Summary of work activities
Natalie Fischer
European Public Health Microbiology Training Programme (EUPHEM), 2019 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 in 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 provides experts in all relevant communicable diseases at the regional, national and international level with the tools they need to mount rapid responses to emerging health threats. This enables them to plan appropriate prevention strategies, assess existing prevention disciplines, develop microbiological guidelines, evaluate/produce new diagnostic tools, assess 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' which is why 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 Natalie Fischer, cohort 2019 of the European Public Health Microbiology Training Programme (EUPHEM) at Sciensano, the public health institute of Belgium.

Natalie Fischer is a microbiologist by training and has extensive research experience in infectious diseases, host-microbe interaction and the human microbiome. She performed her PhD work at the Institut Pasteur in Paris,

where she characterised the expression of antimicrobial peptides and inflammatory molecules in response to bacteria in the gut epithelium. Before joining the ECDC fellowship, Natalie was a post-doctoral fellow at the Stanford University School of Medicine in California, where she conducted human subjects research on the role of the skin and gut microbiota in inflammatory bowel disease and malnutrition in children.

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.

Methods

This report accompanies a portfolio demonstrating 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 and the summary and communication of 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, with the exception of those prohibited for reasons of confidentiality.

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 EUPHEM scientific guide[1].

1. Epidemiological investigations

1.1. Outbreak investigations

Ebola virus disease outbreak in the Democratic Republic of Congo 2020
Supervisor: Marie-Amelie Degail Chabrat, WHO

On 1 June 2020, the 11th Ebola virus disease outbreak in the Democratic Republic of the Congo (DRC) was announced. The fellow deployed through GOARN to the WHO headquarters' epidemiology and surveillance teams within the HIM/FES unit to support colleagues from AFRO, WCO DRC and the WHO sub-office in Mbandaka, Equateur Province, in terms of epidemiological analysis and monitoring of surveillance activities and performances. At the time of deployment on 18 September 2020, 123 EVD cases (117 confirmed, six probable), including 50 deaths, had been recorded in 40 health areas of 12 affected health zones. The primary aims of the team was to provide expert support to field teams from the surveillance pillar and other pillars, monitor the outbreak and provide timely analyses and assessments to inform the response, and effectively communicate these assessments internally and externally to a wide range of audiences, including to senior WHO leadership and international partners. The analysis of surveillance data showed that contact tracing was suboptimal, the transmission chain was fragmented into several short chains across health zones without documented epidemiological link and the number of reported alerts per health zone was suboptimal. This led to increased efforts in review of case reports, additional interviews of cases as well as the attempt to integrate phylogenetic data from whole genome sequencing into the outbreak investigation.

The fellow was involved in maintenance of core case, contact and alerts databases, corresponding data cleaning and review of case reports. She supported daily analyses and epidemiological interpretation of trends and indicators as well as targeted topic specific in-depth analysis such as analysis of the case fatality, modes of transmission, contacts, and health zone specific assessment. She ran, adapted and wrote R code for data analysis and visualisation, and prepared briefing materials and information products. Finally, she applied her microbiology background and expertise to support the visualisation and integration of phylogenetic data from whole genome sequencing of viruses from the outbreak and epidemiological data from transmission chains using R programming. She wrote a post-card from the field about her experience which was published on the ECDC website.

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[1] European Centre for Disease Prevention and Control. European public health training programme. Stockholm: ECDC; 2013. Available from: http://ecdc.europa.eu/en/publications/Publications/microbiology-public-health-training-programme.pdf

Listeria monocytogenes outbreak in Belgium 2020-2021

Supervisor: An Van den Bossche

In 2020, the National Reference Centre for *Listeria* at Sciensano was notified of 54 unique cases of *Listeria monocytogenes* infections in Belgium. Within those, they classified three clusters of serogroup IVb and two clusters of serogroup IIa/b with three to six isolates each. Two clusters from 2020 were targeted for closer investigation. One cluster of three cases of serotype 1/2a in the region of Flanders (CC14) was identified as part of a larger outbreak, counting 13 cases across Belgium since 2014, with very close genetic relation (0-4 allelic differences, as determined by cgMLST). A second cluster of six clonal isolates of serotype 4b (CC4, 0-2 allelic differences, as determined by cgMLST), isolated between April and July 2020, showed close geographical location at the border of the provinces of Antwerpen and Oost-Vlaanderen of four of the cases. Coincidentally, the Belgian authorities for food safety (AFSCA/FAVV) had seven strains of Listeria serotype 4b in their repository, which were sent for sequencing. No link between the human isolates and any food samples could be established, as the closest isolate was 34 AD apart. However, a link with one of the isolates and another 4b cluster could be made. This cluster (CC6) contained four cases spread over Belgium from August to October. An identical strain had been isolated from goat cheese and food authorities had already taken actions in the cheese manufacturing enterprise. In this context, the Flanders health agency (Agentschap Zorg & Gezondheid, AZG) asked Sciensano to develop an updated questionnaire for outbreak investigation of *Listeria* cases in Flanders (Annex). Our team adapted the paper version of the questionnaire and implemented it in the online tool LimeSurvey. The questionnaire assesses timing and symptoms of disease, comorbidities, living situation, food shopping and consumption habits as well as travel. The new questionnaire was also translated to French and shared with the Brussels and Wallonia health agency for their approval. Finally, translations into German and English were also developed. Sciensano is now tasked to investigate all cases and clusters occurring in Belgium in 2021.

The fellow was involved in the review of the paper-based questionnaire from Flanders and Wallonia and developed the online data entry mask in LimeSurvey.

Salmonella Group 07 Braenderup outbreak in Belgium 2021

Supervisors: An Van den Bossche, Dieter Van Cauteren

An outbreak of Salmonellosis occurred among 12 EU/EEA countries and UK comprising 348 confirmed cases of *Salmonella* Braenderup between March and June 2021. In Belgium, 55 cases were reported, and isolates submitted to the NRC between 23 March and 24 June, with a peak around mid-April 2021. All cases were followed up for detailed interviews and food questionnaires with the objective to identify the vehicle of the outbreak with the goal to initiate appropriate control measures. Among cases, 67% (37/55) were female and 33% (18/55) were male, a trend also observed in other countries. The median age was 35 years, with cases ranging from a few months to 97 years. The highest percentage of cases occurred in children aged 5-14 years (18%) and adults older than 85 years (16%). Cases were detected in all three regions of Belgium, with a concentration in Flanders with 41 cases, compared to Wallonia with nine and Brussels with five cases. Of them, 43% (17/40 with information on hospitalisation) needed to be hospitalised. Analysis of the food questionnaire data showed that 67% (29/43 with information on food) cases confirmed eating (some kind of) melon and 33% (14/43 with information on food) cases declare not to have eaten melon. Melon consumption was higher among females (71% versus 58% in males). Most cases which had consumed melon, identified Galia as the type of melon (47%). No analytical study was performed in Belgium, but melon was identified as the most likely vehicle for this outbreak in a case control study in the UK. The team in the UK identified *S.* Braenderup matching the human outbreak strain in 2/200 melons with origin in Honduras. The outbreak strain was also isolated from melons in Austria. In Belgium, several melons have been sampled, but all were negative for the outbreak strain. Furthermore, 7 tracing studies on 14 cases lead to the same origin: FBO Agropecuaria Montelibano SA (Honduras), which is in correlation with results from the UK and the Netherlands. Any products that may remain in circulation in Belgium or other European countries should be taken out of stock. In the meantime, consumers and food handlers should be informed about safe handling of fresh produce.

The fellow developed R code for the analysis and visualisation of case characteristics, as well as questionnaire data on food consumption. This code was shared with the lead epidemiologist and the lead scientist at the NRC for the generation of an outbreak report as well as for future use.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course – During the introductory course, lectures and case studies focused on the 10 steps of outbreak investigation, the conduction of analytical epidemiological studies, collection of data through questionnaires, and communication during an outbreak, as well as the monitoring of epidemiological indicators.

Outbreak Investigation Module - This module focused on the 10 steps of an outbreak investigation, epidemiological data analysis in R, GIS, phylogenetic analysis, and the writing of an outbreak report during lectures and case-studies.

Management, Leadership and Communication in Public Health Module –This module focused on successful communication and management of projects with different partners, as well as the communication with various stakeholders and public health authorities.

Educational outcome

The fellow gained practical experience of working in food-borne and emerging disease outbreak investigations and surveillance in different settings, international teams, and within multilevel organisations. She strengthened her knowledge in monitoring and analysis of epidemiological indicators while applying and developing her skills working in R. She became familiar with the setup of online questionnaires as well as the evaluation of data collected through questionnaires for outbreak investigation.

1.2. Surveillance

Setup of the daily and weekly epidemiological COVID-19 bulletin in Belgium

Supervisor: Javiera Rebolledo Gonzalez

In December 2019, health authorities in Wuhan (China) reported grouped cases of pneumonia of unknown cause. Shortly afterwards, a new corona virus, SARS-CoV2, was identified, spread rapidly internationally and a global pandemic was declared by WHO in March 2020. In Belgium, the first cases of COVID-19, the disease caused by SARS-CoV-2, were reported in the first week of March 2020. Sciensano, the public health institute of Belgium, has a legally determined surveillance mission within the framework of public health as defined by the federal law of 25 February 2018. As part of this mission, Sciensano set up different surveillance systems to follow the evolution of the COVID-19 epidemic in Belgium. The fellow was part of the initial team in the service of 'Epidemiology of infectious diseases' to set up a daily report with the main epidemiological indicators, such as the number of cases, hospitalisation, ICU admission and deaths. The initial report was produced daily in Excel and Word in the two national languages French and Dutch. In the following weeks, the report evolved and was split into a version for the public and a more extensive version for authorities, including more regional data, and was finally automated using R programming. Since then, the report has evolved to contain many more chapters summarising the information and data collected from different surveillance systems in Belgium, for example testing capacity, surveillance of nursing homes, clinical hospital surveillance (giving information on the severity of hospitalised cases), ICU capacity, absenteeism, and serological studies, which were elaborated on in greater depth in a weekly epidemiological bulletin. Moreover, communication strategies regarding authorities and the public were discussed with the communication department at Sciensano, and key numbers and topics were produced to be communicated at the daily press conference.

The fellow was involved in the setup and production of the very first epidemiological bulletin, including decisions on the content and structure of the report, coordination with the different surveillance teams, collection of data from the hospital surveillance, laboratory network as well as on mortality, production of graphics in Excel and explanatory text in a word template in French and Dutch, management of timely daily upload to the Sciensano website, and transition to automation of the report in R. In addition, she was involved in the development of a FAQ document for the public on the data, sources, and indicators reported in the epidemiological bulletin.

Burden of disease of seasonal human coronaviruses in Belgium

Supervisors: Cyril Barbezange and Lorenzo Subissi (Alumni EUPHEM fellow at Sciensano)

Seasonal human coronaviruses (hCoVs) broadly circulate in humans. Their epidemiology and effect on the spread of emerging coronaviruses has been neglected thus far. We aimed to elucidate the epidemiology and burden of disease of seasonal hCoVs OC43, NL63, and 229E in patients in primary care and hospitals in Belgium between 2015 and 2020. We retrospectively analysed data from the national influenza surveillance networks in Belgium during the winter seasons of 2015–20. Respiratory specimens were collected through the severe acute respiratory infection (SARI) and the influenza-like illness networks from patients with acute respiratory illness with onset within the previous 10 days, with measured or reported fever of 38°C or greater, cough, or dyspnoea; and for patients admitted to hospital for at least one night. Potential risk factors were recorded and patients who were admitted to hospital were followed up for the occurrence of complications or death for the length of their hospital stay. All samples were analysed by multiplex quantitative RT-PCRs for respiratory viruses, including seasonal hCoVs OC43, NL63, and 229E. We estimated the prevalence and incidence of seasonal hCoV infection, with or without co-infection with other respiratory viruses. We evaluated the association between co-infections and potential risk factors with complications or death in patients admitted to hospital with seasonal hCoV infections by age group. Samples received from week 8, 2020, were tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). 2 573 primary care and 6 494 hospital samples were included in the study. 161 (6.3%) of 2 573 patients in primary care and 371 (5.7%) of 6494 patients admitted to hospital were infected with a seasonal hCoV. OC43 was the seasonal hCoV with the highest prevalence across age groups and highest incidence in children admitted to hospital who were younger than five years (incidence 9.0 [95% CI 7.2–11.2] per 100 000 person-months) and adults older than 65 years (2.6 [2.1–3.2] per 100 000 person-months). Among 262 patients admitted to hospital with seasonal hCoV infection and with complete information on potential risk factors, 66 (73.3%) of 90 patients who had complications or died also had at least one potential risk factor (p=0.0064). Complications in children younger than five years were associated with co-infection (24 [36.4%] of 66;

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p=0.017), and in teenagers and adults (≥15 years), more complications arose in patients with a single hCoV infection (49 [45.0%] of 109; p=0.0097). In early 2020, the Belgian SARI surveillance detected the first SARS-CoV-2-positive sample concomitantly with the first confirmed COVID-19 case with no travel history to China. The main burden of severe seasonal hCoV infection lies with children younger than five years with co-infections and adults aged 65 years and older with pre-existing comorbidities. These age and patient groups should be targeted for enhanced observation when in medical care and in possible future vaccination strategies, and co-infections in children younger than five years should be considered during diagnosis and treatment. Our findings support the use of national influenza surveillance systems for seasonal hCoV monitoring and early detection and monitoring of emerging coronaviruses such as SARS-CoV-2.

The fellow performed all data cleaning and selection of the study population based on the surveillance data from the National Influenza Centre at Sciensano. She was in charge of all data analysis and visualisation in R, prepared a manuscript for peer review (Reference 1), and prepared an abstract and oral presentation at the Annual Meeting of the Society of Virology in Belgium (BELVIR) 2020.

Prevalence and severity of influenza-associated SARI in children admitted to Belgian hospitals during the winter seasons 2011-2020
Supervisor: Cyril Barbezange

Influenza type A and B viruses cause respiratory infections in humans, which follow a seasonal pattern coinciding with the winter months in temperate regions. Infections can range from mild to severe with requirement of hospitalisation and can lead to development of severe complications and even death. The burden of influenza infections in Belgium is estimated at 500 000 cases of influenza-like illness each year, i.e., about 2% to 8% of the population according to Sciensano. An estimated 1/1 000 patients develop complications requiring hospitalisation and over 90% of deaths occur in patients above 65 years of age. However, currently little is known about children hospitalised with influenza in Belgium, and how the burden of disease relates to underlying risk factors or coinfection with other respiratory viruses. The aim of this project was to analyse data collected by the Belgium influenza sentinel hospital surveillance system for severe acute respiratory infections (SARI) over the last 10 years to assess the prevalence and burden of influenza infections in children in Belgium per age group, influenza type and subtype, as well as the role of comorbidities and coinfections with other respiratory viruses in disease severity and outcome. Identifying patients at risk for complications is crucial to guarantee optimal monitoring and treatment, as well as useful to inform vaccination strategies.

The fellow was in charge of all sample selection and data cleaning, data analysis, preparation of tables, graphs, multivariable logistic regression modelling in R, and writing of the manuscript (Reference 6).

Training modules related to the assignment/projects

EPIET/EUPHEM introductory course – During the introductory course, surveillance systems and data analysis were extensively discussed in lectures and case studies.

Multivariable Analyses module – This module focused on applied statistics for epidemiological analyses, introducing multivariable analysis, stratified analyses, interaction of variables, and building regression models using R.

Time Series Analysis module – This module focused on statistical analysis of time series data, obtained through surveillance systems.

Educational outcome

The fellow gained extensive knowledge of the surveillance of influenza and other respiratory viruses in Belgium through involvement in activities and analysis of data from the National Influenza Centre at Sciensano. She learned about the calculation of important indicators such as the incidence rate, using a catchment population. She participated in several meetings regarding SARI surveillance in Europe organised by ECDC and Epiconcept.

In addition, she experienced the set-up of a new surveillance system and choice of indicators, data sources and collection tools in an emergency setting, as well as the proper communication of surveillance data with the public and authorities. She honed her skills in data analysis in R, including using different regression models and multivariable analyses. She sharpened her writing skills in the preparation of several peer-reviewed manuscripts.

2. Applied public health research

Prevalence estimation of genital Chlamydia trachomatis in the Belgian population
Supervisor: Wim Vanden Berghe

Chlamydia trachomatis (chlamydia) is the most diagnosed sexually transmitted infection in Belgium. Screening programs focus on young women, due to the implications of chronic asymptomatic infections for reproductive health. Thereby, the frequency of infections in men and older adults is underestimated. We aimed to estimate the point prevalence of chlamydia in the broader Belgian population, to inform evidence-based prevention and control

strategies. We conducted two cross-sectional prevalence studies of chlamydia infection in the population of Belgium aged 16-59 years, 2018-2020. In the CT1 study 12 000 representative individuals were randomly selected from the national register and invited by letter to collect a urine sample at home. The CT2 study used urine samples collected through the Belgian Health Examination Study. Molecular detection of chlamydia DNA was performed using Xpert® or Abbott Real-Time CT/NG assays. Weighted estimated prevalence and 95% confidence interval (CI) was calculated per gender and age groups of 16-29, 30-44 and 45-59 years, relative to the general Belgian population. Data collected on socio-demographic variables and sexual behavior were used to identify potential risk factors for chlamydia infection through calculation of the odds ratio (OR). The population-wide weighted estimated prevalence was 1.54% (95% CI 0.78-3) in CT1 and 1.76% (95% CI 0.63-4) in CT2. We observed no difference between men and women or age groups. Civil relationship status, sexual intercourse with a casual partner (OR = 6.31 (95% CI =1.66- 24.1), p < 0.01) and >3 sexual partners in the last 12 months (OR = 4.53 (95% CI = 1.10-18.6), p = 0.02) were associated with higher relative risk for chlamydia infection. Nationwide prevalence studies are relevant to assess the distribution of chlamydia and inform public health actions. The overall low prevalence of chlamydia in the general Belgian population needs to be considered for future strategies and potential harm of testing and treating asymptomatic individuals need to be considered. Effective case management should include appropriate treatment of symptomatic patients and partner notification, and prevention strategies should encourage behaviors such as condom use.

The fellow was involved in writing of the study protocol and other documents required for the Belgian data protection agency and ethics committees, managed sample transport and data exchange between study partners, performed all data analysis, produced tables, and wrote the manuscript for peer review (Reference 3), prepared and submitted an abstract for ESCAIDE 2021 which was selected for an oral presentation, prepared and presented the study results to stakeholders of health authorities responsible for sexual health in the three regions of Belgium.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course- During the three-week course one entire week was dedicated to developing a research study, including how to define research questions, study design and protocol writing. Lectures gave an introduction on basic epidemiological measures, such a prevalence and analytical epidemiological analysis, such as calculation of odds ratios.

Multivariable Analyses module – This module focused on applied statistics for epidemiological analyses introducing multivariable analysis, stratified analyses, interaction of variables, building regression models using R.

Management, Leadership and Communication in Public Health Module –This module focused on successful communication and management of projects with different partners, as well as the communication with various stakeholders and public health authorities.

Project Review Module –During this module basic presentation and communication skills were trained and the oral presentation for the Annual ESCAIDE conference was reviewed and practiced.

Educational outcome
The fellow deepened her understanding of public health aspects of sexually transmitted diseases and the use of prevalence studies to assess burden of disease within a population. She applied her knowledge in writing a study protocol for ethical approval as well as her project management skills to facilitate exchange of samples, data, and discussions between different groups within Sciensano, as well as the NRC for chlamydia at the Institute of Tropical Medicine in Antwerp. She became versed in the analysis of survey data in R, including the incorporation of study weights and applied multivariable logistic regression modelling. She gained practice in communicating results and recommendations to public health authorities as well as to the scientific community at the ESCAIDE conference 2021.

3. Applied public health microbiology and laboratory investigations

Genomic epidemiology of antimicrobial resistance in Shigella sonnei in Belgium
Supervisors: Pieter-Jan Ceyssens

Shigella sonnei resistant to first-line antibiotics azithromycin and ciprofloxacin are on the rise globally. The aim of this study was to describe the epidemiology of multi-drug resistant *S. sonnei* in Belgium and to identify origins and circulating clusters through WGS. We undertook demographic, temporal, and geographic analysis of 930 *S. sonnei* isolates submitted to the Belgian National Reference Center for *Salmonella* and *Shigella* between 2017-2019. Phylogenetic analysis of WGS data, genotyping and identification of genetic markers of antimicrobial resistance was performed on 372 Belgian isolates submitted between 2013-2019. *S. sonnei* was identified in 75% (930/1253) of Belgian *Shigella* isolates submitted 2017-2019. Overall, 7% (69/930) of isolates were resistant to ciprofloxacin alone, 6% (57/930) showed reduced susceptibility to azithromycin alone and 24% (223/930) exhibited both. Men were at higher risk to carry a double resistant *S. sonnei* strain compared to women (risk ratio 8.6, 95% CI 5.4, 13.9). Phylogenetic analysis revealed 4 independent Belgian clusters of persistently circulating multi-drug resistant

strains associated with MSM, and of the same genotypes as previously described international MSM clades. Belgian isolates carried various Incompatibility (Inc) type plasmids, SpA plasmid, as well as ESBL genes. Based on our results we recommend routine country-wide reporting of Shigellosis cases and submission of isolates to the NRCSS, the integration of questionnaires on sexual behaviour as well as routine WGS in *Shigella* surveillance in Belgium. This will be crucial for future research on risk groups, needed to develop targeted prevention campaigns and guidelines for appropriate clinical and public health management in Belgium. Considering the globally observed rise of resistance to ciprofloxacin and azithromycin, as well as the threat of increased exchange and carriage of ESBL genes, the treatment guidelines for Shigellosis should be re-evaluated and AMR profiling should be employed for improved antibiotic stewardship.

The fellow performed the sample selection, was in charge of analysis of epidemiological data, processing and analysis of sequencing data, prepared a manuscript for peer review (Reference 2), prepared an abstract and oral presentation at the ECCMID conference 2021, prepared and presented the study results to stakeholders of health authorities responsible for sexual health in the three regions of Belgium, presented the study in a lecture for graduate students at the University of Aarhus, Denmark, in 2020. In addition, she used the dataset and code for phylogenetic analysis to contribute to an R training course at Sciensano, as well as a chapter in the R for Epidemiologist Handbook and a webinar on molecular epidemiology organised by PAHO in September 2021.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course – During the three-week course, basic lectures and case studies on epidemiology provided training on study design, sample selection, demographic data analysis, and analytical epidemiology. Other lectures introduced the issues around antimicrobial resistance and sequencing technologies.

Outbreak Investigation Module – During the module, a lecture as well as a case study dealt with the analysis of whole genome sequencing data for public health purposes.

Management, Leadership and Communication in Public Health Module – During this module, communication with different stakeholders and public health authorities was practised.

Project Review Module – During this module, basic presentation and communication skills were taught.

Educational outcome
The fellow gained understanding of the Belgian surveillance system for Shigellosis and the contribution of the NRC. She furthered her knowledge about genetic determinants and the public health impact of AMR, learned about representative sample selection and preparation of clinical isolates for sequencing. She extended her background in bioinformatics workflow for next generation sequencing and available tools to detect different genetic markers of AMR and became proficient in mastering the ggtree() package in R for phylogenetic analysis and interpretation. She gained practice in communicating results and recommendations to public health authorities, as well as to the scientific community.

4. Biorisk management

Training to work in BSL-3 with SARS-CoV-2

Supervisor: Cyril Barbezange

The fellow read through the Sciensano internal manual for BSL-3 laboratory work (Procédures générales de travail en BSL3- 5ème étage, SOP 10/37/F) and followed a two-hour theoretical course on working in a BSL-3 environment, as well as taking a two-hour guided visit to the BSL-3 by trained senior personnel at Sciensano. The training included familiarisation with the facilities, the safe use of personal protective equipment, and decontamination and waste disposal strategies. In addition, she observed a half day of experiment for viral neutralisation assay of SARS-CoV-2 in the BSL-3 with a senior scientist, focusing on planning and preparation of a safe experiment in the BSL-3, handling of the virus, and decontamination.

Update on the Indoor Air Quality Index for Belgium

Supervisor: Berdieke Goemaere

Due to the time spent inside buildings in modern society, indoor pollution has become a public health problem and is associated with respiratory disorders, such as an increase of asthma, dyspnoea, wheezing, cough, or respiratory infections like rhinitis and bronchitis. In collaboration with the Belgian regional authorities, Sciensano's Mycology and Aerobiology division carries out sampling and analysis work on a number of microbiological pollutants (bacteria, mites, moulds) in private (residential) and public places. Samples are analysed by culturing the organisms at different temperatures and media, followed by visual inspection under a microscope or molecular diagnostics such as PCR or mass spectrometry. In light of the lack of national or international standards and guidelines, an index of indoor pollution with airborne fungal spores based on several airborne concentrations percentiles was previously developed by Sciensano. The objectives of the fellow's work were to update the existing indices on air, dust on surfaces, and moulds in mattresses and other fabrics, as well as to create a new index for

the presence of Thermoactinomyces. The fellow therefore analysed data from a database containing results from investigations performed by the Cellule Régionale d'Intervention en Pollution Intérieure (CRIPI) in homes of occupants with health problems in Brussels between 2005 and 2020. The new updated indices will be applied in investigations and allow a comparison of results obtained during a survey with those obtained from a large panel of similar environments. This may help to indicate when a measured airborne fungal concentration should be considered as abnormal and poses a potential health risk for its occupants.

The fellow participated in a field investigating and sample collection of indoor air in an office space in Brussels where she learned about the handling and analysis of samples in the laboratory, including classification by microscopy. She was in charge of data cleaning and analysis in generation of the new updated indices for indoor air quality, as well as new indices for fabrics and dust.

Training modules related to the assignment/projects

Biorisk and Quality Management Module – This module was not taught in c2019 due to the COVID-19 pandemic. The training materials were made available for self study and contained information on biorisk and control management, identification and mitigation of biorisks, biological sample packaging and shipment and WHO guidelines on biosafety management in laboratories.

Educational outcome
The training provided the fellow with a practical understanding of the work environment in BSL-3, especially the correct use of all required personal protection equipment (PPE), the preparation for an experiment in BSL-3 conditions, and the management of biorisk through adequate decontamination and waste disposal. In addition, she learned about the risks of indoor air pollution and the generation and application of quality standards.

5. Quality management

External Quality Assessment for Listeria monocytogenes 2020
Supervisor: *An Van den Bossche*

The fellow reviewed protocols, SOPs and presentations about the ring tests performed at Sciensano for food borne pathogens and produced a slide set to be shared with ECDC.

Training modules related to the assignment/projects

Biorisk and Quality Management Module – This module was not taught in c2019 due to the COVID-19 pandemic. The training materials were made available for self-study and contained information on organisation and performance of external quality assessments, methodologies for quantitative and qualitative test controls including WHO guidelines.

Educational outcome
The fellow became familiarised with the role, performance, and evaluation of external quality assessments.

6. Teaching and pedagogy

Facilitation of case study at the Katholieke Universiteit of Leuven, Belgium

In March 2021, the fellow organised, adapted, and facilitated a case at the Katholieke Universiteit Leuven in the scope of a course for Master students in Biotechnology. This course was included in a module about possible career perspectives and included a presentation of Belgium's public health system and the role of Sciensano (by Pieter-Jan Ceyssens). The case study was adapted from the ECDC case study 'An outbreak of gastro-enteritis in Kalundborg, Denmark', and facilitated by Natalie Fischer, Pieter-Jan Ceyssens, Andreas Hoefer (EUPHEM fellow, Spain), Justine Schaeffer (EUPHEM fellow, Austria), and Heloise Lucaccioni (EPIET fellow, Portugal) in a three-hour online session in five groups of 12 students with the purpose of introducing public health outbreak investigation.

Guest lecture at the University of Aarhus, Denmark

The fellow was invited to give a guest lecture on 'Next-generation sequencing for next generation public health and outbreak investigation – from *Shigella* to Ebola' in the scope of a PhD course in Host-microbe interactions - from basic microbiology and immunology to medicine, 22-24th March 2021 at Aarhus University, Denmark.

Facilitation of a case study for the ECDC fellowship cohort 2020

The fellow facilitated a case study, 'Multidrug resistant *Salmonella* Kentucky scenario exercise', which aimed to introduce fellows to working with sequencing data using different free tools. It took place online on 26th of April

2021 during the intro course week 3 for the ECDC Fellowship cohort 2020. The fellow facilitated a group of 6 participants (five EPIET fellows and one EUPHEM fellow) together with Justine Schaeffer (EUPHEM fellow, Austria) over four hours.

R Introductory Course at Sciensano

The fellow was part of a team of five Sciensano scientist under the lead of Brecht Devleesschauwer, to develop materials and teach an 'Introductory course to R programming' at Sciensano, Belgium. The course comprised 5 sessions of 3h each, with a mixture of lectures and exercises and was held online from 19-30th of April 2021 with 15 participants. The fellow developed material for the visualisation of phylogenetic trees as well as geographic maps and aided in the facilitation of the exercises.

The Epidemiologist R Handbook

The fellow was invited to join The Epidemiology R Handbook project as a co-author and reviewer by the editor Neale Batra in December 2020 (https://epirhandbook.com/). The handbook was a collaborative effort produced by epidemiologists from around the world, drawing upon experiences in local, national, academic, and emergency settings. It is a free, open-source reference manual which provides sample R code and brief tutorials addressing common data management and visualisation tasks with epidemiological examples (Reference 7). The fellow was the lead author of the page on phylogenetic analysis, for which she used her EUPHEM project on surveillance of antimicrobial resistance in *Shigella sonnei* using WGS as an example.

PAHO Molecular Epidemiology Webinar Series

The fellow was invited as an expert to present her work on using WGS for genomic surveillance of AMR in *Shigella sonnei* in Belgium and highlight bioinformatic tools she used for data analysis, in a webinar series for laboratory personnel, laboratory technicians and professionals in epidemiology departments from the Ministries of Health, organised by PAHO in September 2021.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course – During the introductory course, several lectures and exercises focused on teaching, including assessing needs of the audience, setting objectives for training, and facilitation of training in different forms, as well as evaluation techniques and self-reflection.

Project Review Module – During this module, basic presentation and communication skills were taught.

Educational outcome:
The fellow learned how to assess training needs and identify knowledge gaps for an audience of different backgrounds and career levels and to prepare a lecture or adapt case study accordingly. She was involved in the planning of a course as well as the development of training materials. She learned to facilitate a lecture and several case studies in the challenging times of online training and keeping her audience engaged. She became acquainted with creating and analysing evaluation forms.

7. Public health microbiology management

Support of the field team and communication with different internal and external partners during the Ebola outbreak in DRC, 2020

During her mission with the field epidemiology support team at WHO, the fellow participated in three level meetings with the Minister of Health, the WHO regional office and WHO country representative. She performed a literature review, wrote a report, and presented data on phylogenetic analysis of WGS data and engaged in scientific discussions with experts in the use of sequencing data during previous Ebola outbreaks at WHO.

Presentation of results from studies on sexual health in Belgium to regional health authorities

During a meeting with the Flemish expertise centre for sexual health (sensoa), as well as other representatives from regional public health authorities in Belgium the fellow presented results from two studies she conducted during her fellowship. She shared data on prevalence estimation of genital chlamydia and the on antimicrobial resistance in *Shigella sonnei* in Belgium with MSM-association. The presentation was followed by a question session and discussion of recommendations and communication strategies for future campaigns.

Collaboration on study of seroprevalence of anti-SARS-CoV-2 antibodies among healthcare workers in Belgium

The fellow participated in a collaborative project comprising colleagues from the service of epidemiology of infectious disease, immunology, and viral diseases at Sciensano, as well as scientists from the Institute of Tropical Medicine in Antwerp. She participated in weekly progress meetings and discussions, management of safe sample aliquoting, storage and processing in the laboratory, data entry and exchange between partners, and gave input to scientific articles prepared for peer review (Reference 4 & 5). The fellow also participated in bi-weekly WHO/ECDC WebEx calls on COVID-19, serology, and laboratory testing.

Training modules related to the assignment/projects

Management, Leadership and Communication in Public Health Module –This module focused on personality traits relevant to successful collaboration and conflict management, as well as methods for project management and communication with different authorities and stakeholders.

Educational outcome
The fellow further developed her project management skills and learned to adapt communication to different levels of authority and knowledge base. She gained experience in communicating data for action and form recommendations in public health.

8. Communication

Publications related to the EUPHEM fellowship

1. **Fischer N**, Dauby N, Bossuyt N, Reynders M, Gerard M, Lacor P, Daelemans S, Lissoir B, Holemans X, Magerman K, Jouck D, Bourgeois M, Dalaere B, Quoilin S, van Gucht S, Thomas I, Barbezange C, Subissi L. Monitoring of human coronaviruses in Belgian primary care and hospitals, 2015–20: a surveillance study. The Lancet Microbe, ISSN 2666-5247 (2021).

2. **Fischer N**, Maex M, Mattheus W, Van den Bossche A, van Cauteren D, Laisnez V, Hammami N, Ceyssens PJ. Genomic epidemiology of persistently circulating multi-drug resistant Shigella sonnei strains associated with men who have sex with men in Belgium (2013-2019). In revision at the Journal of Antimicrobial Chemotherapy, August 2021.

3. **Fischer N**, Peeters I, Klamer S, Montourcy M, Cuylaerts V, Van Beckhoven D, De Baetselier I, Van der Heyden J, Vanden Berghe W. Prevalence estimates of genital *Chlamydia trachomatis* infection in Belgium - results from two cross-sectional studies. https://doi.org/10.1186/s12879-021-06646-y, BMC Infect Dis 21, 947 (2021).

4. Mortgat L, Verdonck K, Hutse V, Thomas I, Barbezange C, Heyndrickx L, **Fischer N**, Vuylsteke B, Kabouche I, Ariën KK, Desombere I, Duysburgh E. Prevalence and incidence of anti-SARS-CoV-2 antibodies among healthcare workers in Belgian hospitals: a prospective cohort study. BMJ Open (2021).

5. Duysburgh E, Mortgat L, Barbezange C, Dierick K, **Fischer N**, Heyndrickx L, Hutse V, Thomas I, Van Gucht S, Vuylsteke B, Ariën KK, Desombere I. Persistence of IgG response to SARS-CoV-2. Comment, The Lancet Infectious Disease. Volume 21, ISSUE 2, P163-164 (2021).

6. **Fischer N** *et al.* Prevalence and severity of influenza-associated SARI in children admitted to Belgian hospitals during the winter seasons 2011-2020: a surveillance study. In preparation.

7. Batra, Neale, et al. The Epidemiologist R Handbook. 2021. doi/10.5281/zenodo.4752646

Reports

1. Daily and Weekly Epidemiological Bulletin on COVID-19 in Belgium.
2. Outbreak Report on a *Salmonella* Group 07 Braenderup outbreak in Belgium 2021

Conference presentations

1. Oral presentation at the annual meeting of the Belgian Society for Virology (BELVIR) 2020: 'Monitoring of human coronaviruses in Belgian ambulatories and hospitals in 2015-2020: a surveillance study'
2. Oral presentation at the 31st European Congress of Clinical Microbiology & Infectious Diseases (ECCMID) 2021: 'Genomic epidemiology of persistently circulating multidrug-resistant *Shigella sonnei* associated with men who have sex with men in Belgium (2013-2019)'
3. Oral presentation at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2021: 'Prevalence estimates of genital *Chlamydia trachomatis* infection in Belgium – results from a cross-sectional study'

Other presentations

1. Presentation of the results from the chlamydia prevalence study and the genomic surveillance study in Shigella sonnei in Belgium to representatives of the Flemish expertise centre for sexual health (https://www.sensoa.be/sensoa-flemish-expertise-centre-sexual-health, 45 min presentation, 15 min questions and discussion).
2. Presentation to the head of ECDC on behalf of the working group (two EUPHEM fellows from C2018 and two EUPHEM fellows from C2019) on the future of the fellowship program, 13 February 2020 (10 min presentation, 10 min questions).
3. Speaker at the PAHO Molecular Epidemiology Webinar Series: 'Genomic surveillance of antimicrobial resistance in *Shigella sonnei* in Belgium 2013-2019', 28 September 2021 (30 min presentation, 15 min questions).

Other activities

1. Postcard from the field published on the ECDC Website, about my remote experience supporting WHO during the 14th EVD Outbreak in DRC in 2020 (https://www.ecdc.europa.eu/en/news-events/postcard-field-field-mission-my-living-room-supporting-who-epidemiology-team-during).

2. Guest editor for the journal *Pathogens*, special issue on 'New Tools in 3D Host-pathogen Interactions' (https://www.mdpi.com/journal/pathogens/special_issues/New_Tools_Host_pathogen_Interactions).

9. EPIET/EUPHEM modules attended

1. *EPIET/EUPHEM introductory course (23.09-11.10.2019), Spetses, Greece.*
2. *Outbreak investigation module (09.12-13.12.2019), Nicosia, Cyprus.*
3. *Leadership, management and communication module (09.-14.02.2020), Stockholm, Sweden.*
4. *Multivariable analysis module (20.-24.04.2020), Online.*
5. *Project review module (24.-27.08.2020), Online.*
6. *Time series analysis module (25.-29.01.2021), Online.*
7. *Multivariable analysis: cox regression and multilevel analysis (18.03.2021), Online.*
8. *Rapid risk assessment and survey methods (27.04.2021, 05.-06.05.2021), Online.*
9. *Vaccinology module (14.-18.06.2021), Online.*
10. *Project review module (23.-27.08.2021), Online.*

10. Other training

1. Biofire Film Array machine for rapid syndromic diagnostic, one day in-house training with certificate by Biomerieux, (14.11.2019), Sciensano, Belgium.
2. Advanced R Course for Outbreak Investigation, by RECON (25-26.11.2019), Stockholm, Sweden.
3. ESCAIDE Conference 2019 (27.-29.11.2019), Stockholm, Sweden.
4. Webinar and panel discussion with China's most leading experts on COVID-19 (19.03.2020).
5. IANPHI webinar on COVID-19 with Prof. Silvio Brusaferro, President of Italy's National Health Institute (24.03.2020).
6. Estimating the burden of Seasonal Influenza, WHO online course (13-17.04.2020).
7. ISV: Covid-19 vaccine virtual congress (22.06.2020).
8. EWSI Webinar: Intervention strategies for COVID-19, influenza and RSV (23.06.2020)
9. LSHTM Webinar: Vaccine confidence in the time of COVID-19 (22.07.2020).
10. Contact tracing in the context of COVID-19 response, ECDC Online Training (06.08.2020).
11. Incident Management Support Training, WHO GO-Ebola (31.08.2020).
12. UN Course on Prevention of Harassment, Sexual Harassment and Abuse of Authority, (31.08.2020).
13. UN Inter-Agency: To serve with Pride – Zero Tolerance for Sexual Exploitation Abuse by our own staff (31.08.2020).
14. UN Human Rights and Responsibilities (31.08.2020).

15. ESCAIDE conference 2020 (24.11-27.11.2020), online.
16. Annual BELVIR Meeting (18.12.2020), online.
17. UN BSAFE training, online with certificate (16.01.2021).
18. ECDC/WHO webinar on SARS-CoV-2 variants (20.01.2021).
19. Online conference, 'Operational & implementation NTD research: A field perspective' organised by the BNITM (20.01.2021).
20. ECDC Fellowship Think Tank: Covid-19 in children (01.02.2021).
21. Masterclass: Introduction to the Behavioural Science of Public Health Emergency Response (05.02.2021).
22. The 2021 ISV Virtual Congress - COVID-19 Vaccine Update (10.02.2021).
23. WHO webinar: Disinformation, Conspiracy Theories, and COVID-19 Vaccines (19.02.2021).
24. MOOC: Implementation Research (IR) with a focus on Infectious Diseases of Poverty (IDP), 5 week MOOC with certificate by Pasteur Institute of Tunis, Tunisia and the Special Programme for Research and Training in Tropical Diseases (TDR) located at WHO (29.03-10.05.2021).
25. IANPHI COVID-19 Webinar: Role and Challenges of NPHIs in Vaccination (14.04.2021).
26. WHO Webinar: Global Consultation on SARS-CoV-2 Variants of Concern and their Impact on Public Health Interventions (10.06.2021).
27. Introduction to Nanopore sequencing and data analysis workshop, in house training by Oxford Nanopore Technolgies (22-23.06.2021), Sciensano, Belgium.
28. Interdisciplinary Symposium on COVID-19 (VIB Conference) (24.06.2021).
29. ECCMID conference 2021 (09.07.-12.07.2021), online.
30. WHO COVID-19 vaccines research webinar: Can booster doses contribute to control this pandemic: what research is needed? (13.08.2021).
31. Two days laboratory training on WGS of SARS-CoV-2 using Oxford Nanopore MinION sequencing (08.& 09.09.2021)

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 Natalie Fischer during her two-year EUPHEM fellowship (cohort 2019) as an EU-track fellow at Sciensano in Brussels, Belgium. An experienced and resourceful researcher, she applied herself to learning epidemiology with gusto over the past two years. She has become an expert in R coding, contributing to capacity development at her site and for the fellowship. The challenges brought by the Covid-19 pandemic provided an opportunity for Natalie not only to support the control efforts but also to demonstrate her talents as a collaborator and mentor, while at the same time widening her knowledge of public health microbiology. Through dedication and hard work, she has been able to produce an impressive portfolio of projects that reflect her interests and ability to network and collaborate widely. As her front-line coordinator, it has been a pleasure to see her developing further over these two years.

Supervisor's conclusions

It was a true honour to supervise Natalie during her EUPHEM fellowship at Sciensano, which almost completely concurred during the pandemic. For us, it did not take long to understand that she is an exceptional scientist who brought and/or developed an extraordinary capacity for multitasking a wide variety of topics spanning bacteriology, virology, epidemiology, and immunology. COVID-19 condemned her for the largest part of the fellowship to her home office in Antwerp, but this did not hinder Natalie in engaging in intense internal, external, national, and international collaborations, among which were a highly valued WHO mission that included outbreak investigation, surveillance, and phylogenetic studies on Ebola virus.

During the last two years, Natalie's knowledge in (among others) virology and antimicrobial resistance greatly expanded, but her footprint will not only consist of the (high-impact) peer-reviewed publications she authored on these topics. As an example, I will focus on the ggtree R package. Natalie not only picked up and developed her capacity to use this package for her phylogenetic analysis of Shigella sonnei isolates. She also teamed up with our Epidemiological department to set up a training for Sciensano personnel, and she actively reached out to design code (and transfer this knowledge) to the Salmonella team during an investigation of an international outbreak of S. Braenderup.

In my opinion, the EUPHEM fellowship truly succeeded in stirring Natalie from an academic to a public health-oriented mind-set. Her maturity, her holistic view, her openness to collaborations in combination with her scientific skills and natural kindness will serve her well throughout her career. I wish her all the best, and I thank the EUPHEM program for the possibility to work with her.

Personal conclusions of fellow

Over the last two years, the fellowship has enabled me to reach my goals in strengthening and developing several skills, such as analysis of whole genome sequencing data, statistical analysis, multivariable modelling, and analytical epidemiology in R, as well as communication and teaching skills. Furthermore, it broadened my knowledge in topics of great interest to me, such as virology, antimicrobial resistance, emerging diseases, serology, and sexually transmitted diseases. I was able to work with incredible scientists and teams across departments, institutes, and countries. Being a fellow during the pandemic has served as a crash course in so many important topics in public health: epidemic intelligence, risk assessment, surveillance, diagnostics, vaccinology, communication – to name just a few. I am truly thankful for this experience, and for the wonderful network I am now a part of!

Acknowledgements of fellow

I would like to thank my host site supervisors Steven van Gucht and Pieter-Jan Ceyssens for preparing a selection of a variety of projects for the start of my fellowship. I am grateful to Pieter-Jan for stepping up to guide and support me during the chaos of the COVID-19 pandemic and for teaching me about AMR and WGS. My special thanks go to Cyril Barbezange, who made me feel welcome and integrated in the viral disease laboratory, invested time and care into my training, and who became an important mentor, unasked.

I want to thank Javiera Rebolledo Gonzales for allowing a microbiologist to step into the epi world at Sciensano and contribute to the important work around the COVID-19 pandemic in Belgium, as well as all the other supportive colleagues in the crisis team.

Thank you to my frontline coordinator Aura Andreasen for always giving me quick feedback and keeping me on track to achieve the fellowship goals, and to all ECDC coordinators, lecturers, facilitators, and the administrative team for the high quality of training they provided, especially in the difficult times of online training during the pandemic.

I would also like to thank all colleagues with whom I worked within Sciensano and who were always welcoming and open to work with me or let me follow them around and observe, as well as my collaborators at the Institute of Tropical Medicine in Antwerp for giving me the opportunity to collaborate and learn about their work.

I want to thank my supervisor Marie-Amelie Degail Chabrat and teammates during my international assignment at WHO for the amazing and fun teamwork experience and for giving me the opportunity to learn so much in such a short time. I further want to thank Neale Batra for involving me in the creation of The Epidemiology R handbook.

Finally, I would like to thank all the fellows from my own Cohort 2019, but also previous cohorts and alumni, for all constructive exchanges during modules and the welcoming community that they all provided. A special thank you to my predecessor at Sciensano, Lorenzo Subissi, who was always available with advice and feedback and who initiated and guided the project on seasonal coronaviruses.