Summary of work activities

Daniela Michlmayr

The ECDC Fellowship Programme
Public Health Microbiology path (EUPHEM), 2020 cohort

Background

The ECDC Fellowship Programme is a two-year competency-based training with two paths: the field epidemiology path (EPIET) and the public health microbiology path (EUPHEM). After the two-year training, EPIET and EUPHEM graduates are considered experts in applying epidemiological or microbiological methods to provide evidence to guide public health interventions for communicable disease prevention and control.

Both curriculum paths provide training and practical experience using the 'learning by doing' approach in acknowledged training sites across European Union (EU) and European Economic Area (EEA) Member States.

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 Daniela Michlmayr, cohort 2020 of the European Public Health Microbiology Training Programme (EUPHEM) at the Statens Serum Institut (SSI), Copenhagen, Denmark.

Pre-fellowship short biography

Daniela Michlmayr completed her PhD in viral immunology at the University of Glasgow in the United Kingdom. She then conducted two post-doctoral fellowships at the Mount Sinai Hospital in New York City and the University of California, Berkeley in the United States of America in the field of infectious diseases. During her time at Berkeley, she studied the immune response to Dengue, Zika and Chikungunya virus infections in children in Nicaragua.

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She also initiated and led a medical expedition to Ethiopia to conduct a seroprevalence study for arboviral infections. After a brief work experience as analytical scientist at VIR Biotechnology in San Francisco, Daniela joined the EUPHEM fellowship at the Statens Serum Institute in Copenhagen, Denmark.

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 project or activity work and partly through participation in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the ECDC Fellowship Manual [1].

1. Epidemiological investigations

1.1. Outbreak investigations

1.1.1. Epidemiological studies to assess the protection from reinfection in Denmark during the COVID-19 pandemic

Supervisor: Steen Ethelberg

Two years into the pandemic, COVID-19 continues to have a pronounced effect on public health globally. To investigate the risk of reinfection after natural infection with SARS-CoV-2, two large population-based studies were performed by the fellow based on 10 and 63 million SARS-CoV-2 PCR test results, respectively. In study one, national PCR test data from the start of the pandemic until December 2020 were investigated, while in the second study, two years of national PCR data were analysed and estimated protection against reinfection with SARS-CoV-2 was calculated.

The results from study one showed that protection against reinfection was 80.5% (95% CI: 75.4–84.5%). However, individuals aged 65 years and older had a significantly reduced protection of 47.1% (95% CI: 24.7–62.8%). In study two, which consisted of two years of COVID-19 data, the estimated protection against reinfection was 83.4% (95% CI: 82.2–84.6%) and still lower for 65+ year-olds (72.2%; 95% CI: 53.2–81.0%). By autumn 2021, when infections were almost exclusively caused by the Delta variant, the estimated protection following a recent first infection was 91.3% (95% CI: 89.7–92.7%) compared to 71.4% (95% CI: 66.9–75.3%) after a first infection over a year earlier. With Omicron, a first infection with an earlier variant in the past three to six months gave an estimated 51.0% (95% CI: 50.1–52.0%) protection, whereas a first infection longer than 12 months earlier provided only 19.0% (95% CI: 17.2–20.5%) protection. Protection by an earlier variant-infection against hospitalisation due to a new infection was estimated at: 86.6% (95% CI: 46.3–96.7%) for Alpha, 97.2% (95% CI: 89.0–99.3%) for Delta, and 69.8% (95% CI: 51.5–81.2%) for the Omicron variant. The fellow found that SARS-CoV-2 infection offered a high level of sustained protection against reinfection, but decreased with the introduction of new main virus variants; dramatically so when Omicron appeared. Protection was lower among the elderly.

Role of the fellow: The fellow collaborated with the statistician, Christian Holm Hansen from SSI in analysing and interpreting population-based PCR test data. Furthermore, the fellow reviewed the current SARS-CoV-2 literature on reinfections and wrote the 'Introduction' and 'Discussion' sections of two published manuscripts (see Section 8.1, references 1 and 2). The findings from this study were also communicated by the fellow to external stakeholders such as the WHO, and she learnt how interact with the press to give oral and written interviews. The fellow also wrote an outbreak report, took part in the study design and participated as an expert in the meetings on reinfections with SARS-CoV-2 held at SSI.

1.1.2. Spread of the SARS-CoV-2 variant N439K in Denmark in 2020

Supervisor: Luise Müller

In late 2019, SARS-CoV-2 emerged in China and caused the current global COVID-19 pandemic. Since the outbreak, several viral variants have evolved from the original SARS-CoV-2 strain. One of these viral variants carries a mutation in the receptor-binding domain (RBD) that results in an amino acid change at position 439 of the spike protein of SARS-CoV-2. This variant is called N439K mutant and has emerged in Europe since August 2020.

[1] European Centre for Disease Prevention and Control. European public health training programme. Stockholm: ECDC; 2020. Available from: https://www.ecdc.europa.eu/en/publications-data/ecdc-fellowship-programme-manual-cohort-2021

By January 2021 the N439K variant has been observed in 34 countries and has become the second most observed mutation in the RBD, and the sixth most common mutation in the S protein. The N439K variant has decreased sensitivity to neutralising antibodies and enhanced capability to bind to the RBD. Therefore, there is a concern about how effective a vaccine will be against this variant.

This viral variant has been found in 1 624 samples between 8 August and 6 December 2020. The proportion of the N439K variant in the total number of SARS-CoV-2 sequenced samples has been around 10% by week 46. The SARS-CoV-2, Spike variant N439K in Denmark was primarily found in and around Copenhagen and in South Jutland. The gender and age distribution were similar to the gender and age distribution for the total SARS-CoV-2 cases in Denmark. The hospital admission rate for the N439K variant was lower compared to the total SARS-CoV-2 rate in Denmark. No deaths have been recorded among people with SARS-CoV-2, Spike variant N439K in Denmark by December 2020. There is no indication that this variant has affected a particular population group or that it exhibits increased pathogenicity.

Role of the fellow: The fellow was involved in the outbreak investigation and conducted descriptive epidemiology to determine the spread of the N439K variant in Denmark. Afterwards, the fellow wrote an outbreak report that was published on the SSI website.

1.1.3. Enterocytozoon bieneusi outbreak at a company in Denmark, November 2020

Supervisors: *Luise Müller and Rune Stensvold*

On 23 November 2020, SSI was informed by the Danish Veterinary and Food Administration about an outbreak of diarrhoeal disease in 77 employees of a company in Denmark. A food-borne outbreak was suspected from the canteen at the company. Stool samples were negative for the common gastrointestinal bacteria and viruses. Additional stool samples were collected from patients and analysed by SSI to investigate for parasites. On 1 December 2020, *Enterocytozoon (E.) bieneusi* was identified in stool samples. Microsporidia is a group of single-celled, spore-forming obligate intracellular organisms, which are rarely reported to cause disease in humans due to the lack of diagnostic testing. Most cases have been reported in immunocompromised individuals including AIDS patients. While there are more than 1200 species of microsporidia, most human infections may be caused by *E. bieneusi* and *Encephalitozoon intestinalis.*

A web-based questionnaire was created, and a retrospective cohort study conducted to examine the stool samples for the duration of spore shedding. Altogether, 195 individuals completed the questionnaire. The fellow identified 52 cases (65% male; median age 45 years [range 25–65 years]). Diarrhoea (90%), fatigue (83%), and abdominal pain (79%) were the most commonly reported symptoms. Eight cases were laboratory-confirmed and had *E. bieneusi* genotype C. The incubation period was between 5–12 days, and polymerase chain reaction (PCR)-detectable spore shedding occurred up to 43 days after symptom onset. Disease was associated with consuming food from the workplace canteen on 4 November 2020 (relative risk [RR], 2.8 [95% confidence interval [CI]: 1.4 – 5.4]) and lunchboxes containing open sandwiches with egg, shrimp and corned beef (RR, 3.2 [95% CI: 1.4 – 7.2). Epidemiological findings advocated lunchboxes containing open sandwiches from 4 November 2020, as a likely source. *E. bieneusi* may be an under-reported cause of outbreaks of diarrhoea, and testing for it might be useful in food-borne outbreak investigations.

Role of the fellow: The fellow collaborated with the EPIET colleague Luis Alvares de Sousa (cohort 2019), was involved from the beginning of the outbreak investigation. She assisted with data cleaning and analysis in R to conduct descriptive epidemiology and uni- and multivariable analysis. She also prepared an outbreak report, presented the findings from this outbreak at ESCAIDE and ECCMID 2021 and published this report in a peer-reviewed journal (see section 8.1, reference 2).

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course: In this module, the concept of outbreak investigation was introduced and epidemiological and microbiological methods of analysis were discussed and practised in case studies.

Outbreak Investigation Module: This module covered in depth the 10 steps of an outbreak investigation. Different tools were introduced to aid outbreaks. Based on a case study, the fellow was able to practise the concepts of outbreak investigation in detail, starting with data cleaning, descriptive epidemiology and univariable and stratified analysis.

Multivariable Analysis Module: During this module, the fellow learnt how to perform a multivariate analysis and the various types of analysis applicable for each study. The modules consisted of theoretical and practical sessions where the fellow got acquainted with different models and types of multivariable analysis. The fellow learnt critical skills in this module for the outbreak investigation.

Vaccinology module: The fellow learnt the different principles of vaccinology and participated in cases studies about vaccine-preventable outbreaks, where she learnt how to calculate vaccine effectiveness, adverse events of vaccinations and communication and control measures. This module was helpful for the SARS-CoV-2 epidemiological studies.

Management, Leadership and Communication module: The fellow learnt techniques for communicating with different stakeholders in emergency and outbreak situations.

Educational outcome

The fellow learnt the 10 steps of an outbreak investigation and the types of analysis that are typically conducted in these situations. She expanded her skills in public health microbiology and epidemiology, and learnt the application of these skills. Furthermore, the fellow learnt how to communicate outbreak results to stakeholders and how to collaborate with cross-functional teams. She also contributed to a scientific article and an outbreak report for the affected company.

1.2. Surveillance

1.2.1. Incidence of pinworm infections in Denmark between 2018 and 2020

Supervisors: Rune Stensvold

The human pinworm, *Enterobius vermicularis*, is the most common parasite encountered in developed countries. It typically affects children between 5 and 10 years of age. Infection occurs by directly ingesting eggs from the rectal area to the mouth. The incidence typically lies around 29% in Denmark. Pinworm infections are treated with one dose of mebendazole. During the COVID-19 pandemic, starting in 2020, social distancing and increased hygiene practices were introduced to curb the spread of SARS-CoV-2. It was noted that during the pandemic the use of mebendazole increased in Denmark. It was hypothesised if the incidence of pinworm infection increased during the pandemic despite increased hygiene practices due to the COVID-19 pandemic. The pinworm data from 2020 was compared to the pre-pandemic years (2018 and 2019) to estimate the burden of pinworm infections.

The fellow analysed 25 682 pinworm test results from 11 clinical microbiology laboratories (KMAs) in Denmark between January 2018 and December 2020. Of these test results 5 532 tests were positive for a pinworm infection. Due to the lack of epidemiological information at the time, it was not possible to conduct descriptive epidemiology with these datasets. The results showed that the median positivity rate of pinworm infections per year decreased from 19.4% in 2018 to 14.9% by the end of 2020, but this result was not statistically significant. In summary, the fellow did not find an increase in pinworm infections in any area of Denmark by the year 2020. Time series analysis revealed a downwards trend from 2018 to 2020.

Role of the fellow: The fellow received the surveillance data on pinworm infections from each KMA, cleaned the data sets and performed the analysis. Furthermore, she used a time series analysis to determine a seasonality of pinworm infections in the Danish population. The fellow created a surveillance report and is preparing a manuscript for publication in a peer reviewed journal.

1.2.2. Surveillance of Mycoplasma pneumoniae in Denmark 2015–2022

Supervisors: Søren Anker Uldum and Hanne Dorthe Emborg

Mycoplasma (M.) pneumoniae, is a small bacterium that can cause respiratory tract infections including pneumonia and is an exclusively human pathogen. *M. pneumoniae* infections can range in severity from mild to life-threatening. These lead to neurological symptoms and sequelae in 6–7% of hospitalised *M. pneumoniae* cases. While most infections with *M. pneumoniae* occur in children and younger adults, infections can also occur in adults. Immunity against *M. pneumoniae* is low in people below 20 years of age. The bacterium is transmitted from person to person by respiratory droplets, and the incubation period ranges from four days to three weeks. The main aim of this study was to determine the periodicity of *M. pneumoniae* cases between 2015 and 2022, and describe the epidemiological situation in each year.

Therefore, the fellow conducted an observational retrospective study based on data from a passive laboratory-based surveillance system for *M. pneumoniae* between 2015 and 2022 in Denmark. Weekly surveillance data was extracted from the clinical microbiology database from 12 clinical microbiology departments. The fellow calculated the incidence of *M. pneumoniae* infections, conducted descriptive epidemiology and performed a time-series analysis. The results showed that outbreaks with *M. pneumoniae* occur annually in the winter season. Furthermore, the incidence and number of *M. pneumoniae* cases almost reached zero since the onset of the pandemic in March 2020, despite a constant testing rate in hospitals, which suggested a genuine decrease in the incidence. It was also found that the main target group of *M. pneumoniae* infections are school children between 6–10 years of age. The low incidence of *M. pneumoniae* infection and the absent seasonality pattern of this pathogen in the past two years is of concern and raises the possibility of a large outbreak in the near future when social distancing and face masks are discontinued.

Role of the fellow: The fellow cleaned the surveillance data sets from the past seven years for *M. pneumoniae* infections. Next, she conducted descriptive epidemiology and performed a time-series analysis in STATA. The fellow also drafted a manuscript to investigate the number of *M. pneumoniae*-positive cases between 2015 and 2022, and to determine the effect of the COVID-19 pandemic on the annual case numbers of *M. pneumoniae*.

1.2.3. Supporting the implementation of a COVID-19 vaccine-effectiveness study to prevent severe respiratory infections in Georgia

Supervisors: Mark Katz, WHO and Giorgi Chakhunashvili, NCDC Georgia

Many critical questions remain about the effectiveness of coronavirus disease (COVID-19) vaccines in the real world. These questions can only be answered in post-introduction vaccine-effectiveness studies. Understanding COVID vaccine effectiveness (VE) in preventing severe disease is critical to guide national and global COVID vaccination campaigns. After the influenza A (H1N1) pandemic in 2009, a number of WHO/Europe Member States implemented sentinel surveillance for severe respiratory infection (SARI) for influenza. In this project, the existing SARI surveillance system was adapted to monitor severe SARS-CoV-2 vaccine effectiveness in Georgia. The study was based on the 'test negative design' (TND), where cases are SARI patients who test positive for SARS-CoV-2, and controls are SARI patients who test negative for SARS-CoV-2. The fellow supported the National Center for Disease Control and Public Health (NCDC) Georgia and WHO/Europe team in implementing this SARI surveillance study on the vaccine effectiveness of COVID-19 in three hospitals in the Georgian cities of Tbilisi, Batumi and Kutaisi. Formal ethical committee waivers for Georgia were obtained prior to the study. The fellow was deployed to Georgia at the Health Emergency Department at the WHO/Europe country office located in Tbilisi, Georgia. There, she worked closely with the NCDC Georgia team and supported them with the design and implementation of the questionnaire in REDCap, to enroll eligible individuals into the SARI study. In addition to this project, the fellow also supported WHO/Europe in designing a case control study to determine the immune correlates of protection in Azerbaijan. She helped with data analysis, descriptive epidemiology and multivariable analysis for this particular project.

Role of the fellow: The fellow supported the WHO/Europe team and the NCDC in Georgia. She developed and adapted a questionnaire to enroll SARI cases into the study and to follow these patients over time. She participated in WHO, CDC and Epiconcept meetings, and learned about problems arising with surveillance systems and analysis of surveillance data. She created a questionnaire for the SARI study, developed training materials for healthcare workers and created a study protocol for the WHO. Furthermore, the fellow drafted manuscripts and reports for different stakeholders and learnt how to communicate with health authorities. The fellow prepared a 'postcard from the field' video that was published on the ECDC website. The fellow was also involved in a second project to determine the immune correlates of protection against SARS-CoV-2 in vaccinated individuals from Azerbaijan where she designed a case control study, conducted descriptive epidemiology and analysed serological data.

1.2.4. Identifying the epidemiological source of a methicillin-resistant Staphylococcus aureus t-4549 lineage since 2018 in Denmark

Supervisors: Andreas Petersen and Jesper Larsen

Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly getting more common worldwide and can be difficult to treat with existing antibiotics. In Denmark, MRSA infection is notifiable, and all clinical isolates are typed at SSI. The MRSA with the spa type t4549 and sequencing type 630 is now among the most commonly found spa types among all MRSA isolates in Denmark. This *S. aureus* t4549 lineage first occurred in Denmark in 2012, in someone who travelled to Asia, where this lineage is endemic. The main aim of this study was to identify the most likely environmental reservoir of *S. aureus* t4549 lineage in Denmark between 2018–2022. The number of *S. aureus* t4549 infections in Denmark increased over the years and peaked in 2019 with 109 cases. This particular lineage of *S. aureus* is not adapted to humans based on the molecular composition. Of note is that the majority of cases between 2016 and 2019 were males (76%), and the median age was between 11–20 years. This supports the hypothesis that certain hobbies or sports activities are at play that are more often carried out by males and therefore, puts them at a higher risk. A better understanding of the risk factors of MRSA t4549 infection will facilitate the implementation of public health measures to prevent MRSA infection with this type. In this study, the fellow worked together with the EPIET fellow Tjede Funk and created a questionnaire for an MRSA case control study between 2018 and 2022. The fellow was not able to complete this project due to time constraints, but it will be continued by Tjede Funk.

Role of the fellow: The fellow created a questionnaire with the EPIET fellow, and discussed key information that needed to be obtained from cases and controls to identify the epidemiological source of infection. Moreover, the fellow engaged in regular meetings and was involved in the application for ethical compliance for the study.

Training modules related to the assignment/projects

EPIET/EUPHEM introductory course: The fellow learnt the theoretical basis of surveillance systems. She learnt how to analyse surveillance data through case studies. The advantages and disadvantages of different surveillance systems was also discussed among the fellows.

Rapid Assessment and Survey Methods module: The fellow participated in a simulation exercise on the early response steps during the COVID-19 pandemic. She also learnt specific details about surveillance systems and type of survey methods that are commonly used in the field.

Time Series Analysis module: This module was focused on teaching the fellows the basic concepts of time series analysis. The fellow used STATA for the analysis and participated in the practical sessions. Hands-on training and statistical methods were discussed and applied to the analysis.

Vaccinology module: The fellow learnt how surveillance systems are critical for the monitoring of vaccine-preventable diseases. Theoretical and practical sessions were included in this module.

Outbreak investigation module: The 10 steps of an outbreak investigation can also be applied to the analysis of surveillance data. In particular, the fellow used descriptive epidemiology to extract basic information from the surveillance data.

Educational outcome

The concept of surveillance was new to the fellow and the multitude of modules and projects gave the fellow an opportunity to learn about setting up, implementing and analysing surveillance data. The fellow also conducted an international deployment to Georgia through the WHO/Europe and assisted in the implementation of a surveillance system for SARI including SARS-CoV-2.

2. Applied public health research

Assessment of SARS-CoV-2 reinfections in Denmark based on whole genome sequencing

Supervisors: Lasse Dam Rasmussen and Sophie Gubbels

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was discovered in Wuhan, China, in late 2019. Coronavirus disease 2019 (COVID-19), the disease caused by SARS-CoV-2, rapidly evolved into a global pandemic. Cases with suspected possible reinfection with SARS-CoV-2 have been reported. It is known that natural infection and/or vaccination against SARS-CoV-2 elicits protective immunity but it wanes over time. The aim of this project was to identify reinfections in the Danish population by analysing real-time reverse transcription polymerase chain reaction (RT-PCR) and whole genome sequencing (WGS) data.

In this population-based retrospective study, the fellow analysed approximately 19 million real-time RT-PCR tests from 4.4 million individuals between March 2020 and March 2021, as part of Denmark's SARS-CoV-2 surveillance system. Suspected reinfection cases were defined as individuals with two or more SARS-CoV-2 PCR positive tests, with more than 60 days between the two tests and confirmation by WGS analysis. Additionally, WGS-confirmed reinfection cases with less than 60 days between the first and second infection were also considered in this study, despite the narrow time window between infections with SARS-CoV-2. Based on real-time RT-PCR positive results, 593 suspected reinfection cases were identified, and 15 of these were confirmed by WGS analysis. In nine persons, confirmed reinfections occurred within 60 days after the first SARS-CoV-2 positive test. A unique set of amino acid changes occurred within the S protein sequence isolated from the first and second infection samples from each confirmed reinfection case. Most reinfections occurred before the recommended 60–90-day threshold between the first and second episode of infection. Therefore, it is suggested, that the case definition for reinfection cases for SARS-CoV-2 be reexamined. This may have important implications for intervention measures to control the ongoing pandemic and gives a better understanding of the risk of reinfection after natural immunity to SARS-CoV-2.

Role of fellow: The fellow designed the study and took the lead of the project. She worked with data analysts, statisticians and researchers to compile the results. She analysed data sets and the WGS data for SARS-CoV-2. The fellow prepared a manuscript that was submitted *to The Lancet Infectious Diseases* where it was under review (see section 8.1, reference 4). Moreover, she created a study protocol, presented the findings in the COVID-19 Think Tank organised by ECDC and got her abstract accepted as a poster presentation at the National Public Health Conference (NPHC) in Iceland in 2022.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course: During the introductory module, the fellow had a lecture and practical sessions about operation research, and learnt about statistical analysis and descriptive epidemiology. The fellow also learnt how to write a study protocol and what study designs are appropriate for different research questions.

Outbreak Investigation Module: This module gave the fellow an insight into data analysis and descriptive epidemiology. In this module, the fellow also learnt how to create a study design and prepare a report for communication.

Multivariable Analysis Module: The fellow learnt how to conduct a multivariable analysis, as part of this module. She also had a practical session where she could try out different models and evaluate the advantages, disadvantages and limitations of each model. The fellow also got acquainted with using R and/or STATA for data analysis.

Management, Leadership and Communication in Public Health Module: This module informed the fellow about project and time management skills, and how to work effectively with multifunctional teams. She also learnt how to communicate public health findings to different audiences, and how to create reports and budgets for a project.

Project Review Module: During this module, the fellow learnt presentation skills and how to communicate scientific findings to other public health professionals.

Educational outcome

Based on the modules and this project, the fellow learnt how to create a study protocol aimed at a broad public health context rather than to an academic audience. She also learnt about ethical principles and compliance issues for research projects. The fellow gained a better understanding of data analysis in R and STATA, and developed an in-depth knowledge of whole genome sequencing analysis. She presented her results in the ECDC COVID-19 Think Tank, shared her findings with a broad public health community at a conference, and authored a manuscript for a peer-reviewed journal.

3. Applied public health microbiology and laboratory investigations

3.1. Implementation of a protocol for heat-shock protein 70 molecular typing of *Leishmania*

Supervisor: Rune Stensvold

Leishmania is a parasite transmitted by the bite of a female sandfly and can cause leishmaniasis. In Denmark, all cases of leishmaniasis are thought to be associated with travel to endemic areas. The disease can either be visceral, cutaneous or mucocutaneous; each with different clinical signs and treatments. Accurate species differentiation can be a challenge depending on the molecular targets used for diagnosis and detection, and is critical for disease prognosis and treatment choice. Currently the reference laboratory at SSI uses a PCR-based sequencing assay targeting the internal transcribed spacer (ITS) region of the ribosomal gene of *Leishmania.* However, this assay cannot discriminate between certain species of *Leishmania*. The aim of this project was to implement a heat-shock protein (hsp) 70 gene-sequencing assay for improved identification of the *Leishmania* species.

This assay was originally developed and further upgraded by a group in Belgium for discrimination of all *Leishmania* species and is routinely used at the parasitology reference laboratory at the Oslo University Hospital, Ullevål, Norway. The *hsp70* gene is arranged as a tandem repeat unit, with almost no sequence variation between the coding sequences of the different copies. The applicability of the *hsp70* assay was compared with the ITS-based assay at SSI. For this assay, the *hsp70* region of *Leishmania* was first amplified by PCR and then sequenced using the Sanger method.

The fellow collaborated with the Oslo University Hospital to learn this technique and analysed 39 travel-associated real-time PCR-positive samples collected between January 2018 and December 2020 in Denmark. The *hsp70* assay was able to discriminate between *Leishmania* species based on single nucleotide polymorphisms at specific locations within the *hsp70* gene. In summary, the *hsp70* assay offers a high-resolution assay for species molecular typing and is recommended for use at SSI.

Role of the fellow: The fellow was trained for the use of *hsp70* typing at the Oslo University Hospital. The fellow selected all leishmaniasis cases between January 2018 and December 2020 and analysed them in the laboratory. She performed PCR to create an *hsp70* amplicon and used Sanger sequencing to obtain the nucleotide sequence of each leishmaniasis patient sample. The fellow was able to correctly type every sample, and wrote an SOP for the *hsp70* molecular typing of *Leishmania*. In addition, the results were also accepted for poster presentation at the conferences – International Congress of Parasitology (ICOPA) and European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) – in 2022 in Denmark and Sweden, respectively.

3.2. Development of a PyroMark sequencing assay for *Mycoplasma genitalium* to screen for fluoroquinolone resistance

Supervisor: Jørgen Skov Jensen

Mycoplasma genitalium causes a sexually transmitted bacterial infection found in 2% of the adult population globally. It causes urogenital infections and leads to infertility. *M. genitalium* is diagnosed using PCR methods and can be treated with macrolide and fluoroquinolone antibiotics. However, antibiotic resistance to both drugs in *M. genitalium* is increasing and reaching an alarming level. Resistance to macrolides has been observed in 70–82% of *M. genitalium* positive samples. The fluoroquinolone-resistant single nucleotide polymorphisms (SNPs) occurred is particularly high in Asia (more than 80% of M. genitalium screened samples carry this mutation) and is below 10% in Europe. Testing for antibiotic resistance has therefore become critical for patient care to avoid treatment failure and prevent further increase of antibiotic resistance. Resistance-guided therapy has demonstrated cure rates of >90%.

In Denmark, *M. genitalium*-positive samples from clinical microbiology laboratories are routinely sent to the reference laboratory at SSI. Here, all clinical samples are currently tested for macrolide resistance, but testing for fluoroquinolone resistance is not yet done on a routine basis. Using the *parC* gene region of *M. genitalium*, the aim of this study was to establish an easy screening assay for fluoroquinolone resistance using pyrosequencing. Pyrosequencing is a cost-effective DNA-sequencing technique that has many applications including rapid SNP genotyping. The fellow first designed four primers targeting the *parC* region, and then she tested these primers on well-characterised *M. genitalium* samples in the laboratory. The analysis revealed that the forward primers worked well for identifying mutations in the *parC* gene region. The reverse primers initially failed to work but after the fellow optimised the primers, these eventually also worked in identifying SNPs within *parC*. The developed protocol will be used to test patient samples in the future and will help to guide clinicians in initiating the appropriate treatment for the infection.

Role of the fellow: The fellow designed and tested four different primers targeting the *parC* gene region of *M. genitalium.* She then analysed the data and identified mutants of *M. genitalium* in the laboratory. The fellow prepared a protocol for pyrosequencing using these validated primers and became familiar with the workflow of this novel technique.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course: During the three-week introductory course, the fellow attended lectures on how to define research questions and the different types of laboratory techniques that are available for use in public health microbiology. Furthermore, the fellow learnt about the limitations of each method.

Biorisk and Quality Management Module: The fellow got acquainted with quality assurance, sample handling and management of clinical samples. These techniques were applied in the lab investigation projects.

Educational outcome

The fellow expanded her knowledge about laboratory investigations in a public health setting, and applied her technical skills to develop a clinical assay. She also gained knowledge of PyroMark sequencing and the steps that are involved in conducting these studies. Lastly, the fellow learnt how to design primers for PyroMark sequencing.

4. Biorisk management

Sample management for a seroprevalence study of Hepatitis B and C in Ukraine

Supervisor: Lasse Dam Rasmussen, Rune Stensvold and Antons Mozalevskis (WHO/Europe)

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are major causes of acute and chronic liver disease globally, and cause an approximate 1.4 million annual deaths. It is estimated that, at present, 248 million people are living with chronic HBV infection, and 110 million persons are HCV-antibody positive, of whom 80 million have active viremic infection. Testing and diagnosis of hepatitis B and C infection is the gateway for access to both prevention and treatment services, and is a crucial component of an effective response to the hepatitis epidemic. Early identification of persons with chronic HBV or HCV infection enables them to receive the necessary care and treatment to prevent or delay progression of the liver disease. Since HBV and HCV cases are typically high in low- and middle-income countries, the WHO decided to conduct a seroprevalence study in Ukraine to estimate the burden of HBV and HCV infection in the general population, based on serum samples obtained from a national seroprevalence study for COVID-19 conducted in 2021. Due to political instability in Ukraine, the results from this study are pending. However, a better understanding of the prevalence of HBV and HCV infection in Ukraine will be critical for targeting populations at risk and to implement public health actions that will lead to the elimination of hepatitis in the country.

Role of the fellow: The fellow assisted the WHO with sample management, handling and shipment recommendations for HBV and HCV at the national public health laboratory in Ukraine. Furthermore, the fellow was involved in developing a laboratory-testing algorithm for HBV and HCV based on varying low sample volumes to determine the stage of infection in each infected individual. The fellow communicated with officers from the WHO/Europe and public health leaders from Ukraine on a regular basis. The fellow was also involved in setting up a collaboration with the public health institute in Georgia to screen Ukrainian blood samples for HBV and HCV infection markers due to lack of WHO-approved equipment available in the Ukrainian public health laboratories.

Training modules related to the assignment/projects

The fellow already had extensive experience working in this area as she worked in BSL-3 and BSL-3+ laboratories in the United States before starting the fellowship at SSI.

Biorisk and Quality Management Module: The fellow learnt about different techniques in biorisk assessment and mitigation efforts based on WHO recommendations on biosafety management laboratories, international shipping and regulations for the transportation of infectious substances and dangerous goods.

Educational outcome

The fellow's previous knowledge about biorisk management and BSL-3 experience was beneficial for assisting the WHO/Europe team with sample management, handling and shipment of samples from Ukraine to Georgia. The fellow applied different principles and practises of biorisk management for this project.

5. Quality management

5.1. External Quality Assessment for SARS-CoV-2 molecular testing

Supervisors: Lasse Dam Rasmussen, Kristina Trætholt Franck

A key pillar of mitigating and containing the impact of the COVID-19 pandemic is to perform extensive and early molecular testing by RT-PCR using patient samples. Since the beginning of the pandemic, several different RT-PCR assays have been developed for clinical diagnostic purposes including commercial and in-house assays. In May/June 2022 an external quality assessment (EQA) was organised by SSI. The aim of this EQA was to compare different RT-PCR platforms for the detection of SARS-CoV-2. For this, all clinical microbiology laboratories in Denmark were invited to participate. A panel of six virus stock samples with a high and low concentration of the SARS-CoV-2 variants – Delta, Omicron BA.1 and Omicron BA.2 – were sent to each participating clinical laboratory and analysed on their platforms. The results were sent back to SSI within two weeks of receiving the samples and compared to the reference values obtained at SSI. An EQA report was sent to each clinical laboratory separately and kept anonymous.

Overall, all the EQA results passed the quality control but some variability between platforms was noted. The results from this EQA allowed us to ensure a high standard of SARS-CoV-2 molecular tests that are in use in Denmark. This was the first EQA that was conducted for SARS-CoV-2 since the start of the pandemic. It helped the clinical microbiology laboratories in Denmark to gain a better understanding of the variability associated with each platform.

Role of the fellow: The fellow designed the EQA, communicated with the clinical microbiology laboratories, analysed the results and created individual and an overall EQA report that was distributed to each clinical laboratory.

5.2. Laboratory audit and Biosafety Risk assessment

Supervisor: Erik Michael Rasmussen

As part of the quality management module homework, the fellow had to conduct a laboratory audit and a biosafety risk assessment using the BioRAM tool. The fellow conducted this lab audit at the tuberculosis (TB) BSL-3 laboratory at SSI under the supervision of Erik Michael Rasmussen. This TB laboratory receives suspected *Mycobacterium (M.) tuberculosis* samples from hospitals in Denmark which are then screened for TB. Samples that are positive for *M. tuberculosis* are subsequently transferred to the BSL-3 part of the laboratory and screened for drug resistance and genetic bacterial markers. The incidence of TB in Denmark is estimated to be around 3.8 cases per 100 000 people, so a considerable number of patient samples are handled at SSI on a daily basis.

For the lab audit, the fellow first received a laboratory introduction, followed by sample handling and storage of *M. tuberculosis*. The fellow was introduced to other laboratory functions and workflows such as species identification of *M. tuberculosis*, resistance pattern and genetic characterisation. Next, the fellow conducted the lab audit focused on process management and quality control in the laboratory, as well as documentation practices for SOPs, protocols and quality control. Specifically, SOPs were inspected for completeness and a final report was created afterwards. Furthermore, the fellow conducted a biosafety risk assessment for working with *M. tuberculosis* samples in the laboratories. The lab audit and biorisk assessment both attested to a high standard of the TB laboratory and did not reveal any major gaps in the laboratory quality. The documentation and sample handling at SSI was performed satisfactorily.

Role of the fellow: The fellow participated in the lab audit and conducted the biosafety risk assessment for the TB reference laboratory at SSI. She created a lab and biosafety risk assessment report that was submitted as part of the homework for the Biorisk and Quality Management Module.

Training modules related to the assignment/projects

Biorisk and Quality Management Module: This module gave an overview of the organisation and performance of EQAs, methodologies for qualitative and quantitative tests and how to conduct lab audits and risk assessments for laboratories. The fellow was also introduced to International Organization for Standardization (ISO) norms as well as handling and shipment of samples.

Educational outcome

This was the first time that the fellow performed an EQA. She learnt how to design and analyse an EQA from beginning to end. Moreover, the fellow created an EQA report and learnt how to apply time management skills and communicate results back to clinical microbiology laboratories. This EQA provided the fellow with different aspects of EQAs ranging from administrative, scientific to logistical tasks. The fellow also conducted a lab audit for the first time, and became familiar with the procedures of accreditation and quality control measures in the laboratory.

6. Teaching and pedagogy

6.1. Lecture series for public health microbiology

The fellow co-organised a lecture series together with Andreas Rohringer (EUPHEM fellow) which was aimed for EPIET and PAE (Postgraduate Training for Applied Epidemiology) fellows (from Germany) to gain a basic understanding of public health microbiology. The one-hour lectures were held weekly via Microsoft Teams, and they were given by different EUPHEM fellows. The fellow gave the lectures, 'Introduction to Parasitology' and 'Introduction to Immunology'.

6.2. Lecture for the MSc course at the University of Copenhagen

The fellow prepared a one-hour lecture for the MSc course in 'Parasitic Zoonoses and One Health control approaches'. This lecture was attended by nine students from the University of Copenhagen on 27 September 2022. The lecture was about the parasitic outbreak of microsporidia in Denmark, and it also gave an introduction to the EPIET/EUPHEM programme. Students were able to ask questions afterwards and were engaged in the lecture.

6.3. Presentations for the Nordic Project Mini Review Module

The fellow prepared two one-hour lectures for the Nordic Project Mini Review Module held online in March 2021, and at the Norwegian Public Health Institute in Oslo, Norway in March 2022. The lectures covered the *E. bieneusi* outbreak in Denmark in November 2020 and the implementation of a heat-shock protein 70 based assay for *Leishmania* genotyping in the Reference laboratory for Parasitology at SSI.

6.4. Developing training materials for SARI studies in Georgia

The fellow was selected for an international assignment in Georgia. She developed training materials which contained presentations about REDCap (a data collection tool) to monitor the surveillance of SARI cases including COVID-19 case studies. She devised a role play that allowed the hospital staff to familiarise themselves with case definitions of SARI patients. The fellow also designed a training agenda. See section 1.2.3 for further details.

6.5. Raising awareness for Microsporidia outbreaks

E. bieneusi caused a food-borne outbreak in November 2020 at a Danish company in Denmark. The fellow was involved in this outbreak investigation and presented this unusual outbreak at a one-hour online seminar at the Department of Bacteria, Parasites and Fungi at SSI to raise awareness for this pathogen. Furthermore, the fellow conducted training of laboratory personnel for *E. bieneusi* infection. See section 1.1.3 for further details.

6.6. Literature review and presentation for the WHO/Europe Health Emergency Team on correlates of immune protection

Reinfections or breakthrough infections with SARS-CoV-2 became increasingly common after natural infection or vaccination, respectively. The fellow supported the WHO/Europe Health Emergency Team with an extensive literature review about the immune correlates of immune protection against SARS-CoV-2 infection. She presented the findings at the local weekly WHO Health Emergency meeting at the WHO/Europe office in Denmark.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course: During this module, ECDC organised a teaching workshop with focus on adult learning, the importance of engaging the audience and alternative teaching formats.

Management, Leadership and Communication in Public Health module: This module gave an introduction to effective communication and individual learning styles. A practical workshop during this module allowed the fellow to apply this knowledge to different situations.

Educational outcome

Daniela Michlmayr has always been passionate about teaching. Not only was she able to put this skill into practice, but she was also able to develop teaching materials for the first time and try out different teaching tools to keep the audience engaged. Daniela trained a wide range of health professionals ranging from epidemiologists, WHO subject matter experts, public health professionals, doctors and hospital workers.

7. Public health microbiology management

7.1. Management and leadership of projects

The fellow was involved as principal investigator or project manager for her projects at SSI. This involved establishing collaborations, research coordination and substantial project management working with cross-functional teams within SSI as well as external stakeholders such as universities, hospitals, regional clinical diagnostic laboratories and governmental institutions such as ECDC, WHO or public health institutes worldwide. Besides managerial tasks, the fellow was also involved in leading research projects, analytical work and communication. The fellowship allowed Daniela to broaden her skills in project and time management, communication and problem solving.

7.2. Deployment at the WHO European regional office (WHO/Europe), Denmark based in Georgia

In October 2021, the fellow was deployed to Tbilisi, Georgia to assist the WHO/Europe team with the implementation of the surveillance system for SARI in Georgia. A detailed description of this project can be found in section 1.2.3.

7.3. Support for the WHO/Europe team to conduct a seroprevalence study for Hepatitis B and C in Ukraine

The fellow assisted the WHO/Europe team for sexually transmitted diseases to set up a seroprevalence study during the COVID-19 pandemic to monitor the prevalence of Hepatitis B and C in Ukraine. A detailed description of the project can be found in section 4.

7.4. Working with the media

In March 2020, the fellow co-authored a population-based epidemiological study about the risk of reinfection in Denmark after natural immunity which was published in *The Lancet*. The media reached out to the fellow and asked for more in-depth explanations about the key highlights of the study. Thus, the fellow had an opportunity to give interviews to the press which were aimed at a broad audience. She also contributed to a press release by *The Lancet* and a WHO scientific brief about reinfections with COVID-19.

7.5. Presentation to the WHO COVID-19 reinfection work group

The fellow was invited as subject matter expert to virtually present at the COVID-19 reinfection work group in Geneva, Switzerland. She presented the key findings and study design of the epidemiological reinfection study in Denmark, which was published in *The Lancet* in March 2021. The fellow learnt how to communicate with officers from WHO and how to convey key messages to policy makers.

7.6. Collaboration with clinical microbiology laboratories in Denmark to conduct an EQA for SARS-CoV-2

In June 2022, the fellow conducted an external quality assurance (EQA) study for the molecular detection of SARS-CoV-2. The fellow was responsible for the direct communication with each clinical microbiology laboratory in Denmark, and collected results from each site. Furthermore, the fellow communicated the EQA results back to each clinical laboratory by writing up an EQA report for each individual laboratory.

7.7. Collaboration with the Oslo University Hospital, Ullevål in Norway for *Leishmania* diagnostics

The parasitology diagnostic laboratory at the Oslo University Hospital in Norway offered an opportunity for the fellow to visit the laboratory and get trained in the heat-shock protein 70 assay, which is the in-house assay in Norway to genotype *Leishmania* infections in travellers, immigrants and refugees. The fellow spent two weeks in Norway and analysed all *Leishmania* samples at the Oslo University Hospital. Next, the fellow implemented this new technique in the reference laboratory for parasitic infections at SSI and wrote an SOP for the use of this method. This collaboration trained the fellow to work with different stakeholders and develop an SOP for a reference laboratory.

7.8. Organisation of a lecture series for public health microbiology and delivering lectures

The fellow co-organised a lecture series with Andreas Rohringer, an EUPHEM fellow from the Norwegian Public Health Institute. She was involved in designing the content of the lecture series, creating an agenda, selecting speakers, and setting up a teaching platform in EVA (the ECDC Virtual Academy) to offer teaching materials. The fellow also tried out a new online presentation tool, SLIDO, which allows the creation of live polls, quizzes and word clouds to keep the audience engaged. The fellow learnt a lot about content creation, time management and creating meeting agendas.

7.9. Support for Santé publique France for the detection of Microsporidia in France

The fellow assisted another EPIET fellow (Athinna Nisavanh) to understand the epidemiology of *E. bieneusi* based on an outbreak in Denmark. The French public health institute (Santé publique France) was seeking assistance with microsporidia cases that they had identified in France. The fellow had a meeting with the French public health team and was engaged in fruitful discussions about microsporidia cases in France. She also offered technical assistance in detecting *E. bieneusi* in the laboratory and shared her study results with the French team. This brief activity encouraged the fellow to collaborate internationally and to assist the French public health institute, Santé publique France.

Training modules related to the assignment/projects

Management, Leadership and Communication in Public Health module: During this module, the fellow was trained in effective communication and aspects of management such as management styles, time management and wheel of success. The fellow also learnt people management and how to provide constructive feedback to colleagues and other stakeholders.

Project Review module: In this module, the fellow learnt different aspects of preparing presentations, such as how to keep an audience engaged during a talk, and how to present results in a meaningful way.

Educational outcome

The fellow learnt how to manage complex and fast-moving public health projects involving international and national stakeholders. She also learnt how to apply effective time management skills to complete projects on time and with a high quality standard. Moreover, the plethora of different projects throughout the fellowship gave the fellow the opportunity to learn how to communicate with stakeholders at different levels of authority, and how to solve problems within teams and projects.

8. Communication

8.1. Publications related to the EUPHEM fellowship

1. Hansen*CH, **Michlmayr* D,** Gubbels SM, Mølbak K and Ethelberg E. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark 2020: a population-level observational study. **The Lancet. 2021.** 397:10280: 1204-1212. DOI: 10.1016/S0140-673(21)00575-4
   ***joint first-authors**

2. **Michlmayr D**, Alves de Sousa L, Mueller L, Jokelainen P, Ethelberg E, Vestergaard LS, Schjørring S, Mikkelsen S, Jensen CW, Rasmussen LD and Stensvold CR. Incubation period, spore shedding duration and symptoms of Enterocytozoon bieneusi infection in a foodborne outbreak in Denmark, 2020. **Clinical Infectious Diseases. 2021**. Ciab949, DOI: 10.1093/cid/ciab949

3. **Michlmayr D***, Holm Hansen C*, Gubbels SM, Valentiner-Branth P, Bager P, Obel N, Drewes B, Holten Møller C, Trier Møller F, Legarth R, Mølbak K and Ethelberg S. Observed protection against SARS-CoV-2 reinfection following a primary infection: A Danish cohort study among unvaccinated using two years of nationwide PCR-test data. **The Lancet Regional Health. 2022** (20) 100452, DOI:10.1016/j.lanepe.2022.100452
   ***joint first-authors**

4. **Michlmayr D,** Asger Andersen M, Meaidi M, Irshad I, Alves de Sousa L, Fonager J, Rasmussen M, Gubbels SM and Dam Rasmussen L. SARS-CoV-2 reinfections in Denmark confirmed by whole genome sequencing. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4054457 (**submitted to The Lancet Infectious Diseases. 2022**)

5. Mirzaaghaei S, **Michlmayr D**, and Mirhendi M. Current Diverse Vaccines Platforms to Combat COVID-19. (submitted, 2022)

6. **Michlmayr D,** Emborg HD and Uldum S. Surveillance of Mycoplasma pneumoniae infections in Denmark between 2015 and 2022. (in preparation)

7. **Michlmayr D,** Træholt Franck K and Dam Rasmussen L. External quality assessment of SARS-CoV-2 molecular detection in Denmark in June 2022. (in preparation)

8. **Michlmayr D**, Jokelainen P and Stensvold R. Surveillance of pinworm infection before and during the COVID-19 pandemic. (in preparation)

8.2. Reports

1. **Standard Operating Procedure (SOP):** '*Leishmania* genotyping using a heat-shock protein 70 gene sequencing assay' (July 2022)
2. **External quality assessment summary report (EQA):** 'SARS-CoV-2 molecular testing by QPCR in June 2022' (July 2022)
3. **Outbreak investigation report:** 'FUD1904- *Enterocytozoon bieneusi* outbreak in Denmark, November 2020' (February 2021)

4. **Outbreak investigation report:** 'SARS-CoV-2, Spike protein variant N439K' (December 2020)
5. **International assignment study protocol:** 'Immune correlates of protection by COVID-19 vaccines in Azerbaijan, Georgia and Albania' (December 2021)
6. **Study protocol:** 'Assessment of SARS-CoV-2 reinfection cases in Denmark confirmed by whole genome sequencing' (September 2021)
7. **Surveillance report** for pinworm infections in Denmark between 2018 and 2020 (July 2022)
8. **Protocol** about pyrosequencing for the detection of *parC* fluoroquinolone resistance gene in *Mycoplasma genitalium* urine samples (July 2022)
9. **Lab audit and Risk assessment** of a tuberculosis laboratory at SSI, Denmark (led by Michael Rasmussen) (April 2022)
10. **Training material** package for Severe Respiratory Infection studies in Georgia (December 2021)
11. **Literature review** summary for the WHO about COVID-19 reinfection cohort studies worldwide (October 2021)

8.3. Conference presentations

1. **Oral presentation:** 'Incubation period, spore shedding duration and symptoms of Enterocytozoon bieneusi infection as observed during an outbreak in Denmark', **ECCMID 2021**, (9–12/07/2021), online
2. **Oral presentation:** 'Incubation period, spore shedding duration and symptoms of Enterocytozoon bieneusi infection as observed in an outbreak in Denmark, 2020', **ESCAIDE 2021**, (16–19/11/2021), online
3. **Poster presentation:** 'Assessment of SARS-CoV-2 reinfection cases in Denmark based on whole genome sequencing', **NPHC 2022**, (28–30/06/2022), Reykjavik, Iceland (attended online)
4. **Poster presentation:** 'Leishmania genotyping based on heat-shock protein 70 sequencing', **ICOPA 2022**, (21–26/08/2022), Copenhagen, Denmark
5. **Poster presentation:** 'Species identification of travel-associated leishmania positive cases in the Danish population between 2018 and 2020 using heat-shock protein 70 sequencing', **ESCAIDE 2022**, (23–25/11/2022), Stockholm, Sweden

8.4. Other presentations

1. Oral Presentation at the MSc course, 'Parasitic Zoonoses and One Health control approaches' at the University of Copenhagen (27/09/2021), Copenhagen, Denmark
2. Oral presentation at the ECDC Think Tank: 'Assessment of SARS-CoV-2 reinfection cases in Denmark in 2020' (11/11/2020), online
3. Oral presentation at the WHO COVID-19 reinfection work group meeting: presentation of the research highlights from *The Lancet* study by Hansen and Michlmayr et al.(09/04/2021), online
4. Oral presentation to the Who Health Emergency group to present the study proposal to investigate the correlates of immune protection against SARS-CoV-2 in vaccinated individuals with breakthrough infections in Georgia (23/11/2021) online
5. Periodical presentation on projects (one hour) at the EPIET/EUPHEM forum at SSI, 2020–2022
6. Oral presentations at SSI Department of Bacteria, Parasites and Fungi, (04/03/2021 and 02/06/2022), online
7. *E. bieneusi* outbreak presentation to SSI Lab personnel from the Department of Bacteria, Parasites and Fungi (10/06/2021), SSI, Denmark
8. 'Introduction to Microbiology' lecture series 2022 created by EUPHEMs. Two presentations: *'Introduction to Immunology'* (19/05/2022) and '*Introduction to Parasitology'* (05/05/2022), online

8.5. Other activities

1. Press release published by *The Lancet* with key highlights from the epidemiological reinfection study from Denmark (see publications) (17/03/2021), online
2. Interview with journalist from the 'Contagion live' website about the epidemiological study findings for SARS-CoV-2 reinfections in Denmark (https://www.contagionlive.com/view/learning-from-denmark-during-covid-19)
3. Interview with the websites – Medscape and The Spiegel (oral interviews) about COVID-19 reinfection study. https://www.medscape.com/viewarticle/947666 https://www.spiegel.de/wissenschaft/medizin/corona-infektion-schuetzt-aeltere-nicht-zuverlaessig-vor-erneuter-ansteckung-a-28c7e891-dadd-41ec-863f-ba7f37f934fd
4. Contribution to the WHO scientific brief about COVID-19 reinfections (March 2021)

5. Review of scientific papers for peer-reviewed journals
   - *BMJ Open*: Manuscript about a study protocol for SARS-CoV-2 and testing the rate of reinfections
   - *Parasite Epidemiology and Control*: Manuscript and review article about surveillance systems of parasitic infections in Europe
6. 'Postcards from the field' video published on the ECDC website about an international assignment in Georgia through WHO/Europe (link: https://www.youtube.com/watch?v=1AvXJ0mN6ZY)

8.6. Other training modules

1. GIS EAN Module (09–10/04/2022), Rome, Italy
2. ECDC Fellowship COVID-19 Think Tank (11/11/2020), online

9. Other activities

Cohort representative for EUPHEMs: Daniela was elected as one the EUPHEM representatives for the fellows from cohort 2020. This involved participation in monthly meetings with other representatives from cohort 2019, 2020 and 2021, training site forums, various ECDC workgroup meetings, meetings with the fellowship office and cohort meetings. As part of the cohort representative team, satisfaction surveys were conducted throughout the fellowship. Key issues arising during the fellowship were addressed with the head of the fellowship programme. This was particularly important during the COVID-19 pandemic when many fellows faced issues with their fellowship. Furthermore, Daniela was also involved in curriculum discussions for the EPIET/EUPHEM fellowship to ensure continuous improvement of the fellowship.

10. EPIET/EUPHEM modules attended

1. EPIET/EUPHEM Introductory Course Part 1 (28/09–6/10/2020), online
2. EPIET/EUPEM Introductory Course Part 2 Inject Days – Operational Research, (9–10/11/2020), online
3. Outbreak investigation Module (7–11/12/2020), online
4. Multivariable Analysis Module (15–19/02/2021 and Inject Day 18/03/2021), online
5. Nordic Mini Project Review Module 2021 (23–24/03/2021), online
6. EPIET/EUPHEM Introductory Course Part 3 (26/04-07/05/2021, except May 3), online
7. Rapid Risk Assessment and Survey Methods Module (05–06/05/2021), online
8. Project Review Module 2021 (23–27/08/2021), online
9. Biorisk and Quality Management Module (17–18/01/2022), online
10. Vaccinology Module (14–18/02/2022), online
11. Nordic Mini Project Review Module 2022 (07–08/03/2022), Oslo, Norway
12. Time Series Analysis Module (04–08/04/2022), Rome, Italy
13. Management, Leadership and Communication in Public Health Module (13–17/06/2022), Stockholm, Sweden
14. Project Review Module 2022 (29/08–02/09/2022), Lisbon, Portugal

10. Other training

1. FETP masterclass: Introduction to behavioural science and public health emergency response (05/02/2021), online
2. FETP masterclass: Public Health Communication (24/02/2021), online
3. EAN Risk communication during the COVID-19 pandemic webinar (02/03/2021), online
4. Introduction to R (30/09/2020 – 09/06/2021), SSI, online
5. PyroMark Q48 Autoprep training by Qiagen, one day in-house training (22/09/2021), SSI, Copenhagen, Denmark
6. REDCap Training, WHO/Europe office, Copenhagen, Denmark (October 2021), online
7. EVD LabNet webinar on emerging alphaviruses (07/10/2021), online
8. Online courses for United Nations and GOARN:
   - BSAFE Security Awareness Training (28/04/2021), GOARN, online
   - Working in an international Multidisciplinary Outbreak Response Team (03/05/2021), GOARN, online
   - Personal Well-being for deployment, GOARN (03/05/2021), online
   - The Public health Emergency and Humanitarian Landscape and Architecture, GOARN (03/05/2021), online
   - The Global Outbreak Alert and Response Network (GOARN) (04/05/2021), online
   - Working with GOARN in the Field (04/05/2021), online
   - UN Human Rights and Responsibilities (04/05/2021), online
   - UN Interagency: To serve with Pride – Zero Tolerance for sexual exploitation abuse by your own staff (04/05/2021), online
   - UN Prevention of Harassment, Sexual Harassment and Abuse of Authority, (04/05/2021), online
   - Introduction to Go.Data (04/05/2021), online
   - WHO Public Health Laboratory Knowledge Sharing Webinars – Laboratory Management Series (September – October 2021), online
   - WHO consultation on COVID-19 vaccines research (25/10/2021), online

9. Conferences

- ESCAIDE 2020 (24–27/11/2020), online
- ECCMID 2021 (9–12/07/2021), online
- ESCAIDE 2021 (16–19/11/2021), online
- NPHC 2022 (28–30/06/2022), Reykjavik, Iceland (online)
- ICOPA 2022 (21–26/08/2022), Copenhagen, Denmark
- ESCAIDE 2022 (23–25/11/2022), Stockholm, Sweden

Discussion

Coordinator's conclusions

One of the main goals of the EUPHEM programme is to expose the fellows to diverse and multidisciplinary public health experiences and activities, thus enabling them to work across different disciplines. This report summarises all the activities and projects conducted by Daniela Michlmayr during her two-year EUPHEM fellowship (cohort 2020) as an EU-track fellow at the Statens Serum Institut in Copenhagen, Denmark. The projects described in this portfolio demonstrate the range of the public health microbiology work of Daniela, who started her fellowship during the full COVID-19 pandemic. Such context posed many challenges to all the fellows, but Daniela rose to the occasion by her resilience and capacity to take on the burden and give support to the training site. Her projects on SARS-CoV-2 reinfection resulted in prestigious publications which attest to the value of her work. Daniela had many outputs from various projects spanning from *Enterocytozoon bieneusi* outbreak investigation to the surveillance of pinworm infections before and after the COVID-19 pandemic. She is an enthusiastic fellow who successfully applied herskills and expertise acquired as a researcher in the field of public health. Her service was not restricted to the training site, but also to the cohort community as a representative of the fellows. Daniela contributed with her cohort fellow Andreas Rohringer, to the lecture series on Public Health Microbiology which was much appreciated by colleagues and scientific coordinators. Daniela emerged out of the programme, an intelligent, determined and independent fellow whose core competencies were fully developed in domains described for mid-career professionals and above. The EUPHEM Frontline Coordinator concludes that the fellow has succeeded in performing all her tasks to a very high standard and with a professional attitude, which indicates her development in leadership. I wish Daniela every success in her future career as a public health microbiologist.

Supervisor's conclusions

Daniela has been very passionate about her fellowship, eagerly engaging in as many tasks as she had the chance to collaborate on without losing control of her tasks/matrix. She has proved herself competent both in contributing to ongoing work where help was needed as well as in leading those projects. Daniela is a highly responsible, well-respected, and well-liked project manager, and she performs her numerous tasks efficiently, in a timely manner, and satisfactorily. She has been remarkably proficient in terms of publishing her data in high-impact journals, and she has also been quick to grasp the opportunities and tools that are specific to Denmark, including data resources such as the various registers and databases available in the country. Without neglecting her scientific or her administrative responsibilities, Daniela has been able to run in parallel her work in different projects and teams. This often involved unexpected situations where she was willing to jump in, such as the *Enterocytozoon* outbreak in Copenhagen only a few months after her arrival, where she quickly took the lead and saw the project through to publication in *Clinical Infectious Diseases*. Moreover, in the second year of her fellowship, Daniela quickly responded to a WHO request and was deployed for a few weeks to Georgia. Daniela is highly skilled in teaching and training. In very little time, she was able to structure and teach textbook materials within parasitology – a field previously unknown to her. Moreover, she is an extremely good presenter with an outstanding ability to get her messages across in a logical way, making sure to take the audience's general knowledge and expertise into account. Finally, Daniela demonstrated good social skills, adapting easily to new living and working environments. She worked with high stability and efficiency even when everything was new and despite the challenges posed by the COVID-19 situation.

Personal conclusions of fellow

For a long time, it was my desire to work in the area of public health and epidemiology. The EUPHEM fellowship has allowed me to pursue this area, and during these two years I have learnt the fundamentals of epidemiology, data analysis and public health microbiology. While it was a challenging two years due to the pandemic and social isolation, I could not have hoped for a better training site. The supervision was outstanding, the cornucopia of projects was mind blowing and the expertise of scientists at SSI was inspiring. I am also truly grateful to be part of this incredible public health network.

The EUPHEM fellowship allowed me to gain insights into other aspects of microbiology such as parasitology and bacteriology. Now I have a much more comprehensive understanding of microbiology and its methods. I am also grateful that I had the opportunity to do an international assignment in Georgia, and through this project I learnt so many aspects of surveillance. It was a dream-come-true to work with the WHO. It was a truly great experience to have been part of the EUPHEM fellowship programme.

Acknowledgements of fellow

I would like to thank ECDC for accepting me into the EUPHEM fellowship and my supervisors Rune Stensvold and Lasse Dam Rasmussen for welcoming me to SSI. You made my time in Denmark very special. It was a pleasure working with you and getting to know you. I would also like to thank Steen Ethelberg, for the great collaborations and for giving me the opportunity to learn so much about epidemiology. I will always remember our great chats, fun and laughter. I would like to thank all of my other supervisors and colleagues at SSI. You all have been an inspiration and I learnt so much from you. Thanks also to my EPIET colleagues from cohorts 2018–2021. You have helped me stay sane and it was great getting through this experience with your help. A special thanks also goes out to my frontline coordinator, Loredana Ingrosso who helped me stay on track and helped me focus on the most important aspects of my fellowship. I am also grateful for having been part of the cohort representative team. Lastly, I would like to thank all my EPIET, EUPHEM and PAE fellows. You rock!