

FINAL REPORT

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European Public Health Microbiology Training Programme (EUPHEM), Cohort 2021

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Background

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

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

According to Article 9 (6), Article 5 (8) and Article 11a (1) of Regulation (EU) 2022/2370 of the European Parliament and of the Council of 23 November 2022 amending Regulation (EC) No 851/2004 establishing a European centre for disease prevention and control (the ECDC Founding Regulation):

Article 9 (6) 'The Centre shall, as appropriate, support and coordinate training programmes, in particular in relation to epidemiological surveillance, field investigations, preparedness and prevention, response to public health emergencies, public health research and risk communication. Those programmes shall take into consideration the need for training to be kept up-to-date, take into account the training needs of Member States and shall respect the principle of proportionality.'

Article 5 (8) 'By encouraging cooperation between experts and reference laboratories, the Centre shall foster the development of sufficient capacity within the Union for the diagnosis, detection, identification and characterisation of infectious agents that have the potential to pose a threat to public health. The Centre shall maintain and extend such cooperation and support the implementation of quality assurance schemes'.

Article 11a (1) 'The Centre shall establish a EU Health Task Force and ensure that there is a permanent capacity and an enhanced emergency capacity to mobilise and use it. The EU Health Task Force shall provide assistance with regard to requests for prevention, preparedness and response planning, local responses to outbreaks of communicable diseases and after-action reviews in Member States and in third countries, in cooperation with the WHO. The EU Health Task Force shall include the Centre's staff and experts from Member States, fellowship programmes and international and non-profit organisations'.

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 final report describes the output of the fellow and the competencies they acquired by working on various projects, activities, theoretical fellowship training modules, other modules or trainings and international assignments or exchanges during the fellowship.

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Pre-fellowship short biography

Margarida Pires Simões is both a registered nurse and a veterinarian with a special interest in zoonoses and a 'One-Health' integrative approach. Since gaining a Masters in Conservation Biology and a PhD in Veterinary Sciences she has worked mainly in research and diagnostics, also focusing on Comparative Medicine strategies, benefiting both humans and animals. She is currently undertaking a residency training at the European College of Veterinary Public Health and has been an active member of 'Veterinarians without Borders' (Portugal) for more than 15 years, collaborating on projects to improve animal and public health, mainly in African countries.

Her motivation is to enhance capacity building and promote harmonised practices, especially in the field of zoonotic diseases, via multisectoral and collaborative efforts, enabling sustainable, equitable and better health for all.

Results

The objectives of these core competency domains have been achieved partly through project and activity work and partly by participating in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the ECDC Fellowship Manual¹.

1. Epidemiological investigations

1.1. Outbreak investigations

Investigation of a cross-border tuberculosis cluster in the Netherlands and Aruba, 2018–2023

Supervisors: Dr. Gerard de Vries

Category: Respiratory diseases (including influenza and TB)

In June 2023 a Whole Genome Sequencing (WGS) cluster, referred as 'TB - cluster B230', was identified, with an increasing number of cases both in the Netherlands and Aruba. WGS investigation was performed by the National Institute for Public Health and the Environment (RIVM), while contact tracing involved several municipality health services in Aruba. The aim of the outbreak investigation was to have a full understanding of transmission of the outbreak strain in the two countries over time and to mitigate its spread.

Municipal health teams from both countries conducted interviews as part of contact tracing and investigation of WGS-clustered cases. RIVM supported the teams to further investigate *Mycobacterium tuberculosis* isolates and cross-border epidemiological relatedness of cases. Microbiological investigations were based on comparison of Single Nucleotide Polymorphisms with a reference strain, using in-house protocols and pipeline.

WGS information suggests that the first case in this cluster, diagnosed in Aruba in 2018, infected the second case (diagnosed in the Netherlands in 2022). Having been sick for some time, Case 2 emigrated from Aruba to the Netherlands a few months before diagnosis. The timeline and WGS evidence suggest that Case 2 is the source of infection for Case 3 (Aruba) and for other cases, including Case 7 (in the Netherlands) which proved to be highly infectious and the source of several other TB disease cases.

Cross-border investigations pose additional challenges in terms of collaboration and methodology, mainly due to the variability of information available and being able to link and identify cases in a timely manner. Being able to better understand and contain this TB outbreak depended on good communication and coordination among all those professionals involved.

Margarida was part of the outbreak investigation team (co-investigator), contributing to descriptive data analysis and combining epidemiological information and WGS outputs. Together with the outbreak investigation coordinator, she prepared the cross-border outbreak report [1] and proposed the cluster network (WGS data).

Outbreak investigation of gastrointestinal illness during the Project Review Module, 2022

Supervisors: Dr. Kostas Danis, Dr. Ivo Foppa

Category: Food and waterborne diseases

At the end of September 2022, noting that several participants of the Project Review module (PRM) 2022 had reported gastrointestinal illness, ECDC encouraged an investigation of this possible outbreak. FETP (PAE) EPIET fellow colleague, Dr. Franziska Hommes was the main investigator, aiming to confirm the existence of an outbreak, find and count cases and determine the potential source of infection. Dr. Anna-Lisa Behnke (PAE fellow) completed the outbreak investigation team.

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

A cohort study was performed based on the 94 PRM participants. Interviews were conducted with those who had reported being sick and a request was sent to all PRM participants to answer an online questionnaire (symptoms and food items consumed) which was completed by 71%. The collected data revealed that 10 (14%) fulfilled the case definition. One of two cases with stool samples analysed tested positive for *Campylobacter jejuni*. In addition, one case tested positive for SARS-CoV-2 14 days after the module. The risk of developing gastrointestinal illness was five-times higher among those who consumed fruit/fruit salad during breaks at the PRM venue compared to those who did not eat fresh fruit (95% CI 0.71–40.16, p-value = 0.057). The epidemiological investigation suggested fruit/fruit salad contaminated with *C. jejuni* as the most likely vehicle for the outbreak. The main constraint was the fact that the investigation was initiated several weeks after the outbreak event (impairment for food and environmental testing, recall of eating habits).

Margarida was part of the inter-institutional and multidisciplinary outbreak investigation team. As a co-investigator she contributed to data collection from the caterers (food items offered during meals at the hotel and PRM venue throughout the week) by establishing good communications and feedback with the stakeholders. As part of the team effort, and based on her professional background, she helped to search for supporting evidence and reviewed the draft outbreak report [2].

MPox 2022 outbreak in the Netherlands

Supervisors: Dr. Bettie C.G. Voordouw

Category: Emerging and re-emerging diseases, vaccine-preventable diseases, hepatitis B and sexually transmitted diseases.

The global monkeypox (MPox) outbreak, which led to the declaration of a global health emergency by WHO in July 2022, began in April 2022. In the Netherlands, the first case of MPox infection was reported on 6 May 2022 and the country was considered to have one of the highest cumulative incidence rates worldwide. Epidemiological and laboratory data (next-generation sequencing) were analysed to explore how viral genomic sequencing could be used to track transmission, genomic rearrangements and viral evolution.

Results from the selected Dutch isolates revealed that all sequenced genomes appear to have originated from Clade IIb, lineage B.1, in a similar situation to that in most other countries, as only a few non-endemic countries reported other lineages. This evidence strongly suggests a single (outbreak) source for this global outbreak, disseminated mainly among the MSM (men who have sex with men) community. Poxviruses are known to show low SNP rates, with large-scale genomic rearrangement. Curiously, during this outbreak, strains revealed high divergence compared to the previous outbreak (6–12 fold higher than previous estimated mutation rates). In the Dutch cohort, a minor insertion into the inverted repeats and a major deletion event in the flanking region of the left ITR proved to be a large-scale genomic rearrangement event (full impact on viral evolution to be further investigated). Education, awareness, surveillance and immunity largely contributed to the control of the outbreak in the Netherlands. However, improvements are needed on data sharing, in compliance with data protection regulations, whilst allowing for a timely public health response.

Margarida collaborated on this inter-institutional effort by managing communications among intra-institutional and inter-institutional teams. In addition, she contributed to aggregate datasets from diverse sources for further analysis and helped to review the manuscript [1].

Educational outcome

In the diverse investigations she was involved in, Margarida applied all the steps of an outbreak investigation. She broadened her knowledge of epidemiology and the role of a public health microbiology expert and enhanced her communication and data analysis skills. In addition to the useful experience of participating in outbreak investigations, Margarida appreciated the opportunities to collaborate with professionals from other countries and institutions, with varied backgrounds and expertise, promoting harmonised practices and the need for appropriate communication and coordination. Margarida further developed her understanding of the use of genetic markers to assess pathogen transmission and evolution, and improved her skills in the reporting and presentation of data to different audiences.

1.2. Surveillance

Surveillance of respiratory disease associated with non-polio enteroviruses, part of ENPEN (European Non-Polio Enterovirus Network) study protocol

Supervisors: Dr. Kimberley Benschop

Enteroviruses (EVs) are known to infect millions of people every year. In general, EV infection causes mild and self-limited disease, however the majority of viral meningitis/encephalitis cases involve non-polio enteroviruses (NPEV) infections. EV-D68 is mainly known to cause respiratory disease but has also been implicated in encephalitis and acute flaccid myelitis (AFM). EV-D68 infections are a cyclical phenomenon, typically recurring every two years in Europe, although the COVID-19 pandemic shifted the expected upsurge period (main due to non-sanitary measures). As part of ENPEN activities, partners were made aware that from late July to mid October 2021, an upsurge of EV-D68 infections had been noted (139 cases in eight European countries). Following a rapid alert, surveillance was enhanced

with the aim of investigating the epidemiological and molecular characteristics of EV-D68 and its clinical impact during the autumn/winter season of 2021–22 in Europe.

A total of 58 institutes from 19 European countries reported 10 481 (6.8%) EV-positive samples, of which 1 004 (9.6%) were identified as EV-D68. Clinical data was reported for 940 cases in 2021 and 29 additional cases in 2022. Overall, 78.9% of infections were reported in children (0–5 years) half of whom were under two years; 37.9% of the cases were hospitalised. Acute respiratory distress was commonly noted (93.1%) while neurological problems were observed in 6.4% of cases, six being reported with acute flaccid myelitis (AFM). Phylodynamic and phylogenetic analyses showed the emergence of two novel B3-derived lineages. The study describes a large-scale EV-D68 European upsurge with severe clinical impact, and reinforces the need for standardised clinical and molecular surveillance of EV to improve knowledge of disease burden and enhance public health preparedness.

As a co-investigator, Margarida contributed to data collection by managing communications among partners and performing clinical and epidemiological descriptive analysis. She wrote the manuscript, which was submitted to a peer-reviewed journal [II], and prepared various communications [i] and presentations.

Sewage surveillance project in the Netherlands (data on enteric viruses) and comparative analysis with syndromic surveillance data from 2019 to 2022

Supervisors: Dr. Erwin Duizer, Dr. Harry Vennema and Dr. Kimberley Benschop

Enteric viruses are considered to be a 'complex community' of viruses, found in the intestinal tract of both humans and animals. The faecal-oral route is the main transmission path, strongly linked to the rapid dissemination of enteric viruses outbreaks, commonly associated with food- and waterborne transmission. Enteric virus infections can translate into more than gastrointestinal illness, also causing respiratory infections, conjunctivitis, hepatitis and neurological diseases, such as encephalitis and paralysis. Given that enteric viruses are mainly shed in faeces in very high loads ($>10^5$ virions/g of stool), an environmental surveillance system, based on molecular detection of enteric viruses in sewage, can provide valuable information on the viral-infection status in the community. This project therefore aimed to clarify patterns and seasonality of viruses detected by environmental surveillance and compare findings with the syndromic/clinical surveillance results for a four-year period.

Sewage samples were periodically collected at several sampling sites in the municipalities of Yerseke, Tholen, Ouddorp, Opheusden, Kesteren, Barneveld, Nunspeet, Elspeet and Staphor (known as the 'Bible Belt' region of the Netherlands). These samples were further tested for adenovirus, enterovirus, noroviruses, sapovirus and rotavirus. It should be noted that during the COVID-19 pandemic, enteric viruses detections decreased overall, both in environmental surveillance and clinical surveillance. For 2019 and 2022, similar patterns and overlapping detections were observed in both surveillance systems, although an apparent lag effect was noted for some viral agents in environmental surveillance detections. Environmental surveillance can be used as a predictive tool for virus circulation and distribution (time and space), contributing to public health monitoring and preparedness, similar to its use for COVID-19 community infection estimations.

Margarida collaborated with the environmental surveillance team and gained experience on comparative data analysis, reinforcing the arguments in support of the continued use of environmental surveillance. She also improved her knowledge of multiplex molecular diagnostic/detection tools and the added value of combining diverse surveillance systems to better understand the health of communities. Margarida prepared a report [3] and an additional presentation.

Antiviral resistance – starting a collaborative effort for data collection in the Netherlands

Supervisors: Dr. Adam Meijer, Dr. Kimberley Benschop

Nowadays, the monitoring of antimicrobial resistance is a relevant tool to better understand the challenges of pathogen evolution and treatment efficiency. At present, information on antiviral resistance (AVR) is scarce and only partial data on influenza antiviral susceptibility is available in the Netherlands. The intention of this project was to help establish a collaborating network with harmonised practices (capacity building) and initiate a baseline recording of AVR/antiviral susceptibility data, including influenza viruses, human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). In most countries, AVR data is collected as a result of further laboratory characterisation (phenotypical and genetic markers) in cases where patients fail to respond to treatment.

A collection data tool (questionnaire/form), developed by the team on phenotypic resistance and genetic markers of HBV and HCV, was prepared and sent out to identified experts. AVR trends, mutation characterisation, multi-resistance occurrence and noteworthy events were aggregated. A report of this pilot study will be shared with experts who contributed and within the institution. Concurrently, data on HIV AVR was selected from the national report (SHM)² and presented as a summary, integrating the NethMap³ annual report.

² HIV monitoring report 2022 (Netherlands), [Home | Stichting hiv monitoring \(hiv-monitoring.nl\)](https://www.hiv-monitoring.nl)

³ Report on surveillance of human antibiotic use and resistance development in the Netherlands (NethMap), [NethMap | SWAB](https://www.nethmap.nl)

There is still a need to establish systematic collection (AVR trends) for other viral groups where treatments are available, and to propose harmonised practices to assess and report susceptibility/resistance and share information.

The fellow coordinated the network with national experts on the designated viral diseases, drafted the data collection form and aggregated and analysed datasets before preparing the reports. She also summarised the antiviral resistance data on HIV and compiled the General Introduction chapter for the NethMap annual edition [4-6]. Combining this AVR project with the study on hepatitis, the fellow was able to contribute material used by Dr. Kimberley Benschop at a meeting of the Global Action Plan to eliminate viral hepatitis and STI (WHO expert group).

Educational outcome

Margarida worked mainly on event-based surveillance systems, both at national and international levels. In addition, she participated in the weekly early-warning meetings (*Signaleringsverleg*) which enabled her to experience first-hand outbreak detections, control and mitigation measure decision-making, and national and international signalling. Margarida also accompanied the preparatory work for these meetings, helping to detect signals relevant for public health in the Netherlands during the final stages and aftermath of the COVID-19 pandemic. The different projects contributed to a better understanding of the advantages and limitations of surveillance systems and disease notifications (and the data-sharing difficulties, even in public health emergency responses).

2. Applied public health microbiology and laboratory investigations

***Brucella canis* diagnostics, adapting a serology test to improve diagnosis and enable surveillance**

Supervisors: Dr. Titia Kortbeek and Dr. Karen Kerkhof

Human brucellosis is caused mainly by the infection of *Brucella melitensis*, *B. abortus*, *B. suis*, and *B. canis*, and is one of the leading 'neglected zoonotic diseases' in the developing world, which is of significant social, economic, animal and public health importance. What started as a project to develop a diagnostic tool for an emerging pathogen (*B. canis* imported into the Netherlands in rescued dogs from Eastern Europe, soon became a more comprehensive approach, aiming to develop a specific and highly sensitive pan-*Brucella* immunoassay. Therefore, the main objective of this project was to identify and select high-quality biomaterials, able to detect brucellosis with pan-reactive peptides.

Through literature queries, the team acknowledged that *Brucella* OMP31, Bp26, OMP2a, OMP2b, BCSP31, CuZn, hisD and GroEs proteins have a considerable potential for serological application. Comparative analysis of epitope data from all four *Brucella* species against the selected protein regions, was followed by the selection of highly conserved peptides (10-40 amino acids in length) used for validation with Luminex immunoassay. From the initial 38 pan-reactive peptides from regions OMP31, Bp26, OMP2a, OMP2b, BCSP31 and hisD, 15 showed the best results (specificity and sensitivity) in the multiplex immunoassay. It is expected that this diagnostic tool will facilitate the timely diagnosis of cases with an infection by clinically relevant *Brucella* species, advancing laboratory capacity not only in non-endemic countries, but also in countries aiming to eradicate this disease. Improving the diagnosis will also contribute to targeted therapeutics and a better understanding of disease burden.

The fellow developed the study protocol, carried out the literature review (identifying relevant immunoreactive proteins of the pathogen) and collaborated on the in silico and genetic analysis. Margarida presented the evolving project to team colleagues, fostered inter-institutional collaboration and, as co-author, prepared a poster [ii] and a manuscript (to be submitted to a peer-reviewed journal [III]).

Educational outcome

The research aimed to address a public health microbiology issue, enabling the development of new laboratory-related skills and competences. Having more experience in virology, by undertaking work in bacteriology Margarida was better able to understand the methodological advancements, tools and challenges of culturing a biohazard pathogen. The highlight of this project was the design steps and in silico analysis, a fantastic experience, using diverse tools and resources, performed as a team effort. Most of the peptide selection and validation process was performed by a colleague, however the outputs were presented by both as equally contributing authors (poster and manuscript).

***Babesiosis* molecular diagnostic validation project**

Supervisors: Dr. Titia Kortbeek, Dr. Jeroen Roelfsema and Dr. Karen Kerkhof

Babesiosis is a zoonosis caused by several species of intraerythrocytic parasites of the *Babesia* genus, mainly transmitted by tick bites (blood transfusions and vertical infections can occur). Most infections are believed to be asymptomatic but clinical signs can range from mild to life-threatening symptoms, especially in

immunocompromised/splenectomised people. In the Netherlands, many people enjoy an outdoor lifestyle, meaning that they have a greater risk of being exposed to ticks. Although there have only been around 60 cases reported (since 1950), the true incidence and disease burden in Europe is unclear. The need for a diagnostic test to identify human infections with *Babesia spp.* originated from the increase in cases of animal babesiosis (with zoonotic potential) being detected in the Netherlands. The aim was to validate a system designed in house and a molecular assay to confirm human babesiosis (resulting from infection with *Babesia divergens*, *B. microtii*, *B. venatorum*), to carry out further testing and to assess experimental systems already in use (detection of pathogen in animal and vector samples). As diagnostics of tick-borne diseases usually involves other pathogens, the diagnostic system was built as a multiplex qPCR.

Although no human samples were initially available, validation steps relied on animal samples, which required secondary testing (PCR) and subsequent sequencing. To clarify, this last step was necessary because for each organism the qPCR amplicons were designed in highly-conserved regions, valid only for the zoonotic species. Participation in a proficiency test using human standards enabled the assay to be validated (100% accurate results even for those deemed as 'educational' specimens). Implementation of the *Babesia spp.* diagnostic assay (part of the tickborne multi-target diagnostic tool) is considered very pertinent as human babesiosis is an increasing public health concern.

The fellow carried out the literature review, laboratory activities and data analysis, collaborated on the EQA and prepared the internal report [7].

Educational outcome

The fellow had the opportunity to practice basic but diverse bioinformatics methodologies, while improving her design skills for molecular (multiplex) diagnostic tool development and validation. In addition, Margarida worked on the interpretation of laboratory results and the integration of interlaboratory comparison trial results. Other projects (brucellosis immunoassay and hepatitis type-specific study) also had a strong laboratory investigation component, providing a further opportunity for the fellow to learn new methodologies and scientific approaches for addressing public health issues. The communication among multidisciplinary teams, feedback and shared learning experiences provided experience of a team-working approach which will be useful to apply in the future. The fellow also further enhanced her presentation skills to different audiences, based on an integrative approach.

3. Biorisk management

Biosafety level 3 training and ISO 17025

Supervisor: *Dr. Titia Kortbeek*

Having extended her BLS-3 working experience (e.g. *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *African swine fever virus*), Margarida was offered the opportunity to enrol in a BSL-03 course for all laboratory staff at the RIVM. The intensive BSL-03 course enabled the fellow to update her knowledge and be fully prepared to work with biohazard pathogens, as planned with the research project on brucellosis immunoassay development.

Having worked at the Portuguese National Animal Health Laboratory (applying ISO 17025 standards), the fellow was offered the chance to undertake continuous development training at the RIVM, which was a great opportunity to revise knowledge of ISO standards, institutional practices and obligations (different to those for animal health reference laboratories) and compare ISO 17025 and ISO15189. The fellow prepared reports on each activity [8-9], and was able to adhere more closely to the quality standards in practice at each laboratory in the institution.

Educational outcome

Margarida consolidated safe and standard practices, complying with national, European and WHO rules and regulations. The learning experiences from the RIVM training course, combined with the ECDC Biosafety and Quality module, and the possibility to shadow colleagues performing activities in accordance with standard operating procedures and accredited procedures provided the opportunity to learn and apply consistent practices. This working example has taught the fellow how to maintain strong and consistent performance and reduce errors while prompting optimised results and communication. In addition, the fellow performed a biosafety risk assessment (group work) and a quality assessment at the site, together with colleagues from the Parasitology Unit [10].

4. Quality management

Clarifying the impact of hepatitis B virus (HBV) genotype F circulation in the Netherlands

Supervisor: Dr. Kimberley Benschop

According to the most recent data, hepatitis B virus (HBV) represents a global health burden, with 296 million people chronically infected worldwide and approximately 900 000 dying annually due to complications. HBV is endemic in humans and can be divided into 10 genotypes, with distinct geographical distributions. In most European countries, genotype A is the prevalent genotype but since 2019, genotype F has been increasingly detected, even replacing genotype D (which was the second most common genotype). This detection was possible because in the Netherlands, notification of acute HBV infections is mandatory and, since 2004, all cases are sampled for viral typing. In order to ascertain whether diagnostic panels detect all HBV genotypes equally, it is necessary to improve understanding of HBV genotype F occurrence in the Netherlands. For this reason we set up an External Quality Assessment (EQA) trial with approval from the experts, and invited collaborating laboratories nationwide to perform serological tests on HBV type-specific samples.

A serological EQA subpanel, using WHO reference materials, was distributed to 27 Dutch laboratories to provide testing efficiency parameters (Se/Sp, PPV, NPV) of each diagnostic system. This interlaboratory proficiency test was established to help determine the sensitivity and specificity of the commercial immunoassays.

Hepatitis B infections have a considerable burden of disease which is the reason for the national vaccination plans. The influx of migrants from areas with diverse prevalent genotypes and the current genotype diversification in the Netherlands raises concerns about possible vaccine evasion. The results obtained will help to maintain/improve the screening process (e.g. HBV acute infections, blood donations) and improve preparedness (e.g. improvement of immunoassays and vaccine evolution).

Margarida coordinated the project, managing the laboratory and procedural logistics, creating the EQA panel reporting form and composing the EQA instructions letter. She also maintained communications and feedback with all participating laboratories and presented the project design and EQA set-up at the PRM 2023 training module.

Participation in an internal audit (ISO 15189)

Accompanying RIVM colleagues Kim van der Zwaluw and Mark Jonker

ISO 15189:2012 specifies requirements for quality and competence in medical laboratories. ISO 15189:2012 is a regulatory standard for medical laboratories. Its criteria help laboratories to develop quality management systems and assess their own competence. The standard is also used by regulators, accreditation bodies and customers to confirm or recognise laboratory competence. The common components of the standard include document control, internal audits, occurrence management, and risk management. Compliance with ISO 15189 improves the overall quality of a laboratory's services and products, which directly affects the quality of healthcare.

The Diagnostics and Laboratory Surveillance Unit (IDS) at The Centre for Infectious Disease Research is currently accredited according to EN ISO15189 standards. The fellow participated in internal audits of protocols and procedures and a laboratory meeting to provide feedback on the audit results. The purpose of an ISO internal audit is to assess the effectiveness of an organisation's quality management system and overall performance. In addition, an internal audit promotes its own system vigilance, detecting failures and errors before they become a problem, thereby reducing errors by getting things right the first time. In addition, an audit helps identify opportunities for improvement by involving and empowering its staff (involvement in problem-solving and solution implementation). At the RIVM, all those involved in laboratory work are expected to actively participate (leading or supporting) in quality assurance activities. The fellow accompanied colleagues as part of the learning-by-doing process and prepared a final report [11].

Educational outcome

During the preparations for the internal audit, Margarida became more aware of the basic differences and similarities between ISO15189 and ISO17025. The former aims to be a standard quality assurance, mainly for medical laboratories with an advanced topic for continuous improvement. The observation activity helped the fellow to better understand the complex system for ensuring quality and accreditation standards at human diagnostic laboratories. By participating (human babesiosis molecular detection EQA) and coordinating the EQA set-up on HBV type-specific detection, Margarida improved her communications and organisational skills.

5. Public health microbiology management

Early warnings meetings

The fellow participated in the weekly infectious diseases early warnings meetings (*Signaleringsoverleg Infectieziekten*) bringing together epidemiology, public health microbiology, infectious diseases, infectious disease control, veterinary and environmental science and other related experts to discuss events of national interest. The meetings were held in a hybrid format, and all discussions and outputs were confidential. All participants were able to present a selected public health issue from the Netherlands and abroad and after discussion, a summary of signals considered to be of public health importance would be presented, to be shared in the weekly bulletin for public health professionals (early warning for preparedness). The fellow participated in these weekly meetings, contributed to signals and discussions within her area of expertise, and assisted the management group in the preparation of a meeting (18–21 July 2022).

ENPEN management assistance

Supervisor: Dr. Kimberley Benschop, Dr. Heli Harvala

The European Non-Polio Enterovirus network (ENPEN) aims to perform research activities (retrospective and prospective), using multi-centre and cross-sectional pilot study designs, proposing harmonised practices such as diagnostics, detection, case definition, characterisation and reporting of NPEV infections. The network has more than 30 sites in various European countries, covering both the clinical and laboratory perspective. Although activities were disrupted during the COVID-19 pandemic, communication between partner sites enabled the early detection of an upsurge in EV-D68 infections from late summer 2021 onwards, mostly related to respiratory illness in children under five years. This initial alert turned into a large-scale European study, describing epidemiological, clinical and genetic features of the outbreak.

Alongside the 2021 upsurge study, the fellow assisted the ENPEN respiratory surveillance group coordinator with data management and analysis (datasets on clinical, epidemiological and viral genetic characterisation), helped with communication and the management of data sharing agreements among the 58 partners, and ensured inter-institutional coordination of all ENPEN partners. The fellow also participated in the ENPEN steering committee meetings, where ongoing studies, outreach activities and preparation of proposals for funding calls were discussed.

Educational outcome

As a public health professional, the importance of communication and management skills cannot be underestimated. Throughout the training modules, Margarida had the opportunity to learn about public health topics and to apply the competencies acquired. Conveying public health control and mitigation recommendations along with preventive measures ('right time and adequate fashion') were practised both in training modules and at the RIVM within the diverse projects. Margarida feels confident that she can formulate and deliver information to different audiences (experts and non-expert communities), having gained valuable insight into national, regional and global public health practices. Margarida obtained valuable management experience coordinating a surveillance set-up project and a surveillance evaluation project, mainly being responsible for the logistics, communication and analytical work, and for engaging with the stakeholders involved.

6. Teaching and pedagogy

Case study facilitation and guest lecture at Radboud UMC

Salmonella in the Caribbean (ECDC, 2016)

Ongoing collaboration between EPIET/EUPHEM fellows and the Radboud University Medical Centre in Nijmegen gave the fellow the opportunity to present her career path, information on research in subjects with public health relevance and an overview of projects developed within the scope of EUPHEM training. 'Salmonella in the Caribbean' was a case study prepared and facilitated on the basis of WHO and ECDC case study materials in November 2021. It was a face-to-face activity, in a practical class context, with third-year medical and biomedical science students.

An outbreak of gastroenteritis in Kalundborg, Denmark (ECDC, 2015)

In a combined effort to facilitate another example of outbreak investigation, both fellow EPIET colleague Dr. Katja van Ewijk and Margarida worked with third-year students studying Medical and Biomedical Sciences (BSc course Control of Infectious Diseases) at the Radboud University Medical Centre in early December 2021. Despite the COVID-19 restrictions, with students both on-site and online, and having adapted the study material, we facilitated the learning process with a classical example of outbreak investigation.

Case study facilitation at Netherlands School of Public and Occupational Health (NSPOH)

Outbreak of hepatitis A virus in Scandinavian countries, 2013 (ECDC, 2013).

The fellow participated in a session for medical doctors in training (specialising in public health for infectious diseases) held by the Netherlands School of Public and Occupational Health in June 2022. The facilitation of the case study also entailed the practical application of ECDC toolkits and EpiInfo resources. The online session allowed for small working groups, with students grouped in a break-out room (online meeting platform), enabling interaction from start to finish of the simulated outbreak investigation.

Infodemics 101 and Communication Management

Following a short course on infodemics offered by the EPIET Alumni Network (EAN), the fellow shared the highlights of her experience with RIVM colleagues (April, 2023). WHO material was adapted and combined with other sources to deliver a concise overview of current infodemic and communication management challenges. The presentation was held in a hybrid format (with colleagues online and on-site), with questions and appeals for interaction [report 12].

Educational outcome

Margarida combined her previous teaching experience with up-to-date communication strategies, refining communication skills to address diverse audiences, from undergraduates to experts, and also communities other than public health professionals. The fellow reviewed these teaching activities and compiled a report [13].

7. Communication

8.1 Publications related to the EUPHEM fellowship

8.1.1 Manuscripts published in peer-reviewed journals

- I Circulation, viral diversity and genomic rearrangement in Mpox virus during the 2022 outbreak in the Netherlands [review process].
- II. Epidemiological and clinical insights into the enterovirus D68 upsurge in Europe 2021/22 and the emergence of novel B3-derived lineages, ENPEN multicentre study [manuscript submitted to JiD on 6 June 2023].
- III. In silico analysis-based identification of novel biomarkers for the serological diagnosis of pan-*Brucella* [in preparation].

8.1.2 Other reports

1. Investigation of a cross-border tuberculosis cluster in the Netherlands and Aruba, 2018-2023 (report).
2. Outbreak investigation of gastrointestinal illness during the Project Review Module, 2022 (report).
3. A retrospective study based on environmental surveillance – detecting community viruses during a four-year period (2019-2022) (review process).
4. Antiviral drug resistance data - HIV summary as part of the national NethMap report (review process).
5. General introduction on antiviral resistance, inclusion as NethMap chapter (review process).
6. Antiviral drug resistance of HBV and HCV, report on pilot data assembly (report in preparation).
7. Laboratory investigation: babesiosis molecular diagnostic validation project (activity report).
8. BSL-03 course.
9. ISO 17025 course.
10. Process Management and Quality Control – Biorisk and quality management activity report.
11. Internal audit (ISO 15189:2014) observation report.
12. Infodemics 101 and preparedness.
13. Notes on teaching.

8.2 Conference presentations

- i. Simões MP, Hodcroft E, Simmonds P, Fischer TK, Harvala H, Benschop K. Enterovirus D68 upsurge in Europe during 2021: highlights of a collaborative effort. Epidemiological and clinical insights into the enterovirus-D68 upsurge in Europe 2021/22 and the emergence of novel B3-derived lineages, ENPEN multicentre study. ECCMID, 15–18 April 2023, Copenhagen, Denmark [ePoster with six-minute flash presentation].
- ii. Smits T, Simões MP, Çelik G, Kortbeek T, Kerkhof K. In silico analysis-based identification of novel biomarkers for the serological diagnosis of brucellosis. Scientific Spring Meeting KNVM NVMM 2023, Papendal, Netherlands. 5 April 2023 [poster on-site].

8.3 Other presentations

- M. Simões. Enhanced laboratory surveillance of HBV infections in The Netherlands, 2023. Presentation at the Project Review Module, session 8; Lisbon, Portugal, 31 August 2023
- M. Simões. Infodemics 101 and Preparedness. Seminar format presentation at RIVM, Bilthoven, the Netherlands, 24 April 2023 [hybrid format].
- M. Simões. EUPHEM C2021 – overview of the ECDC fellowship programme and developed projects. Presentation at RIVM, Bilthoven, the Netherlands, 22 June 2023 [hybrid format].
- K. Benschop (On behalf of ENPEN steering committee Heli Harvala, Thea Kølsten Fischer, Peter Simmonds, Audrey Mirand, Natasa Berginc, Sofie Midgley, Andrés Antón Pagarolas, Caroline Klint Johannesen, Jean-Luc Bailly, Margarida Simões, Jelte Hellferich). EV-D68; a new public health threat on the brink of poliovirus eradication. ECCMID, 2023 15–18 April 2023, Copenhagen, Denmark.
- K. Benschop and M. Simões. ENPEN: EV surveillance in Europe in the 21-century with EV-D68 as a new public health threat on the brink of poliovirus eradication. EID, 2022, Atlanta, United States of America. 7–10 August 2022.
- K. Benschop and M. Simões. Re-emergence of enterovirus D68 in Europe after easing the COVID-19 lockdown, 2021. EUROPIC, 2022, Harrogate, United Kingdom, 5–9 June 2022.
- M. Simões. Sewage surveillance on enteric viruses in the Netherlands. Introductory course part 2, Spétses, Greece. 21 April 2022.
- M. Simões. Development of a Serodiagnostic test for human brucellosis. Presentation of project evolution and prospect activities to BacSer, Bacteriology and IDS project team. RIVM, 13 January 2022.

9. EPIET/EUPHEM modules attended

1. Introductory course (part I), 20 September 2021 – 8 October 2021, online.
 - a. IC Inject Day – Phylogeny – 20 October 2021, online.
 - b. IC Inject Day - Operational Research – 27–28 October 2021, online.
 - c. IC Inject Day - Data Management – 10 November 2021, online.
2. Introductory course (part II), 20–29 April 2022, Spétses, Greece.
3. Biorisk and Quality Management (BQM) module, 17–18 January 2022, online.
4. Multivariable analysis, 14–18 March 2022, online.
 - a. MVA Inject Day – 30 March 2022, online.
5. Outbreak investigation, 6–10 December 2021, online.
6. Rapid Assessment & Survey Methods (RAS) module, 6–10 June 2022, Solna, Sweden.
7. Project Review Module, 29 August 2022 – 2 September 2022, Lisbon, Portugal.
8. Time Series Analysis (TSA) module, 7–11 November 2022, Bilthoven, the Netherlands.
9. Qualitative Research (QR) module, 31 January 2023 and 3 February 2023, online.
10. Vaccinology, 13–17 February 2023, online.
11. Management, Leadership and Communication in Public Health (MLCPH) module, 8–12 May 2023, Stockholm, Sweden (attended online).
12. Project Review Module 2023, 28 August 2023 – 1 September 2023, Lisbon, Portugal.

10. Other training

- ESCMID post-graduate training course: Emerging laboratory and point-of-care technologies for detection of AMR and bacterial infection in veterinary medicine (online), 9 March 2022.
- TB Surveillance Systems and Inventory Studies Workshop. Organised by Robert Koch Institute, Berlin and National Tuberculosis and Lung Disease Research Institute, Warsaw (online), 31 March 2022–1 April 2022.
- Current and future challenges in veterinary virus genomics (One Health EJP Consortium online workshop) 21–22 June 2022.
- Second acute flaccid myelitis ENPEN virtual symposium, 18 and 25 August 2022.
- Genomic surveillance of viral pathogens: are we prepared for emergent global threats across One Health? (ESCMID -EFWSIG third webinar) (online), 20 September 2022.
- New roles of microbiology laboratories in public health surveillance and epidemic response (ESCMID - ESGPHM web symposium) (online), [27 October 2022.
- One Health EJP Consortium Diagnostic Satellite Workshop 2022 (online participation) 2 and 4 November 2022.

EAN mini-module on media and infodemic management (Stockholm) 21–22 November 2022.

ESCAIDE 2022 (Stockholm) 23–25 November 2022. *Rejected abstract*: Simões MP, Hodcroft E, Simmonds P, Fischer TK, Harvala H, Benschop K. Enterovirus D68 upsurge in Europe during 2021: an example of early warning from a voluntary network and evidence to harmonise surveillance practices.

Symposium Interpretatie Lyme Diagnostiek. RIVM, Bilthoven, the Netherlands, 23 June 2022.

ESCMID/IDSA joint web symposium on monkeypox (webinar) - Monkeypox virus: Epidemiological updates and latest recommendations for laboratory testing (online), 24 May 2022.

Highlights WHO Public Health laboratories knowledge sharing webinar: Monkeypox Virus Epidemiological Updates and Latest Recommendations for Laboratory Testing (online), 1 June 2022.

WHO Public Health Laboratories knowledge sharing webinar series: acute hepatitis of unknown aetiology in children: epidemiological situation and lab testing strategy guidance (online), 22 June 2022.

ESCAIDE 2021 (online) 16–19 November 2021.

RIVM IDS VIR, Epi *refereer* and BAC-PAR; EPIET-EUPHEM joint seminars and webinars series. Participation throughout the entire fellowship period both on-site (Bilthoven, the Netherlands) and online attendance.

12. Other activities

European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Member of the Study Group for Public Health Microbiology and the Study Group for Veterinary Microbiology. The fellow participated in group activities, proposed training events and is currently involved in the development of an online course 'Training for an outbreak response: simulation exercises to strengthen local preparedness'; helping to disseminate the table-top exercise event among other professionals (e.g. residents of veterinary specialities).

European College of Veterinary Public Health (ECVPH) residency. The fellow started her training in the sub-speciality of population medicine in June 2020 and was able to further her knowledge in epidemiology and related themes through the EUPHEM fellowship.

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