EUPHEM REPORT

Summary of work activities
Emma Sáez López
European Public Health Microbiology Training
Programme (EUPHEM), 2017 cohort

Background

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

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

According to Articles 5 and 9 of ECDC's founding regulation (EC No 851/2004) 'the Centre shall, encourage cooperation between expert and reference laboratories, foster the development of sufficient capacity within the community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health' and 'as appropriate, support and coordinate training programmes in order to assist Member States and the Commission to have sufficient numbers of trained specialists, in particular in epidemiological surveillance and field investigations, and to have a capability to define health measures to control disease outbreaks'.

Moreover, Article 47 of the Lisbon Treaty states that 'Member States shall, within the framework of a joint programme, encourage the exchange of young workers. 'Therefore, ECDC initiated the two-year EUPHEM training programme in 2008. EUPHEM is closely linked to the European Programme for Intervention Epidemiology Training (EPIET). Both EUPHEM and EPIET are considered 'specialist pathways' of the two-year ECDC fellowship programme for applied disease prevention and control.

This report summarises the work activities undertaken by **Emma Sáez López**, **cohort 2017** of the European Public Health Microbiology Training Programme (EUPHEM) at the National Health Institute Doutor Ricardo Jorge (Instituto Nacional de Saúde Doutor Ricardo Jorge, INSA), Lisbon, Portugal. All EUPHEM activities aim to address different aspects of public health microbiology and underline the various roles of public health laboratory scientists within public health systems.

Pre-fellowship short biography

In 2009, Emma Sáez López completed her studies at the University of Salamanca (Spain) with a Bachelor's degree in Biology and Biochemistry, having spent one year in Leuven (Belgium), with an Erasmus scholarship. After graduation, she spent the following three months in Argentina working for the Spanish Agency of International Cooperation for Development (AECID) and then, she worked as a European volunteer for one year in Guinea-Bissau. She returned to Spain to take a Master's degree in International Health at the University of Barcelona. After the Master thesis in the ISGlobal-Barcelona Institute for Global Health (Hospital Clinic-University of Barcelona), former CRESIB, she undertook the PhD in Microbiology related to obstetric and neonatal infections caused by *Escherichia coli*. During the development of her PhD project, she participated in three scientific internships in the two African countries of Morocco and Mozambique.

Methods

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

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

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

Results

The objectives of these core competency domains were achieved partly through projects or activities (on-job services) and partly through participation in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the EUPHEM scientific guide[1].

1. Epidemiological investigations

1.1. Outbreak investigations

A. Lessons learned from a prolonged Norovirus GII.P16-GII.4 Sydney 2012 variant outbreak in a long-term care facility in Portugal, 2017

Supervisors: Rita de Sousa (National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases) and Ricardo Mexia (Department of Epidemiology)

In October 2017, an outbreak of acute gastroenteritis began caused by norovirus (NoV) occurred in a long-term care facility (LTCF) in Mafra, Portugal. The objectives of this study were to investigate, describe and estimate the outbreak extent and implement control measures. The outbreak was caused by NoV GII.P16-GII.4 Sydney 2012, affecting 146 people and is the first report of the circulation of this lineage in Portugal. Higher attack rates were observed in residents (29%; 97/335), and in nurses (19%; 16/83). All of the 11 resident wards were affected. Data on cases and their working or living areas suggested that movement between wards facilitated the transmission of NoV, likely through person-to-person transmission. The delay in the identification of the causative agent, lack of restrictions on resident and staff movement between wards and ineffective deep cleaning procedures resulted in outbreak lasting for more than one month. The outbreak ended only after implementation of strict

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

control measures. Recommendations for controlling future NoV outbreaks in LTCFs include emphasizing the need to control resident's movements and restrict visitors, timely and effective environmental cleaning and disinfection, leave of absence for ill staff and encouraging effective hand hygiene. This project was executed in close collaboration with the Public Health Unit of ACES South West, Regional Health Administration of Lisbon and Tagus Valley.

The fellow participated in all stages of the outbreak investigation, from contributing to designing questionnaires, conducting interviews, performance of the microbiological investigation, analysing the data, to writing the manuscript. Results were published in the Infection Control & Hospital Epidemiology Journal (see Publications section below). Results were presented at ECCMID 2019 (European Conference of Clinical Microbiology and Infectious Diseases) as a mini-oral presentation (see Conference presentations section below).

B. Three measles outbreaks in three Health Regions of Portugal, February to April 2018

Supervisor: Paula Palminha (National Reference Laboratory for Vaccine Preventable Diseases, Department of Infectious Diseases)

Three simultaneous measles outbreaks with 112 confirmed cases in three Health Regions of Portugal occurred from February to April 2018. Measles case definition used for epidemiological surveillance in Portugal was based on the EU case definition. The mean age of cases was 30 years, 79% worked in a healthcare setting and 87% were vaccinated. Primary cases in each outbreak were imported and represented three different chains of transmission from France, Italy and Africa. Among the 112 confirmed cases, 83 (74.1%) were vaccinated with two or more doses of a measles-containing vaccine. Fifty (44.6%) confirmed cases did not meet the clinical criteria from the EU case definition; among them, 24 of 50 had a maculopapular rash and fever as clinical presentation and 13 cases (11.6%) only had a maculopapular rash. Twenty-one cases (18.8%) were confirmed through laboratory results of second samples, where an increase of either IgM, IgG (≥ 4x) or both was verified. Genotype B3 was identified in 84 cases from all the three chains of transmission, although the four cases from the Africa-related chain of transmission had a 5 nucleotide difference from the genotype B3 identified in the other two chains of transmission, which was phylogenetically indistinct. The outbreaks described here included a number of cases with modified measles and a large number of cases among vaccinated healthcare workers. Nearly half of the cases would not have been identified using the current EU case definition, highlighting the importance of rethinking the measles case definition for vaccinated cases.

The fellow learned the laboratory tests performed in the confirmation of possible measles cases and was involved in the methods of measles genotyping. Results were published in the Eurosurveillance Journal, 2018 (see Publications section below).

C. Acute gastroenteritis outbreak caused by calicivirus during international festival, Portugal, July 2018

Supervisors: Rita de Sousa (National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases) and Ricardo Mexia (Department of Epidemiology)

Approximately 30,000 people from 147 countries attended the BOOM Festival between 21st and 29th of July 2018, in Idanha-a-Nova, Portugal. Incidence of acute gastroenteritis (AGE) illness considerably increased on 26th July. Epidemiological and microbiological investigations were undertaken to identify the source and prevent further cases. Active and syndromic surveillance was performed by a field team of 17 health-care workers (BOOM Epidemiological Team) collaborating with the BOOM Medical Service (BMS). A total of 3,100 healthcare episodes occurred, including 451 (14.6%) AGE. Data from 448 cases was collected. Five of the 448 cases had onset of gastrointestinal symptoms before arrival at BOOM Festival coming from another large festival in Spain. Thirteen stool samples were collected, being all negative-confirmed for bacteria. Calicivirus (Norovirus and Sapovirus) were detected in 12 patients´ stools. Four (30.8%) were positive for norovirus (NoV) genogroup I (G.I), one (7.7%) for NoV G.II, one (7.7%) for sapovirus (SaV), four (30.8%) showed a NoV G.I/SaV coinfection and, two (15.4%) showed a NoV G.II/SaV coinfection. Control measures such as strengthening food safety at food and beverage facilities and water testing were implemented in collaboration with the local public health authorities.

The fellow participated in all stages of the outbreak investigation, from being part of the BOOM epidemiological team, collecting the samples, carrying out the laboratory work, analysing the data, to writing the manuscript (in preparation)

(see Publications section below). Results were presented in two meetings: IMED 2018 (International Meeting in Emerging Diseases and Surveillance) and ECCMID 2019 (European Conference of Clinical Microbiology and Infectious Diseases) (see Conference presentations section below).

Training modules

- "Introductory course", Spetses, Greece, September-October 2017. The EPIET/EUPHEM introductory course provided participants with the basic concepts of logistical and analytical approach to outbreak investigations, including the ten steps of an outbreak investigation.
- "Outbreak investigation" Berlin, Germany, December 2017. The module 'Outbreak investigations' taught fellows how to perform analytical epidemiological studies within outbreak investigations using various software packages. During the module fellows performed all steps involved in outbreak analysis from creation of a data entry file to the stratified analysis using EpiData, Stata and Microsoft Excel. They were also given practical training in when and how to perform analytical studies for an outbreak investigation, including descriptive, cohort and case-control studies.
- "Management, leadership and communication in public health", Stockholm, Sweden, February 2018. This module focussed on understanding and applying the role and responsibilities of effective Management within a Public Health environment relating to a variety of situations and circumstances, including outbreaks investigations.
- "Multivariable analysis", Nicosia, Cyprus, April 2018 (one week). The 'Multivariable analysis' module provided a more comprehensive understanding of the principles of statistical analyses, and how to build an optimal model using linear, logistic, Poisson and Cox regression in STATA.
- "Rapid Assessment and Survey methods", Athens, Greece, May 2018. It was possible to explore other tools for questionnaires and mapping, using for the investigation on the field. Moreover, fellows produced a rapid risk assessment in a complex emergency situation with guidance from experts.

Educational outcome:

The fellow gained substantial experience in healthcare and vaccine preventable outbreak investigations from the start of an outbreak to the implementation of control measures. She was involved in all steps of an outbreak investigation including epidemiological and microbiological analyses as well as operate microbiological support on surveillance systems. In addition, the fellow had the possibility to perform field epidemiology and microbiology tasks. The fellow used molecular typing methods in reference laboratories for specific pathogens. Moreover, the fellow understood the roles and responsibilities of local and national organisations in different outbreaks and events and she worked in multidisciplinary teams with epidemiologists, public health doctors and microbiologists through the different outbreaks.

1.2. Surveillance

A. Performance of surveillance case definitions for respiratory syncytial virus infections through the sentinel Influenza Surveillance System in Portugal, 2010-2018

Supervisor: Raquel Guiomar (National Reference Laboratory of Influenza and Other Respiratory Virus, Department of Infectious Diseases)

As in many EU/EEA countries, respiratory syncytial virus (RSV) cases are detected through the National Influenza Surveillance Program (NISP) in Portugal. The NISP was established in 1990 to monitor the virus circulation in Portugal. Since 2009 other respiratory viruses, including RSV, are being monitored in the scope of the influenza and other respiratory viruses surveillance. However, one of the main identified problems to implement a global RSV surveillance system is the lack of a suitable case definition for RSV disease and RSV cases are usually detected trough ILI/ARI (influenza like illness/acute respiratory infection) surveillance systems. Moreover, there is a lack of studies combining clinical, laboratory and epidemiological data related to RSV, and especially in Portugal, no data have been reported apart from virological data or the notified cases in the weekly bulletins due to the NISP. Current RSV vaccines are progressing in phase III clinical trials being expected to become available in the coming 5-10 years. In consequence, evidence-based support for vaccination policies at the national, regional and global levels is necessary. Such evidence shall include the documentation of RSV epidemiology, seasonality, virology, and identification of high-risk groups. We evaluated clinical predictors and performance of three case definitions to detect RSV infections through the Portuguese influenza surveillance system. Epidemiological and laboratory surveillance data (October 2010-May 2018; n=7085 subjects) were used as dataset. A total of 190 (2.9%) samples were RSV positive cases, being highly

significant (P < 0.0001) among children aged under 5 years (12.6%) and adults ≥ 65 years (24.6%). Cough (OR = 2.29, 95% CI: 1.17-6.49) and shortness of breath (OR = 2.14, 95% CI 1.58-2.88) were the best predictors for RSV infection considering all age groups. However, systemic symptoms were significantly associated (P < 0.01) with RSV-negative and influenza-positive cases. A more sensitive case definition than ILI and, even a different case definition according to the age, would be more suitable to avoid the underestimation of RSV disease burden in Portugal in the primary care health units. An enlargement of RSV surveillance in the hospital-based network may add value for RSV surveillance.

The fellow carried out the data analysis and wrote a final manuscript. The manuscript was accepted in the Eurosurveillance Journal (see Publications section below). Results were presented at the 11th Meeting Portuguese Laboratory Network for the Diagnosis of Influenza Infection and ESCAIDE 2018 (European Scientific Conference on Applied Infectious Disease Epidemiology) (see Conference presentations section below). The results of this project were also presented by the supervisor Raquel Guiomar at the RSV surveillance workshop by invitation on behalf of ECDC, academic partners and public health agencies in RESCEU project (March 20th-21st 2019, Copenhagen, Denmark).

B. Epidemiological surveillance of *Clostridium difficile* infection in Portugal, 2016-2018

Supervisor: Mónica Oleastro (National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases)

Clostridium difficile is the main cause of nosocomial antibiotic-associated diarrhoea in developed countries and was the 8th most frequently detected pathogen in healthcare associated infections in Europe between 2011-2012. Moreover, reports from the "European, multi-centre, prospective bi-annual point prevalence study of *C. difficile* infection in hospitalised patients with Diarrhoea" (EUCLID), showed that during 2011 to 2013 an estimated 40,000 inpatients with *C. difficile* infection (CDI) were potentially undiagnosed every year in 482 European hospitals. This fact was mainly due to the absence of clinical suspicion and the use of suboptimal diagnostic algorithms at local microbiological laboratories. For all these reasons, CDI surveillance at country level was encouraged by the European Centre for Disease Prevention and Control (ECDC). In Portugal, no data on CDI was available before 2014. However, the Directorate-General of Health (DGS) issued a regulation in 2013 whereby all clinical laboratories (hospital and community), which belong to the network "Infection Prevention and Control and Antimicrobial Resistance", must notify cases of CDI every three months maximum regardless the pathogen's antimicrobial susceptibility.

The retrospectively data analysis on CDI in Portuguese clinical laboratories was performed every year during the 2016-2018 period. The results showed that the number of notifier centres (PT) increased from 46 (2016) to 99 (2017) and 90 (2018). The two-fold increase of the numbers of notifier centers from 2016 suggested a good adjustment to the database for collecting information. On laboratory diagnostic, 38 PT performed the optimal algorithm in 2016, whereas 44 PT did in 2017 and 42 in 2018. The number of patients with CDI in Portugal slighted increased from 1021 (2016) to 1104 (2017) and to 1117 (2018), which was likely due to the increase in the number of PT that notified. Nevertheless, the number of PT that notified and performed the optimal algorithm for diagnostic was around half of the PT, and thereby, the positivity rates may be underestimated if we extrapolate the results to the whole country.

The fellow learned about hospital acquired infections, compiled the data files, performed the data analysis and wrote the report for every year (2016-2018). All reports were sent to the DGS to be disseminated to the healthcare workers from the clinical laboratories and hospitals.

C. Epidemiological surveillance at a mass gathering event: Festival Andanças, August 2018, Portugal

Supervisor: Ricardo Mexia (Department of Epidemiology)

Andanças is a festival that promotes folk music and dance in order to preserve traditions in the community. It took place in Castelo de Vide, Portugal and around 10,000 participants (an average of 2,000 participants per day) attended the festival between the 1st and 5th of August 2018. Healthcare to all festival participants was delivered by "Espaço Saúde", provided by voluntary healthcare workers (HCW) including doctors, nurses and other public health professionals (e.g epidemiologists, microbiologists). An epidemiological surveillance team (including 6 HCW)

performed active and syndromic surveillance during the festival. Descriptive analysis was performed and reported daily to relevant stakeholders. A total of 361 health related episodes were recorded, most of them among women (235, 65.1%). The 20-29 years (93/361, 25.8%) and 30-39 years (85/361, 23.5%) age groups were the most affected (specific demographic data is not available but most of the participants at the festival likely belong to these age groups). The most common complaints included bruises and wounds (191, 52.9%), especially in the lower limbs, and osteoarticular disease (39, 10.8%), likely due to the fact that many participants danced and walked barefoot. In addition, 9.1% (33/361) presented gastrointestinal (GI) complaints but no epidemiological link was identified. Indeed, several participants with GI complaints came from another festival (BOOM, 22-29 July 2018) that took place also in Portugal and in which, an acute gastroenteritis (AGE) outbreak had occurred. HCW were informed and at the beginning of Andanças, control measures including strengthening food safety at food and beverage facilities and hand hygiene measures were implemented to avoid spread of a potential AGE outbreak. This presents as an example of how communication among HCW and enhanced surveillance during and after festivals are essential.

The fellow was part of the Andanças epidemiological team and participated in data collection and analysis.

D. Evaluation of respiratory syncytial virus laboratory-based surveillance system, Portugal, 2017-2018

Supervisor: Raquel Guiomar (National Reference Laboratory of Influenza and Other Respiratory Virus, Department of Infectious Diseases)

The Portuguese Laboratory Network for the Diagnosis of Influenza Infection (PLNDII), which is coordinated by the National Institute of Health Doutor Ricardo Jorge (INSA) and comprises 14 hospital-based laboratories, is the most important setting to detect and monitor the respiratory syncytial virus (RSV) circulation in Portugal. It might perform two tasks for RSV surveillance in Portugal during the 2017-2018 season: (A) all reported the RSV-positive cases of every age weekly and, (B) all were invited to participate in the genetic characterization of RSV-positive cases from children <5 years and adults ≥65 years by sending RSV-positive samples and a form with epidemiological characteristics to the INSA. Improvements are needed to a better oriented RSV surveillance and thereby, three attributes were evaluated in order to improve RSV laboratory-based surveillance system in the country. RSV data analysis from the PLNDII during 2017–2018 was performed and on-line questionnaires were sent to the laboratories (n=14). Eleven of fourteen laboratories (78.6%) were involved in the genetic characterization of RSV and eight (57.1%) answered the questionnaire. All laboratories (8/8) perceived the public health relevance of the disease and 64.3% (9/14) reported cases for >90% weeks. The data collection form, devised by INSA, was reported as easy to fill in by five laboratories (62.5%, 5/8) for component A and by four (50%, 4/8) for component B. For genetic characterization, five laboratories (45.5%) completed all variables for 127 patients whereas six laboratories (54.5%) had >50% unknown or incomplete signs/symptoms for 188 patients. RSV laboratory-based surveillance system was evaluated as acceptable and simple. However, the format of the data collection form requires improvement while preserving the contents.

The fellow designed the questionnaire, carried out the data analysis and wrote a report that was disseminated to the hospital-based laboratories of the PLNDII. An abstract presenting the results was accepted for presentation at ESCAIDE 2019 (see Conference presentations section below).

E. Georeferencing influenza in Portugal

Supervisor: Raquel Guiomar (National Reference Laboratory of Influenza and Other Respiratory Virus, Department of Infectious Diseases)

Influenza epidemiological surveillance in Portugal is performed by the National Influenza Surveillance Program (NISP) that integrates clinical and laboratory surveillance components. The clinical component enables the calculation of influenza-like illness (ILI) incidence rates to describe the intensity of the influenza epidemic and its evolution over time. The virological component is based on laboratory diagnosis of influenza viruses including detection and characterization of influenza in circulation during each winter. The NISP has the collaboration of sentinel and non-sentinel networks that weekly reports ILI cases and send nasopharyngeal swabs to the National Reference Laboratory (NRL) for Influenza and other Respiratory Viruses diagnosis.

The knowledge of spatial and temporal distribution by the use of Geographical information systems (GIS) has been reported to be very useful in many diseases including influenza. This information support intervention at local level

in the allocation of infrastructures to respond to the demand for health services and to implement disease transmission preventive measures. Therefore, the aim of the project was to decide the best indicators for georeferencing influenza in the current influenza surveillance system in Portugal in order to enhance the influenza surveillance at a regional and national level in the country. A total of 1,136 ILI cases were reported and the flu activity was considered of moderate intensity during the 2016-2017 season by the NISP in Portugal. Data from this season that were used to develop the study proposal were the intensity of influenza activity, the number of ILI reported cases and the influenza positive/negative cases by region in each week.

The fellow participated in meetings with the Department of Epidemiology and the Head of the NRL for Influenza and other Respiratory Viruses to collaborate with the implementation of GIS to the current influenza surveillance system in Portugal. This included decisions on the best indicators for georeferencing and writing a proposal for the Institute. In addition, she learned how to create the maps with the data obtained from the NISP using ArcGIS.

Training modules

- ▪ "Introductory course", Spetses, Greece, September-October 2017 (three weeks). The introductory course provided participants with the basic concepts of logistical and analytical approach to analyse epidemiological and laboratory data, even in light of limitations of the surveillance system.
- ▪ "Management, leadership and communication in public health", Stockholm, Sweden, February 2018 (one week).
- ▪ "Multivariable analysis", Nicosia, Cyprus, April 2018 (one week). The fellow was provided with basic definitions and concepts for the different types of regression models. It will also provide the skills needed to perform and interpret multivariable analysis and to communicate the results. Therefore participants acquired competencies in the areas of statistical data analysis, inferential statistics and written communication.
- ▪ "Rapid Assessment and Survey methods", Athens, Greece, May 2018 (one week). This module focused on field surveys and investigations and taught surveys and sampling methods adapted to the population. Moreover, it included a field excercise with the use of mobile tool for data collection (KoBoCollect) and the utilization of GIS tools (GPS).

Educational outcome: The fellow applied the learned skills in the different modules and gained substantial experience in several surveillance systems. She collaborated in active and syndromic surveillance for two mass gathering events (BOOM and Andanças Festivals), carried out data analysis of sentinel surveillance systems (combined syndromic and laboratory based and only laboratory-based) and evaluated a voluntary laboratory surveillance system.

2. Applied public health microbiology research

A. Men who have sex with men: What is the risk factor for transfusion-transmissible human immunodeficiency virus infection in male blood donors in Portugal?

Supervisors: Baltazar Nunes (Department of Epidemiology) and Helena Cortes Martins (Reference Unit and Epidemiological surveillance, Department of Infectious Diseases)

Since the acquired immune deficiency syndrome (AIDS) epidemic in the 80's, in most industrialized countries, men who had sex with men (MSM) are permanently deferred from blood donation to ensure universal access to safe, quality and efficacious blood and blood products for transfusion. In Portugal, MSM were not allowed to donate blood until the current regulation issued by the Directorate-General of Health (DGS) in 2016 (Norma 09/2016) and revised in 2017, whereby the deferral period is limited to 12 months after the last occurrence of male to male sex. Moreover, the same regulation envisions the development of an epidemiological study in the Portuguese setting to assess the transfusion-transmitted human immunodeficiency virus (HIV) infection risk.

The project aimed to conduct a pilot study to evaluate the risk associated to sexual behaviour in MSM and HIV transmission through transfusion in Portugal. Therefore, after a systematic literature review, the National Health Institute Doutor Ricardo Jorge developed two proposals of observational prospective studies: a case-control study and a cohort study. In the case-control study, HIV-positive MSM donors would be compared with HIV-negative MSM donors regarding the frequency of the last intercourse. The duration of the study would be 6 years, which include 6 months to set up the study, 5 years of data collection and 6 months to end and write the report. In the cohort study, MSM would be recruited in donation centres and Non-Governmental Organizations (NGO's) or centres to support

MSM in the community. These will be compared with men who had sex with women only (MSWO) regarding the HIV incidence. The duration of the study would be 5 years, which include 6 months to set up the study, 4 years of data collection and 6 months to end and write the report.

Both of the proposals were presented to a steering committee formed by the DGS, Portuguese Institute of Blood and Transplantation (IPST), health parliament group, civil society and specific experts in the area. This group selected a proposal to perform a one-year pilot study in order to decide afterwards if it is necessary and feasible to conduct a full-scale research project.

The fellow participated with the Portuguese Working Group to develop the proposals of both of epidemiological studies, cohort and case-control.

B. Evaluation of the serum bactericidal antibody to serogroup C *Neisseria meningitidis* in the Portuguese population, 2015-2016.

Supervisor: Maria João Simões (National Reference Laboratory of *Neisseria meningitidis*, Department of Infectious Diseases)

Invasive meningococcal disease (IMD) caused by *Neisseria meningitidis* is associated with high morbidity and mortality. The incidence of IMD varies geographically ranges reaching 0.73 cases per 100,000 population in Portugal in 2014. Meningococcal C (MenC) disease has been substantially reduced over the last decade in Portugal. This fact may be explained by two events: i) the routine vaccination with the meningococcal serogroup C conjugate (MCC) vaccine since 2006 and ii) the herd protection acquired by non-immunized population. MCC vaccine induces a production of serum bactericidal antibodies and avoids the nasopharyngeal colonization. Consequently, it induces herd immunity and indirect protection to non-vaccinated individuals. MenC antibody persistence data are important in indicating the duration of protection in otherwise vulnerable groups and the point at which booster doses become necessary.

Up to the date, seroprevalence of MenC studies have never been performed in the Portuguese population. Therefore, the aim of this study was to evaluate the MenC serum bactericidal antibody level in the Portuguese population in order to understand the relevance of a booster dose of MenC in adolescents and thereby, ensures the indirect protection of unvaccinated children under one year old. A cross-sectional and retrospective study will be performed with the serum specimens collected for the national vaccine preventable diseases surveillance programme in Portugal during 2015/16. Stratified sampling was performed according to age groups and the vaccination schedule they followed.

The fellow colaborated in the design of some of the project steps, calculated the sample size, collaborated in the sample selection and preparation, and will contribute with the data analysis and manuscript writing.

Training modules

- "Introductory course", Spetses, Greece, September-October 2017 (three weeks). The course provided participants with the basics of project planning and writing a research proposal.
- "Rapid Assessment and Survey methods", Athens, Greece, May 2018 (one week). This module focused on field surveys and investigations and taught surveys and sampling methods adapted to the population.

Educational outcome: The fellow participated in a multidisciplinary team with epidemiologist, statisticians and specialists in HIV in order to develop two proposals of epidemiological studies: cohort and case-control to answer the research question.

3. Applied public health microbiology and laboratory investigations

A. Epidemiology and genetic variability of respiratory syncytial virus in Portugal, 2014-2018.

Supervisor: Raquel Guiomar (National Reference Laboratory of Influenza and Other Respiratory Virus)

Considering the availability of the respiratory syncytial virus (RSV) vaccines in the coming years, molecular understanding in RSV is necessary. We characterized circulating RSV strains during the 2014/15-2017/18 period in Portugal. Epidemiological data and RSV-positive samples from patients with a respiratory infection were collected through the influenza surveillance system (ISS). RSV detection, subtyping in A and B, and sequencing of the hypervariable region (HVR2) of G gene were performed by molecular methods. Phylogenetic trees were generated using the Neighbor-Joining method on MEGA 7.0. RSV prevalence varied between the sentinel (2.52%, 98/3892) and the non-sentinel ISS (17.68%, 3066/17344). The HVR2 was sequenced for 562 samples. RSV-A (46.4%, 261/562) and RSV-B (53.6%, 301/562) strains clustered mainly to ON1 (89.2%, 233/261) and BA9 (92%, 277/301) genotypes, respectively, although NA1 and BA10 were also present until 2016. The sequence and phylogenetic analysis reflected the diversity of Portuguese RSV isolates.

The fellow characterized the RSV isolates from 2017/18 season by genome sequencing. In addition, she performed the epidemiological and phylogenetic analysis of 562 samples that were collected during the 2014/15-2017/18 period in Portugal, and drafted the manuscript. The manuscript was accepted in the Journal of clinical Virology (see Publications section below). Results were presented at ECCMID 2019 (European Conference of Clinical Microbiology and Infectious Diseases) (see Conference presentations section below).

B. Genomic insights into Mediterranean spotted fever disease: profiles of *Rickettsia conorii* strains associated with clinical outcomes

Supervisor: Rita de Sousa (National Reference Laboratory for Vector-borne diseases, Rickettsial Laboratory)

Mediterranean spotted fever (MSF) caused by *Rickettsia conorii* is is the most important tick-borne disease of public health concern in Portugal. In Portugal, *R. conorii* Malish and Israeli spotted fever (ISF) strains, have been detected in both patients and ticks. Both strains are transmitted by the same vector, which is *Rhipicephalus sanguineus,* the brown dog tick. The incidence of MSF in Portugal has one of the highest incidence rates (1989−2005: 8.4/10^5 inhabitants) when it is compared with other endemic countries in the Mediterranean basin. Malignant forms of MSF have been described every year and a higher case fatality rate (CFR) was observed in comparison with other endemic countries such as France or Spain. Significant advances and progress in understanding the mechanisms underlying host-pathogen interactions and rickettsial pathogenesis/immunity have been made. Analyses comparing the genomes of pathogenic and non-pathogenic strains detected many factors that are related to pathogenicity. However, some aspects remain to be clarified, namely the different rickettsial genomic traits that may influence the virulence of Rickettsia conoriistrains and host interaction. Therefore, the aim of this study was to perform an extensive comparative genomic analysis to identify specific pathogenicity traits inherent to different *R. conorii* strains. For this purpose, we performed genomic analysis of *R. conorii* strains based on Whole genome sequencing (WGS) methodologies.

The fellow has learn how to isolate and propagate Rickettsia from patients samples and participated in optimization of the laborious DNA extraction protocols for NGS. Since Rickettsia is an obligatory intracellular bacteria DNA extraction required specific procedures. The fellow learned to analyse the data (including comparative genome sequencing and alignment) and will contribute to the preparation of the manuscript.

Training modules

- "Introductory course", Spetses, Greece, September-October 2017 (three weeks). The course provided an overview of laboratory diagnostic methods.
- "Multivariable analysis", Nicosia, Cyprus, April 2018 (one week). This module provided a more comprehensive understanding of the principles of statistical analyses, and how to build an optimal model using linear, logistic, Poisson and Cox regression in STATA.
- "Rapid Assessment and Survey methods", Athens, Greece, May 2018 (one week). Sampling methods adapted to the population were provided.

Educational outcome: The fellow applied sampling strategies, perform sequence analysis and alignment, identified the limitation of typing methods and their interpretation in surveillance. In addition, the fellow was involved in all the steps regarding the whole-genome sequencing of an intracellular bacteria since its culture until the genome analysis.

4. Biorisk management

A. Biosafety and biosecurity level 2 (BSL-2) and 3 (BSL-3) training

The fellow participated in a course on Biosafety and Biosecurity in Laboratories level 2 (BSL-2) and 3 (BSL-3), which was organised by the Bio-preparedness and Emergencies Response Unit from the Department of Infectious Diseases at INSA.

B. Certificates

- Biosafety and Biosecurity in Laboratories level 2 (BSL-2) and 3 (BSL-3)
- United Nations training online: Basic Security in The Field II (UNDSS)
- United Nations training online: Advanced Security in The Field (UNDSS)
- International Transport of Infectious Substances (WHO)

Training modules

- Biorisk and quality control/quality management", Stockholm, Sweden, February 2018 (one week). On biorisk management, this course was developed in collaboration with international experts appointed by WHO/IHR/LBS, under the overall supervision of the WHO project leader in Biosafety and Laboratory Biosecurity. It provided training on topics related to biorisk assessment and mitigation and international shipment of dangerous substances. Moreover, it included a visit to a biosafety level 4 (BSL4) facility.

Educational outcome: The fellow gained understanding of principles and applied practices for biorisk management, including the use of personal protection equipment in BSL3 facilities, working with known and unknown infectious agents, understanding processes associated with BSL3 and BSL4 work environments, and biosafety and security risk assessments and mitigation procedures to maintain a safe and responsible work environment.

5. Quality management

A. Observer at external quality audit on parasitic infections

Supervisor: Maria João Gargaté (National Reference Laboratory for Parasitic and Fungal Infections)

An external quality audit was performed to the National Reference Laboratory for Parasitic and Fungal Infections at INSA in February 2019. This laboratory has the management system NEN-EN-ISO 15189: 2012.
The audited specific techniques were serology and RT-PCR for *Toxoplasma gondii* and RT-PCR for *Plasmodium spp*. The audit included processes management, quality control, technical references, documentation, storage of reagents, data analysis and communication of results. The fellow observed the external audit and familiarized with the required process management and quality control and documentation.

Training modules

- "Biorisk and quality control/quality management", Stockholm, Sweden, February 2018 (one week). On quality control and quality, management, the module provided training on topics quality management in biomedical as well as public health laboratories according to ISO 15189.

Educational outcome: The fellow got acquainted with standardized ISO protocols for quality assurance accreditation and certification of National Reference Centres and WHO-Collaborating Centres.

6. Teaching and pedagogy

A. Seminar: Prevalence of carbapenem-resistant Enterobacteriaceae and carbapenemase-producing Enterobacteriaceae in Europe

The fellow participated in a seminar series on Carbapenem-resistant Enterobacteriaceae with the other EUPHEM fellows during the introductory Course in Spetses, Greece, 12.10.2019.

B. Facilitating of several case studies of outbreak investigations

The fellow facilitated case studies of outbreak investigations in the courses: "Epidemiology and biostatistics applied to health research" and "Outbreak investigation course", which were organised by the Department of Epidemiology at INSA. In addition, she facilitated a case study in the University of Algarve for the students of Medicine in the Faculty of Medicine. 5-6.6.2018, 30-31.5.2019 and 4-5.6.2019.

C. Deliver a lecture on study design in the "Outbreak investigation course"

The fellow delivered a lecture on study design in the course: "Outbreak investigation course", which was organised by the Department of Epidemiology at INSA, 31.05.2019.

D. Develop, adapt and facilitate a case study on a cholera outbreak

The fellow developed a case study on an outbreak of cholera in Portuguese. It was an adaptation of "An outbreak of cholera. West Bengal, India", which is a case study that was developed by the WHO. In addition, she facilitated it in the course: "Outbreak investigation course" which was organised by the Department of Epidemiology at INSA for public health doctors. 4-5.6.2019.

Educational outcome: The fellow delivered a seminar within a series for other EUPHEM. On case studies, she facilitated several case studies public health doctors and other interested health care workers. Moreover, she adapted a case study to Portuguese for a specific audience for a course at INSA.

7. Public health microbiology management

Public health microbiology management was an integral component of all projects (e.g. Norovirus, measles, HIV) and activities during the fellowship. This included establishing networks and contacts, communication within and between multidisciplinary work teams, ethical and integrity considerations, security issues with confidential information, team building, research collaboration and time management.

She collaborated with different National Reference Laboratories in the Department of Infectious Diseases at the institute as well as with the Department of Epidemiology during her two years programme. Particularly, she worked with her colleagues from INSA and Public Health Unit ACES South West to implement the control measures and conduct the outbreak investigation during the gastroenteritis outbreak in the LTCF. During the surveillance projects, especially in the development of an epidemiological study protocol for HIV, the fellow collaborated with epidemiologists, statisticians and microbiologists specialized in HIV. In both of the mass gathering events, she was part of the epidemiological surveillance team and collaborated with other healthcare workers. She also worked as a volunteer staff in the XXXVI Reunión Científica de la Sociedad Española de Epidemiologia (SEE) and XIII Congresso da Associação Portuguesa de Epidemiologia (APE) "Epidemiología em un contexto global".

She communicated effectively to disseminate results in the different projects through publications, reports and presentations.

Training modules

- "Management, leadership and communication in public health", Stockholm, Sweden, February 2018 (one week). The module gave tools to develop management skills of the fellows at different and distinct levels in order to effectively support their own personal development by focusing on the fundamentals of effective Management, Leadership and Communication.

Educational outcome: The fellow adjusted to different situations that involved working in a multidisciplinary public health team. She applied management and communication skills for several projects.

8. Communication

Publications

1. Augusto Gonçalo Figueiredo, Diogo , Andreia Silva, Natália Pereira, Bárbara Aguiar, Ana , Elisabete Serrada, Paula Valente, Teresa Fernandes, Fernando Guerra, Paula Palminha, Elsa Vinagre, Sílvia Lopo, Rita Cordeiro, **Emma Sáez-López**, Maria Neto, Paulo Jorge Nogueira, Graça Freitas. *Challenging measles case definition: three measles outbreaks in three Health Regions of Portugal, February to April 2018*. EuroSurveillance. 2018;23(28):pii=1800328

2. **Emma Sáez-López**, Rodrigo Farinha Marques, Nuno Santos Rodrigues, Mónica Oleastro, Helena Andrade, Ricardo Mexia, Rita de Sousa. *Lessons learned from a prolonged Norovirus GII.P16-GII.4 Sydney 2012 variant outbreak in a long-term care facility in Portugal, 2017*. Infection Control & Hospital Epidemiology. 1-6. doi:10.1017/ice.2019.201.

3. **Emma Sáez-López**, Pedro Pechirra, Inês Costa, Paula Cristóvão, Patrícia Conde, Ausenda Machado, Ana Paula Rodrigues, Raquel Guiomar. *Performance of surveillance case definitions for respiratory syncytial virus infections through the sentinel Influenza Surveillance System in Portugal, 2010-2018*. Accepted in the Eurosurveillance Journal for publication.

4. **Emma Sáez-López**, Paula Cristóvão, Inês Costa, Pedro Pechirra, Patrícia Conde, Raquel Guiomar, Portuguese Laboratory Network for the Diagnosis of Influenza Infection. *Epidemiology and genetic variability of respiratory syncytial virus in Portugal, 2014–2018*. Accepted in the Journal of Clinical Virology for publication.

5. **Emma Sáez-López**, Ricardo Mexia, Vítor Cabral Veríssimo, Agostinho Moreira de Sousa, Marta Fazendeiro, Miguel Ribeiro Oliveira, Sérgio Santos Serra, Patrícia Cunha Correia, Regina Sá, Manuela Marta de Castro, Ana Catarina Ribeiro, Joana Miranda, Patrícia Saraiva,Vasco, Ricoca Peixoto, Rita de Sousa. *Acute gastroenteritis outbreak caused by calicivirus during international festival, Portugal, 2018: results from active and syndromic surveillance at a mass gathering event.* Manuscript in preparation.

Reports

1. Evaluation of the Respiratory Syncytial Virus laboratory-based surveillance system in Portugal, 2017-2018.

2. Project proposal: "Implementation of georeferencing influenza in the National Influenza Surveillance Program in Portugal".

3. Epidemiological surveillance of Clostridium difficile infections (CDI) in Portugal, 2016-2018 (Three reports).

Conference presentations

1. Agostinho Moreira de Sousa, Regina Sá, **Emma Sáez-López**, Ana Sottomayor, Vítor Cabral Veríssimo, Patrícia Cunha Correia, Vasco Ricoca Peixoto, Gisela Leiras, Ricardo Mexia. "*Active and Syndromic Surveillance for Mass Gatherings: BOOM Festival 2018, Portugal".* Selected as poster presentation. Conference IMED 2018 (International Meeting in Emerging Diseases and Surveillance). Vienna, Austria, 9-12 November 2018.

2. **Emma Sáez-López**, Pedro Pechirra, Inês Costa, Paula Cristóvão, Ausenda Machado, Ana Paula Rodrigues, Raquel Guiomar. *"Evaluation of ECDC Influenza-like illness (ILI) case definition to detect respiratory syncytial virus (RSV) infection through the Influenza Surveillance System in Portugal".* Selected as an Oral Poster presentation. ESCAIDE 2018 (European Scientific Conference on Applied Infectious Disease Epidemiology). St Julian, Malta. 21-23 November 2018.

3. **Emma Sáez-López**, Rodrigo Farinha Marques, Nuno Santos Rodrigues, Mónica Oleastro, Helena Andrade, Ricardo Mexía, Rita de Sousa. *"Lessons learned from a prolonged Norovirus GII.P16-GII.4 Sydney 2012 variant outbreak in a long-term care facility in Portugal, 2017"*. Selected for Mini-oral flash session. ECCMID 2019 (European Conference of Clinical Microbiology and Infectious Diseases). Amsterdam, Netherlands. 13-16 April 2019.

4. **Emma Sáez López**, Rita Marques de Sousa, Vítor Cabral Veríssimo, Agostinho Moreira de Sousa, Marta Fazendeiro, Miguel Ribeiro Oliveira, Sérgio Santos Serra, Patrícia Cunha Correia, Regina Sá, Manuela Marta de

Castro1, Ana Catarina Ribeiro, Joana Miranda, Patrícia Saraiva, Vasco Ricoca Peixoto, Ricardo Mexia. *"Acute gastroenteritis outbreak caused by calicivirus during international festival, portugal, July 2018"*. Selected as a Paper Poster presentation. ECCMID 2019. Amsterdam, Netherlands. 13-16 April 2019.

5. **Emma Sáez-López**, Paula Cristóvão, Inês Costa, Pedro Pechirra, Patrícia Conde, Raquel Guiomar, Portuguese Laboratory Network for the Diagnosis of Influenza Infection. *"Epidemiology and genetic variability of respiratory syncytial virus (RSV) in Portugal, 2017–2018"*. Selected as a Paper Poster presentation. ECCMID 2019. Amsterdam, Netherlands. 13-16 April 2019.

6. **Emma Sáez-López**, Pedro Pechirra, Inês Costa, Raquel Guiomar, Portuguese Laboratory Network for the Diagnosis of Influenza Infection. *"Evaluation of the Respiratory Syncytial Virus laboratory-based surveillance system in Portugal, 2017-2018"*. Selected as an Oral Poster presentation. ESCAIDE 2019 (European Scientific Conference on Applied Infectious Disease Epidemiology). Stockholm, Sweden. 27-29 November 2019.

Other presentations

- Presentation in the 11th Meeting Portuguese Laboratory Network for the Diagnosis of Influenza Infection. (INSA, Lisbon, Portugal). "*Respiratory Syncytial Virus: genetic characterization in Portugal, 2017/2018".*

9. EPIET/EUPHEM modules attended

1. "Introductory course", Spetses, Greece, September-October 2017 (three weeks).
2. "Outbreak investigation", Berlin, Germany, December 2017 (one week).
3. "Biorisk and quality control/quality management", Stockholm, Sweden, February 2018 (one week).
4. "Management, leadership and communication in public health", Stockholm, Sweden, February 2018 (one week).
5. "Multivariable analysis", Nicosia, Cyprus, April 2018 (one week).
6. "Rapid Assessment and Survey methods", Athens, Greece, May 2018 (one week).
7. "Project review", Lisbon, Portugal, August 2018 (one week).
8. "Project review", Prague, Czech Republic, 2019 (one week).

10. Other training

1. Rotation in the National Reference Laboratories from the Department of Infectious Diseases (INSA) (October 2018).
2. Attendance to the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2017). Sweden, 6-8.11.2017.
3. Preparation for Primary Departure course: Médecins Sans Frontières (MSF), OCA (Operational Centre Amsterdam). Germany, 25-31.01.2018.
4. Attendance to the XXXVI Reunión Científica de la Sociedad Española de Epidemiologia (SEE) and XIII Congresso da Associação Portuguesa de Epidemiologia (APE) "Epidemiología em un contexto global". Portugal, 12-14.9.2018.
5. Workshop: "The Ixodes ricinus group of ticks in the western Mediterranean region and North Africa: new insights in to their population genetics and microbiome fauna-TickGenoMi". Portugal, 16.1.2019
6. Massive Open Online Course in "Medical entomology: insect vectors and transmission of pathogens". Organised by the Institute Pasteur. 1-14.5.5019.
7. Microbiology laboratory training focus on Cholera and other bacterial gastrointestinal pathogens. July 2019.
8. Laboratory training in Parasitology: focus in helminths and malaria. July 2019.

Discussion

Coordinator's conclusions

Dr Emma Sáez López joined the EUPHEM fellowship as an EU track fellow, bringing with her a wide range of skills gained during her career as a scientist and field researcher. Over the past two years she supported capacity

development by carrying out projects at INSA, whilst at the same time, acquiring experience in Public Health Microbiology. The quality and number of her outputs exemplifies what is possible when a very motivated, hard-working fellow develops the EUPHEM fellowship under very competent site supervision. Under their guidance Emma achieved an excellent portfolio of projects and activities. Of note are the opportunities she had to participate in enhanced surveillance activities at mass gatherings, which suited perfectly an energetic and enthusiastic Emma. Her ability to communicate widely was also an asset for project management activities at INSA and elsewhere. In addition, she was extremely generous with her time, organising and hosting extracurricular activities for her colleagues during modules. As her front-line coordinator, it has been a privilege to share her experience of the EUPHEM fellowship and I look forward to hearing about her successful next steps.

Supervisor's conclusions

Emma Sáez López came to INSA with experience in microbiology and with the ambition to broaden her scope and skills in Public health microbiology and epidemiology. Given this background, she was introduced to a challenging list of projects that required in-depth knowledge of laboratory work, even if this was not in her own area of expertise. She also combined this with new insights from the training modules. She approached her tasks in microbiology and epidemiology projects with great enthusiasm and a scientific rigour that led to interesting discussions with her colleagues and supervisors, thus creating a mutual learning experience, in the spirit of the EUPHEM programme. Emma was the first fellow to carry out the EUPHEM programme at INSA, and with her excellent technical skills, positive attitude, management and dedication to her work, she easily integrated into the institute, which facilitated the beginning of this programme at INSA. I have no doubt that Emma will be a strong advocate and role model for future EUPHEM fellows, and will continue to do excellent work in the public health arena.

Personal conclusions of fellow

The EUPHEM fellowship gave me the opportunity to acquire many knowledge and gain experience through a learning-by doing process. I was involved in several projects of microbiology and epidemiology related to public health topics. I felt very fortunate to work at INSA because of the wide-range of projects to carry out and the people's willingness to collaborate. The fellowship has broadened my experience in the microbiology field and added new competencies in the field of epidemiology and other public health disciplines. EUPHEM modules gave to the fellows a lot of knowledge and tools for developing the projects during the programme and onwards. It offered me the possibility to work with people from many different backgrounds and live different experiences, opening my view to a dynamic and international setting. Moreover, the fellowship showed me the relevance of working in a multidisciplinary team and the essential link between the epidemiology and microbiology in public health. Furthermore, it expanded my personal and professional network in Europe, which I think it is one of the most positive aspects of the programme.

Acknowledgements of fellow

First, I would like to acknowledge my supervisor Rita de Sousa for her support, closeness and offer me all the possibilities for projects during the fellowship. You made me think out of the box and I really appreciated your laissez-faire and your wise advices/suggestions at the same time. I just can say thank you very much. I also would like to acknowledge my co-supervisor Maria João Simões, because you were always available for me and tried to do my fellowship better. Special thanks to Ricardo Mexia for being an excellent host in the Department of Epidemiology. You offered me great opportunities and experiences and I am really grateful. Furthermore, thanks to all the supervisors of the projects (Paula Palminha, Raquel Guiomar, Mónica Oleastro, Baltazar Nunes, Helena Cortes Martins, and Maria João Gargaté) for your support, patience, time, effort and work because without you I would not have carried out this fellowship. In addition, thanks to all colleagues from the Department of Infectious Diseases, the Department of Epidemiology and outside the Institute I worked with, but also to those ones I did not work with albeit they helped me at any time I asked for. I also want to specially thank to the National Reference Laboratory of Parasitology I shared the space with, every day. In addition, I would like to thank you Aura for your work, availability and closeness as frontline coordinator, Aftab for caring of every fellow's progress and to all the facilitators for creating friendly environments for learning during the modules. Finally, meeting the colleagues from the programme (EPIET/EUPHEM/PAE/UK-FETP cohort 2017) and sharing all the modules and congresses has been a pleasure. I am sure our relationship will continue in the future. Again, thank you so much to all the people who did my fellowship a great experience. Muito obrigada!