

## FINAL REPORT

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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.

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Stockholm, September 2023

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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.

## Pre-fellowship short biography

The fellow, Camille Jacqueline, holds a bachelor's degree in Biological and Environmental Sciences from the University of Aix-Marseille (France) as well as a Master's degree specialised in Ecology and Evolution of Tropical Diseases from the University of Montpellier (France). Following her Master's degree, she completed a PhD in Health Science on the interactions between infectious diseases and cancer where she performed fungal and bacterial infection experiments on genetically modified *Drosophila* and applied cutting-edge transcriptomic and metagenomic analyses. Her post-doctoral research was performed in the University of Pittsburgh (USA) where she was responsible for developing a preventative cancer vaccine based on previous epidemiological data showing an association between previous febrile infections and a decrease in cancer risk. In this context, she identified shared molecular targets between infected and cancer cells through proteomics methods and participated to one clinical trial to test the efficacy of preventive cancer vaccines.

## Results

The objectives of these core competency domains were achieved partly through project and activity work and partly by participating in the training modules. Results are presented in accordance with the EPIET core competencies, as set out in the ECDC Fellowship Manual<sup>1</sup>.

### 1. Epidemiological investigations

#### 1.1. Outbreak investigations

##### 1.1.1. 2021 Norovirus Outbreak in Muxia, Galicia, Spain

Supervisor: Maria Dolores Fernandez-Garcia (CNM, Spain)

Category: Food and waterborne diseases

On 30 September 2021, the city council of Muxia reported an unusual increase of patients with acute gastroenteritis (AGE). Because geographically widespread villages belonging to the same water supply were affected, a waterborne outbreak was suspected. Overall, 115 probable cases were ascertained during epidemiological investigations carried out by the local health authority (attack rate, 5.7%). Primary cases peaked on 29 September and subsided on 1 October, compatible with a point-source outbreak followed by possible secondary cases until 7 October.

The case-control study included 62 cases and 46 controls. Univariate analysis showed that cases had a higher exposure to tap water (odds ratio [OR] = 86; 95% confidence interval [CI], 18–409). Norovirus GII was detected in two terminal points of the water supply system, and 14 cases were laboratory confirmed after detection of GII in stool samples. A unique genotype (GII.3[P12]) was identified in stool samples but genotyping could not be conducted in water samples. On 1 October, a tap water ban was put in place and the water was purged and chlorinated. The rapid increase in the number of cases and its decline after implementing control measures confirmed the hypothesis of a waterborne point-source outbreak among the residents of Muxia sharing the same water distribution system.

Effective norovirus detection and the early recognition of water as a possible source of infection are important to reduce morbidity as appropriate steps are taken to control the source. In our study, we combined epidemiological, environmental, and microbiological investigations to demonstrate that it was a waterborne outbreak caused by norovirus. Detection of faecal indicator bacteria and the fact that the drinking water was not chlorinated suggest a breakdown in chlorination as the cause of the outbreak. This outbreak investigation also demonstrated the importance of timely communication to the public about the risk linked to tap water consumption.

Role: The fellow was a co-investigator but lead the microbiological analyses related to the outbreak and applied her microbiological and epidemiological knowledge on outbreak investigation. She performed all steps of the genotyping of the clinical samples as well as the phylogenetic analyses. The fellow also wrote the outbreak report, drafted the manuscript and submitted it to a peer-reviewed journal and presented this work with a poster at ESCAIDE 2022.

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

### **1.1.2. 2021 Salmonella Enteritidis ST11 in Europe and its association to Spanish eggs.**

Supervisor: Silvia Herrera-León (CNM, Spain)

Category: Food and waterborne diseases

On 2 September 2021, France reported an increase of cases with *S. Enteritidis* ST11 (sequence type 11) infections in EpiPulse, a platform hosted by the European Centre for Disease Prevention and Control (ECDC). Several countries, including Spain, reported cases with genetically close isolates using cgMLST analyses. In collaboration with the affected countries, the European Food Safety Authority (EFSA), and the European Commission, ECDC initiated an international outbreak investigation to identify the outbreak source and vehicle.

A confirmed case was defined as follows: a laboratory-confirmed *S. Enteritidis* case with symptoms onset on or after 1 January 2021 and the isolates:

- a) clustered within five allelic differences (AD) of one of the three French representative outbreak strains using cgMLST, or
- b) were part of the HC5\_2301- cluster using Enterobase scheme, or
- c) were within five AD in a centralised single linkage cluster analysis, or
- d) were part of the 5-single nucleotide polymorphism (SNP) single linkage cluster according to the UK Health Security Agency's (UKHSA) pipeline.

Historical cases were defined as those reported before 2021 and fulfilling the laboratory criteria. ECDC collected demographic, sequencing, and exposure data on human cases. EFSA collected sequences and background information on non-human isolates for centralised whole genome sequencing (WGS) analysis.

In 2021, 272 confirmed cases were reported in five EU/EEA countries and the UK. All 60 interviewed cases reported consumption of eggs or dishes containing eggs. Traceback analyses of eggs pointed to a packing centre supplied by two Spanish laying hen farms positive for the outbreak strain. Between 2013 and 2020, 801 historical cases were reported in seven EU/EEA countries and the UK. The geographical spread and outbreak duration suggested continuous circulation of the outbreak strain in the egg production chain.

**Role:** The fellow was mostly involved in the discussion of the microbiological study group established by ECDC to determine the case definition. She uploaded the Spanish sequences on Enterobase and analysed maximum likelihood phylogenetic trees generated with Snippy and used Microreact to present the results in the study group meetings. She worked alongside interdisciplinary local public health officials. She was listed as a co-author in the Rapid Outbreak Assessment produced by ECDC and EFSA

### **1.1.3. Investigation of two tuberculosis outbreaks in Pais Vasco: deciphering hypotheses of transmission using whole genome sequencing**

Supervisor: Laura Herrera-León (CNM, Spain)

Category: Respiratory diseases

In January 2022, and after receiving two additional strains for a cluster in Pais Vasco (Spain), the regional epidemiological department asked the CNM for help in deciphering the chains of transmission and index case of this outbreak, as contact tracing information were limited. From 2019 to 2023 11 cases (eight were sub-Saharan immigrants) from Bilbao were identified as belonging to the outbreak. No other country reported related cases following the alert in EpiPulse. The 11 confirmed cases were within 5 AD using cgMLST and within 4 SNPs.

Even though WGS coupled with cgMLST was key to identify this outbreak in real-time, SNP analysis was insufficient to fully resolve chains of transmission or to orient the hypothesis on index case identity. However, this outbreak was particularly interesting as microbiological methods were key in its identification, and because of the public health actions taken to control the outbreak. Indeed, the regional public health institute used different communication strategies to reach the hospitals and the community. Notably, they created infographics in six different languages to overcome potential language barriers in the immigrant's population and work with multicultural association to decide on the better approach to communicate with the cases about their contacts and travels.

In parallel, the epidemiological department of Pais Vasco, also asked for a retrospective analysis of an outbreak associated with the transmission of susceptible strains in a university in 2016. While the pattern obtained by RFLP was identical in the 6 strains studied, the cgMLST analysis showed that one strain had high AD with the main cluster. Interestingly, this case had no known contact with the index case and might be part of a different outbreak. SNP analysis showed that the remaining sequences were identical and therefore we could not resolve the chain of transmission further.

Role: The fellow participated in each step of the study: work in a BSL-3 laboratory, DNA extraction and library preparation for WGS, analyses of the raw reads using Enterobase, interpretation of the results from cgMLST and SNP analyses. She presented the results to the local epidemiologists, drafted the manuscript and submitted an abstract to ESCAIDE.

### **Educational outcome**

Over the course of the three outbreak investigations, the fellow was able to develop a wide range of outbreak related competencies. Working alongside diverse stakeholders and public health authorities, both at local and international level, from the initial characterisation to the dissemination of findings and recommendations, both from the epidemiological and laboratory side. The competencies acquired during these outbreak investigations covered all stages of an outbreak investigation and have provided the fellow the necessary skills to confidently participate in future outbreak investigations.

## **1.2. Surveillance**

### **1.2.1. Non-coding region MF-NCR reveals unknown events of importation of measles cases in Spain, 2017-2020**

Supervisors: Josefa Masa Calles (CNE, Spain), Noemi Lopez Perea (CNE, Spain), Juan E. Echevarria (CNM, Spain), Aurora Fernandez-Garcia (CNM, Spain)

Category: Vaccine preventable diseases

In countries entering the post-elimination phase for measles, the study of variants by sequencing 450 nucleotides of N gene (N450) does not always allow the tracing of chains of transmission. Indeed, since 2017, when measles was declared eliminated from Spain, sporadic outbreaks have been observed, and most reported sequences belong to the Mvs/Dublin.IRL/8.16/-variant (B3-Dublin) and the MVs/Gir Somnath.IND/42.16/-variant (D8- Gir Somnath). Thus, increasing the sensitivity and specificity of the surveillance of measles cases is primordial to ensure an accurate estimation of the transmission of the virus (MeV) in countries entering the post-elimination phase.

Researchers have focused on the MF-NCR which is the longest non-coding region of the MeV, GC-rich and highly variable. However, standard protocols are still not available for the analysis of MF-NCR sequences and for the criteria used to associate sequences in order to distinguish chains of transmission. Therefore, we aimed to evaluate the analyses of the MF-NCR as a method to discriminate strains with a higher resolution, and infer case origin, chains of transmission and outbreaks in Spain.

We obtained 115 samples from Spanish patients infected with either B3-Dublin or D8-Gir Somnath between 2017 and 2020. We performed the phylogenetic and phylodynamic analyses of the MF-NCR region and applied a recently published mathematical model to determine relatedness of identified clades. Our results suggested that the analysis of the MF-NCR region was able to decipher outbreaks and chains of transmission in accordance with the epidemiological data available. In addition, it allowed the detection of concomitant introduction of the virus during two outbreaks in 2017 and 2019 that were not captured by the study of variants and epidemiological analyses by time and place. We suggested considering the use of the MF-NCR region in conjunction with the study of N-450 variants in the future WHO recommendations for the MeV microbiological surveillance.

Role: The fellow participated to every step of the work both in the laboratory and during the analyses: RNA extraction, PCR, sequencing, editing, phylodynamic and phylogenetic analyses. She presented the work to the collaborators in the epidemiology department and at ECCMID as a poster flash presentation. She drafted the manuscript and participated actively in its revision and submission to a peer-reviewed journal.

### **1.2.2. Circulation of multi-resistant strains of *Shigella sonnei* and *S. flexneri* in the Iberic peninsula from 2015 to 2022: a retrospective study**

Supervisors: Silvia Herrera-León (CNM-Spain) and Ângela Pista (INSA-Portugal)

Category: Food and waterborne diseases

Resistance to first-line antibiotics is on the rise globally which led WHO to include fluoroquinolone-resistance (FQR) in *Shigella* among serious antimicrobial resistance (AMR) threats. In Europe, circulation of extensively-resistant *Shigella* species was recently reported. We established a collaboration with Portugal to study the epidemiology and determinant of resistance of FQR *S. sonnei* and *S. flexneri* isolates in Spain from 2015 to 2022. We determined the AMR profiles of 184 *S. flexneri* and 232 *S. sonnei* isolates collected in both countries. FQR isolates, were subjected to whole-genome sequencing to assess AMR determinants and to perform phylogenetic analysis.

In Spain, the proportion of *S. sonnei* isolates resistant to fluoroquinolone only increased in 2021 (90%) compared with 2018, and remained high in 2022 (88%). In Portugal, this pattern of MDR was only observed in 2021 (40%) and 2022 (50%). Regarding *S. flexneri*, FQR isolates were relatively uncommon in both countries until 2022, when we observed a duplication in the number of cases in Spain. The relative risk of carrying a FQR *S. sonnei* strain was higher in male cases (RR = 4.9, 95% CI = 2.7- 9.0) compared with female cases. Such association between FQR and male cases was not significant for *S. flexneri*. The increase in FQR was associated with the presence of two plasmids IncFII and IncB/O/K/Z.

Although *Shigella* species mostly cause self-limiting diarrhoea, it can lead to severe symptoms in immunocompromised patients. Therefore, the circulation of resistant strains to both first and second line of treatment raises concerns about the clinical management of those cases. The spread of extensively resistant *Shigella* infection, unnoticed before 2021, highlights the need to strengthen surveillance of shigellosis in both Portugal and Spain. In addition, we concluded that there was a need to improve data collection about exposure to further explore why men are more at risk than women for FQR *Shigella* infection.

Role: The fellow participated to every step of the work both in the laboratory and for the analyses: DNA extraction, WGS, cgMLST and phylogenetic analyses. She presented the work to the collaborators and at ECCMID and FETP Nights as posters. She worked in collaboration with the fellow from Portugal to draft the manuscript.

### **1.2.3. Mapping of surveillance systems for diseases and nutrition in the Great Horn of Africa (international assignment, Nairobi, Kenya)**

Supervisors: Tobias Homan (WHO, Switzerland)

Category: Vector-borne diseases

The seven countries within the Great Horn of Africa (GHOA) have been significantly affected by ongoing conflicts, extreme weather events, such as drought and flooding, food price inflation and insecurity as well as loss of livelihoods and increased population displacement. Subsequently, the number of malnourished people has increased significantly in the GHOA. This food insecurity crisis is accompanied by a health crisis which has increased the morbidity and mortality of the populations. All seven countries in the GHOA have been malaria-endemic countries for many years but outbreaks due to other pathogens are multiplying. Currently, six of the seven countries are dealing with measles outbreaks and four are dealing with cholera outbreaks. In this context, the surveillance systems are challenged to provide accurate and timely data.

The purpose of this exercise was to draw an overview of existing disease and nutrition surveillance systems in the Greater Horn of Africa and highlight gaps and challenges at the country and region levels. We focused on the surveillance systems for five key diseases in the context of food-insecurity (measles, cholera, dengue, meningitis and malaria) and COVID-19. We also included the surveillance of severe acute malnutrition (SAM) with a specific interest in the outcomes indicators used by each system. We combine two approaches: i) desk reviews of the available documents and sources available online or shared with WHO and ii) interviews of key informants. Data were extracted and summarised to allow visualisation of the main characteristics of each surveillance system.

We also identified the gaps and challenges associated to the surveillance systems and drew recommendations to address them. Especially, we found that the Integrated Disease Surveillance and Response framework was not implemented uniformly across the seven countries and that the harmonisation and compilation of data from different surveillance systems was the most commonly reported challenge.

Role: The fellow conducted all the interviews with the key informants, collected and summarised the information into a report. The fellow also did two presentations during the GHOA incident management support team meetings. During the international assignment, the fellow supported routine activities such as: revision of situation report, realisation of epi-curves, assist to IMST meetings from the seven countries, data scrapping, update of the table compiling the key data for each outbreak of the region.

### **Educational outcome**

Over the course of these surveillance projects, the fellow participated in a wide range of surveillance-related competencies, such as evaluating the previously established laboratory techniques for surveillance, as in the case of measles, performing large-scale sample management and data analysis as well as using the data collected to formulate action-oriented recommendations. The fellow also had the unique opportunity to participate in an emergency response with WHO and to learn about disease and nutrition surveillance in low- and middle-income countries. The combined experience provided by the training modules and the experience in the lab and on field have left the fellow with the skills required to confidently interpret and evaluate surveillance systems and the data they provide in a wide array of future applications.

## 2. Applied public health microbiology and laboratory investigations

### ***2.1. Comparison of genetic and phenotypic characteristics of Salmonella enterica serovar Choleraesuis isolated in humans and animals from 2006 to 2021: a One Health approach for antimicrobial resistance surveillance.***

Supervisors: Silvia Herrera-León (CNM, Spain) and Julio Alvarez Sanchez (VISAVET-UCM, Spain)

Category: Food and waterborne diseases

The serovar Choleraesuis is a well-known host-adapted pathogen in swine and can lead to invasive diseases in 56.4% of human cases, for which antimicrobial therapy is necessary. Multidrug-resistant (MDR) Choleraesuis clones are increasingly reported in swine and wild boars in Europe and worldwide, with 20% being extended-spectrum beta-lactamase producing. Through a collaboration with the veterinarian public health institute, we compared Choleraesuis isolates from different origins (human and animal) in order to characterise the circulation of MDR strains in Spain and inform clinical guidelines for antimicrobial treatment.

We found that several MDR clones of *S. Choleraesuis* were circulating in Spain from 2006 to 2021 and that 19% of them were resistant to fluoroquinolones. We identified that the plasmid Inc-HI2/Inc-HI2A was more frequent in swine than human isolates and that certain phylogenetic clusters accumulated genes of resistance. Finally, WGS allowed the identification of a small cluster of colistin-resistance strains associated with an outbreak in a pig farm. The team from VISAVET organized meetings with the farm managers to inform them and communicated on the possible control measures.

The project was at the interface between animal and human health which are two fundamental actors of salmonellosis transmission. Therefore, the study of *S. Choleraesuis* transmission and resistance provides a compelling example of the One Health paradigm. Reducing human infections will require the adoption of new prevention strategies such as control measures on hygiene and biosafety in pig farms and during transportation that will not only seek to reduce *Salmonella* transmission in humans but also in animals and from the environment. By focusing on a serovar with a high impact on public health, our results could permit the adaptation of clinical guidelines for antimicrobial treatment in patients infected with *S. Choleraesuis*, by prioritising the use of ciprofloxacin over other quinolones.

Role: The fellow participated in every step of the work both in the laboratory and for the analyses: DNA extraction, WGS, cgMLST and phylogenetic analyses. She presented the work to the collaborators at VISAVET and at ECCMID as a poster. She drafted the manuscript and included revisions from the co-authors.

### ***2.2. Long-term impact of chronic HCV infection on immunological markers in HIV+ patients: a prospective study in Spain from 2016 to 2018***

Supervisor: Amanda Fernandez Rodriguez and Veronica Briz (CNM, Spain)

Category: Sexually-transmitted diseases

The development of direct acting antiviral (DAA) has revolutionised chronic hepatitis C management and almost all treated patients achieve a sustained virological response (SVR). The aim of this work was to analyse whether HCV clearance may partially restore both humoral and cellular immune profile to levels similar to those found HIV mono-infected patients. Furthermore, we explored the changes over time in the immune profile of patients that cleared HCV spontaneously to distinguish the impact of chronic infection and/or of the treatment.

Ninety HIV patients with different status of HCV infection were enrolled: 1) CHC group: HIV patients with active HCV-chronic infection naïve to any HCV treatment; 2) SC group: HIV patients who had been acutely infected with HCV and experienced spontaneous viral clearance during the first 6 months after HCV infection; 3) control group: HIV patients without previous HCV infection. CHC patients were evaluated at baseline and 48 weeks after the achievement of SVR with DAAs treatment. SC was sampled with the same follow-up. We used spectral flow cytometry and ELISA multiplex immunoassays to assess inflammation, immuno-senescence, and activation markers.

One year following DAA treatment, we found that CHC patients had lower levels of circulating senescence markers and naïve central memory CD4 and CD8 T cells compared to controls. SC patients had a higher level of effector memory CD4 and CD8 T cells than controls. In the CHC group, we found a decrease in pro-inflammatory cytokines, checkpoint inhibitors and immune cell activation after treatment compared to baseline. Alternatively, we found an increase in exhausted immune cells and immuno-senescence markers in SC group over time to level similar of those in controls. In conclusion, the HCV elimination with DAAs after chronic HCV infection led to an improvement of cellular senescence profile and spontaneous HCV clearance could have been associated with a transient improvement of immuno-senescence.

Role: The fellow was responsible for performing spectral flow cytometry on all the patients at baseline and follow-up which included adapting the protocol for flow cytometry to optimise cost and time. The fellow collected the data and analysed the results of the flow cytometry and performed the statistical analyses. Finally, the fellow drafted the manuscript.

### **2.3. Genotyping of *P. falciparum* in the context of hospital-acquired malaria infections**

Supervisor: José Miguel Rubio (CNM, Spain)

Category: Vector-borne diseases

While malaria is still endemic in 97 countries, it has been considered eradicated in Spain since 1964 and is a statutorily notifiable disease. Nevertheless, between 2019 and 2021, 18 autonomous communities reported 1 477 confirmed cases principally due to *P. falciparum*. Malaria cases in non-endemic countries may be due to introduced malaria, imported malaria, airport malaria, congenital or induced (blood transfusions and blood products, post-transplant, parenteral, and health care related) malaria. Even though they represent rare events, cases of hospital-acquired malaria transmission have been described in the literature. It is generally described as non-vector-borne malaria acquired in a healthcare facility by a patient in whom the infection was not present or incubating at the time of admission.

In Spain, the first reported case of nosocomial malaria was in 1978. Subsequently and until 2016, three further cases were reported with an epidemiological link to a hospital, but it was not possible to establish the mechanism of infection in any of those cases. As of 2023, eight cases of nosocomial malaria infection were reported in Spain. Genotyping has therefore been introduced in many epidemiological investigations to study population diversity and establish links between cases. Commonly used markers for the genotyping are regions of genes coding for three surface antigens: the merozoite surface protein (MSP) 1 and 2 and the glutamate-rich protein (GLURP). The fellow was invited to a training exercise to perform the genotyping method on a random selection of five unrelated samples and to interpret the results of the genotyping. In this activity, the method gave the expected results and showed that the samples did not share the same allelic profiles nor the same populations. Therefore, in a real-life situation, we would have concluded that those samples were infected independently, and this would have not supported the hypothesis of nosocomial infection.

Role: The fellow performed qPCR, automated electrophoresis and the analyses of the results for the generation of an activity report.

#### **Educational outcome**

The first two research projects allowed the fellow to acquire and strengthen a very broad spectrum of knowledge and techniques including WGS and immunology. The One Health approach was a new experience for the fellow and working alongside multidisciplinary stakeholders was enriching and has helped prepare the fellow to undertake such tasks in the future. In addition, the exploration of the long-term effect of DAA treatment in HIV/HCV co-infected patients highlights the importance of longitudinal studies of prospective cohorts in applied public health research. Finally, the laboratory investigation on hospital-acquired malaria infections was a great opportunity for the fellow to learn about *P. falciparum* genotyping and malaria surveillance and the epidemiological situation in Spain.

## **3. Biorisk management**

Previously to the project described below, the fellow was trained to access and conduct experiments in the BSL-3 facility. Since then, the fellow conducted several activities on *M. tuberculosis* in BSL-3 conditions as described in other sections.

### **3.1. Highly pathogenic pathogens EQAE**

Supervisors: Raquel Escudero Nieto (CNM, Spain)

Category: Imported and emerging diseases

The External Quality Assurance Exercise (EQAE) targets at the identification of Risk Group 3 bacteria and is embedded in the Joint Action on Strengthened International Regulations and Preparedness in the EU (SHARP JA) co-funded by the European Commission (Strengthened International Health Regulations and Preparedness in the EU– 848096). The laboratory analysis was performed by the Reference Laboratory on Special Pathogens (RLSP). Before the EQAE, the laboratory had worked extensively to validate their panel of PCRs used to identify the different bacterial species and subspecies. Three sets of samples are sent to the RLSP comprising living (infectious), inactivated (non-infectious) and serological (potentially infectious) samples.

The exercise included several tasks: i) to report arrival of the parcel and shipment conditions; ii) to identify or to rule out the target inside of all coded samples; iii) to specify the *Brucella* species and the *Francisella tularensis* subspecies, if identified and v) to report the methods chosen for analysis. The panel of potential bacterial species included: *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis* subspecies (included are: *F.t. tularensis*, *F.t. holarctica*, *F.t. mediasiatica*, *F.t. novicida*) *Burkholderia pseudomallei*, *Burkholderia mallei*, *Brucella* species and *Coxiella burnetii*.

The deadline for completion was 24 of May 2022 and the participating diagnostic laboratories were notified about the contents of the samples in July 2022. Furthermore, a meeting to discuss the EQAE, potential problems, new techniques and the improvement of SHARP was held in September 2022 in Berlin.

Role: BSL-3 diagnostic activity, biorisk management, laboratory sample management; application of microbiological knowledge to complete EQAE; molecular characterization (qPCR) and MALDI-TOFF.

### **3.2. Contact investigation following case of *Corynebacterium* spp and ELEK testing for the rapid identification of toxigenic *Corynebacterium* spp.**

Supervisors: Laura Herrera-León and Silvia Herrera-León (CNM, Spain)

Category: vaccine preventable diseases

Diphtheria is an acute infectious disease that affects the upper respiratory tract and occasionally the skin caused by the production of the diphtheria toxin (Dtx) of certain species of *Corynebacterium*, usually, *C. diphtheriae* and *C. ulcerans*. While the incidence in Spain resembles that of the rest of EU, diphtheria has been increasing in relevance due to surges in mass relocation events of travellers, refugees, asylum seekers and immigrants from diphtheria endemic countries. This mobilisation in combination with a growing vaccine hesitancy in non-endemic countries, gives diphtheria a high potential for re-emergence.

In July 2023, a case of non-toxigenic Diphtheria was reported following the arrival of a boat of immigrants in the Canary Islands. Because of the proximity on the boat, nasal and oropharyngeal samples were collected from the passengers and sent to the CNM for testing. From over 100 samples, we identified five positive samples for non-toxigenic *Corynebacterium diphtheria*. All the cases were treated to avoid further spread.

In addition, with an increased amount of suspected diphtheria cases being submitted to the national reference centre, the phenotypical determination of toxigenicity is crucial. The ELEK test is used in the CNM to confirm the expression of the *tox* gene and to reduce the time of diagnosis. The fellow was trained on the method and how to apply it to respond to outbreak cases. Over the course of the fellowship, a number of clinical samples of suspected toxigenic diphtheria cases were submitted for confirmation to the CNM and the fellow was ready to assist with the realization of an ELEK test if the presence of the toxin was confirmed by PCR.

**Role:** Performing bacterial culture from nasal and oropharyngeal samples, identification of bacterial colonies and conventional PCR for species identification as well as toxin determination. Application of ELEK in outbreak samples and working in BSL+ conditions.

#### **Educational outcome**

It was a unique opportunity for the fellow to learn about the biology and the symptoms associated with highly pathogenic bacteria. In addition, the fellow was given the chance to learn how to culture and inactivate high-risk bacteria in BSL-2+ and BSL-3 conditions as well as the diagnostic workflow. The fellow learnt the advantages and disadvantages of the MALDI-TOF method. Finally, this activity allowed the fellow to have a glimpse of what preparedness to bioterrorism attacks and outbreaks of high threat pathogens looks like and how quality is implemented in the RSLP.



## 4. Quality management

### 4.1. 11th External Quality Assessment scheme for typing of Shiga toxin-producing *Escherichia coli* in 2022-2023

Supervisors: Silvia Herrera-León (CNM, Spain)

Category: Food and waterborne diseases

The ECDC Food and Waterborne Diseases and Zoonoses Programme organise an annual External Quality Assessment for the typing of Shiga toxin-producing *Escherichia coli* (STEC). The Reference Laboratory for Food- and Waterborne diseases (FWD) of the Instituto de Salud Carlos III (ISCIII) was invited to participate as member of the FWD network. Since 2012, the unit of Foodborne Infections at Statens Serum Institut (SSI) in Denmark has arranged this EQA. The aim is to harmonise typing methods used by European laboratories in order to produce comparable data and ascertain high quality data in the European Surveillance System (TESSy).

The EQA had three objectives: i) serotyping, ii) detection of virulence genes, and iii) molecular typing-based cluster analysis. We used WGS to assign correct O groups and H types. Virulence genes detection and subtyping of the *stx* genes were realized with conventional and real-time PCR but also confirmed using the CGE tool VirulenceFinder. Finally, cluster of closely related isolates were identified using WGS and additional sequences provided by the EQA coordinator.

The fellow had an active participation to this EQA, performing all steps of the EQA: PCR assays, WGS, cluster analysis and to the upload of the results. For the fellow, it was a unique opportunity to conduct an EQA in a semi-independent manner. The deadline for completion was set in October 2022. The final results were communicated through an online form and raw reads were also uploaded on a server. The participating diagnostic laboratories were notified about the content of the samples in December 2022. The evaluation report showed 100% correct assignment of O groups and H types and correct identification the cluster of closely related strains. A meeting was held to discuss the EQA, potential problems, new techniques and improvement.

Role: The fellow applied knowledge on biorisk management, laboratory sample management, and microbiological techniques to complete the EQAE. She performed the molecular characterisation (qPCR), WGS and bioinformatic analyses (cgMLST) and submission/validation of the results.

### 4.2. Validation of the MTplex MONODOSE dtcc-qPCR for the diagnostic of the *Mycobacterium tuberculosis* complex and of a qPCR (VITRO) for the rapid diagnostic of tuberculosis and non-tuberculosis *Mycobacteria*.

Supervisor: Laura Herrera-León (CNM, Spain)

Category: Respiratory diseases

The *Mycobacterium tuberculosis* complex (MTBC) refers to a genetically closely related group of *Mycobacterium* species which includes *M. tuberculosis*, *M. africanum*, *M. bovis* and *M. bovis* BCG, *M. caprae*, *M. microti*, *M. canettii*, *M. pinnipedii* and the newly described *M. mungi*. While TB culture is regarded as the gold standard method, TB culture is normally performed as a back-up test method because of the long periods of time required for TB growth in culture (weeks to months). To address this issue, gene amplification techniques were developed and can now achieve superior performance over TB culture-based methods such as quantitative polymerase chain reaction (qPCR).

MTplex MONODOSE dtcc-qPCR was developed by Genetic PCR Solutions™ for MTBC detection. This kit contains ready-to-use tubes containing all the components needed for detection of the MTBC. Several advantages are conferred by MONODOSE, it is less time-consuming, and it can be stored at room temperature (dehydrated). The aim of our study was to validate this new qPCR test using clinical samples previously characterised in the laboratory and to compare the results with an in-house PCR which was published for the differential diagnostic of *M. tuberculosis*. We assessed/determined sensitivity, specificity, positive and negative predictive values of the test as well as to calculate the efficiency, linearity and detection limit.

The second validation was performed for the qPCR kit from Vitro Master Diagnostica. The kit determines if a sputum is positive for *M. tuberculosis*, MTBC species or non-tuberculosis species in one assay. The aim of our study was to validate this new qPCR test using clinical samples previously characterised in the laboratory. We also test the cross-reactivity of the qPCR test with other bacterial and fungal species, and we characterised the limit of detection by doing serial dilutions.

**Role:** The fellow was responsible of DNA extraction and to perform the qPCR assays. The fellow was also in charge in analysing the results and communicating them with the different stakeholders.

### 4.3. Culture and antifungal susceptibility testing of dermatophytes: implementation of EUCAST protocol

Supervisor: Ana Alastruey-Izquierdo (CNM, Spain)

Category: Antimicrobial resistance

Dermatophytosis is the most common fungal infection in the world affecting a large proportion of the population especially in tropical and subtropical regions. There are a limited number of drugs that can be used to treat dermatophytosis and terbinafine (TRB) is widely used and is considered to be a first-line drug in the therapy of dermatophyte infections. Antifungal drug-resistance in dermatophytes was rare but since 2017, the problem has become increasingly concerning with several cases of TRB-resistant *Trichophyton rubrum* or *T. interdigitale* both in Europe and Asia. Therefore, dermatophytosis and management of drug resistance in this group is a major public health challenge as it can lead to therapeutic failures.

Antifungal susceptibility tests (AFST) are performed when infections are invasive, relapsing or failing therapy or when susceptibility cannot reliably be predicted from the species identification alone. In dilution methods, fungi are tested for their ability to produce sufficient growth in microplate wells of broth culture medium containing serial dilutions of the antifungal agents. This method allows the determination of the antifungal Minimum Inhibitory Concentration (MIC) and the resistance of the isolates to a given antifungal product. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) recently developed a new protocol of AFST for dermatophytes to determinate the MIC *in vitro*.

The Mycology Reference Laboratory (MRL) at the National Centre for Microbiology (CNM) is aiming to implement this new protocol and to apply it for a retrospective study. However, during this activity we observe a low recovery rate of samples after long-term storage and the retrospective study was not performed. The protocol was therefore tested on samples from 2022 and 2023.

**Role:** The fellow was responsible of establishing the best method to recover samples and was trained on the preparation of growth media and microdilutions plates but also on how to perform MIC assays.

#### Educational outcome

It was the first time that the fellow actively participated to an EQA and it was a unique opportunity to learn about the biology of *E. coli* and the definition of the different pathotypes (EAEC, EIEC, EPEC, STEC, etc.) associated with the virulence genes tested. The fellow learnt the methods for STEC *E. coli* identification and more generally about the biology of enterobacteria. In addition, it was the first experience of the fellow with qPCR validation for start-ups and companies. This was a great opportunity to learn more about concepts such as sensitivity, specificity, and limit of detection and to work with clinical samples. Finally, the activities on *E. coli* and dermatophytes gave to the fellow a glimpse of what surveillance of these pathogens implies and how diagnostic is performed routinely.

## 5. Public health microbiology management

While the fellow conducted an activity which was centred in public health management, this competence was also developed across multiple projects and activities. Indeed, the three outbreak investigations have required a high level of communication with collaborators and other stakeholders. In addition, the project conducted during the international assignment had also a big component of management as the fellow had to not only coordinate with the teams but also with different actors from different organizations and countries. Finally, she was given a glimpse of what the management of a laboratory entails during the internal audit of the food and water-borne disease reference laboratory.

### 5.1. Epidemic intelligence in the Centro de Coordinación de Alertas y Emergencias Sanitarias (CCAES) in the Ministry of Health

Supervisors: Bernardo Guzmán (CCAES, Ministry of Health, Spain)

The goal of epidemic intelligence is to obtain rapid information on events or situations that may require actions from the CCAES or other public administrations. The CCAES is principally concerned by i) events that affect multiple autonomous communities, and which will therefore need a coordination at the national level; ii) events that could affect multiple countries such as events related to travellers or foreign citizens; iii) events with a capacity to disseminate internationally.

Every day, the result of the epidemic intelligence is presented during a round table, where all CCAES employees are present, and a risk evaluation is performed. For each signal, the team can take a number of actions: to discard the signal, to follow-up the situation, to do a rapid risk assessment or a situation report and/or to propose responses measures.

Following the presentation and evaluation of the signals extracted from the epidemic intelligence work, two reports are prepared. The daily report of signals is organised in international and national sections and is shared internally and with Department of Foreign Health. It can also contain updates on the epidemiological situations of different notifiable diseases. The daily alert report is shared broadly to all potential stakeholders and contains only new information on public health alerts.

An event related to a case of measles, who had recently taken an international flight, was identified in January 2023 and the CCAES then informed all countries and Spanish regions where flight contacts resided and provided them advice for contact tracing. The CCAES also collaborated with the National Center for Epidemiology to elaborate surveys to collect information on contact tracing protocols in each autonomous community.

Role: The fellow was given the opportunity to conduct epidemic intelligence and to follow the response to an event related to measles cases. The fellow assisted to a series of presentation on the different function of the CCAES and to two days of the steering committee of the joint action TERROR (<https://www.jaterror.eu/>).

### ***Educational outcome***

This two weeks in the CCAES was a unique opportunity for the fellow to learn about public health management and to be familiarised with the different institutions involved in public health management at the national, European and international level. The fellow performed epidemic intelligence which was greatly beneficial to her understanding of signal detection and risk evaluation. In addition, the fellow learned about the management of imported measles cases and how to deal with contact tracing when international flights are involved. Finally, assisting to the steering committee of the joint action TERROR, in which Spanish Ministry of Health leads one Work Package allowed the fellow to be familiarised with European projects.

## **6. Teaching and pedagogy**

### ***6.1 Case study for students of the 'Microbiology Applied to Public Health and Infectious Diseases Research' degree at the University of Alcala***

The ECDC case study (Gastroenteritis in Kalundborg, Denmark) was facilitated for Masters students of the University of Alcala, in 2021 and 2022. Both case studies required two hours of preparation and ran for about four hours each. Verbal feedback from the students was very positive and throughout the case study a productive and dynamic conversation was maintained.

### ***6.2. Facilitation of the exercise on descriptive analysis of surveillance data for tuberculosis, meningitis and legionellosis in Spain for students of the 'Microbiology Applied to Public Health and Infectious Diseases Research' degree at the University of Alcala***

During this exercise, databases containing anonymised surveillance data were provided to the students as digital copy and the instruction was given orally during a 1h presentation of the exercise. Groups of six students were formed and they organised their work independently. At the end of the exercise, the students conducted a short descriptive analysis based on time, place and person but also to generate hypotheses based on the descriptive analyses. They summarised their results in an oral presentation with informative tables and figures. An anonymous evaluation was given as a hard copy to evaluate the content of the whole module (not only the exercise). Even though the students would have liked more time to perform the exercise, the feedback was overall really positive.

### ***6.3. Factors influencing quality in laboratories for EPIET and EUPHEM fellows participating in the Biorisk and Quality Management Module***

The lecture on factors influencing quality in laboratories was performed for EPIET 2020 and EUPHEM 2021 fellows in 2022 for the Biorisk and Quality Management Module. The lecture was one hour long and virtual. The learning needs assessment was performed by the team that developed the module. Active participation of the learners was insured by prompting them to answer SLIDO questions and by inviting them to share personal experiences on quality management. The presentation was formally evaluated through a questionnaire on EVA and received an average satisfaction score of 9/10.

### ***6.4. Mentoring fellows, Master and PhD students***

Alongside their host-site supervisor, the fellow, co-supervised MediPIET and EUPHEM fellow who came to the National Center of Microbiology for a short stay, a Medical Biology undergraduate student from the University of Alcala in the context of the HIV/HCV project and finally a PhD student from Ethiopia that came to Spain to realise a project on tuberculosis. This included assistance with laboratory activities for fellows and the supervision for flow cytometry experiments for the master student. The fellow was involved in the support to the PhD student in the realization of the libraries for his project as well as support with the bioinformatics analyses. As a result the fellow was listed as co-author in 2 publications of which one has been accepted in Frontiers in Microbiology.

## 6.5. Update of the ECDC case study Gastroenteritis in Kalundborg, Denmark

After facilitating the ECDC case study (Gastroenteritis in Kalundborg, Denmark) for Masters students of the University of Alcalá for two years, the fellow proposed to update the case study and use her recent experience with norovirus outbreaks. Therefore, we prepared a new case study based on the norovirus outbreak in Muxia, Galicia, Spain (see outbreak section). The aim was to use this case study with the Master student as they might be able to relate better with a Spanish outbreak but also to propose the update to ECDC for teaching EPIET/EUPHEM fellows.

### Educational outcome

These activities provided valuable experience in modern pedagogical techniques to the fellow. The fellow was able to practice three effective ways to achieve diverse learning objectives, such as lectures, practical exercise and case-study, as well as different teaching environments, virtual and in-person but also in Spanish and English. Teaching in an online format required some innovative strategies to make sure students participate and are following actively, but using SLIDO ensured that students are achieving the set learning objectives. Finally, the fellow was given the opportunity to evaluate the presentation of the surveillance exercise as a member of the jury which was a first experience and help her gain new pedagogical skills.

## 7. Communication

### 8.1 Publications related to the EUPHEM fellowship

#### 8.1.1 Manuscripts published in peer-reviewed journals

- **Paper 1:** Jacqueline C\*, Arrojo M del V\*, Moreira PB, Feijóo MAR, Cabrerizo M, Fernandez-Garcia MD. **2022** Norovirus GII.3[P12] Outbreak Associated with the Drinking Water Supply in a Rural Area in Galicia, Spain, 2021. *Microbiol. Spectr.* 10. **(Published)**
- **Paper 2:** Jacqueline C, Gavilan AM, Lopez-Perea N, Penedos AR, Masa-Calles J, Echevarria JE, Fernandez-Garcia A. **2023**. Utility of MF-non coding region for measles molecular surveillance during post-elimination phase, Spain, 2017–2020. *Front. In Microbiology*. **(Published)**
- **Paper 3:** Mekonnen D., Munshea A, Nibret E., ..., Jacqueline C., Aseffa A., Herrera-León L. **2023**. Comparative Whole Genome Sequence Analysis of *Mycobacterium tuberculosis* Isolated from Pulmonary Tuberculosis and Tuberculous Lymphadenitis Patients in Northwest Ethiopia. *Front. In Microbiology*. **(Published)**
- **Paper 4:** Jacqueline C, ... Herrera-Leon S. Genetic characterization of extensively-drug resistant *Shigella sonnei* infections in Spain, 2021-2022. *Emerging Infectious Diseases*. **(Published)**
- **Paper 5:** Mekonnen D., Munshea A, Nibret E., ..., Jacqueline C., Aseffa A., Herrera-León L. *Mycobacterium tuberculosis* Sub Lineage 4.2.2/SIT149 as dominant drug resistant clade in Ethiopia. *Infection and Drug Resistance*. **(Submitted)**
- **Paper 6:** Jacqueline C.\*, Minetti C\*., Pista A.#, Herrera-Leon S.# Circulation of fluoroquinolone-resistant strains of *Shigella sonnei* and *S. flexneri* in the Iberic peninsula from 2015 to 2022: a retrospective study. *Eurosurveillance*. **(Submitted)**
- **Paper 7:** Jacqueline C., Samper Cativiela C., Alvarez Sanchez J. #, Herrera-Leon S.# Comparison of phenotypic and genetic characteristics of *Salmonella enterica* serovar Choleraesuis isolated in humans and animals in Spain from 2006 to 2021: a One Health approach for antimicrobial resistance surveillance. *Journal of Antimicrobial Chemotherapy*. **(Submitted)**
- **Paper 8:** Jacqueline C., Beraza X., Castells Carrillo C., Garros Garay F. J., Unzaga Barañano J., Quiros Fernandez S., Bilbao Calvo M., Herrera Leon L. Multidrug-resistant tuberculosis outbreak associated with African immigrants from 2019 to 2022 in Bilbao, Spain. **(In preparation)**
- **Paper 9:** Jacqueline C.\*, Llamas M\*, ..., Fernández-Rodríguez A.#, Briz V.# T Cell Immune Phenotypic and Senescence Profile in People living with HIV co-infected with HCV or who spontaneously cleared HCV. **(In preparation)**
- **Paper 10:** Jacqueline C.\*, ..., Fernández-Rodríguez A.#, Briz V.# Changes in the immune phenotype and senescence profile of T Cell after spontaneous HCV elimination or DAAs therapy in People living with HIV: a longitudinal study. **(In preparation)**

#### 8.1.2 Other reports

- **Report 1:** 2022 Multi-country outbreak of *Salmonella* Enteritidis sequence type (ST)11 infections linked to eggs and egg products – 8 February 2022. EFSA Support. Publ. 19. (doi:10.2903/sp.efsa.2022.en-7180)

## 8.2 Conference presentations

1. Poster presentation: Norovirus GII.3[P12] Outbreak associated with the drinking-water supply in a rural area in Galicia, Spain, 2021. *ESCAIDE, 2022, Stockholm*
2. Poster presentation: Genetic characterization of extensively-drug resistant *Shigella sonnei* infections in Spain, 2021-2022. *ESCAIDE, 2022, Stockholm*
3. Flash-Poster presentation: Utility of MF-non coding region for measles molecular surveillance during post-elimination phase, Spain, 2017–2020. *ECCMID, 2023, Copenhagen*
4. Flash-Poster presentation: Comparison of genetic and phenotypic characteristics of *Salmonella enterica* serovar Choleraesuis isolated in humans and animals from 2006 to 2021: a One Health approach for antimicrobial resistance surveillance. *ECCMID, 2023, Copenhagen*
5. Flash-Poster presentation: Circulation of fluoroquinolone resistant strains of *Shigella sonnei* and *S. flexneri* in Spain from 2015 to 2022. *ECCMID, 2023, Copenhagen*
6. Poster presentation + Flash Oral presentation: Circulation of fluoroquinolone resistant strains of *Shigella sonnei* and *S. flexneri* in Spain from 2015 to 2022. *FETP Nights, 2023, Atlanta*

## 8.3 Other presentations

1. Oral presentation: Description of non-toxicogenic vibriosis cases in Spain from 2012 to 2022. ECDC Food and Waterborne Network, 2023, Helsinki.
2. Oral presentation: Multidrug-resistant tuberculosis outbreak associated with migrants from 2019 to 2023 in Bilbao, Spain

## 9. EPIET/EUPHEM modules attended

1. EUPHEM/EPEIT Virtual Introductory Course, 20/09/2021-08/10/2021, Virtual
2. Inject day: Phylogeny, 20/10/2021, Virtual
3. Inject days: Operational Research, 27/10/2021 –28/10/2021, Virtual
4. Inject days: Data Collection and Management, 10/11/2021 – 11/11/2021, Virtual
5. Outbreak investigation Module, 06/12/2021 –10/12/2021, Virtual
6. Biorisk and quality management Inject days , 17/01/2022 – 18/01/2022, Virtual
7. Multivariate analyses module, 14/03/2022-18/03/2022, Virtual
8. Inject days: Cox regression, 30/03/2022, Virtual
9. Project review activity, 20/04/2022-22/04/2022, Spetses, Greece
10. Introductory Course Part II, 25/04/2022-29/04/2022, Spetses, Greece
11. Rapid assessment survey module, 06/06/2022-10/06/2022, Stockholm, Sweden
12. Project review module, 29/08/2022-02/09/2022, Lisbon, Portugal
13. Time Series Analyses module, 07/11/2022-11/11/2022, Bilthoven, Netherlands
14. Inject days: Qualitative studies, 31/01/2023, 03/02/2023, Virtual
15. Vaccinology module, 13/02/2023-17/03/2023, Virtual
16. Leadership, Management and Communication in Public Health, 08/05/2023-12/05/2023, Stockholm, Sweden
17. Project review module, 28/08/2023-01/09/2023, Lisbon, Portugal

## 10. Other training

1. Addressing online vaccination misinformation, EVA platform
2. Introduction to Infectious Disease Preparedness micro learning, EVA platform
3. BSAFE, WHO online
4. UN Prevention of Harassment, Sexual Harassment and Abuse of Authority (PSEA), WHO online
5. Introduction on the prevention of and response to sexual exploitation, abuse, and harassment (PRSEAH), WHO online
6. United to Respect: Preventing Sexual Harassment and Other Prohibited Conduct, WHO online
7. UN Human Rights and Responsibilities, WHO online
8. WHO SOP for Emergencies, WHO online

## 11. Missions

21/02/2023 – 14/04/2023, Nairobi, Kenya in the IMST Drought and food-insecurity in the Great Horn of Africa, Health Information Management Team: Mapping of surveillance systems in the Great Horn of Africa

## 12. Other activities

During the fellowship, Camille was elected as one of the EUPHEM representatives. She participated in training sites forums, various ECDC fellowship workgroups, meetings with the fellowship office and a variety of other tasks to best represent Cohort 2021. As a liaison between the fellows and the fellowship office, it was particularly important to facilitate communication during the COVID-19 pandemic. In addition to many meetings with the cohort representatives from Cohort 2020 and 2022, satisfaction surveys were also key to support the continuous improvement of the EPIET/EUPHEM fellowship.

## Acknowledgements

First, I want to thank the amazing team of the supervisors/Wonder Women that surrounded me during those two years, Silvia, Laura, Ana and Lola. I appreciated all the hard work you have done for me and the opportunity you gave me inside the laboratory to be independent and learn from you as well as outside the laboratory to represent our work at international conferences. Thank you also for being good mentors and discussing what were the best options for me professionally and personally. I can say that this fellowship changed my career, and you had a huge part in the success of my transition towards public health microbiology.

I also want to thank all the supervisors that opened the doors of their laboratory to me and dedicated time and energy to the project we had in common. It was a pleasure and an honour to work with such a team of motivated and brilliant people.

I am very grateful to have been the last fellow of frontline coordinator Aftab Jasir and I want to thank her for her kindness, support and reactivity. This made our work so much easier and ensure I was always reaching my learning objectives and professional goals. I also want to thank Jennifer Bender for taking over and helping me to give the last push for the exit interview.

Finally, I was so lucky to be surrounded by wonderful people during those two years and especially by the cohort 2021. They were a constant source of inspiration and comfort and I know our paths will cross again very soon. I want to give a special shout-out to the EUPHEM fellows which were both a technical and emotional support during those two years. I'd like to give a special thanks to Alba Mendez and Lea Franconeri who shared every minute of my journey in Kenya and who made it even better. Thank you to my fellow cohort representatives and Adam Roth it was a real pleasure to work in improving the fellowship experience for all.