

The main title 'Summary of work activities' in a bold, white, sans-serif font, set against a blue background.The author's name 'Zsófia Iglói' in a white, sans-serif font, positioned below the main title.The subtitle 'European Public Health Microbiology Training Programme (EUPHEM), 2016 cohort' in a white, sans-serif font, positioned below the author's name.The section header 'Background' in a bold, blue, sans-serif font.

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

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

According to Articles 5 and 9 of ECDC's founding regulation (EC No 851/2004) 'the Centre shall, encourage cooperation between expert and reference laboratories, foster the development of sufficient capacity within the community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health' and 'as appropriate, support and coordinate training programmes in order to assist Member States and the Commission to have sufficient numbers of trained specialists, in particular in epidemiological surveillance and field investigations, and to have a capability to define health measures to control disease outbreaks'. Moreover, Article 47 of the Lisbon Treaty states that 'Member States shall, within the framework of a joint programme, encourage the exchange of young workers'. Therefore, ECDC initiated the two-year EUPHEM training programme in 2008. EUPHEM is closely linked to the European Programme for Intervention Epidemiology Training (EPIET). Both EUPHEM and EPIET are considered 'specialist pathways' of the two-year ECDC fellowship programme for applied disease prevention and control.

This report summarises the work activities undertaken by Zsófia Iglói, cohort 2016 of the European Public Health Microbiology Training Programme (EUPHEM) at the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

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.

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Pre-fellowship short biography

Zsófia completed her university education in biology at the University of Szeged in Hungary in 2007 and moved to the UK to undertake a PhD at the University of Leeds working on molecular and cellular aspects of hepatitis C virus. She followed this up with a postdoctoral fellowship remaining in the same laboratory. In 2015-16 she spent three months with Public Health England in the ebola affected Sierra Leone as part of the diagnostic laboratory team. The following year she spent four months in Brazil as part of an ongoing collaboration testing natural compounds against chikungunya and hepatitis C virus.

Methods

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

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

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

Results

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

1. Epidemiological investigations

1.1. Outbreak investigations

Supervisor: Lapo Mughini Gras

Outbreak of *Salmonella* Typhimurium MLVA type 3-12-9-0-211 in the Netherlands, 2017

A significant increase ($p < 0.001$), compared to the same period last years, of *S. Typhimurium* (monophasic variant) with MLVA type 3-12-9-0-211 from the north east part of the Netherlands involving three municipalities was detected in the national *Salmonella* surveillance system. The RIVM was asked to take the lead in the investigation and an outbreak investigation team was formed. A general *Salmonella* questionnaire was modified to suit the food items and activities typical for this serotype and sent out to the patients. Median age was 26 years, 50% female and suspected food items were meat products purchased in certain supermarket chains. There was no travel history amongst the cases. The source of the outbreak could not be identified and the outbreak resolved naturally. This is often the case with foodborne outbreaks as the food item will be out of stock and consumed normally within a relatively short timeframe. Although there are several *Salmonella* outbreaks every year, the cause of the outbreak varies. By investigating each event we gather information and evidence which can be used during the next outbreak. Fellow was part of the outbreak team, entered the questionnaires and analysed the data with statistical software.

Supervisor: Harry Venema

Molecular-epidemiological tracing of hepatitis A virus using nucleotide variants of three strains in the 2016-2018 outbreak amongst MSM in six European countries

Between June 2016 and March 2018, 22 EU/EEA countries reported hepatitis A virus (HAV) genotype IA and sequences with $\geq 99.3\%$ homology to one of three outbreak strains (named Event 1, 2, 3), mostly amongst men who have sex with men (MSM). Focusing on the sequences with $< 100\%$ homology, we gathered molecular information

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

of the circulating strains in order to see the extent of sequence diversity and track cross border transmission. Data used was gathered through contacting experts in countries involved in the outbreak (Belgium, France, Germany, Italy, Netherlands, Portugal) through HAVNET and EPIS. VP1/2A region sequences (n=829, 21.1% of the total 3,924 sequenced) were compared using alignment and unweighted pair group method with arithmetic mean to detect clustering using BioNumerics 7.6.2. Country of isolation and date of sampling/diagnosis were also collected and used to prepare epidemiological curve. Outbreak strains Event 1 and 2 (n=404 and n=395) were more widespread than strain Event 3 (n=30) and the numbers of variants were directly proportional to their prevalence. Up to the date of this summary, 26 variant sequences amongst Event 1, and 11 amongst Event 2 could be identified, of these 22 were isolated multiple times across countries. Based on the timeline of the incubation period, epidemiological curves show both continuous and one-to-one transmissions. HAV has a low mutation rate therefore the presence of this many variants is probably due to the high incidence and the duration of the outbreak. Re-occurrence of the same variant strongly suggests a link by transmission. This data can contribute to the understanding of the generation of HAV variants and can help in tracing transmissions when epidemiological information is lacking, however we believe that complementing molecular data with epidemiological information and vice versa (molecular-epidemiology) is the way forward in the future. Furthermore, this study highlighted the importance of harmonized typing methods. Fellow was responsible for contacting experts and sending periodical updates of the analysis, selected best method for visualising data, analysed the data and wrote the manuscript.

Outbreak of hepatitis A in South-West Netherlands, possibly food related

There were 8 confirmed cases of hepatitis A (strain IA, VRD_521_2016) with a first day of illness at weeks 12 and 13 in 2018 in the South Holland region of the Netherlands. What made the situation more complicated was the fact that this strain was one of the three circulating strains in the large hepatitis A outbreak amongst men who have sex with men (MSM) in Europe, during 2016-2018. However, cases in this current outbreak in the South Holland region were majority not MSM. The female to male ratio was 2.6, median age 48 years and the cases did not cluster by place. The outbreak was suspected to be food related, point source based on the shape of the epi curve and based on the suspected index case who was MSM and worked in a food production facility. Unfortunately the questionnaires did not show a clear food source and the suspected index couldn't be confirmed. He stated that he did not work in production while ill, but of course the virus can be transferred in other ways (eg. use of the same toilet). However the outbreak resolved without source identification, it highlighted the importance of surveillance and typing and the combination of this information. There were several spin-off outbreaks like this where conventional typing methods could link the cases to the same outbreak but like in our case, to be able to trace the source/index case with 100% confidence, typing by whole genome sequencing (WGS) should have been used. This would have been the next step if more cases would have been detected. Fellow was part of the outbreak team, analysed the data, mapped the patients location and prepared epi curve.

Training modules

The EPIET/EUPHEM introductory course provided participants with the basic concepts of logistical, microbiological and analytical approach to outbreak investigations, including the ten steps of an outbreak investigation.

The module, outbreak investigations, taught fellows how to perform analytical epidemiological studies within outbreak investigations using various software packages. During the module fellows performed all steps involved in outbreak analysis from creation of a data entry file to the stratified analysis using EpiData, Stata and Microsoft Excel. They were also given practical training in when and how to perform analytical studies for an outbreak investigation, including descriptive, cohort and case-control studies and how to write an outbreak report. A brief session on phylogeny also provided the basics in this technique.

The multivariable analysis module provided a more comprehensive understanding of the principles of statistical analyses, and how to build an optimal model using linear, logistic, Poisson and Cox regression in STATA.)

The rapid assessment module, provided the principles of intervention epidemiology and touched upon the roles and responsibilities on a public health laboratory in outbreak situations. Fellows produced a rapid risk assessment with guidance from experts and got familiar with GIS software.

Project review module taught how to communicate results and give feedback and how to write good quality scientific paper.

Educational outcome: The Fellow was able to apply microbiological and epidemiological knowledge in outbreak investigations, considered the uses and limitations of diagnostic and typing methods and their interpretation in outbreak investigation, participated in multidisciplinary outbreak team (involved in case definition generation, data collection, data analysis, laboratory typing and communication), appreciated the roles and responsibilities of the involved parties (municipal health services, RIVM, ECDC, reference laboratories and specialised infectious disease networks), and communicated with the European network of hepatitis A to facilitate data collection and writing a scientific article.

1.2. Surveillance

Supervisors: Leo Schouls, Thijs Bosch

Whole genome sequencing of carbapenemase producing *Acinetobacter baumannii* isolates in the Netherlands 2015-2017

Acinetobacter baumannii is an important nosocomial pathogen that can develop multidrug resistance, from which the most alarming is the last line antibiotic carbapenem. Currently in the Netherlands *A. baumannii* is part of the national antimicrobial resistance (AMR) surveillance but not of the molecular-surveillance system of carbapenemase producing microorganisms, hence there is no information beyond species and the limited resistance profile of the circulating strains. In this study we subjected 80 carbapenem resistant *A. baumannii* strains, isolated between 2015-2017 in the Netherlands to whole genome sequencing in order to characterise the circulating strains, determine resistome and detect transmission events especially in healthcare facilities. Besides a wide variety of carbapenemase encoding OXA genes, all isolates contained one of the types of the novel cephalosporinase-encoding gene *bla*ADC. We found a genetically very diverse group of isolates based on wgMLST analysis and no clustering with available whole genome sequences on NCBI. Eight clusters were identified by wgMLST and two was a result of possible ongoing transmission. The genetic diversity of the isolates points to slow but continuous introduction of new highly resistance strains in the Netherlands. Given the small numbers and cases with non-severe outcomes, we do not find it necessary to uptake *A. baumannii* into the national molecular surveillance. The available epidemiological information was incomplete for most patients, and hence this information alongside with WGS data is key in understanding origin, length of carriage and linking strains in an outbreak, quality of collected data should be improved. Fellow was responsible for the assembly and analysis of raw sequence data using several appropriate softwares in order to investigate the carried AMR genes and clustering of isolates, analysis of epidemiological data and writing the manuscript.

Supervisor: Titia Kortbeek

Voluntary laboratory surveillance monitoring of *Cryptosporidium* and data analysis 2013-2017

Cryptosporidiosis is not a notifiable disease in the Netherlands, it is part of a voluntary surveillance system. Participating laboratories send in samples for further typing to the RIVM which are entered into a database. The objectives of this project were to monitor the submission numbers and trends for the 2013-2017 period. Furthermore, in order to provide an update and feedback to the participating laboratories about their performance, a descriptive and statistical analysis of the data was performed and a newsletter prepared. Data was analysed as a whole and per laboratory. The results briefly: in total 1,549 samples were submitted from four laboratories with a 65% success rate of typing. Majority of patients were female (46%) and agegroups 0-5, 6-12 and 26-50 were most affected. The proportion of the two most common species (*C. parvum* and *C. hominis*) varied over the years, but this was noticed earlier. This analysis is communicated to the involved parties in order to ask and give feedback on performance and to give an overview of the number of tested samples by other laboratories thereby also encouraging cooperation and collaboration. Reports like this are crucial in maintaining and improving the surveillance. As fellow worked on other *Cryptosporidium* projects, she had thorough understanding of the data and the database. She carried out the analysis and prepared text and figures for the news letter.

Supervisor: Ingrid Friesema

The effect of STEC notification criteria and diagnostic method changes over time on the number and type of the reported cases in the Netherlands, 1999-2017

The aim of this project was to describe the effects of notification criteria changes using the data from the national STEC surveillance. In the Netherlands, the STEC surveillance was initiated in 1999 and expanded in 2007 from O157 only to all STEC serotypes, concurrent with the introduction of PCR techniques besides the existing cultures. However previous analyses of the available data showed that a large part of the reported cases had no or only mild symptoms and/or had these symptoms for a long period. Therefore in July 2016, the notification criteria were narrowed to acute, more severe, STEC infections. Overall these changes resulted in fluctuation in numbers, with 388 cases/year for the whole period on average (range varies from 32 to 1173/year) and 54, 656 and 466 cases respective of first, second or third period of notification on average. The changes in reporting also presented a challenge and extra workload for all involved parties (GP, hospitals, laboratories, municipal health services) especially in the second notification period which resulted in delay in reporting, more missing demographic or symptoms data and more untyped samples. However less delay in the first and third period can also be explained by mainly acute disease reported with shorter and clearer course of the disease. Furthermore, there were less samples in general and in the first period only O157 had to be reported. In conclusion, the moderate/severe acute cases are the most relevant for public health and the latest criteria change seems to be capturing these. Other

countries could also adapt the same model. Fellow was responsible for data analysis which will be incorporated in the comprehensive analysis of the surveillance system. Fellow also spent time in the STEC reference laboratory and understanding the typing practices helped with the data interpretation considerably.

Training modules

The EPIET/EUPHEM introductory course included sessions on surveillance system development and evaluation. Several modules (eg. introductory course, outbreak investigations, rapid assessment) highlighted the role of public health laboratory in disease surveillance.

The multivariable analysis module provided a more comprehensive understanding of the principles of statistical analyses.

The rapid assessment module taught various surveillance and sampling techniques and included a field exercise with mobile tool and GPS.

Educational outcome: Fellow gained substantial experience in evaluation of surveillance data - both epidemiological and molecular, gained solid understanding of the strengths and weaknesses of surveillance systems, she experienced the uses and limitations of diagnostic and typing methods and their interpretation in surveillance systems evaluation and understood the role of laboratories in surveillance. She applied the principles and the basic algorithms for the evaluation of surveillance systems and their crucial parameters. She analysed whole-genome sequencing data and used molecular typing methods in reference laboratories for specific pathogens. Fellow wrote several reports, scientific papers and formulated public health recommendations.

2. Applied public health microbiology research

Supervisors: Gerard de Vries, Dick van Soolingen

Quantifying tuberculosis transmission between Netherlands-born and foreign populations in the Netherlands using variable number tandem repeat (VNTR) typing between 2009-2017

In the Netherlands TB incidence and epidemiology is strongly influenced by migration from high-endemic areas (29.7 vs. 1.3/100.000 TB incidence amongst foreign-born vs. Netherlands-born in 2017) and it is further characterised by a low rate of transmission with occasional outbreaks. The aim of this study was to investigate transmission between Netherlands-born and foreign-born populations in order to investigate whether the high number of foreign-born patient represent a risk for transmission to general population. We included in our study pulmonary TB patients with *Mycobacterium tuberculosis* infection. Clusters were defined if strains had 100% identical VNTR types, the index case occurred in 2009-2015 and their secondary cases followed within two years in order to restrict to recent transmissions. The direction and amount of transmission was quantified based on date of sampling and country of birth (referred to as Netherlands-born or foreign-born). 8,392 TB patients were notified in 2009-2017; 6,153 (73%) were foreign-born; 5,757 (69%) had VNTR typing results and 2,801 (49%) had unique fingerprints. 458 patients met our inclusion criteria (165 new clusters). Transmission between groups was comparable and bidirectional: 21.8% of cases resulted from Netherlands-born to foreign and 27.4% from foreign-Netherlands-born transmission. Transmission index was similar for Netherlands-born and foreign-born indexes (1.95 and 1.72). Due to the majority of foreign-born patients 50.8% of the Netherlands-born secondary cases presumably were infected by a foreign-born TB patient. Transmission from foreign-born patients to the Netherlands-born population is relevant in a low-incidence country, due to the high proportion of foreign-born patients. However, transmission occurred as frequent from a Netherlands-born patient to a foreign-born person, as vice versa. The fear that TB control and elimination are greatly hampered by transmission from the foreign to the Netherlands-born population and that migration strongly influences the TB burden among existing residents of the countries is not supported by the available evidence. We recommend to stay vigilant with TB control independently of origin of the patient and in order to further decrease incidence amongst foreign population, possibilities of diagnosis and treatment of LTBI should be further explored. Fellow was the leader of the project, requested data from the National TB registry and choose appropriate methodology for analysis. She consulted numerous experts, wrote an abstract for a conference presentations and a manuscript.

Supervisor: Kim Benschop

Seroprevalence study in the general population to determine the potential threat of Echovirus 6 associated outbreaks in the Netherlands

In Europe in 2016, an increase in the number of non-Polio Enteroviruses (EV) associated with severe disease was reported. The Dutch molecular virus surveillance platform (VIRO-TypeNed) also reported a small outbreak that was associated with a new E6 (Echovirus 6) strain (12% of all reported and typed EV infections). To investigate whether the E6 increase was related to an immunity gap due to the emergence of a new strain to which the

population is not immune, we measured the neutralizing activity of intravenous immunoglobulin (IVIg) batches obtained from the general Dutch population against 25 E6 isolates of representative subgenogroups (C1 and C9). E6 outbreaks were identified in 2000, 2009, and 2016 therefore we collected IVIg batches from the years 2005, 2010 and 2016. The expected patterns of protection was seen in the neutralization assays with variation depending on the year of the IVIg and on the virus strain. This project also contributed to a follow-up study where a wide panel of EV isolates were tested against IVIg from 2010 and 2017. They found a good agreement of neutralisation against most EV subtypes with the viruses frequently detected by molecular surveillance. For the lack or no increase in neutralisation between years, one explanation may lie in different viruses targeted by genotypic and serological surveillances. Since genotypic data is obtained mostly from hospitalized patients, virulent viruses are detected more often compared to less virulent subtypes. This might make the prevalence data based on genotyping data mainly reliable for virulent viruses, but to a lesser extent for non-virulent viruses. This and the follow up project together will contribute to better understanding of EV circulation and will lead to improved treatment options. The project was carried out at another research institute. Fellow selected the EV strains, cultured them and carried out the neutralisations assay. Phylogeny of isolates using WGS data and data analysis of the results from the neutralisation assays were integral part of the project.

Supervisors: Titia Kortbeek and Lapo Mughini Gras

Long-term sequelae of sporadic cryptosporidiosis: a follow-up study

To determine the frequency of occurrence of sequelae following cryptosporidiosis a follow-up study was performed during a case-control study for sporadic cryptosporidiosis in the Netherlands (2013-2016). Cryptosporidiosis cases were invited to complete a follow-up questionnaire four months after diagnosis. Using a case-crossover study design, we compared the frequencies of reported symptoms four months after the acute phase to those reported four months before the onset of illness and during illness. Frequencies of symptoms in the pre- to post-infection phases were also compared with those of a population control group. *Cryptosporidium* species-specific effects were also studied. Logistic regression was used to calculate adjusted odds ratios (aOR) for symptoms occurrence. Of 731 available cases, 443 (60%) responded and 308 (42%) could be included in the follow-up study. Median age was 26 years (range 1-80); 58% were female; 30% were infected with *C. hominis* and 70% with *C. parvum*. Compared to before illness, cases were significantly more likely to report dizziness (OR=2.25), headache (OR=2.15), fatigue (OR=2.04), weight loss (OR=1.82), diarrhoea (OR=1.50), abdominal pain (OR=1.38) or joint pain (OR=1.84). However, symptoms of joint pain and headache occurred among cases after illness at a rate that was not significantly different from that observed in the general population. There were no significant differences in post-infection symptom occurrence between *C. hominis* and *C. parvum*. The disease burden of cryptosporidiosis extends beyond the acute phase of the infection, with cases reporting both intestinal and extra-intestinal symptoms up to four months following infection. This study adds to the growing body of evidence for the presence of sequelae following cryptosporidiosis, so far represented by four published studies. Although our results do not change the general advice for patient care, awareness of medical personnel should be raised that non-gastrointestinal symptoms can be the onsequence of enteric infection. As there is no treatment for cryptosporidiosis, the focus should be on preventive measures. Fellow was responsible for the data analysis from merging and cleaning the data through selecting the most appropriate epidemiological analysis and writing the manuscript.

Training modules

The EPIET/EUPHEM introductory course provided participants with the basics of project planning and writing a research proposal.

The project review module thought qualitative research methods.

The multivariable analysis module provided the principles of statistical analyses.

Educational outcome: Fellow learnt to lead and conduct all stages of a research project, from developing ideas, planning and writing detailed study protocol to completion of project and scientific papers. She interacted with a wide range of people from various institutes and disciplinary backgrounds. Work was also presented at the ESCAIDE.

3. Applied public health microbiology and laboratory investigations

Supervisors: Kristin Kremer, Daan W. Notermans

Validation of a two-tier diagnostic testing for *Francisella tularensis* ssp. in the Netherlands

After six decades of absence, tularemia re-emerged in the Netherlands since 2011 and as of November 1st 2016, the disease is reportable. Therefore, we wanted to validate tularemia serological diagnostics for future

implementation in order to increase the diagnostic capacity at RIVM. A commercial enzyme-linked immunosorbent assay (ELISA) with an in-house confirmatory western blot (WB) were selected based on specificity, sensitivity, and throughput. Specimens included positive controls, previously positive tested tularemia sera from Sweden and the Netherlands (n=27); negative controls, sera from blood donors from before 2011 (n=10); specimens from Dutch patients infected with diseases with symptoms similar to tularemia (EBV, CMV, Bartonella (n=10 each) and Toxocara, Ascaris (n=5 each)) and two cross-reactivity controls, positive Brucella sera. All samples were tested by ELISA and WB. Samples were considered borderline if the results were within 20% of the pre-determined cut-off. The results showed that all positive controls were detected by both ELISA and WB and all negative controls were negative by ELISA and WB. All but one specimen from diseases similar to tularemia were found to be ELISA and WB negative. In conclusion, the combination of the ELISA with WB enables specific (98.44%) and sensitive (100%) detection of tularemia and is suitable for routine diagnostic and larger seroprevalence studies. In daily practice, only ELISA positive samples will have to be confirmed by WB. Until now testing was only offered at one location in the Netherlands. Tularemia testing now is available at the RIVM by this improving patient care. Fellow was responsible for researching possible new methods, sample acquisition and for consulting experts. She carried out the experiments and prepared SOP and validation reports.

Laboratory rotations: identification and characterisation of pathogens in bacteriology and virology

Fellow did two types of rotation, visiting different types of laboratories to observe work and time spent in laboratories for the duration of the projects. Fellow spent one or more days at several different laboratories including: a local hospital laboratory, Wageningen biovetenary facility, STD clinic, *Salmonella* reference laboratory, MRSA and carbapenem resistant *Enterobacteraceae* reference laboratory, medical virology laboratory at the Amsterdam medical centre. During each project fellow was introduced to procedures and methods used in the laboratory to identify and characterise various pathogens. The activities included observation and execution of methods such as: isolation/identification techniques for virus and bacteria, serotyping, neutralisation assay, MLST, MLVA, antibiotic resistance profiling, PCR, whole genome sequencing, ELISA, microagglutination assay, western blot and many more techniques.

Training modules

The EPIET/EUPHEM introductory course provided an overview of laboratory diagnostic methods. Biorisk and quality management module provided training on techniques for biorisk and biosafety assessment and mitigation.

Educational outcome: Fellow learnt to apply basic concepts of virology, bacteriology, parasitology and immunology in relation to different diagnostic tests, public health disciplines and translate it to advice. She developed a novel test and identified the use and limitation of diagnostic and typing methods and their interpretation in patient diagnosis, outbreak investigation, surveillance and epidemiological studies.

4. Biorisk management

Biorisk and quality management module

The objective of the module was to provide training on three related topics: quality management in biomedical laboratories according to ISO 15189 norm; training for international shipping of infectious substances; biosafety management in biomedical laboratories according to WHO guidelines. In each session the content was presented first then discussed as part of a group exercise. During the module the BLS-4 laboratory at the Public Health agency of Sweden was visited. Fellow has extensive previous experience working with high containment pathogens working in category 3 facilities.

Educational outcome: Fellow familiarized herself with national, European and WHO rules and regulations regarding biosafety and biosecurity, she learnt and experienced the principles and practices of biorisk management, biorisk assessment and mitigation. She obtained a certification on international shipping of infectious substances from WHO.

5. Quality management

Supervisor: Titia Kortbeek, Kristin Kremer, Daan W. Notermans

Preparation of SOP and validation report for *Francisella tularensis* ssp. diagnostic

SOPs and validation reports for the "Validation of a two-tier diagnostic test for *Francisella tularensis* ssp. in the Netherlands" project (described under point 3) were prepared by the fellow in order to facilitate the implementation of the test at the RIVM. Tularemia diagnostic is only being offered at one location in the

Netherlands and the test developed by us offers better sensitivity and specificity therefore more reliable outcome for the patients.

Supervisor: Hannelore Gotz

Evaluation of the usefulness of the Alere HIV combo point-of-care test at the Rotterdam sexual health clinic.

HIV has a low incidence in the Netherlands with the highest number of cases amongst MSM. For HIV patients, at the Rotterdam sexual health clinic, an opt-in rapid testing is performed (thus clients have to accept the rapid test). MSM, those notified for HIV and other high risk patients are offered rapid testing at first consultation followed up by laboratory testing. The aim of this study was to assess the rapid test performance in terms of usage frequency, correlation (if any) between using the test and waiting time from initial diagnosis to treatment and agreement of rapid test results with laboratory HIV tests. We found that the test was used in only 48% of consultations. The results of the rapid test and the laboratory test were in concordance in all but one case where the outcome was non-conclusive due to control error. We did find shortened waiting time for laboratory confirmation or treatment when test was performed however it was not statistically significant probably due to small sample size. Having an almost immediate answer instead of having to wait approximately one week for the results is important for both the physical and the psychological wellbeing of the patient. It could significantly improve the treatment outcome for the patient and also for any recent sexual partners as they still might fall within the time period suitable for post-exposure prophylaxis (PEP) treatment. Furthermore, positive test probably results in decreased risk behaviour thus reducing further transmissions. Given the low price and ease of use we recommend to make rapid testing part of normal testing procedures at the Rotterdam sexual health clinic for high risk patients (including in person or online consultations) to improve quality of care. Fellow participated in patient consultations and followed the procedures in order to understand the information flow and the potential issues around test acceptance. She analysed the data and presented the results for the medical staff to prompt discussion about how to address the issues around the low acceptance. Both logistical and patient well being concerns were discussed.

Supervisor: Titia Kortbeek

Observer at external audit at RIVM

National Institute for Public Health and the Environment (RIVM) as any institute offering patient diagnostic has to comply to certain standards. The assessment concerned the diagnostics according to the specified scope. The centre for infectious disease research, diagnostic and laboratory surveillance (IDS) has adapted the management system to NEN-EN-ISO 15189: 2012 and prepared the quality manual that follows the classification of this standard.

This audit was a follow up on the audit in 2016 therefore this time only the laboratories/topics where shortcomings were noted were re-visited. Fellow familiarized herself with the documents and reports from the previous year prior to the audit and consulted involved personell in order to understand the nature of the shortcomings and actions taken to solve the problems. On the day of the audit, the procedures of the external audit were observed.

Training modules

The biorisk and quality management module included lectures and group discussions on quality management in biomedical laboratories according to ISO 15189 norm.

Educational outcome: Fellow became familiar with different quality management systems and evaluation procedures. She prepared SOP and validation/verification report in preparation to implement a new diagnostic assay. Workflow at a sexual health clinic was evaluated to inform patient treatment policy.

6. Teaching and pedagogy

Organisation of "Infection and Immunity" one-day course at RIVM

Fellow actively participated in the organisation of a one day course at the RIVM. This is a recurrent event, part of an annual 4 week summer course for master students on Infection and Immunity. Fellow was the main contact between the main organiser of the course and the speakers and other involved parties at RIVM and was involved in the decision about the composition of the scientific program. Also, a case study was developed (see below). Students could learn about the roles and responsibilities and ongoing research at the RIVM and through two activities the process of decision making was also demonstrated. Thus a broad overview of the roles and responsibilities of the RIVM was covered. Fellow assessed the course based on the feedback from previous year by looking at the comments from students and changes where necessary were implemented. Students wanted more specific example on public health research and more activities. As the feedback form for this day at the RIVM was too short, a more extensive feedback for each activity with both scale and free text option was included in the course evaluation to capture the details for further improvements for next year's course.

Preparation of a case study on “early warning and response procedures”

The case study was prepared as part of the Infection and Immunity summer course. It is an interactive exercise set up to demonstrate how infectious disease incidents are handled at the national level. Participants play the role of one of the members of the early warning and response committee, who in real life are representatives of all departments of the centre for infectious disease control at RIVM and the Dutch Food Safety Authority (NVWA) and any other relevant person like representatives from the municipal health services. The committee is responsible for verifying the signal by discussing the facts and bringing any extra information to the group to debate. Working together as a team participants have to come up with a plan and approaches how to deal with the incoming information. A recent hepatitis A outbreak in the Netherlands was used as an example.

Facilitation of the “Salmonella in the Caribbean” case study.

Fellow facilitated the case study for a group of bioscience undergraduate students at Nijmegen University in November 2017. Fellow put extra emphasis on the role of laboratory surveillance and typing.

Facilitation of the “Giardia in Bergen” case study.

Fellow facilitated the case study for a group of epidemiology master students at Utrecht Medical Centre in June 2017 as part of the Epi Summer course.

Educational outcome: The fellow evaluated the feedback of a course and introduced changes accordingly; she was involved in the planning of the content and in the identification of educational needs of the target group. She developed a case study in collaboration with epidemiologists and advised on content of lectures based on educational needs. She facilitated case studies for students with diverse backgrounds about the importance of public health microbiology and the collaboration of epidemiology.

7. Public health microbiology management

Management during projects and outbreak investigations

In all projects the fellow needed to communicate with experts from various disciplines to effectively progress and finish the projects. She learned to plan, coordinate and lead projects and communicate with involved people who often were more senior or people from various authorities. For example, during the *Salmonella* outbreak, she was part of the outbreak team and communicated with epidemiologists and the food safety authority and represented the link between the laboratory and other disciplines. During the hepatitis A outbreak she participated in an ECDC teleconference and frequently communicated with other members of the HAV network from across Europe.

Training modules

Management, Leadership and Communication in Public Health module, ECDC, Stockholm, Sweden

The module provided educational outcomes such as describing and understanding the role of different EU agencies, the role and responsibilities of leaders and managers within a public health environment, different management styles, roles and team evolution and how it can inform team success, how to motivate teams to improve performance and generate loyalty and commitment, effective task delegation, providing structured feedback.

There were several opportunities to deliver written and oral communication (such as meetings, discussions, presentations, reports and publications) with people from diverse, multidisciplinary and multicultural backgrounds. Engaging in and experiencing a debate with the Director and the chief microbiologist at ECDC was the main challenge of the week.

8. Communication

Publications

1. Iglói Z, Mughini-Gras L, Nic Lochlainn L, Barrasa A, Sane J, Mooij S, Schimmer B, Roelfsema J, van Pelt W, Kortbeek T. Long-term sequelae of sporadic cryptosporidiosis: a follow-up study. *Eur J Clin Microbiol Infect Dis.* 2018 Jul;37(7):1377-1384. doi.org/10.1007/s10096-018-3268-9

2. Fanoy E, Pattipeilohy J, Braks M, Morroy G, van Drunen K, Iglói Zs, van de Water J, Sigurdsson V, Wintermans B. Added value of scabies PCR in outbreaks in healthcare institutions. *Infectieziekten Bulletin*, 2017 Nummer 10, Jaargang 28:391-396.
3. Zsófia Iglói, Dick van Soolingen, Gerard de Vries. Quantifying tuberculosis transmission between native and foreign populations in the Netherlands between 2009 and 2017 using variable number tandem repeat (VNTR) typing (in preparation).
4. Zsófia Iglói, Lieke Wielders, Sandra Witteveen, Marga van Santen, Thijs Bosch, Leo Schouls. Genetically diverse carbapenemase producing *Acinetobacter baumannii* strains harbouring a wide range of antibacterial resistance genes are present in the Netherlands. Results of a two year molecular surveillance (2015-2017) (in preparation).
5. Zsófia Iglói, R. Bruni, AR. Ciccaglione, SL Ngui, AM. Roque-Afonso, R. Sousa, L. Subissi, J. Wenzel, H. Venema. Molecular-epidemiological tracing of hepatitis A virus strains using nucleotide variants of three strains in the 2016-2018 outbreak amongst MSM in seven European countries (in preparation).

Reports

1. Newsletter on continuous *Cryptosporidium* surveillance in the Netherlands 2016-2017.
2. SOP of *Francisella tularensis* ssp. *holarctica* antibody detection using immunoblot (IgG).
3. SOP of *Francisella tularensis* ssp. *holarctica* antibody detection using SERION ELISA classic *Francisella tularensis* IgG/IgM.
4. Validation report for *Francisella tularensis* ssp. *holarctica* antigen detection with SERION ELISA classic *Francisella tularensis* IgG/IgM.
5. Validation report for *Francisella tularensis* ssp. *holarctica* antigen detection with IgG immunoblot assay.
6. Outbreak report of Salmonella Typhimurium (MLVA type 3-12-9-0-211) outbreak in the Netherlands in 2017.
7. Outbreak report of possibly food related hepatitis A outbreak in South-West of the Netherlands in 2018.

Conference presentations

1. Iglói Zs, Kremer K, Kroese M. V, Kortbeek T, Notermans D. W. Validation of a two-tier diagnostic testing for *Francisella tularensis* ssp. in the Netherlands. Poster presentation at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2017) congress, Stockholm, Sweden, 6-8 November 2017.
2. Iglói Zs, Nic Lochlainn L, Barrasa A, Sane J, Mooij S, Mughini-Gras L, Schimmer B, Roelfsema J, van Pelt W, Kortbeek T. Investigation of long-term sequelae among sporadic cryptosporidiosis cases in the Netherlands, 2013-2016. Poster presentation at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2017) congress, Stockholm, Sweden, 6-8 November 2017.
3. Zsófia Iglói, Gerard de Vries, Dick van Soolingen. Quantifying tuberculosis transmission between native and foreign populations in the Netherlands between 2009 and 2017 using variable number tandem repeat (VNTR) typing. Late breaker poster presentation at The TB Science pre-conference, Den Hague, The Netherlands, 23-24 October 2018 (accepted).

Other presentations

1. Zsófia Iglói. Long-term sequelae of sporadic cryptosporidiosis: a follow-up study. Presented at the RIVM and at the multivariate analysis module module, March 2017 and September 2018.
2. Zsófia Iglói. The ebola outbreak from the laboratory perspective. Presented to undergraduate students in Nijmegen, November 2017.
3. Zsófia Iglói. Capacity and capability of national laboratories in the Netherlands in regards of WGS. EUPHEM module: Initial management in public health microbiology, ECDC, February 2018, Stockholm.
4. Zsófia Iglói. Tuberculosis transmission between foreign- and native populations in the Netherlands 2009-2017. Presented to involved parties at RIVM, June 2018.
5. Zsófia Iglói. Results of the evaluation of the usefulness of the Alere HIV combo point-of-care test at the Rotterdam sexual health clinic. Presented to medical staff and nurses at the Rotterdam sexual health clinic, July 2018.

Other

1. Qualitative Public Health Risk Assessment of the emergence of Seoul virus in the Netherlands 2017, RIVM.
2. Study protocol on antimicrobial efficacy of lens care solutions against *Fusarium spp.* and *Acanthamoeba spp.* in the presence and absence of contact lenses and lens cases.

3. Cohort representative 2017-18. Initiated and participated in teleconferences with EAN and ECDC scientific coordinators, organised several social activities and with the other cohort representatives conducted a survey amongst fellows.

Training modules

Initial management in public health microbiology, ECDC, February 2018, Stockholm, Sweden.

9. EPIET/EUPHEM modules attended

1. EPIET/EUPHEM introductory course, Spetses, Greece (15 days)
2. Outbreak investigation module, Berlin, Germany (5 days)
3. Multivariate analysis module, Zagreb, Croatia (5 days)
4. Rapid assessment of complex emergency situations, Athens Greece (5 days)
5. Project review module 2017, Lisbon, Portugal (5 days)
6. Biorisk and quality management, Stockholm, Sweden (5 days)
7. Initial management in public health microbiology, Stockholm, Sweden (5 days)
8. Project review module 2018, Lisbon, Portugal (5 days)

10. Other training

1. Online course on vaccinology provided by Institut Pasteur/MOOC
2. Online course on basic and advanced security in the field provided by UNDSS

Discussion

Coordinator's conclusions

One of the main goals of the EUPHEM programme is to expose the fellows to different public health experiences and activities, thus enabling them to work across various disciplines. This portfolio includes laboratory and epidemiological projects covering bacterial, parasitic and viral pathogens across a variety of disease programmes, such as vector-borne diseases, food and waterborne diseases, respiratory tract infections, vaccine-preventable disease and sexually transmitted diseases. Outbreak, surveillance, research and laboratory activities contributed to the understanding of important public health issues. During the two-year fellowship, Zsófia, supervisors and the training site have demonstrated the capability of addressing communicable disease threats in a structured joint approach between public health microbiology and epidemiology such as the national salmonella outbreak investigation or the Hepatitis A outbreak taking place amongst MSM, or the investigations on the *Cryptosporidium* infection sequelae at national level. Activities were in line with the 'learning by doing' and 'on the job training' ethos of the EUPHEM programme and fulfilled the core competency domains described for professionals in their mid-career. Projects had a clear educational outcome, with results communicated in scientific journals and at conferences. Zsófia had been successfully integrated with professionals at RIVM in effective multidisciplinary team work with colleagues having different roles and specialities either inside or outside the training site as well as with colleagues from different member states when multi countries study has been undertaken (hepatitis A OI among MSM). Activities were complemented by 10 weeks of training modules that have provided theoretical knowledge. The coordinators team concludes that the fellow has succeeded in performing all her tasks and in the mean time wishes her all the best for her future career as public health microbiologist.

Supervisor's conclusions

Dr. Zsófia Iglói came to RIVM as an experienced microbiologist, being trained in virology research and Ebola response, and with the ambition to broaden her scope and skills in public health microbiology. Zsófia started working on a variety of projects that required old and new skills both in laboratory work and epidemiological tools. She used in her projects the knowledge and insights that came with the training modules. She approached her work with enthusiasm and persistence and she was able to do this in different settings: in our public health

institute but also in an academical hospital and municipal health service setting. She has been part of multidisciplinary teams, collaborating with epidemiologists, veterinarians, in early warning meetings etc. She was able to use her knowledge in all the different themes of public health microbiology and collaborate with epidemiologists and clinicians in projects for surveillance and outbreak response. She can assess what is needed in specific situations, shows initiatives while keeping her deadlines and is diplomatic. She showed great flexibility and was very active in participating and organizing training of students. I am very confident that Dr. Zsófia Iglói will be of great value for future programs in public health microbiology and for the EUPHEM fellowship.

Personal conclusions of fellow

In 2015, I was deployed with Public Health England to Sierra Leone to support the laboratory diagnostics during the ebola outbreak. That mission changed the course of my life, it made me developed a strong interest in public health and epidemiology and led to my application and acceptance to the EUPHEM fellowship. I wanted to gain insights into public health microbiology and field epidemiology, and get hands on experience. I have a busy two years behind me, busy and successful I believe. I could work in numerous exciting projects, gaining invaluable cross-disciplinary experience. Trough the program, I also became part of a professional network among public health actors in the European region which will be very valuable in my future professional roles. I'm looking forward to work in a cross-disciplinary public health field, with this continue to help bringing microbiology and field epidemiology closer together and to improve the health of the public.

Acknowledgements of fellow

First of all, I would like to thank ECDC for giving me the opportunity to be part of the program. Furthermore, I would like to thank the fellowship office, the frontline and scientific coordinators for all their dedication. A special thanks goes to my fantastic supervisor, Titia Kortbeek who was always there for me, both personally and professionally, and from who I learnt a lot! Several people made my stay at the RIVM very pleasant and productive and I'm eternally thankful for all your help, contribution and guidance what led to outstanding scientific outcomes. I would also like to thank the EPIET fellows at the RIVM (from C2015 Roan Pijnacker, Gudrun Frijdl; from C2016 Diederik Brandwagt, Susana Monge and from C2017 Anna Loenenbach, Jossy van den Boogaard) for their help and great lunch discussions. Lastly I would like to acknowledge the cohort representatives of C2015 (Amrish Baidjoe, Patrick Keating), C2016 (Alastair Donachie and myself) and C2017 (Timothee Dub and Lucia Reh) with who we worked a lot together trying to bring on changes and representing the fellow's interests.