 20% of the global cancer burden is attributable to infectious agents, including bacteria. In a nationwide epidemiological study a 3-fold increased colon cancer risk has been shown in patients with a history of (severe) *S*. Enteritidis infection as compared to the general Dutch population. Given those results, we aimed to test the association between non-typhoid salmonellosis and colon cancer in an independent dataset (a Danish cohort) as validation and to increase the power of the analysis. Analyses are performed within the group led by S. Ethelberg. During the mission, we started with an explanatory data analysis and explored the possibilities of additional analysis on the confounding effects of e.g. IBD. An analysis plan was drawn and we started with the first survival analyses.
Next, to explore the possible association between repeated mild *Salmonella* infections and colon cancer, we aimed to investigate whether individual seroincidence rates of *Salmonella* (defined as the number of infections/person-year) in colon cancer patients differs from that of controls. To this end, in collaboration with K.A. Krogfelt, we performed a validated indirect ELISA method on 108 Dutch sera (i.e. 36 cases and 72 controls) to determine serum IgG, IgM and IgA levels. Knowledge was gained on the exact procedure of the ELISA method as well as on the interpretation of kinetics of antibody production in relation to time since last infection and seroincidence rates. The results from both parts of the mission will be disseminated in peer-reviewed scientific journal articles.

2018_STM_06_Lorente

Bioinformatics training to perform epidemiological studies on bovine tuberculosis

Victor Lorente Leal *from VISAVET Health Surveillance Centre, Departamento de Sanidad Animal, Faculty of Veterinary Medicine, Universidad Complutense in Mardrid, Spain visited the Animal &Plant Health Agency, new Haw, Addlestone, UK from February 23rd – April 7th 2019.*

OBJECTIVE: The main objectives of the STM were: 1)to learn the main method required to obtain genomic data from biological samples 2) to understand the functioning of the illumine (short-read) and Oxford nanopore (long-read) technologies, 3) to gain practical experience in

the use of different WGS packages, including variant calling pipelines (such as the in-house developed Bov-TB and Snippy pipelines), 4) to learn about the infrastructure requirements for the set-up of bioinformatics projects and 5) to learn about the use of WGS in other areas of study such as antimicrobial resistance or *Salmonella* control and surveillance.

REPORT: Bovine tuberculosis (bTB) is an economically important infectious disease that is under stringent surveillance in the EU, with well-established eradication campaigns set up in different non Officially Tuberculosis-Free (OTF) regions. The development of Next Generation Sequencing platforms have positively influenced the study of infectious diseases, giving rise to a completely new perspective in the study of pathogens. The use of WGS for the study of bTB will deeply benefit its surveillance and control, improving outbreak investigations and increasing the overall knowledge of the evolution and distribution of this microorganism throughout Europe and the world.

The APHA has long time experience in WGS research in many different pathogens, including bTB and *Salmonella*, as well as in the study of AMR. The aim of the STM was to visit the Central Sequencing Unit in the Animal & Plant Health Agency in Addlestone, United Kingdom, in order to achieve hands-on experience in the analysis of Whole Genome Sequencing data in the context of bTB. The mission was organised so that all steps required to obtain WGS data and its processing were covered, including sequencing protocols for both Illumina and Oxford Nanopore platforms, sequence pre-processing, practical experience in the use of bioinformatic pipelines, such as an in-house variant calling pipeline (Bov-TB) for the analysis of bTB, and learning about the infrastructure requirements for WGS projects.

2018_STM_07_Pinol

Use of Whole Genome Sequencing of Spanish *Mycobacterium bovis* Strains to Investigate Transmission Dynamics of Bovine Tuberculosis among Wildlife and Livestock

Pilar Pozo Piñol *from VISAVET Health Surveillance Centre – UCM, Madrid visitied Suelee Robbe-Austerman from the National Veterinary Services Laboratories (NVSL) U.S. Deparment of Agriculture, Animal and Plant Health Inspection Service (USDA), Ames, Iowa, USA from January 8th – February 8th 2019.*

OBJECTIVE: The key aims of this mission were: i) Development of skills on the use of the in-house whole genome sequencing (WGS) USDA APHIS Veterinary Services pipeline called vSNP for *Mycobacterium tuberculosis* complex. ii) Optimization of the pipeline to work in a Windows environment. iii) Validation of SNP (single nucleotide polymorphisms) calls and interpretation of the WGS data of *M. bovis* isolates recovered from wildlife and cattle from Spain. iv) Understanding the genetic diversity of *M. bovis*, its evolution and the spatio-temporal patterns of spread between and within species in Spain.

REPORT: *Mycobacterium bovis*, the main causative agent of animal tuberculosis, has a broad host range, causing economic loss and infecting humans, livestock and wildlife.

Despite efforts invested on its eradication in Spain, herd prevalence has remained constant for the last 15 years (~2.1-2.8%) due to a combination of epidemiological factors impairing disease control. During the training at the NVSL, WGS analysis of 55 *M. bovis* isolates

recovered from different animal species and locations in Spain during 2005-2017 was conducted to assess the patterns of spread and evolution in the cattle-wildlife interface.

A variable diversity was found among the 55 isolates, as isolates were within a range of 1-67 SNPs of their common ancestors. However, genetic heterogeneity was geographic rather than host species-specific, as isolates recovered from cattle and wildlife sharing recent common ancestors were more closely related within same provinces. Limited within-herd genetic diversity was found for isolates coming from 5 out of 9 herds, with the majority of isolates having ≤3 SNPs.

The presence of local diversity among isolates from cattle and wildlife suggested the existence of several sources of infection in these host species within provinces over time. The genetic relatedness found between isolates from different host species demonstrated the complex between-host transmission cycle present in endemic areas in Spain, which will be further assessed with the addition of 50 additional samples.

These results have been submitted for oral presentation at the Annual Scientific Meeting of the One Health European Joint Programme on Food-borne Diseases, Antimicrobial Resistance and Emerging Threats.

2018_STM_09_Franssen

Next generation technologies and application

Frits Franssen from the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands visited Dr. Simone Cacció and Dr. Paolo Vatta at the Istituto Superiore di Sanità, Viale Elene Regina, Rome from 16th – 20th September 2019.

OBJECTIVE: Metagenome analysis for parasites

REPORT: The specific challenges and requirements for parasite DNA preparation for whole genome sequencing were discussed, as well as a genomic workbench for read mapping and genome reconstruction.

Bio-informatics pipelines (both commercial and in-house) and the principles of metagenome (MG) analysis were discussed to obtain a better understanding of the available computer programs that are necessary tools to interrogate metagenome databases. Also statistics to validate the results and consequent automated blasting of Genbank, were discussed.

Previous metagenome analysis results that had been obtained by the applicant, revealing the presence of *Cryptosporidium* sequences in metagenome databases based on 18S, were challenged by interrogating the same MG project numbers with a whole genome sequence (WGS) of Crypto. The hypothesis was that using a longer query sequence could yield more DNA reads from the MG project numbers interrogated earlier, and a better statistical separation between noise and specific reads. Indeed, MG analysis with the Crypto WGS yielded several hits, identified by basic statistical analysis of the results, which will be blasted in Genbank for confirmation. These results will be incorporated in an already prepared joint publication concerning metagenome analysis for parasites.

Use of ribosomal RNA, instead of rDNA, could be an alternative strategy for MG analysis. A suitable database to search for bait reference sequences could be the SILVA database, which is considered better curated than Genbank.

NGS and metagenome analysis concerning bacteria were discussed with Dr. Stefano Morabito of the EU Reference Laboratory for E. coli.

2018_STM_10_Stollberg

Training course on "Identification of DNA of Toxoplasma gondii in Food Matrices (Meat and Meat products) by LAMP

Kaya Stollberg *from the German Federal Institute for Risk Assessment (Bundestinstitut für Risikobewertung,BfR), Berlin, Germany visited Dr. Simone M Cacciò and Dr. Marco Lalle from the Istituto Superiore di Sanita European Union Reference Laboratory for Parasites Unit of Foodborne and Neglected Parasites from 18th February – 24th February 2019.*

OBJECTIVE: Key aims of the training were to be trained in a LAMP assay for detection of *T. gondii* DNA in meat that is routinely used at the EURLP in Rome and to gain experience in said method. Training on effective methods for DNA extraction from meat was included as well. The technique will be established at the German Federal Institute for Risk Assessment and later compared to other detection methods that are already used at the German Federal Institute for Risk Assessment (BfR).

REPORT: *Toxoplasma gondii* is a protozoan zoonotic parasite with a worldwide distribution, and the causative agent of toxoplasmosis. *T. gondii* ranks among the most important foodborne pathogens. Human can be infected by either consumption of meet containing tissue cysts or by accidental ingestion of water and food contaminated by the parasite oocysts. Despite parasite relevance and several molecular test available, standardized procedures for the detection of *T. gondii* in food are not available. Among the available detection methods, loop-mediated isothermal amplification (LAMP). A LAMP-based assay, targeting a 529 bp repetitive region unique to *T. gondii,* has been developed at EURLP (European Union Reference Laboratory for Parasites) at the Istituto Superiore di Sanita (ISS) in Rome, to isolate and detect *T. gondii* DNA in foodstuffs (Test method MI-12 https://eurlp.iss.it/2018/02/01/test-methods; Lalle M et al., Food Microbiol. 2018,70:137-142).

After being introduced to the theory of the LAMP and having performed the LAMP with reference material, DNA was extracted from a batch of samples (digested wild boar and roe deer meat) that were previously tested at BfR by qPCR (targeting the same genetic locus). These samples were subjected to LAMP. As some samples had shown signs of inhibition in the qPCR, these samples were also tested following spiking with *T. gondii* positive control DNA to check for inhibition in LAMP. However one of the spiked samples showed signs of inhibition in the LAMP assay.

Noteworthy, some of the samples that tested positive for *T. gondii* using qPCR, tested negative using the LAMP assay. To evaluate the possibility that T, gondii DNA was below the sensitivity of the method, reaction time was extended from 90 to 120 min. The extended

reaction time lead to all positive results and, in addition, some of the samples that were tested negative for *T. gondii* using qPCR showed positive results in LAMP.

These preliminary results are in favor of a superior performance of LAMP vs qPCR to detect *T. gondii* in wild boar and roe deer meat, both in term of sensitivity and resistance to inhibitors. To corroborate the LAMP results, all DNAs extracted at the ISS, and eluted in the Qiagen Elution buffer, will be re-tested at BfR using qPCR. This step is necessary to properly compare the results of the LAMP vs qPCR performed at BfR, as routinely DNA is eluted in ddH_2O.

This training has been a relevant step in our aim to establishing the LAMP assay at BfR enabling our laboratory to plan future comparative studies with other detection methods for *T. gondii* in meat.