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**EUPHEM****EUPHEM REPORT**

Summary of work activities

Nina Lagerqvist
European Public Health Microbiology Training
Programme (EUPHEM), 2017 cohort

Background

According to the European Centre for Disease Prevention and Control (ECDC) Advisory Group on Public Health Microbiology ('national microbiology focal points'), public health microbiology is a cross-cutting area that spans the fields of human, animal, food, water, and environmental microbiology, with a focus on human population health and disease. Its primary function is to improve health in collaboration with other public health disciplines, in particular epidemiology. Public health microbiology laboratories play a central role in detection, monitoring, outbreak response and the provision of scientific evidence to prevent and control infectious diseases.

European preparedness for responding to new infectious disease threats requires a sustainable infrastructure capable of detecting, diagnosing, and controlling infectious disease problems, including the design of control strategies for the prevention and treatment of infections. A broad range of expertise, particularly in the fields of epidemiology and public health microbiology, is necessary to fulfil these requirements. Public health microbiology is required to provide access to experts in all relevant communicable diseases at the regional, national and international level in order to mount rapid responses to emerging health threats, plan appropriate prevention strategies, assess existing prevention disciplines, develop microbiological guidelines, evaluate/produce new diagnostic tools, arbitrate on risks from microbes or their products and provide pertinent information to policy makers from a microbiological perspective.

According to Articles 5 and 9 of ECDC's founding regulation (EC No 851/2004) 'the Centre shall, encourage cooperation between expert and reference laboratories, foster the development of sufficient capacity within the community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health' and 'as appropriate, support and coordinate training programmes in order to assist Member States and the Commission to have sufficient numbers of trained specialists, in particular in epidemiological surveillance and field investigations, and to have a capability to define health measures to control disease outbreaks'.

Moreover, Article 47 of the Lisbon Treaty states that 'Member States shall, within the framework of a joint programme, encourage the exchange of young workers. Therefore, ECDC initiated the two-year EUPHEM training programme in 2008. EUPHEM is closely linked to the European Programme for Intervention Epidemiology Training (EPIET). Both EUPHEM and EPIET are considered 'specialist pathways' of the two-year ECDC fellowship programme for applied disease prevention and control.

This report summarises the work activities undertaken by Nina Lagerqvist, cohort 2017 of the European Public Health Microbiology Training Programme (EUPHEM) at the Public Health Agency of Sweden.

All EUPHEM activities aim to address different aspects of public health microbiology and underline the various roles of public health laboratory scientists within public health systems.

Pre-fellowship short biography

Nina Lagerqvist holds a Master of Science in Engineering Biology (2006) and a PhD in Medical Science (2013) from Karolinska Institutet. She has been working at the Public Health Agency of Sweden (PHAS, previously Swedish Institute of Communicable Disease Control) since 2013. Her focus has been in emerging infectious diseases, primarily in virology. Her personal objectives for entering the EUPHEM fellowship was to broaden her knowledge in public health microbiology, especially within bacteriology.

Methods

This report accompanies a portfolio that demonstrates the competencies acquired during the EUPHEM fellowship by working on various projects, activities and theoretical training modules.

Projects included epidemiological investigations (outbreaks and surveillance); applied public health research; applied public health microbiology and laboratory investigation; biorisk management; quality management; teaching and public health microbiology management; summarising and communicating scientific evidence and activities with a specific microbiological focus.

The outcomes include publications, presentations, posters, reports and teaching materials prepared by the fellow. The portfolio presents a summary of all work activities conducted by the fellow, unless prohibited due to confidentiality regulations.

Results

The objectives of these core competency domains were achieved partly through projects or activities (on-job services) and partly through participation in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the EUPHEM scientific guide¹.

1. Epidemiological investigations

1.1. Outbreak investigations

Outbreak of gastroenteritis at a conference venue, Halland, Sweden, 2017

Supervisors: Adam Roth and Cecilia Jernberg

On 13 and 14 November 2017, local health authorities were notified of several people reporting gastroenteritis after a visit to a conference venue. The outbreak was investigated with the aim to identify the mode and source of transmission in order to implement control measures.

A retrospective cohort study was performed among individuals who had consumed food served at the venue 8-10 November. Information on demographics, days of attendance, consumption of food items, and symptoms were collected using an online questionnaire. Descriptive analysis of cases was performed and attack rates, risk ratios, confidence intervals, and p-values were calculated for all exposures.

The epidemiological investigation identified 83 cases who had experienced gastrointestinal disease after visiting the venue and suggested contaminated leafy greens to be the transmission vehicle. Stool samples collected from three cases were analysed for enteric pathogens and all were PCR-positive for the *ipaH* gene, exclusively found in enteroinvasive *Escherichia coli* (EIEC) and *Shigella*. EIEC was isolated from stool samples and whole genome sequencing (WGS) revealed almost genetically indistinguishable genomes based on single nucleotide polymorphism (SNP)-phylogeny and confirmed EIEC of molecular serotype O96:H19 to be the disease-causing pathogen.

¹ European Centre for Disease Prevention and Control. European public health training programme. Stockholm: ECDC; 2017. Available from: <http://ecdc.europa.eu/en/publications/Publications/microbiology-public-health-training-programme.pdf>

The public health response to this outbreak involved communication with the public and government sectors and the investigation into this outbreak will lead to further studies on the differential diagnostics of EIEC and *Shigella* within the National Reference Laboratory network.

The fellow analysed the WGS result and was part of the team that set up the questionnaire, analysed the results from the cohort study, and wrote the outbreak investigation report. In addition, the fellow contributed to the communication with other authorities and authored a manuscript regarding the investigation into this outbreak.

Training modules

The EPIET/EUPHEM Introductory course introduced the fellow to the ten steps of an outbreak investigation and familiarised the fellow with the analytical and logistical approach to outbreak investigations as well as scientific writing. During the Outbreak Investigation module the fellow performed all steps in an outbreak investigation from the creation of data entry files to the interpretation and communication of findings. In addition, the fellow was introduced to various software packages used for data management and analysis during this module. The fellow learned how to perform more comprehensive statistical analyses and how to best communicate results obtained from such analyses during the Multivariable Analysis module.

Educational outcome: application of bacteriology concepts to the public health discipline; understanding the use and limitations of typing methods and their interpretation; application of microbiological and epidemiological knowledge in outbreak situations; participating in a multidisciplinary team; involvement in an outbreak investigation including case definition, case-finding, data collection, data analysis, and communication; writing of outbreak report and scientific publication.

1.2. Surveillance

Update on the epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, assessment by national experts from 37 countries, 2018

Supervisors: Alma Brolund and Sara Byfors

Part of the ECDC initiated and funded project 'Genomic-based surveillance of carbapenem-resistant and/or colistin-resistant Enterobacteriaceae at the EU level (EURGenCCRE)'.

The rapid spread of carbapenemase-producing *Enterobacteriaceae* (CPE)/carbapenem-resistant *Enterobacteriaceae* (CRE) represents a global public health threat as these pathogens are resistant to several antibiotics. In 2017, ECDC established the European Antimicrobial Resistance Genes Surveillance network (EURGen-Net) to perform structured surveys of carbapenem- and/or colistin-resistant *Enterobacteriaceae* (CCRE) in Europe. With many documented cases of cross-border import not only globally but also within Europe, the future successfulness of European CPE/CRE control will be dependent on all countries having strong surveillance and control measures in place.

In order to update the epidemiological situation of CPE and gather information on the current national capacity for surveillance, containment, and laboratory detection and characterization of CRE, a self-assessment questionnaire was sent to national experts representing 37 European countries in 2018.

The results from this capacity evaluation showed that the epidemic situation had worsened in 11 of 37 countries since 2015 and all countries had now identified CPE cases. Twenty-seven countries reported to have a dedicated national surveillance system for CRE, 24 countries had national recommendations or obligations for notification of CRE cases to health authorities, and 24 countries had issued guidance on control measures. A majority of the countries had national reference or expert laboratories (NRLs) and good laboratory capacity for genotypic identification and characterisation of CRE.

The fellow performed the survey data analysis, drafted the manuscript, and presented the result of the survey during the EURGen-Net training workshop in Stockholm, Sweden, 2018.

Laboratory surveillance of Rotavirus after introduction into the national childhood immunisation program

Supervisor: Mia Brytting

Rotavirus infections are the leading cause of severe diarrhoea in young children and almost all children are infected by the age of 5 years. Sweden will include rotavirus in the national childhood immunisation program by the autumn of 2019. The genotypes circulating in Sweden have been monitored within the national microbiological surveillance program since 2008 and on a yearly basis, 100 to 300 samples are submitted to and typed at the Public Health Agency of Sweden (PHAS). However, structured laboratory-based surveillance is currently lacking. As laboratory-based surveillance is one of the key elements for monitoring the effect of vaccine introduction on national as well as EU level a new strategy for the laboratory surveillance needs to be produced and implemented.

The fellow analysed the current set-up of the microbiological surveillance, reviewed the literature, analysed the genotyping data collected 2008-2018, proposed strategies for the future microbiological surveillance, and was involved in planning of the introduction of laboratory notification. The fellow was invited speaker at the 6th European Expert meeting on Rotavirus Vaccination joint with 8th European Rotavirus Biology meeting (EEROVAC) 2019 meeting in Riga, Latvia, where she presented the currently available Rotavirus surveillance in Sweden and the strategy for the future.

Training modules

The fellow was familiarised with different attributes of surveillance systems and the development, evaluation, and analysis of surveillance systems during the EPIET/EUPHEM Introductory course. In addition, participants were trained in scientific writing. The Rapid Assessment and Survey Methods module introduced the fellow to sampling methods adapted to study populations. The Management, Leadership, and Communication module trained the fellow in communication and presentation techniques.

Educational outcome: analysis of survey results to assess surveillance practices; review and improve a survey aimed for EU/EEA national/regional reference laboratories; interpret and summarize survey results; analysis of microbiological surveillance data; review literature; communication with different partners; presentation for public health specialists; writing scientific publication.

2. Applied public health microbiology research

Retrospective investigation into an outbreak of invasive *Streptococcus pyogenes* type *emm66* disease in Sweden, 2013-2015

Supervisor: Thomas Åkerlund

An outbreak of *Streptococcus pyogenes* (*S. pyogenes*) *emm66* type infections was described in a largely homeless population in United Kingdom in 2016. Three years earlier, in 2013, the first invasive *emm66* case was reported in Sweden. This particular *emm*-type suddenly disappeared by the end of 2015, and no more invasive cases due to *S. pyogenes emm66* has since been reported in Sweden. An investigation into this three-year long outbreak was conducted in order to get a better understanding of invasive *S. pyogenes emm66* disease transmission.

The study was retrospective in design and included patients reported to the national notification system with invasive disease due to *S. pyogenes emm66* type infection. The medical records for these patients were reviewed and information on demographics, clinical data, and lifestyle risk factors were collected. The isolates of these patients were sequenced and whole genome SNP-analysis was performed in order to investigate the genetic similarities among isolates.

The median age of the patients were 60 years (range 25-90), 70% were male and the cases were residents of seven Swedish counties. Preliminary data suggest that 30% of the cases were homeless and 57% had problematic alcohol use and/or used drugs. Two 0 SNP difference clusters, each consisting of four isolates, were detected and a majority of the isolates not represented in such a cluster showed relatively high genomic sequence similarity (< 10 SNP difference).

Preliminary data suggests that the population affected by this unusual *emm*-type in Sweden has similar lifestyle characteristics to that reported in United Kingdom. More detailed analysis of the sequence data and investigations into possible transmission links between cases are currently ongoing. This study will generate knowledge about unusual *emm*-types and possible transmission routes which can be used to implement control strategies when new *emm*-types emerge.

The fellow wrote the study protocol and the application for the ethical permit, participated in the collection of data from patient records, performed WGS and analysed genomic sequence data, performed analysis of epidemiological data, and drafted the manuscript.

Genetic relationships within geographic clusters of invasive *Streptococcus pyogenes* *emm1* and *emm89* infections in Sweden

Supervisor: Thomas Åkerlund

In Sweden, invasive *S. pyogenes* infections are notifiable and data collected within the national microbiological surveillance program indicates geographic *emm*-type-specific clusters. In order to gain further knowledge of possible invasive *S. pyogenes* disease-transmission in Sweden, WGS was used to increase the genetic resolution of the *emm89* 2013- and the *emm1* 2016-collections facilitating investigations of observed clusters.

The genomes of 79 *emm89* and 64 *emm1* isolates collected from all but one county in Sweden were sequenced and whole genome SNP-analysis was performed to investigate genetic similarities between the isolates.

Preliminary sequence analyses indicate high genomic sequence diversity within the *emm1* and *emm89* types, however, five geographically distinct genetic clusters (<6 SNPs difference) containing two or more isolates were observed for *emm89* and 11 were observed for *emm1*. Data from patient records were collected for the cases identified within the genetic clusters to investigate possible epidemiological links. Analyses of these data are currently ongoing.

In Sweden, data on outbreak-like situations for invasive *S. pyogenes* infections are scarce and the knowledge gained from this study can support in the introduction of targeted control strategies and in the formulation of recommendations. In addition, this study will generate information on the potential additional value of WGS as a surveillance tool for *S. pyogenes*.

The fellow wrote the study protocol and the application for the ethical permit, performed WGS and analysed genomic sequence data, participated in the collection of data from patient records, and performed analysis of epidemiological data. The fellow gave an oral presentation about this work at a Microbiology forum meeting at PHAS and presented the initial findings at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2018 (poster presentation). The fellow will draft the manuscript.

Training modules

The fellow was trained in study design, writing of study protocol, and scientific writing during the EPIET/EUPHEM Introductory course and was introduced to the concepts of phylogeny and relevant software for multilocus sequence typing (MLST) and data obtained from WGS during the Outbreak Investigation module. The fellow was trained in time management and communication during the Management, Leadership, and Communication module. The Project Review module trained the fellow in how to structure abstracts and data in poster/oral presentations and present data in scientific publications.

Educational outcome: identification of a public health problem; reviewing literature; preparation of study protocol; adherence to ethical principles and preparation of ethical permit; analysis of WGS data and minimal spanning trees analyses; cleaning of data; data analysis; communication with different partners; presentation at conference; writing scientific publication.

3. Applied public health microbiology and laboratory investigations

Investigation into suspected horse-to-human transmission of *Streptococcus equi*

Supervisor: Thomas Åkerlund

In contrast to *Streptococcus equi* subspecies *equi* (*S. equi*), which mainly infect horses, *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) is a zoonotic pathogen that can be transmitted from horses to humans. *Streptococcus equi* was isolated from a child presenting with bacterial meningitis. The child had been in contact with a horse and a pony and *Streptococcus equi* was isolated from nostril swabs obtained from the pony.

In order to investigate possible horse-to-human transmission, the genomes of the isolates were sequenced. The genomic sequence analysis revealed different MLST-profiles and the SNP-based phylogeny showed high genomic sequence differences (> 100 SNPs) between the two isolates. The data obtained from WGS showed that the two isolates were not related. This study demonstrate the usefulness of WGS for investigating zoonotic disease transmission.

This project was performed in collaboration with EPIET alumna Maria-Louise Borg, Malta.

The fellow communicated transportation conditions and shipment regulations for *Streptococcus* isolates, was involved in the preparation of a WGS pipeline for *Streptococcus equi*, performed sequence-analysis, wrote a short report describing the laboratory findings, and communicated the result of the analysis.

Investigation into a case of Puumala virus infection in a non-endemic region in Sweden

Supervisors: Jonas Klingström and Pontus Jureen

Puumala virus (PUUV) causes hemorrhagic fever with renal syndrome (HFRS) in humans and is endemic in Europe including the northern and central regions of Sweden, whereas the southernmost region of Sweden is considered non-endemic. In 2018, a farmer residing in the southernmost region of Sweden was diagnosed with HFRS. The patient had no history of travel to endemic regions within the recognized incubation time for HFRS and had not imported any livestock fodder from PUUV-endemic countries.

In order to explore the origin of this PUUV, a region (775 bp) of the S genome segment was sequenced using Sanger methodology. Phylogenetic trees were constructed using relaxed Bayesian analysis with 100 million generations. In addition, as PUUV is transmitted to humans by inhalation of contaminated excreta from infected bank voles, a small number of voles trapped in close proximity to the farmer's home were analysed for PUUV.

The PUUV strain infecting this HFRS patient genetically resembled strains from eastern Finland and western Russia, and differed substantially (12-14%) from PUUV strains in central and northern Sweden as well as strains from the geographically close Fyn in Denmark (17%). No PUUV was detected in the sampled voles using real-time reverse transcriptase (RT)-PCR.

The result of this initial study shows that the farmer was infected by a PUUV not genetically linked to known PUUV strains circulating in the proximity of the farmer's residential area, hence the source of this virus is still unknown and necessitates further investigations. Investigations into unexpected HFRS cases in previous non-endemic regions are of importance for timely identification of possible changes to the PUUV epidemiology, which could affect clinical diagnosis and the notification of cases.

The fellow developed the Sanger sequencing method, performed the sequencing and analysed sequence data, participated in the team communicating the results to the regional Communicable Disease Control Unit and treating physicians, wrote the risk-assessment for handling, storing, and preparation of vole tissue, prepared and analysed vole tissue for the presence of PUUV RNA, and wrote a short report describing the laboratory findings.

Evaluation of a commercial micro-immunofluorescence assay for diagnostics of leptospirosis

Supervisor: Andreas Bråve

Leptospirosis is a bacterial disease affecting humans and animals and as one of the most widespread and prevalent zoonotic diseases, is of high public health concern. When diagnosed and treated appropriately leptospirosis cause minor complication, however, when left untreated, the disease can cause severe symptoms and even death.

The most common way to diagnose leptospirosis is through serological tests and the golden standard is Microscopic Agglutination Test (MAT). At PHAS, a commercial IgM ELISA is currently used, and samples testing positive using ELISA are sent for laboratory confirmation using MAT at Statens Serum Institut (SSI) in Denmark. Among those samples sent to SSI, all of which have tested positive in ELISA, a majority are negative in MAT, indicating low specificity of the ELISA. The aim of this project was to evaluate a commercial micro-immunofluorescence (MIF) assay to determine the possibility to introduce this assay as a complement to ELISA testing at PHAS. This would allow for a timelier laboratory diagnosis of suspected leptospirosis cases.

The MIF assay was evaluated using a collection of clinical samples submitted to PHAS for leptospirosis laboratory diagnosis. The assay correctly classified true-positive samples as positives, but the number of false-positives was similar to that observed for the ELISA, and hence, the MIF assay will not be implemented at PHAS.

The fellow performed the laboratory analyses, evaluated the results, drew conclusions from the data, and wrote a short report describing the findings.

Is detection of viral RNA in samples from patients presenting with tick-borne encephalitis a marker for disease severity?

Supervisor: Jonas Klingström

Tick-borne encephalitis (TBE) is a viral disease of the nervous system transmitted to humans from infected ticks. The number of reported cases has increased during the last decade and TBE poses a growing public health problem in several endemic European countries. In Sweden, TBE is primarily diagnosed using serology at clinical laboratories, however, when vaccine failure is suspected, samples are sent to PHAS for confirmation serology. In addition, PHAS implemented a specific real-time RT-PCR in 2013/2014.

Information on how to use PCR in the diagnostics of suspected TBE cases is scarce, and this study aimed at investigating when in the course of illness and for which patients (e.g. co-morbidities, immunosuppression, clinical symptoms) molecular methods are relevant to apply in order to generate knowledge for clinicians as well as clinical and public health laboratories performing TBE laboratory diagnostics.

The study was retrospective in design and included all patients who were tested for TBE using real-time RT-PCR at PHAS during 2014-2018. After ethical approval, laboratory data was collected from PHAS. Clinical data from medical records are currently being collected.

The fellow collected, verified, analysed, and cleaned laboratory data and will be a co-author on a manuscript.

Genotyping of *Cryptosporidium parvum* using PCR-based amplicon sequencing

Supervisor: Jessica Beser

Cryptosporidiosis, caused by the intestinal protozoan parasite *Cryptosporidium*, is endemic worldwide and two species, *Cryptosporidium parvum* (*C. parvum*) and *Cryptosporidium hominis* (*C. hominis*) account for a vast majority of reported human cases. *Cryptosporidium* is known for its ability to cause large food- and waterborne outbreaks and as *C. parvum* is responsible for a majority of detected infections in cattle, this species has repeatedly been associated with outbreaks among veterinary students, farmers, and individuals visiting farms. Molecular characterization with good sequence resolution is essential for establishing evidence for causative links in outbreak investigations and the predominant typing marker currently used, gp60, is not optimal for this purpose as the resolution is low.

For this reason, an amplicon sequencing panel for genotyping of *C. parvum* has been developed by PHAS in collaboration with the Swedish Defence Research Agency (FOI) and the National Veterinary Institute (SVA). The assay was designed using 19 genomic sequences representing 16 subtypes of *C. parvum* and has been evaluated using approximately 100 isolates from both sporadic human cases as well as animal and human outbreak cases.

Preliminary analyses indicate that the assay has higher resolution than genotyping using gp60 and hence, will add value in outbreak investigations.

The fellow became familiar with *C. parvum* typing methodology and she will analyse the results of the amplicon sequencing and author a manuscript.

Epidemiology and diagnostics of schistosomiasis in Sweden

Supervisor: Tore Lier

Schistosomiasis, caused by parasitic flatworms of the genus *Schistosoma*, is one of the most devastating parasitic diseases in terms of public health. In Sweden, people who are diagnosed with schistosomiasis are returning travellers, expatriates, or migrants from endemic countries. At Karolinska University Hospital, the laboratory diagnostics consists of microscopy to detect eggs in samples of faeces and urine and samples are sent for confirmation serology to PHAS. The aim of this study was to assess the epidemiological and clinical characteristics, and the laboratory diagnostics of patients with suspected schistosomiasis in Sweden in order to optimize the diagnostics and management of these patients.

Patient data was retrieved from PHAS and from medical records at Karolinska University Hospital after ethical approval. Data were collected for 587 individuals seeking medical attention during 2012-2018 with suspected schistosomiasis. Preliminary analysis show that 37% were migrants from endemic countries and 59% were tourists, expatriates, or migrants from non-endemic countries, and for 4%, the reason for travel was not documented. Four

hundred and eighty-nine patients were tested using both serology and microscopy and 30% was positive in serology and 3% positive in microscopy, all of whom were also positive in serology. Among serologically positive patients a majority were exposed to the parasite in Sub-Saharan Africa.

There are studies describing the diagnostics and management of schistosomiasis in European travellers and migrants, however, this is the first study addressing these topics in Swedish travellers. As tourism to endemic areas has increased during the last decades, it is important to increase the knowledge of this patient group in order to provide efficient clinical management and diagnostics. The study was a joint project with Theresa Enkirch, EUPHEM fellow cohort 2016, and data collection will be continued by clinicians at Karolinska University Hospital.

The fellow became familiar with the laboratory workflow of schistosomiasis diagnostics and the travel-related epidemiology of schistosomiasis in Sweden. She collected, compiled, and verified laboratory data, and helped to develop an EpiData entry template for structured data collection.

Training modules

During the Biorisk and Quality Management module the fellow received training in international regulations and good practices for biological specimen shipment and obtained the World Health Organization (WHO) certificate for International Transport of Infectious Substances. Likewise, this module trained the fellow in performing risk assessment and proposing mitigation measures and how to assess laboratory methods to improve diagnostic procedures. The fellow was introduced to EpiData during the EPIET/EUPHEM Introductory course and the Outbreak Investigation module.

Educational outcome: application of virology, bacteriology, parasitology, and immunology concepts to the public health discipline; understanding the use and limitation of diagnostic and typing methods and their interpretation; assessment of laboratory methods to improve diagnostic procedures; developing expertise in analysis of genomic sequence data.

4. Biorisk management

The fellow has several years of experience from work in biosafety level (BSL)-3 laboratories and is trained for work in suited BSL-4 laboratory from her previous work on emerging viruses of risk groups 3 and 4. For this reason, her focus for the biorisk management core competency domain has been on educating others including practical and theoretical training of laboratory personnel for work in BSL-3 laboratory.

Retrospective laboratory diagnosis of the first haemorrhagic fever case in Sweden, 1990

Supervisor: John Pettersson

Viruses from several families can cause haemorrhagic fever which are associated with an acute onset of fever and haemorrhagic manifestations that can be relatively minor to life-threatening. In 1990, a Swedish resident presented with severe haemorrhagic fever after returning from travel to Africa. No conclusive diagnosis was reached at that time, however, infection with Marburg or Ebola virus was suspected because of severe symptomatology. In an attempt to reach a diagnosis of the first haemorrhagic fever case in Sweden, the samples were reanalysed using modern molecular techniques.

As the initial analyses (electron microscopy examinations and serology) performed in 1990 indicated pathogens of risk group 4, patient samples were exclusively handled in a glovebox (biosafety cabinet class III) located in the BSL-3 laboratory until the absence of risk group 4 pathogens could be confirmed.

Dengue virus (DENV) was detected in serum samples collected within the first week after symptom onset by sequencing of all RNA of non-human origin present in the sample, and the finding was confirmed by a specific real-time RT-PCR. In addition, samples collected one to two weeks after symptom onset, tested positive for DENV IgM and IgG antibodies. These findings suggest that the patient suffered from severe dengue.

This project provided the fellow the opportunity to train a PHAS staff member in performing sample inactivation and serological methods in the BSL-3 laboratory, both in biosafety cabinet class II and glovebox (biosafety cabinet class III). The fellow will be a co-author on the manuscript.

Laboratory preparedness in Sweden

The fellow prepared and gave presentations to external audiences regarding the 24/7 preparedness diagnostics in Sweden (risk group 4 pathogens) and the laboratory diagnostics available at PHAS for emerging and re-emerging risk group 3 pathogens.

Certificates

- Certificate of Achievement: Basic Security in the Field II (United Nations Department of Safety & Security, UNDSS)
- Certificate of Achievement: Advanced Security in the Field (UNDSS)
- International Transport of Infectious Substances (WHO)

Training modules

The Biorisk and Quality Management module introduced the fellow to quality management practices and trained the fellow in performing risk assessments and proposing mitigation measures. In addition, the fellow was trained in shipment practices for infectious substances.

Educational outcome: pedagogical training; preparing seminar material, delivering presentations to a multidisciplinary audience; the use of appropriate decontamination strategies/personal protection; applying principles and practices of biorisk management, assessment, and mitigation.

5. Quality management

External quality assessment for detection and characterization of carbapenem- and/or colistin resistant *Enterobacteriaceae*

Supervisors: Alma Brolund and Sara Byfors

Part of the ECDC initiated and funded project 'Genomic-based surveillance of carbapenem-resistant and/or colistin-resistant Enterobacteriaceae at the EU level (EURGenCCRE)'.

The spread of carbapenem-and/or colistin resistant *Enterobacteriaceae* (CCRE) has been occurring at an alarming rate, and these pathogens have become a critical threat to human health worldwide. These organisms are often resistant to many antibiotics, leaving very few treatment options.

In 2017, ECDC established the EURGen-Net to perform structured surveys of CCRE in Europe. A CCRE survey is being conducted within the EURGen-Net in 2019 and approximately 300 European hospitals will collect CCRE isolates with related epidemiological information. The isolates will be characterized using WGS which will allow for investigations into the presence and distribution of high-risk CCRE clones and epidemic clades in European hospitals and their potential cross-border spread. A more in-depth understanding of molecular epidemiology and transmission routes of CCRE in Europe can inform risk assessment and allow better targeting of prevention and control efforts.

The purpose of this EQA was to evaluate the capacity of the NRLs within EURGen-Net to perform carbapenem and colistin antibiotic susceptibility testing and detection of corresponding resistance mechanisms. The results of the EQA will be used to identify laboratories in need of additional training and/or help with updating available diagnostic methods for participation in the CCRE-survey.

The EQA panel was distributed to 38 NRLs in 37 European countries and consisted of six well-characterized colistin or carbapenem-resistant *E. coli* or *K. pneumoniae* isolates.

The fellow was involved in the preparation of isolates for the EQA panel, participated in the distribution of the EQA panel, analysed the individual laboratory's results, prepared a template for reporting of EQA results, and was part of the team finalising and distributing the individual country reports. In addition, the fellow will participate in the writing of a report summarizing the countries EQA results and a manuscript.

External quality assessment for the molecular diagnostics of Influenza virus A and B

Supervisor: Mia Brytting

An EQA for molecular detection and characterization of circulating influenza viruses is yearly performed by the diagnostic laboratories in Sweden. By monitoring the standard of diagnostic performance, this EQA plays a key role in strengthening national diagnostic capacity for influenza virus. Participating laboratories receive a panel of 10 representative influenza specimens (influenza A and B), which they identify and subtype using their methods of choice.

A total of 19 laboratories participated in the EQA. The result of the EQA showed that most laboratories had difficulties in detecting one or more samples. The samples for which false negative results were reported contained either low levels of Influenza A or Influenza B (subtype B/Victoria). Of note was that one commercial assay used by six different laboratories failed to detect Influenza B (subtype B/Victoria) strains that have a deletion in the hemagglutinin gene, a genetic variant that has emerged globally in the past years. All laboratories performing subtyping of influenza viruses determined the subtypes correctly. No participating laboratory submitted false positive results.

In conclusion, the overall result of this panel indicated that the laboratories in Sweden was well prepared for the coming flu season, however, there were some laboratories that had difficulties in detecting circulating Influenza B viruses.

The fellow evaluated and summarized the results, made improvements to the reporting format, and wrote parts of the report of the 2018 influenza EQA.

Training modules

The Biorisk and Quality Management module provided an overview of quality management concepts in diagnostic laboratories and introduced the fellow to different proficiency tests, internal and external quality control, factors influencing quality in laboratories, and the importance of adequate documentation. Also, the fellow used a laboratory quality assessment tool to evaluate process management, quality control, and documentation available at the training site during this module. The fellow gained theoretical knowledge of different concepts within antibiotic resistance in the problem-based learning session held during the EPIET/EUPHEM Introductory course.

Educational outcome: importance of quality control; evaluate results of an EQA; assessment of laboratory methods to improve surveillance and diagnostics procedures; application of bacteriology concepts to the public health discipline; understanding the use and limitations of typing methods and their interpretation.

6. Teaching and pedagogy

Laboratory diagnostics of emerging infectious diseases

Supervisor: Andreas Bråve

The fellow was responsible for identifying training needs, prepare teaching material, organizing, and delivering a course for public health professionals at the Unit of Emerging Infectious Diseases at the National Institute of Health (INS) in Maputo, Mozambique. The purpose of the course was to provide the audience with the theoretical and practical knowledge on principles and methods used in the laboratory diagnostics of emerging infectious diseases with focus on viruses in risk groups 3 and 4. The course contained theory and practical laboratory exercises.

Microbiology in surveillance and outbreak investigations

The fellow was responsible for preparing and delivering a lecture on 'Microbiology in surveillance and outbreak investigations' to master students at Södertörn University, Huddinge, Sweden.

An outbreak of gastrointestinal illness in Sweden

The fellow facilitated a case-study, 'An outbreak of gastrointestinal illness in Sweden', for master students at Södertörn University, Huddinge, Sweden.

An outbreak of trichinosis in France

The fellow facilitated a case study 'An outbreak of trichinosis in France' for veterinary students at the Swedish University for Agricultural Sciences, Uppsala, Sweden.

External quality assessment

The fellow was responsible, together with another EUPHEM fellow, for updating and delivering a lecture during the Biorisk and Quality Management module on 'External quality assessment - principles and the role in the quality management system'.

Workshop on molecular detection methods

The fellow was responsible for preparing and delivering two lectures and facilitated practical assignments advanced design of molecular detection methods to bioinformaticians and laboratory personnel at the next generation sequencing platform at the Public Health Agency of Sweden.

Educational outcome: Identify training needs; defining learning objectives; plan and organize a course for public health professionals; give lectures for public health professionals; assessing a course; structuring a case study; preparing lecture material; delivering lectures to university students and public health professionals; facilitating group work around case studies; use diverse pedagogical techniques to stimulate learning.

7. Public health microbiology management

Public health microbiology management during projects and activities

Public health management was a central component of all projects and activities undertaken throughout the fellowship. All projects required careful ethical and integrity considerations, in particular the schistosomiasis, tick-borne encephalitis, and *S. pyogenes* projects during which the fellow handled and analysed patient data. Time management and organisational skills were acquired from working in multiple projects spanning different microbiological disciplines simultaneously. During several of the projects including the outbreak investigation and the projects regarding Puumala virus and *S. pyogenes*, the fellow was part of multidisciplinary teams consisting of epidemiologists, laboratory technicians, local health authorities, physicians, statisticians, veterinarians, and/or bioinformaticians. National and international collaborations during the fellowship such as the outbreak investigation and the *Streptococcus equi* project, respectively, relied on effective communication with experts from various disciplines. In addition, the fellow was engaged in scientific communication to peers, stakeholders, and public health authorities.

Implementation of relevant laboratory methods enabling diagnostics of emerging diseases at the National Institute of Health in Mozambique

Supervisor: Andreas Bråve

Infections with pathogens in risk groups 3 and 4 rarely cause pathogen-specific symptoms, rendering clinical diagnosis difficult. The current availability of diagnostic methods are limited in Mozambique, where the diagnosis of malaria and primary HIV are the focus in febrile patients. For this reason, a well-functioning battery of laboratory tests and diagnostic routines are needed allowing for correct diagnosis of additional pathogens, the understanding of the epidemiology of circulating pathogens, and the timely introduction of preventive measures in order to limit disease transmission and prevent outbreaks that may have serious consequences in the low-income setting that Mozambique represents.

The aim of this project was the transfer of theoretical and practical knowledge between the National Institute of Health (INS), Maputo, Mozambique and PHAS regarding principles of infectious disease control, diagnostics, and surveillance of emerging infectious diseases. The fellow was responsible from the PHAS side for two of the six focus areas listed within the workplan of the memorandum of understanding (MoU) between INS and PHAS:

'Emerging zoonotic diseases' with the aim of supporting the implementation of laboratory surveillance of emerging zoonotic diseases and 'Diagnostics for emerging infectious diseases, methodology development and transfer of methods'.

The fellow organized a two-week capacity building workshop at PHAS for two public health specialist from INS. The workshop spanned both topics included in the workplan. Structure and focus areas (with regards to scope of pathogens and methodologies) of the workshop were discussed and decided together with the Scientific Director at INS and the supervisor at PHAS.

Implementation of interim routines for Ebola laboratory diagnostics

Supervisor: Andreas Bråve

Because of the ongoing Ebola outbreak in the Democratic Republic of the Congo, PHAS received a request from INS in Mozambique regarding the development and implementation of biosafety and diagnostic routines for Ebola.

The fellow communicated with representatives from INS and together with a biosafety officer and colleagues at PHAS, planned the training and developed routines, standard operating procedures, and risk assessments. **The fellow participated in the implementation of these routines and was responsible for the training of laboratory staff at site.**

Training modules

The Management, Leadership and Communication in Public Health module familiarised the fellow with the roles and responsibilities in public health management settings and trained the fellow in time management and communication to different audiences and how to tailor the main message depending on audience. In addition, during this module and the Rapid Assessment and Survey Methods module, the fellow was trained in how to perform and communicate the results of rapid risk assessments. During the Biorisk and Quality Management module, the fellow received guidance on how to perform biosafety risk assessments.

Educational outcome: working in a multidisciplinary public health team; understanding team management; planning, scheduling and organizing; understanding roles and responsibilities to be an effective manager within public health environment; understanding different management styles; recognising the role of different agencies.

8. Communication

Publications

1. Worsening epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, assessment by national experts from 37 countries, July 2018. A. Brolund*, N. Lagerqvist*, S. Byfors, M.J. Struelens, D.L. Monnet, B. Albiger, A. Kohlenberg, European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) capacity survey group. *Eurosurveillance*, 24 (9) 2019.*shared first author
2. An outbreak of gastroenteritis highlighting the diagnostic and epidemiological challenges of enteroinvasive *Escherichia coli*, Sweden, 2017. N. Lagerqvist, E. Löf, T. Enkirch, P. Nilsson, A. Roth, C. Jernberg. *Manuscript submitted*
3. Retrospective investigation into an outbreak of invasive *Streptococcus pyogenes* type emm66 infections, 2013-2015, Sweden. N. Lagerqvist, T. Wahab, J. Darenberg, B. Mäkitalo, T. Åkerlund, XXX. *Manuscript in preparation*
4. **Retrospective laboratory diagnosis of the first haemorrhagic fever case in Sweden, 1990. XXX. Manuscript in preparation**

Reports

1. Outbreak of EIEC gastroenteritis at a hotel and conference venue in Halland, Sweden, 2017. E. Löf, N. Lagerqvist, T. Enkirch, A. Roth.
2. External quality assessment (EQA) for the molecular diagnostic of Influenza viruses, 2018. N. Lagerqvist, M. Brytting.
3. Evaluation of a commercial *Leptospira* micro-immunofluorescence assay, PHAS, 2019. N. Lagerqvist.
4. Investigation into suspected horse-to-human transmission of *Streptococcus equi*, PHAS, 2018. N. Lagerqvist.

5. Investigation into a case of Puumala virus infection in a non-endemic region in Sweden, PHAS, 2019. N. Lagerqvist, P. Jureen, J. Klingström

Conference presentations

1. Whole genome sequencing of *Streptococcus pyogenes* type *emm89* from 2013 in Sweden reveals geographically distinct outbreak clusters. N. Lagerqvist, T. Wahab, J. Darenberg, B. Mäkitalo, T. Åkerlund. Poster presentation at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Malta, 2018.
2. Rotavirus surveillance in Sweden. 6th European Expert meeting on Rotavirus Vaccination joint with 8th European Rotavirus Biology meeting (EEROVAC), Riga, Latvia, 2019.

Other presentations

1. Laboratory capacity for carbapenem and colistin testing and surveillance in Europe. Oral presentation delivered during the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) training workshop, Stockholm, Sweden, 2018
2. Can the implementation of whole genome sequencing improve the microbiological surveillance of *Streptococcus pyogenes* in Sweden? Oral presentation at the Nordic Project module, Oslo, Norway, 2018.
3. Preparedness diagnostics for risk group 3 and 4 pathogens. Oral presentation for clinical microbiologists, Solna, Sweden, 2017
4. Difficulties in diagnosing high consequence pathogens. Oral presentation for clinical microbiologists, Solna, Sweden, 2017
5. Molecular diagnostics of emerging infectious diseases. Oral presentation for clinical microbiologists, Solna, Sweden, 2018
6. Whole genome sequencing in Surveillance and Outbreak Investigations, Sweden. Panel: Head of ECDC, chief microbiologist, and chief scientist. Followed by 10 min questions. Presentation delivered jointly with EUPHEM fellow T. Enkirch and EPIET fellows F. Chereau and E. Löf, ECDC, 2018.
7. The Swedish preparedness diagnostics. Oral presentation for clinical microbiologists, Solna, Sweden, 2018
8. Whole genome sequencing of *Streptococcus pyogenes* types *emm1*, *emm66*, and *emm89* and the implementation of WGS into the microbiological surveillance program. Microbiology forum, PHAS, Solna, 2018.
9. Molecular diagnostic methods. Oral presentation for laboratory staff from Karolinska University Hospital, Solna, Sweden, 2018
10. Epidemiology and diagnostics of schistosomiasis in Sweden. Oral presentation at the Nordic Project module, Copenhagen, Denmark, 2019

9. EPIET/EUPHEM modules attended

1. EPIET/EUPHEM Introductory Course, Spetses, Greece, 25 September – 13 October 2017
2. Outbreak Investigation Module, Berlin, Germany, 4 – 8 December 2017
3. Biorisk and Quality Management Module, Stockholm, Sweden, 5 – 9 February 2018
4. Management, Leadership, and Communication in Public Health Module, Stockholm, Sweden, 12 – 16 February 2018
5. Multivariable Analysis Module, Nicosia, Cyprus, 16 – 20 April 2018
6. Rapid Assessment and Survey Methods Module, Athens, Greece, 14 – 19 May 2018
7. Project Review Module, Lisbon, Portugal, 27 – 31 August 2018
8. Project Review Module, Prague, Czech Republic, 26 – 29 August 2019

10. Other training

1. Site-visit at the Unit for Epidemic Intelligence at ECDC. The training included presentations, participation at round table meetings, and screening exercises. 23 – 27 April 2018
2. Theoretical training in incidence emergency measures, BSL-3 laboratory, PHAS, 15 Nov 2017 and 30 Jan 2019
3. The Global Outbreak Alert and Response Network (GOARN), online course 7 May 2018
4. Working with GOARN in the Field, online course 7 May 2018
5. Nordic Project module, Oslo, Norway 2018
6. Nordic Project module, Copenhagen, Denmark 2019

7. EPIET/EUPHEM meetings at PHAS. The weekly meetings were organized by fellows and supervisors and included discussions on both epidemiological and microbiological aspects of their projects

Coordinator's conclusions

Nina started her fellowship as an expert in emerging infectious diseases, working primarily in virology. She was involved in the fellowship field assignments and activities contributing to mission of the Public Health Agency of Sweden to strengthen and develop public health, including two outbreak investigations of EIEC O96:H19 and invasive *Streptococcus pyogenes emm66*, *emm89*, and *emm1* types. Nina has been collaborating with different departments at the agency, with other Swedish agencies, clinical laboratories, and with national and international reference laboratories.

She is highly organised, able to work independently and effectively. She learned new epidemiology skills in outbreak investigation and surveillance systems data analysis and set-up. With her scientific knowledge, she could be involved in assignments with several bacterial pathogens of public health importance and gain new experience in public health microbiology. Her projects and activities within the fellowship addressed the EUPHEM core competencies and fulfilled the criteria of the EUPHEM matrix.

Nina has been a pleasure and a highly valuable experience to work with and I wish her all the success in her further career.

Supervisor's conclusions

The EUPHEM programme was a great opportunity for Nina and has provided her with many tools to find her way in the field of public health microbiology and epidemiology. Nina had already before the program started a broad knowledge about different methods and pathogens. During the past two years it has been interesting to see her develop and grow within the programme and broaden her knowledge from diagnostics to a more public health perspective. Nina was fully engaged in each project and actively involved in planning. Nina was open for new projects and areas. She has been able to plan her work well and run her projects independently.

During these two years, Nina's work has contributed in many ways to the agencies mission to strengthen and develop public health. She has been involved in outbreak investigations (both epidemiological and microbiological) with whole genome sequencing of EIEC O96:H19 (outbreak at a conference venue), outbreaks of invasive *Streptococcus pyogenes emm66*, *emm89*, and *emm1* types (retrospective investigations), and excluded a horse-to-human transmission of *Streptococcus equi*.

Nina has been collaborating with different departments at the Public Health Agency of Sweden, with other Swedish agencies, clinical laboratories, and with national and international reference laboratories. These collaborations have enabled projects such as sequencing of a Puumala virus in a non-endemic region in Sweden, a survey and an EQA panel in Europe concerning carbapenemase-producing *Enterobacteriaceae*/carbapenem-resistant *Enterobacteriaceae*, and the implementation of relevant diagnostic methods for emerging infectious diseases at the National Institute of Health in Mozambique. She has analysed the current set-up of the microbiological surveillance of rotavirus and proposed strategies for the future microbiological surveillance, and was involved in the planning of the introduction of laboratory notification of rotavirus in Sweden. She has been involved in the national EQA for influenza virus detection. Nina has been able to evaluate and improve the diagnostics for schistosomiasis and tick-borne encephalitis in Sweden by collaboration with clinical laboratories.

Nina's broad knowledge within different methodologies made it possible to evaluate a commercial micro-immunofluorescence assay for leptospirosis diagnosis and improve the molecular characterization of *Cryptosporidium parvum*. In addition, she was involved in the retrospective laboratory diagnosis of the first haemorrhagic fever case in Sweden (1990).

The projects she was involved in covered all areas within the scope of EUPHEM programme and showed that she was able to work on all these different projects independently. Thanks to Nina outbreaks have been investigated and handled, diagnostics improved and transferred, surveillance system evaluated and the projects involved both nationally and internationally collaborations. Nina's scientific knowledge, technical and organizational skills have been appreciated by all supervisors and colleagues. It was a great pleasure to have Nina as a EUPHEM fellow within PHAS and we highly appreciate her contribution and achievements within the fellowship programme.

Personal conclusions of fellow

The EUPHEM fellowship program provided a unique opportunity to receive professional training and working experience in public health microbiology. During the past two years I have had the great pleasure of working in

diverse projects incorporating the many public health aspects of infectious diseases. I have broadened my knowledge on different pathogenic organisms and experienced new bioinformatic tools and laboratory techniques. In addition, my epidemiological and statistical skills have strengthened due to training at modules and from close interaction with, and transfer of knowledge between, EUPHEM and EPIET fellows based at PHAS. The fellowship offered a great opportunity for multidisciplinary collaborations and expanded my professional as well as personal network in Europe. I truly hope that with my newly acquired knowledge and established network, I can continue to contribute to the field of public health microbiology.

Acknowledgements of fellow

I would like to thank my local EUPHEM supervisor Mia Brytting and my frontline coordinator Frantiska Hrubá for their excellent support and encouragement throughout the programme. I also would like to thank the head of EUPHEM, Aftab Jasir for her constructive feedback and continuous support. I am grateful to my employer, the Public Health Agency of Sweden that I was given the opportunity to participate in the programme. I am especially grateful to all the local project supervisors and the EPIET/EUPHEM alumnae at PHAS for dedicating their time and experience and making these two years an excellent learning experience. My warmest thanks go to the EUPHEM and EPIET fellows at site, Theresa Enkirch (EUPHEM cohort 2016), Emma Löf (EPIET cohort 2017), and Fanny Chereau (EPIET cohort 2016) for their team spirit, valuable discussions, and practical guidance.

I would also like to acknowledge the members of the EUPHEM forum, the EPIET coordination team, and the fellowship programme office for their administrative assistance throughout the fellowship. Last but not least, thanks to all EPIET/EUPHEM/PAE/UK-FETP fellows of cohort 2017.