EUPHEM

EUPHEM REPORT

Summary of work activities

Andreas Petersen

European Public Health Microbiology Training Programme (EUPHEM), 2015 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 Andreas Petersen, cohort 2015 of the European Public Health Microbiology Training Programme (EUPHEM) at Statens Serum Institut, Denmark.

Stockholm, 2017

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

Andreas Petersen holds a Master in Biology (1995) and a PhD in Microbiology (2003). He has been working at the Royal Veterinary and Agricultural University (now merged with University of Copenhagen) 1996-2009 and since 2009 at Statens Serum Institut (SSI). His focus has been on antimicrobial resistance in bacteria, including environmental bacteria and veterinary and human pathogens. His time at the University was spent mostly with basic research and some teaching. At SSI he has been working mostly with surveillance in the National Reference Laboratory for antimicrobial resistance and staphylococci and doing research in related areas. His personal objectives for entering the EUPHEM fellowship was to broaden his knowledge about public health microbiology and develop interdisciplinary skills.

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 guide[1].

1. Epidemiological investigations

1.1. Outbreak investigations

A. Outbreak of Legionnaires' disease in Denmark, October 2015 — February 2016

Supervisors: epidemiologist Charlotte Kjelsø and Senior Researcher Søren Uldum.

In the autumn of 2015, a clustering of cases/isolates characterised as *Legionella pneumophila* serogroup 1, subgroup Knoxville and belonging to sequence type (ST) 9 was observed. Four patients were admitted to hospital in October, and another five patients were admitted in November. The outbreak was confined to the municipality of Odense (population size, 198,912 [October 1, 2015]). All cases either lived or worked in this area. In late November 2015, the Department of Infectious Disease Epidemiology formed an outbreak group in order to assist the municipality of Odense and the Danish Health Agency in the identification of a suspected environmental transmission point. The fellow was part of the SSI outbreak team.

The fellow met with the local authority in Odense municipality and the Regional Health Agency to discuss the process. Later a meeting was arranged with all stakeholders to discuss the outbreak.

Personal and telephone interviews were performed using a standard questionnaire. The fellow used the geographic information obtained from the interviews as input in a dispersal model developed by Centre for Biosecurity & Biopreparedness (CBB), SSI. Another geographic software tool developed by ECDC was also used in its beta version.

Log files on water temperatures from the local district heating company were requested and reviewed to determine temperatures of inbound household water.

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

All clinical and relevant environmental Legionella isolates in Denmark are whole genome sequenced (WGS) at SSI. The fellow compared WGS sequences from the outbreak isolates with WGS sequences of previous ST9 isolates from Denmark and abroad to determine which isolates most likely originated from this outbreak.

The fellow contacted companies supplying and maintaining wet cooling towers in Denmark in order to locate units placed in the greater area of Odense. In addition, Google Earth/Map was used to identify location of possible cooling towers in the same area.

The fellow summarized the results in an outbreak report. The suspected environmental source was never found but nevertheless the theory of an environmental source remained. There were no new cases after February 2016. The outbreak was also reported in EPI-news, the weekly bulletin letter from Statens Serum Institut. To increase the possibility to locate any future outbreak sources it was recommended that wet cooling towers should be registered so that environmental samplings could be taken as quickly as possible.
B. Outbreak of gastroenteritis in a company canteen in Greater Copenhagen, November 2016 Supervisors: epidemiologist Luise Müller and senior researcher Steen Ethelberg, Department of Infectious Disease Epidemiology.

An outbreak of gastroenteritis in a company canteen involving more than 300 employees and kitchen staff was investigated. A total of 292 patients met the case definition. Norovirus genogroup II, subtype GII.Pe-GII.4 Sydney 2012 was identified in patient stool samples. The fellow developed a questionnaire (using Enalyzer) to identify possible sources of infection. The fellow performed descriptive and analytical analysis of the responses from the questionnaire using Stata and identified that having eaten in the canteen on October 31, 2016 was associated with a higher risk of being ill (risk ratio 4.97). No specific food item was found that could explain the outbreak fully. It was concluded that one or more food items sold in the canteen on October 31, 2016 were responsible for the outbreak and general recommendations on hygiene for both kitchen staff and guests in the canteen was given. An outbreak report in Danish was shared with Food Control Office Copenhagen, The Danish Veterinary and Food Administration. The fellow also produced an outbreak report in English.

Training modulesThe EPIET/EUPHEM introductory course, Outbreak module and the Multivariable analysis module trained participants in the principles, techniques and logistical aspects of outbreak investigations. The introductory course familiarised the participants with the ten steps of an outbreak investigation, the principles of intervention epidemiology and the function and importance of public health microbiology. The outbreak and multivariable analysis modules taught the participants how to apply this knowledge in practice using various software packages (STATA and EPI info). Fellows were taught essential data management skills including data entry, validation and cleaning as well as dataset management. They were also given practical training in how to perform analytical studies for an outbreak investigation, including descriptive, cohort and case control studies and stratified analyses.

Educational outcome:

The fellow participated in two multi-disciplinary outbreak investigation teams and was involved in all ten steps of an outbreak investigation. The fellow applied microbiological and epidemiological skills and acquired new knowledge on data analysis in Stata, GIS mapping, and sequence variation in *Legionella pneumophila*. The fellow communicated with patients, clinicians, and colleagues at different institutions in Denmark and internationally (ECDC).

1.2. Surveillance

A. Implementing whole-genome sequencing (WGS) as a surveillance tool for invasive group A streptococci (GAS) in Denmark

Supervisors: Chief physician Steen Hoffmann and bioinformatician Marc Stegger

The aim of the project was to evaluate the potential additional value of implementation of WGS as a surveillance tool for invasive group A streptococci (GAS) in Denmark. The objectives were to obtain a broad overview of the diversity and clonality of current invasive GAS strains in Denmark, to confirm and expand the current typing using genome sequencing data, to identify known resistance and virulence genes and to evaluate the feasibility of using WGS to improve the Danish surveillance of invasive GAS infections. The fellow analysed all submitted GAS isolates from 2015. *Emm* types were available for some of the isolates, generated by PCR and traditional Sanger sequencing. The fellow produced the remaining *emm* types in the laboratory by the same approach. In addition, Illumina generated whole genome sequences were available. The fellow imported the raw reads in CLC Genome Workbench, and assembled the sequences. Contigs were BLASTed against a downloaded database containing all known *emm* types. The contigs were also BLASTed against a database with resistance genes. Resistance gene profiles were compared to phenotypic resistance to penicillin, erythromycin and clindamycin, which had been generated in the lab. Sequence types were identified by BLASTing against the multi-locus sequence typing scheme for S. pyogenes. The emm types generated from WGS were 100% in concordance with the types obtained through PCR and Sanger sequencing. Phenotypic and genotypic resistance corresponded also completely. By combining *emm* and MLST typing, large clusters comprising the same *emm* type could be divided in subgroups. By applying phylogentic analysis of SNP trees, an even further subgrouping of *emm* types could be obtained. The results were promising and suggested that WGS could replace traditional laboratory methods for typing and resistance profiling

for more or less the same cost and in addition provide a much more detailed picture of the clusters of GAS in Denmark. The results were presented at ESCAIDE 2016 as a poster and a manuscript, which also include GAS from 2016 and 2017 (first half year) is in preparation. The fellow continued assigning *emm* types from WGS data from received isolates in 2016 and onwards. In 2017 the national reference laboratory for streptococci received more isolates than expected and approximately 50% of them belonged to *emm* type 1.0. These isolates were compared to all *emm* type 1.0 from 2015 and 2016 by SNP analysis to clarify if a specific clone of *emm* 1.0 was responsible for the increase. The 2017 isolates of *emm* 1.0 were distributed over the entire phylogeny but one group contained half of the 2017 *emm* 1.0 isolates, together with few *emm* 1.0 from 2015 and 2016. No special geographic correlation were found. The increase and the SNP analysis were communicated in EPINyt.

B. Livestock-associated meticillin-resistant Staphylococcus aureus (MRSA) among MRSA from humans, EU/EEA countries, 2013

Principal investigator: Pete Kinross, ECDC

The project objective was to map the LA-MRSA detection in EU/EEA Member States and the MRSA subtyping capacity/availability in EU/EEA national/regional reference laboratories in 2013 by sending out a survey to national/regional reference laboratories. Member states are experiencing different prevalence of LA-MRSA and their laboratory capacities may thus differ. This study was aiming to describe the surveillance capacities across the member states. The result from this survey is important for EU in order to identify areas and laboratories that might benefit from capacity strengthening in detection and typing of LA MRSA, an increasingly important clone of MRSA. The fellow entered the project prior to the fellowship but continued working on it infrequently during the fellowship. The fellow tested and revised the first draft of the survey questionnaire and based on the detailed national surveillance of MRSA in Denmark was able to contribute substantially to a list of spa types considered surrogates for ST398 (LA-MRSA). The fellow co-authored a manuscript that has been accepted for publication in Eurosurveillance.

C. mecC methicillin resistant Staphylococcus aureus surveillance in Denmark

Supervisors: Anders Rhod Larsen, Anders Koch

As part of a EUPHEM fellow Céline Barnadas' research project in public health microbiology the fellow participated in two ways: He introduced Céline to the methicillin resistant Staphylococcus aureus (MRSA) surveillance system in Denmark. The fellow gave support in the analysis of the mecC-MRSA epidemiological and laboratory data. Inferred epidemiological links from a phylogenetic analysis of strains was confirmed or dismissed by check in different registers. The fellow was a co-author of the resulting manuscript (draft).

Training modules

The EPIET/EUPHEM introductory course familiarised participants with many aspects and concepts associated with surveillance, including the principles of surveillance and how to develop, validate, evaluate and operate a surveillance system. The rapid assessment and survey module introduced techniques for surveillance in complex emergencies, including morbidity and mortality surveys. Prior to the module the fellow completed the two United Nations Departments of Safety and Security (UNDSS) online courses Basic Security In The Field II and Advanced Security In The Field.

Educational outcome:

The fellow learned about *S. pyogenes*, including the typing of the organism. The fellow learned how to handle raw data from whole genome sequencing, and learned how WGS contributes with much more detailed typing. Based on the experiences from the project typing of GAS in Denmark is now done by WGS and the fellow collaborate with the section at SSI which perform the surveillance. The fellow also learned how to review and improve a survey aimed for EU/EEA national/regional reference laboratories. The fellow also learned about the importance of personal safety and security in the field.

2. Applied public health microbiology research

A. Transmitted drug resistance among newly diagnosed Danish HIV-1 patients

Supervisor: Molecular biologist Jannik Fonager Collaborator: Head of section Susan A. Cowan

Transmission of human immunodeficiency virus (HIV) harbouring resistance mutations is associated with impaired outcomes from antiretroviral therapy (ART). Guidelines therefore recommend baseline genotypic testing in patients newly diagnosed with HIV to guide the choice of the first line of ART. Surveillance of the transmission of HIV drug resistance (TDR) is essential to inform treatment policy making and guidance. Transmitted HIV resistance is surveyed in Denmark through the SERO project. Samples from newly diagnosed HIV patients are sent to SSI for genotypic characterization of the POL (Pr and RT) gene along with epidemiological information. Public health actions on HIV prevention and treatment rely on updated information on patients and strain characteristics. The

aims of the project were to describe I) Transmitted PI, NRTI and NNRTI resistance among newly diagnosed Danish HIV-1 patients in relation to risk group and country of infection and II) To identify active transmission networks in relation to drug resistance, risk group, country of infection, age and geography in Denmark. The fellow analysed POL sequences from 1,789 patients and identified resistance mutations and transmission clusters. TDR according to the WHO 2009 list were found at a frequency of 6.7% while mutations leading to treatment implications according to HIVdb were demonstrated at 12.1%. 197 transmission clusters containing 707 patients were demonstrated. Results were shared with the epidemiological department, communicated in the weekly bulletin EPINews and a research paper aimed for Journal of Antimicrobial Chemotherapy is in preparation. The surveillance report printed in EPINews from this year presented transmitted resistance according to the interpretation of HIVdb. Previously resistance prevalence were based on the WHO list from 2009. However, new ART have been introduced since then and knowledge on the clinical impact of mutations in the POL gene has increased. It was therefore decided that it was more relevant to describe resistance prevalence according to the HIVdb algorithm.

B. Co-presence of tet(K) and tet(M) in livestock-associated methicillin-resistant Staphylococcus aureus clonal complex CC398 is associated with increased fitness during exposure to sublethal concentrations of tetracycline

Principal investigator: Senior researcher Jesper Larsen, SSI.

LA-MRSA CC398 is the major MRSA clone in Denmark, affecting not only pig farm workers but also persons with no documented contact to farming or farm workers. The emergence of LA-MRSA CC398 has been linked to the intensive use of antimicrobial drugs in food animals. Tetracycline is one of the most commonly used antibiotic classes in food animals. In some LA-MRSA CC398 isolates, the tetracycline resistance gene *tetK* is found. However, it remains unclear whether acquisition of *tetK* plays a role in the co-selection of methicillin resistance, given that virtually all LA-MRSA CC398 isolates are already resistant to tetracycline due to the presence of another tetracycline resistance gene, *tetM*. To gain insights into the role of *tetK* in LA-MRSA CC398, the prevalence and fitness effect of *tet*K during in vitro exposure to tetracycline was investigated. Results demonstrated that tet(K) significantly improved fitness at sub-lethal concentrations of tetracycline in vitro. Because tet(K) is genetically linked to SCCmec, the use of tetracycline in food animals may have contributed to the successful spread of LA-MRSA CC398. The results are important public health information for the control of the spread of LA-MRSA CC398 in pig production. The fellow entered the project prior to the fellowship but continued working on it infrequently during the fellowship. The project involved two master students. The fellow extracted information from the national registry of MRSA on the prevalence of *tetK*. The fellow co-supervised the students during their laboratory work with establishing fitness assays. The fellow co-authored a manuscript that has been published in Antimicrobial Agents and Chemotherapy.

Training modules

The module Bioinformatics and phylogeny provided the knowledge and tools for participants to perform robust maximum likelihood phylogenies and to reliably interpret the results. It further served as an introduction into common software packages and bioinformatics platforms for participants to build on.

Educational outcome:

The fellow learned about viruses in general and HIV typing and resistance in particular. The fellow learned to analyse large sequence data sets and making phylogenetic trees. The fellow learned how to extract and interpret resistance mutations in POL gene sequences of HIV virus strains.

3. Applied public health microbiology and laboratory investigations

A. Genotyping of Respiratory Syncytial Virus (RSV) in Denmark, 2006-16

Supervisor: Researcher Ramona Trebbien and Head of Department Thea Kølsen Fischer

Respiratory syncytial virus (RSV) is a respiratory virus that infects the lungs and breathing passages. It is one of the most common causes of acute bronchiolitis in young children and may develop into pneumonia. It is an enveloped RNA virus which can be divided into two subgroups, RSV-A and -B, which further can be divided in a range of genotypes, the clinical significance of which is unclear. Studies have shown that there are continuous changes in the predominant RSV genotypes.
Historically it has been challenging to produce a safe vaccine against RSV. However, currently several RSV-vaccine candidates are in development and in various phases of clinical testing, respectively. In preparation of future introduction of vaccines to national immunization programs solid data on circulating viruses are important for evaluating the effect of introducing a RSV vaccine. So far no central surveillance of RSV has been performed in Denmark. The aim of the project was to determine the distribution of RSV genotypes in Denmark. The objective

was to get an overview of the RSV genotypes circulating in Denmark, to obtain baseline data for future surveillance and immunization programs.
The fellow learned how to perform a real-time RT-PCR differentiating between the subgroups A and B and sequence RT-PCRs amplified products of surface glycoprotein (G). The fellow made maximum-likelihood phylogeny of the generated and downloaded reference sequences. This demonstrated an apparent confusion and lack of harmonization in designating subtypes and one recommendation from the study will be to achieve an international agreement on subtyping of RSV.
Parts of the results was included in an accepted abstract for the 20th Annual Meeting of the European Society for Clinical Virology, 13-16 September 2017, Stresa, Italy. An abstract has been accepted for poster presentation at ESCAIDE 20017. A full manuscript of all of the results is in preparation.

B. Serotyping of Toxoplasma gondii in symptomatic and asymptomatic individuals

Supervisors: Head of section Henrik Vedel Nielsen and site supervisor Rune Stensvold, SSI

Toxoplasma gondii is a common but under-detected, highly prioritized yet not systematically controlled zoonotic pathogen in Europe and globally. WHO has estimated that toxoplasmosis accounts for up to 20% of the total foodborne disease burden in Europe, which calls for attention and action. Three clonal lineages (types I, II and III) of *T. gondii* were described in the 1990s, and new methods have revealed further polymorphisms. Type II strains are endemic in Europe and appear to cause mainly mild clinical manifestations in immunocompetent individuals. In contrast, type I/III strains have caused severe clinical toxoplasmosis, also in immunocompetent individuals. Typing of *T. gondii* strains has not yet been undertaken in Denmark. The aim of the project was to validate an immunoblotting method in the lab to differentiate between type I and type II genotypes of *Toxoplasma gondii* and to investigate the distribution of *T. gondii* genotypes among small groups of patients with symptoms and serological test results compatible with acute toxoplasmosis in Denmark and in asymptomatic, but seropositive pregnant women. The fellow started validating the method using eleven DNA samples of *T. gondii* which had been genotyped based on 15 polymorphic markers in another project and the genotype of these strains was therefore known. The immunostrips produced clear bands from each sample but unfortunately, and unexpected, the method could not separate the genotypes from each other. The results were discussed with a collaboration partner (and producer of the immune blot assay) in France but the conclusion of the validation remained. The project was therefore terminated before the planned sub-sequent testing of clinical and screening samples from Denmark was performed. A small report was written about the laboratory experiments.

C. Survey of Tick-borne encephalitis virus (TBE) in a previously known micro focus in North-Zealand, Denmark

Supervisors: Professor Anders Fomsgaard, MD, DMSc, and senior researcher Maiken Worsøe Rosenstierne, PhD, Virus & Microbiological Special Diagnostics, SSI and Entomologist René Bødker, DTU-Vet.

In Scandinavia, the incidence of tick-borne encephalitis (TBE) is increasing and expanding its geographic range. TBE virus (TBEV) types TBEV-Eur and TBEV-Sib occur in Estonia and Finland, along with 2 tick species, *Ixodes persulcatus* and *I. ricinus*. In Denmark, TBE has been reported since the 1950s only from the isolated Bornholm Island in the Baltic Sea with an incidence of ≈4 cases per 100,000 persons per year. Statistical climate-matching models based on the known spatial distribution of TBEV indicate that the present North Zealand climate also would support TBEV-Eur transmission cycles. Indeed, in 2009 and 2010 two clinical cases of TBE were hospitalized after infection from the same microfocus called Tokkekøb Hegn in Zealand, right north of Copenhagen. The presence of TBE was established by flagging, PCR, virus culture and sequencing in 2010 and again in 2011 where 1/200 ticks (both nymphs and adults) were found TBE infected. This suggested that a micro-endemic focus had been established.

The microfocus in Tokkekøb Hegn has not been examined since, and no further clinical cases has been detected from this area. Moreover epidemiological statistics of numbers of Clinical TBE Cases in Denmark (from Bornholm or neighbour countries) show a peak every 6 year which is believed to be caused in part by mast years, years when more mast (fruits of forest trees like acorn and other nuts) are produced. A peak was noted in DK in 2004 and 2010, and thus it was feared that year 2016 could result in yet another peak. Thus, 2016 was a preferred year to perform a repeated examination of the presence, frequency of TBE in ticks (nymphs and adults) in the known microfocus and by sequencing and phylogeny determine the TBE type, sequence variation and most likely origin(s).

The fellow collected ticks (nymphs and adults) by flagging the known location in Tokkekøb Hegn during August and September 2016 and again in June, July and August-September 2017. I collected 800+500+1250+1000 ticks. RNA/DNA was extracted from pools of nymphs and adults and screened for presence of TBE by PCR. Nymphs and adults were pooled separately and pool size was 50 individuals. Adult females and adult males were pooled and examined separately. Ticks collected in 2016 were negative in the TBE specific PCR and a subset of four pools (2 pools of nymphs and one pool of adult females and one pool of adult males) were screened for all known viruses using a Pan-virus Microarray. No other viruses were detected. The results of the flagging will be described in EPINews in September 2017 and a brief note will be prepared for Eurosurveillance.

D. Simple method for correct enumeration of Staphylococcus aureus

Principal investigator: Researcher Jakob Haaber, University of Copenhagen

Optical density (OD) measurement is applied universally to estimate cell numbers of microorganisms growing in liquid cultures. It is a fast and reliable method but is based on the assumption that the bacteria grow as single cells of equal size and that the cells are dispersed evenly in the liquid culture. When grown in such liquid cultures, the human pathogen Staphylococcus aureus is characterized by its aggregation of single cells into clusters of variable size. The project aim was to provide a simple and efficient sonication procedure, which can be applied prior to optical density measurements to give an accurate estimate of cellular numbers in liquid cultures of S. aureus. The fellow entered the project prior to the fellowship but continued working on it infrequently during the fellowship. The fellow set up a screening system in the National reference laboratory for antimicrobial resistance and staphylococci, SSI, to determine level of aggregation among the clinical samples of S. aureus received during two months. The screening demonstrated that aggregation was not an uncommon phenomenon (32%). Co-workers then developed a simple sonication procedure to dissolve aggregates before enumeration. The fellow co-authored a manuscript that has been published in Journal of Microbiological Methods

Educational outcome:

The fellow implemented a new screening method for aggregation of *S. aureus* in the laboratory and instructed the lab technicians how to perform and read the test. The fellow validated a new immune blot assay and concluded that it could not be used for the purpose it was intended. A valuable educational outcome! The fellow participated in field work to collect ticks and learned the methods for extracting RNA/DNA. The fellow learned how negative findings can have an important public health importance and how to communicate them. The fellow learned real-time RT and RT PCR for virus, learned to make maximum-likelihood phylogenies and learned how to be critical on the sequence information from Genbank.

4. Biorisk management

A. Laboratory preparedness in Denmark

The fellow was responsible, together with EUPHEM fellow Céline Barnadas, of preparing a short presentation on laboratory preparedness in Denmark and emerging or re-emerging pathogens during the IMPHM module, February 2016. The presentation was given to acting head of ECDC, chief scientist of ECDC and chief microbiologist of ECDC. Fellows conducted interviews with different actors of infectious disease surveillance and public health microbiology in Denmark. They prepared a presentation focused on infectious disease surveillance and reporting systems in Denmark, and on the risk posed by antimicrobial resistance. They also provided information on the laboratory capacity in Denmark (containment levels available).

A. Biosafety level 3 – laboratory activity

Supervisor: Senior Researcher Michael Rasmussen. The project was undertaken together with EUPHEM fellow Céline Barnadas

The fellow was during his stay in the International Reference Laboratory of Mycobacteriology, Statens Serum Institut, trained in all general Biosafety level 3 (BSL3) procedures and protocols. All relevant SOPs on laboratory behaviour, waste disposal and routine experiments were studied and signed in order to authorize the presence in the laboratory. The procedure in case of an accident or fire were thoroughly explained. The fellow were instructed in the procedure of redressing in the locker room before entering the restricted area.

During the stay in lab the fellow was trained in the phenotypic methods used to identify resistance in Mycobacterium spp and DNA preparation for WGS. The fellows wrote a brief report about their activities.

Training modules

A five-day module focusing on biorisk/biosafety assessment and mitigation was completed. The module included WHO recommendations on biosafety management in laboratories and international regulations for the transportation of dangerous goods, as determined by ICAO (International Civil Aviation Organization). The fellow acquired the WHO certificate of International Transport of Infectious Substances during the module. The module also included a short visit to BSL3 and BSL4 laboratories, Folkhälsomyndigheten (FHM), Solna, which allowed the fellow to observe the special conditions needed when working with highly virulent pathogens. After the module homework was completed and a small report on the visit to BSL3/4 lab was written.

Educational outcome:

Understanding the processes associated with BSL2/BSL3/BSL4 laboratories, gaining experience in different types of personal protective equipment, understanding and applying the principles and practices of biorisk management, biorisk assessment and biorisk mitigation. The fellow achieved practical experience working in a BSL3 lab.

5. Quality management

A. External quality assessment scheme for typing of verocytotoxin-producing E. coli (VTEC), self-funded participants, 2015-2016

Supervisors: Researcher Susanne Schjørring and researcher Mie Frid Jensen. The project was undertaken together with EUPHEM fellow Céline Barnadas.

External quality assessment (EQA) is an important aspect of quality management systems. An EQA provides an objective evaluation of a laboratory's performance. It constitutes an early warning for systematic problems, provides objective evidence of testing quality, and identifies areas needing improvement and specific training needs amongst participants. An EQA also allows for comparison among different test sites. Standardised laboratory techniques and national and international comparison of results have many benefits for public health including fostering the rapid detection of dispersed international clusters/outbreaks, facilitating the detection and investigation of transmission chains and relatedness of strains globally. Also, detecting an emergence of new evolving pathogenic strains, supporting investigations to trace-back the source of an outbreak and to identify new risk factors and aid in studying the characteristics of a particular pathogen and its behaviour in a community of hosts.

The EQA contained Pulsed Field Gel Electrophoresis (PFGE) as the main part. In addition serotyping, genotyping (including subtyping) and phenotypic testing (verocytotoxin/Shiga toxin, Extended Spectrum Beta Lactamases (ESBL), β-glucuronidase, enterohaemolysin and sorbitol) was part of the EQA.

The fellows evaluated the quality of the gel by seven parameters and the performance using the specialised analysing tool BioNumerics in five parameters. The evaluation lead to an overall conclusion whether the gel could be used for inter laboratory comparisons. Recommendations for improvements were described. The sero-, geno- and phenotyping were all evaluated by a correct or incorrect result. The fellows made a participation certificate for all participants and wrote an individual report summarising the parts the laboratories participated in. A final report of all the results of the EQA was written by the fellows.

B. WHO audit of measles and rubella laboratory, SSI

Together with EUPHEM fellow Céline Barnadas the fellow was allowed to accompany WHO auditors Dr Myrian Ben Mamou and Dr. Sabine Santibanez at their audit of the Measles and Rubella laboratory, SSI. The audit took place in two parts. The first part consisted of presenting to the auditors the laboratory activities, the research and development activities conducted by the reference laboratory and the surveillance activities (conducted jointly with the epidemiology unit). The second part consisted of a visit of the different sections of the laboratories involved in the processing of the samples, as part of the measles and rubella surveillance activities.

The fellows followed most of the audit and the debriefing session on the last day. The fellow found it very interesting and relevant to follow the audit. It is good to be reminded about that there are so many details that needs to be correct and documented. The fellows wrote a brief report about their experiences.

Training modules

The Quality Management module provided an overview of quality management concepts in diagnostic laboratories, according to the ISO 15189 standard. Topics covered included factors influencing quality in laboratories, internal and external quality control, norms and accreditation, assessments and audits, documentation and record keeping, sample management, stock purchase and inventory management, management of equipment and temperature controlled devices, process improvement, customer service and international health regulations. After the module, EUPHEM fellow Céline Barnadas and the fellow made an audit of the reference laboratory for antimicrobial resistance and staphylococci at SSI, evaluating both process management and quality control as well as the documentation. The outcome was discussed with the group members and existing procedures were discussed to increase in particular quality control.

Educational outcome:

The fellow learned about the importance of quality control and quality management and the importance of documentation. The fellow learned how to evaluate the result of an EQA and writing a report about the results to the participants. The fellow learned how a WHO audit of a reference laboratory was performed.

6. Teaching and pedagogy

A. Ph.D course on Infection epidemiology

Supervisors: Head of Department of Infectious Disease Epidemiology, Kåre Mølbak, senior researcher Steen Ethelberg, Department of Infectious Disease Epidemiology.

All EUPHEM and EPIET fellows at SSI participated in the planning and preparation of teaching materials for a new 3 days Ph.D course for Copenhagen University on Infection epidemiology. The course was held at Statens Serum Institut's department of epidemiology in week 43, 2016. The course gave an introduction to the application of epidemiology to the field of infectious diseases. Main topics were the current global challenges as to communicable diseases and what can be done to respond to them. What makes a good surveillance system and how can its data be used as information for taking action. How outbreaks of communicable disease are discovered, handled and learned from – on a global and on a local basis. The form of the course included a combination of lectures and group work based on real life case studies.

The fellow planned the course and the course content together with the other fellows and senior staff from the department of epidemiology infections. The fellow prepared and presented the lecture 'The main principles of microbiological typing in epidemiology' together with EUPHEM fellow Céline Barnadas. Keywords in the presentation included: pheno- and genotypic typing, advantages and disadvantages of main typing methods (serotyping, PFGE, MLST, WGS/SNP), and their application (identifying clusters, chains of transmission, outbreak investigation). The fellow co-facilitated two case studies and two exercise sessions. The participants in the PH.D course were asked to evaluate the course and after the course the evaluations were addressed. Overall the course was very well received by the participants. The fellows also evaluated the process of preparing this course from scratch and the fellow wrote a reflective note on his teaching experience.

B. Lecture: Laboratory considerations during complex emergency situations (CES). Given at the RAS module in Athens, June 2016.

The lecture was prepared together with EUPHEM fellow Céline Barnadas. Due to private matters the fellow was not able to participate in the module and Céline gave the lecture by herself. The fellows adapted the lecture from the previous year (EUPHEM fellows Kyriaki Tryfinopoulou and Horacio Gil Gil). The purpose of the lecture was to give an overview of the practical considerations and restrictions when setting up a laboratory in complex emergency situations. The lecture included the role and setup of laboratories, specimen logistics, options for diagnostics and risk management.

Educational outcome:

The fellow learned how to plan, organise, deliver, evaluate and reflect on a three-day Ph.D. course.

7. Public health microbiology management

Public health microbiology management was an integral component of all projects and activities during the fellowship. The two outbreak investigations relied on effective communication with people from various backgrounds like microbiologists, medical doctors, laboratory technicians, epidemiologists, statisticians, and public health officers.
Laboratory investigations as in the TBE and Toxoplasma projects required good time managing skills to plan and conduct practical lab work. Time management and organisational skills were further trained by working on multiple projects at a time.
The Legionella outbreak, the increase of emm 1.0 in GAS 2017, the flagging of ticks for TBE and analysis of transmitted resistance mutations in HIV patients were communicated in EPI-NEWS, the weekly bulletin from SSI.

Training modules

The Initial Management in Public Health module focussed on the understanding of roles and responsibilities in public health management. Topics included the identification of different management styles, team roles and team evolution, the delegation of tasks and the provision of structured feedback.

Educational outcome:
The fellow gained experience of working in multidisciplinary national and international public health teams, to understand team management; understand roles and formal responsibilities in public health microbiology; plan, schedule and organise research projects; managing multiple projects at the same time.

8. Communication

Publications

1. Petersen A, Stegger M, Hoffmann S. Two years surveillance of invasive group A streptococcal infections in Denmark, using WGS sequencing for typing. Manuscript in preparation.
2. Petersen A, Cowan SA, Fischer TK, Fonager J. Transmitted drug resistance and identification of transmission chains among newly diagnosed Danish HIV-1 patients 2004-2016. Manuscript in preparation for Journal of Antimicrobial Chemotherapy.
3. Petersen A, Schmidt DJ, Fischer TK, Trebbien R. Genotyping of RSV isolates in Denmark 2006-2016.

Manuscript in preparation.
4. Kinross P, Petersen A, Skov R, Van Hauwermeiren E, Laurent F, Voss A, Kluytmans J, Struelens MJ, Heuer O, Monnet DL. Livestock-associated meticillin-resistant Staphylococcus aureus (MRSA) among MRSA from humans, EU/EEA countries, 2013. Accepted Eurosurveillance
5. Larsen J, Clasen J, Hansen J, Paulander W, Petersen A, Larsen AR, Frees D. Copresence of tet(K) and tet(M) in livestock-associated methicillin-resistant Staphylococcus aureus clonal complex CC398 is associated with increased fitness during exposure to sublethal concentrations of tetracycline. Antimicrob Agents Chemother. 60(7):4401-3
6. Haaber J, Cohn MT, Petersen A, Ingmer H. Simple method for correct enumeration of Staphylococcus aureus. Journal of Microbiological Methods 125 (2016) 58–63.
7. Barnadas C, Stegger M, Petersen A, Møller CH, Koch A, Larsen AR. Molecular epidemiology of mecC-positive methicillin-resistant Staphylococcus aureus infections in Denmark, 1975-2016. Manuscript in preparation
8. Uldum S, Petersen A, Løgstrup L, Madsen AMR, Kjelsø C. Legionella pneumonia 2015. EPI-NEWS 42-43 2016 (http://www.ssi.dk/English/News/EPI-NEWS/2016/No%2043%20-%202016.aspx)
9. Petersen A, Stegger M, Eriksen, HB, Hoffmann S. Increase in infections with invasive group A streptococci in January-April 2017. EPI-NEWS 27a+b-2017. http://www.ssi.dk/English/News/EPI-NEWS/2017/No%2027a%20-%202017.aspx
10. Christiansen AH, Cowan S, Petersen A, Fonager J. HIV 2016. EPI-NEWS 36 2017. https://www.ssi.dk/English/News/EPI-NEWS/2017/No%2036%20-%202017.aspx
11. Ocias L, Petersen A, Krogfelt KA, Rosenstierne MW, Fomsgaard, Bødker R. Infection with TBE virus in Denmark 2013-2016. EPI-NEWS 40 2017. https://www.ssi.dk/English/News/EPI-NEWS/2017/No%2040%20-%202017.aspx

Reports

1. Petersen A. Outbreak of Legionnaires' disease in Denmark, October 2015 — February 2016. Outbreak report.
2. Petersen A. Outbreak of gastroenteritis in a company canteen in Greater Copenhagen, November 2016. Outbreak report.
3. Petersen A, Müller L. Results of cohort investigation: Outbreak of gastroenteritis. Outbreak report FUD1536 (in Danish).
4. Barnadas C, Petersen A, Schjørring S and Scheutz F. External quality assessment scheme for typing of verocytotoxin-producing E. coli (VTEC), self-funded participants 2015-16 (submitted to project supervisors for acceptance)
5. Barnadas C, Petersen A. BSL3 laboratory activity: Characterisation of nontuberculous mycobacteria, Statens Serum Institut, August 2017.
6. Petersen A, Barnadas C. An insight into the WHO Euro office activities, September 2017.
7. Petersen A. Project report: Serotyping of Toxoplasma gondii in symptomatic and asymptomatic individuals report on laboratory experiment. August 2017

Conference presentations

1. Møller CH, Lassen SG, Petersen A, Skov RL, Koch A. Characterization of community-acquired CC398 methicillin-resistant Staphylococcus aureus cases without pig contact in Denmark, 2007-2014. Poster presented at 26th ECCMID, 9 - 12 April 2016, Amsterdam, Netherlands.
2. Petersen A, Stegger M, Hoffmann S. Introduction of whole genome sequencing to the nationwide surveillance of invasive group A streptococcal infections in Denmark in 2015. Poster presented at European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2016), 28-30 November 2016, Stockholm, Sweden.
3. Trebbien R, Jepsen MT, Emborg H-D, Krause TG, Schønning K, Petersen A, Voldstedlund M, Smith DJ, Nielsen J, and Fischer TK. The youngest infants at the highest risk for severe RSV disease: epidemiology and molecular profile of respiratory syncytial virus (RSV) among young children < 5 years-of-age in Denmark, 2010 to 2015. Accepted for oral presentation at the 20th Annual Meeting of the European Society for Clinical Virology, 13-16 September 2017, Stresa, Italy
4. Petersen A, Schmidt D.J., Fischer T.K., Trebbien R. First insight into the distribution of A and B subtypes and G genotypes of respiratory syncytial virus (RSV) in Denmark 2006-2016. Poster presented at European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2017), 6-8 November 2017, Stockholm, Sweden.
5. Petersen A, Cowan S.A., Fischer T.K., Fonager J. Transmitted drug resistance among newly HIV-1 infected in Denmark 2004-2016. Oral presentation at European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2017), 6-8 November 2017, Stockholm, Sweden.

Other presentations

1. EPIET-EUPHEM forum, SSI, December 16, 2015: Presentation of Legionella outbreak.

2. EPIET-EUPHEM forum, SSI, March 9, 2016: Journal club: Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk. Ryan et al, JAMA 258;22
3. EPIET-EUPHEM forum, SSI, May 4, 2016: Introduction of WGS in the surveillance of invasive group A streptococci infections in Denmark
4. EPIET-EUPHEM forum, SSI, August 17, 2016: Presentation at PRM, Lisbon, 2016
5. EPIET-EUPHEM forum, SSI, November 16, 2016: Outbreak of gastroenteritis, preliminary results
6. EPIET-EUPHEM forum, SSI, February 1, 2017: New project: Transmitted drug resistance among newly diagnosed Danish HIV-1 patients
7. EPIET-EUPHEM forum, SSI, March 22, 2017: New project: Genotyping of Respiratory Syncytial Virus (RSV) in Denmark, 2006-16
8. Monday meeting at Infectious Disease Epidemiology, February 29, 2016: Legionella outbreak, Odense, October 2015 – January 2016 (?)
9. Lecture on MRSA, given to lab technicians at Queen Ingrids hospital, Nuuk, Greenland, February 15, 2017.
10. Classification of carbapenemase-resistance genes in Enterbacteriaceae. Lecture given during the PBL exercise at the Introductory course, Spetses, 2015
11. Laboratory preparedness of emerging and re-emerging pathogens in Denmark. Lecture given during IMPHM module to a panel of head of ECDC, chief microbiologist of ECDC and chief scientist of ECDC, February 12, 2016.

Other

1. Feedback to the laboratory on the audit of the reference laboratory for antimicrobial resistance and staphylococci at SSI. Together with EUPHEM fellow Céline Barnadas. September 2016.

9. EPIET/EUPHEM modules attended

1. Introductory course, Spetses, Greece, 28. September – 16. October 2015
2. Bioinformatics and phylogeny, Stockholm, Sweden, 16. – 18. November 2015
3. Outbreak investigation, Berlin, Germany, 7.-11. December 2015
4. Biorisk and Quality Management, Stockholm, Sweden, 1. – 5. February 2016
5. Initial Management in Public Health Microbiology, Stockholm, Sweden, 8. – 12. February 2016
6. MVA, Vienna, Austria, 14. – 18. March 2016
7. Project review, Lisbon, Portugal, 22. – 26. August 2016
8. Rapid assessment and survey, Athens, Greece, 8. – 13. May 2017
9. Project review, Lisbon, Portugal, 28. August – 1. September 2017

10. Other training

1. WHO certificate of International Transport of Infectious Substances
2. UNDSS courses Basic Security In The Field II and Advanced Security In The Field

Discussion

Coordinator's conclusions

One of the main goals of the EUPHEM programme is to expose fellows to diverse and multidisciplinary public health experiences and activities, thus enabling them to work across different disciplines. This report summarises all activities and projects conducted by Andreas Petersen during his two-year EUPHEM fellowship (cohort 2015) as an MS-track fellow at the Statens Serum Institut in Copenhagen, Denmark. The projects described in this portfolio demonstrate the depth and breath of the public health microbiology work of Andreas. Epidemiological studies included participation in local and national outbreak investigations with clear public health outcomes while surveillance activities extended from evaluating the potential additional value of WGS as a surveillance tool for invasive group A streptococci (GAS) in Denmark to an investigation of live –stock associated MRSA surveillance methods and capacity across European member states. His research project on transmitted drug resistance in HIV provided valuable information of relevance to clinicians and public health specialists alike. The laboratory and epidemiologically based projects covered a diverse range of disease programmes working in all domains of microbiology and also exhibiting the fellows strengths in effective multidisciplinary team work with colleagues in both similar and different roles and specialities; clinicians, epidemiologists, laboratory technicians, entomologists, public health officers and government officials and colleagues in different member states.

Activities were in in line with the 'learning by doing' and 'on the job training' ethos of the EUPHEM programme and fulfilled the core competency domains described for professionals in their mid-career and beyond. Activities were

complemented by nine training modules providing theoretical knowledge. Projects had a clear outcome, with results communicated in technical reports, scientific journals and at conferences. Andreas has been actively involved in the training of others during his fellowship, contributing to capacity building beyond the programme. In maintaining a commitment to duties related to his previous post and in participating in projects across different study groups he has exercised time-management and prioritisation skills which will be important to any future career.

The contributions made by this EUPHEM fellow towards public health in Denmark and also within Europe indicates the importance of developing and maintaining a critical mass of highly skilled field public health microbiologists within Member States to contribute towards national preparedness as well as being available for responses in the interest of the EU. The EUPHEM Coordinator Team concludes that the fellow has succeeded in performing all his tasks to a high standard and has conducted himself in a highly professional and effective manner throughout. We wish the fellow every success in his future career as a public health microbiologist.

Supervisor's conclusions

Andreas Petersen was already professionally very experienced in research within infectious diseases when he initiated his EUPHEM fellowship at SSI. During the course of the fellowship, Andreas developed a good understanding of all major core domains of the programme, the usefulness of the EUPHEM fellowship and of Public Health Microbiology altogether. He has been keen to take on projects related to topics that were outside his comfort zone, exemplified in his involvement in projects on virus (tick-borne encephalitis virus) and parasites (*Toxoplasma gondii*). He contributed to analyzing an outbreak of Legionnaire's disease in Denmark in 2015–2016 and an important outcome of the extensive work resulted in recommendations on the future management of outbreaks of Legionella.

Other results from Andreas's work has fed directly into numerous publications (some of which are still in preparation), such as conference abstracts, journal articles, SSI bulletins (EPI-NYT) of major public health microbiology relevance. Teaching-wise, together with EUPHEM and EPIET fellows he participated in developing of a well-recommended three-day Ph.D course for Copenhagen University on Infection Epidemiology.

Of particular note, Andreas has managed to juggle all of his activities related to the EUPHEM programme along with his duties at SSI with a remarkably practical approach and realistic sense of allocation of work load, which is one of Andreas's outstanding qualities. He is open to new ideas and quickly grasps the logic behind new technologies and methodologies. He benefits from good communication skills and is particularly able to tailor his presentation of data and other types of information to his audience. It has been very useful for the SSI to have Andreas as a member-state EUPHEM fellow, as his all-round EUPHEM public health microbiology training and good eye for sound projects and collaborations undoubtedly will have positive impact for future internal as well as external collaborations and international network.

Personal conclusions of fellow

I feel very lucky to have been part of the two years of training in the EUPHEM programme. The training, including the modules and the many projects I have been involved in, has broadened my knowledge on different organisms, including viruses and parasites and provided me with experience in new laboratory techniques as well as bioinformatic tools. I have been part of multi-disciplinary outbreak investigations with colleagues from other institutions both nationally and internationally. My epidemiological skills has been substantially increased due to the training at modules, participation in outbreak investigations and close interaction among all EUPHEM and EPIET fellows based at SSI. I have learned to prioritise my time when tasks from my ordinary position at SSI was demanded to be solved. My personal network has increased both locally on SSI, to other institutions in Denmark and internationally. I have acquired a deeper insight into the importance of my work in relation to public health and the management of infectious diseases. It is my hope that I in the future will continue to have this international outreach and contribute to the field of public health microbiology.

Acknowledgements of fellow

I am grateful to my department at SSI for letting me have this opportunity to participate in the program. I want to thank my front line coordinator and the ECDC, EUPHEM and EPIET coordinators and facilitators for organising all the modules. I am happy to have met so many fine colleagues in my cohort and also from cohorts C2014 and C2016. I am especially happy to have worked together with EU- and MS-track EPIET and EUPHEM fellows based at SSI and for the weekly meetings we have had. I wish to thank all project participants and my site supervisors at SSI, who have worked with me in the projects and helped me with various tasks during the two years. My warmest feelings goes out to my fiancée Helle and the kids: thanks for going without me, especially during module times.