

The main title "Summary of work activities" in a bold, white, sans-serif font, followed by the author's name "Paulo Gonçalves" and the program name "European Public Health Microbiology Training Programme (EUPHEM), 2018 cohort" in a white, sans-serif font, all set against a blue background.The section header "Background" in a bold, blue, sans-serif font.

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

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

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

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

This report summarises the work activities undertaken by Paulo Gonçalves, cohort 2018 of the European Public Health Microbiology Training Programme (EUPHEM) at the National Institute of Health Doutor Ricardo Jorge (INSA), Lisboa, 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 2001, Paulo Gonçalves joined the Portuguese National Institute of Health (INSA) in Lisboa, Portugal, after completing his B.Sc. in Applied Biology (Biotechnology) at the De Montfort University in Leicester England and having had, in this context, an internship year at the Institute working on human enteroviral infections. From 2001 to 2012 he focused his work on diagnosis, surveillance and outbreak preparedness and response to Influenza and other viral respiratory infections and, since 2013, he is the head of the National Reference Laboratory for Legionnaire's Disease and National Focal-Point for Microbiology for the European Legionnaire's Disease Network. From 2015 to 2018, he has also worked on emergency and outbreak preparedness and response linked to international cooperation programmes in Western Africa (Guinea-Bissau). These were first dedicated to emergency response to the 2014 Ebola outbreak and, since then, to building capacity for surveillance and laboratory diagnosis of infectious diseases, in collaboration with the National Institute for Public Health of Guinea-Bissau and the Centers for Disease Prevention and Control in Atlanta, USA.

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 project or activity work and partly through participation in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the EUPHEM scientific guide¹.

1. Epidemiological investigations

1.1. Outbreak investigations

Two simultaneous outbreaks of measles in Portugal were linked to travelling to two European countries, October to December 2018

Supervisors: Carlos Orta Gomes (Department of Public Health, Regional Administration of Health of Lisboa and Vale do Tejo – ARSLVT) and Paula Palminha (National Reference Laboratory for Vaccine Preventable Diseases, Department of Infectious Diseases, INSA)

On November 8th 2018 the *Hospital Particular de Cascais*, a private hospital in Cascais, a municipality of the Lisboa district, reported one measles case to the National Epidemiological Surveillance System. This was a Ukrainian citizen, male, 22 years, non-vaccinated, living in Cascais, who travelled to Ukraine between September 26th and October 30th and presented fever on arrival to Lisboa on October 30th. The patient was admitted at the hospital on November 6th

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

presenting maculopapular rash but, before being isolated, he contacted and transmitted the disease to health staff at the hospital, other patients and visitors, two of which generated 3 additional transmission chains. While investigation of this case was taking place and secondary cases started to emerge, three probable measles cases were reported on November 14th by *Hospital da Luz*, a private hospital in the nearby municipality of Oeiras. One of these cases corresponded to a 19-years old female working-student, non-vaccinated, who had travelled to the Czech Republic between 12-15th October. She initiated symptoms (fever, fatigue and sore throat) on October 19th, but travelled with her family to the south of Portugal and attended several health units with different diagnosis before finally being diagnosed with Measles at the hospital, 18 days after the initial symptoms. The two other cases were her siblings, who in turn infected one hospital health staff each. A total of 26 confirmed (21 in Cascais and 5 in Oeiras) and 2 probable (in Cascais) cases were identified by onset of symptoms between October 19th and December 15th, and a total of 1756 contacts (1184 in Cascais and 572 in Oeiras) were investigated.

Epidemiologic investigations supported the existence of two separate, but simultaneous, outbreaks, as no epidemiological links were found between cases and contacts from both municipalities. In addition, there were 2 clearly identified index cases, one linked to Ukraine (Cascais outbreak) and the other linked to the Czech Republic (Oeiras Outbreak). However, laboratory investigations did not provide sufficient information to confirm the two separate outbreaks. Both outbreaks were associated with infection with Measles virus genotype D8, a common genotype circulating in Europe. All viruses shared 3 aminoacid substitutions, also found in viruses from the Czech Republic, and the one aminoacid difference between them, that could explain the different epidemiological origin, could not be traced back to any of those two countries.

Albeit all the efforts of health authorities for a rapid intervention, which is key in controlling the spread of the disease and avoiding the collapse of the health system, particularly at local level, the investigations of these two outbreaks revealed that improvements are still necessary in 3 main areas: promote the timely and correct diagnosis and notification of cases; promote the effective and timely implementation of control measures, prioritising the vaccination and post-exposure prophylactics of health professionals and susceptible population, and; promote the effective circulation of information. Considering the epidemiological situation in other countries and the increasing popularity of Portugal as a travel destination over recent years, health staff and authorities must comply and advocate for the vaccination strategies adopted in the country, and remain clinically alert to avoid future outbreaks.

The fellow carried out the Measles virus genotyping, data cleaning and analysis, collaborated on the preliminary report for the Oeiras outbreak, sent to the National Health Authority (see section 8), and wrote the final report for the two outbreaks, submitted to the Regional Health Authority for sharing with Local and National Health Authorities, health institutions and other stakeholders (see section 8). Results of the analysis of the two outbreaks were also presented at the Project Review Module 2019 and submitted for presentation at ESCAIDE 2019 (see section 8).

Training modules

The three-week Introductory Course (IC2018; Spetses, Greece, September-October 2018) provided participants with the basic concepts of descriptive and analytical approach to outbreak investigations, including the ten steps of an outbreak investigation. This systematisation approach was quite useful for the understanding of the field work carried out by Local Health Authority and to the management of the outbreak by the Regional Health Authority in this project. In the one-week Outbreak Investigation module (OIM2018; Berlin, Germany, December 2018) fellows learned how to perform outbreak investigations, including questionnaire design, data entry and management, descriptive and univariate analysis in cohort and case-control studies, using software packages such as EpiData and STATA. Data management and use of STATA for descriptive analysis was particularly useful for this project, being the first time that the Fellow experienced this software. A deeper knowledge of STATA functions, useful for data analysis in this project, was obtained during the one-week Multivariable Analysis module (MVA2019; Madrid, Spain, March 2019), where there was an opportunity to go in-depth into statistical analysis and types of regression models, including linear, logistic, Poisson and Cox regression. The tools for a more systematic and pragmatic approach in writing the final outbreak report for this project, and communicating it to health authorities, were acquired during the Management, Leadership and Communication in Public Health module (MLCPH2020; Stockholm, Sweden, February 2020). In this module, communication skills targeted to experts and authorities in public health and to general public, in different circumstances, including emergency scenarios, were explored, contributing to the successful communication of the findings.

Educational outcome:

By integrating a multidisciplinary team managed by a Regional Health Authority, the Fellow had the opportunity to apply the principles of outbreak investigation gained during the modules, particularly in data management and analysis, in communicating to health authorities, and in applying laboratory methods to support epidemiological links. By working with datasets generated in the field, the Fellow also had the opportunity to experience and understand limitations in the field work and data collection processes, proposing alternatives to overcome those limitations. The Fellow gained knowledge in the roles and responsibilities of local, regional and national health entities, working in multidisciplinary teams with epidemiologists, public health doctors and microbiologists.

1.2. Surveillance

Prevalence and mechanisms of azole resistance in *Aspergillus fumigatus* isolated from environments with high fungal loads: risk to public and occupational health

Supervisors: Raquel Sabino and Cristina Veríssimo (National Reference Laboratory for Mycology, Department of Infectious Diseases, INSA)

Aspergillus are ubiquitous in nature and the *Fumigati* section is one of the most prevalent *Aspergillus* sections. They can be isolated from air, water or soil, and can easily contaminate indoor environments originating mycotoxicosis, allergies, and invasive infections. This is particularly important in health-care settings, where environmental contamination can result in nosocomial outbreaks of fungal respiratory disease, or in highly contaminated environments (as it happens in agriculture, in wood and food, particularly animal, industries, and in waste handling), where the frequency of exposure increases the risk of infection, thus becoming an important occupational health problem.

Fumigati cryptic species show intrinsic resistance to several antifungals. However, resistance acquisition in *A. fumigatus* sensu stricto is emerging due to selective pressure caused by prolonged azole treatment of chronic aspergillosis or due to environmental selective pressure, which is particularly problematic in environments where the use of azoles is a requirement, such as in health care, agriculture, in preservation industries and sawmills, where an increased probability of emergence of specific health and occupational-health problems has been observed. After infection of individuals with resistant strains, subsequent treatment failure with triazole therapy (the first choice for treatment and prophylaxis of aspergillosis) may occur. Consequently, higher morbidity and mortality rates associated with azole resistance are likely to become a major public health concern.

Monitoring the emergence of resistant *A. fumigatus* strains to antifungal drugs, particularly to medical triazoles, becomes essential for the adoption of prevention and control strategies with impact in public health. In this context, 3 studies were performed, briefly described next.

Study 1. Frequency of azole resistance in *A. fumigatus* sensu stricto strains isolated from environmental samples collected in Portugal during 2018 and 2019

This study focused on determining the frequency and mechanisms of resistance in *A. fumigatus* sensu stricto strains in Portugal. The fellow analysed 99 isolates of the *Fumigati* section, obtained from 142 environmental samples, 94% (93/99) of which were classified as *A. fumigatus* sensu stricto. From these, 8.6% (8/93, isolated from air from a dairy and a hospital, and from filtering respiratory protective devices (FRPD) routinely worn by workers of a waste sorting plant) grew on the presence of at least one azole-supplemented media (with itraconazole, voriconazole or posaconazole), and 3.2% (3/93) were confirmed resistant by broth microdilution and sequencing of the *cyp51A* gene and its promotor, revealing the TR₃₄/L98H mutation.

The TR₃₄/L98H mutation, commonly found in the *cyp51A* gene of azole-resistant isolates, had only been reported in clinical specimens in Portugal. Other mutations in the *cyp51A* gene were reported in Portuguese environmental azole-resistant *A. fumigatus* isolates, but none undoubtedly described as being associated with resistance. Thus, in this study, we presented the first evidence of environmental pan-azole resistant strains circulating in Portugal harbouring the TR₃₄/L98H mutations. These resistant strains were isolated from FRPDs worn by waste sorting workers during their working shift and from one air sample from a dairy, which is a strong indicator of the increased risk of disease to which workers from these environments are exposed.

The Fellow performed the molecular identification of the *Fumigati* section isolates to the species level, the screening for azole resistant strains by culture on azole-supplemented agars, and the molecular confirmation of resistance by real-time PCR targeting the TR₃₄/L98H and the TR₄₆/T289A/Y121F mutations and by sequencing and sequence analysis of the *cyp51A* gene and its promotor. The Fellow also learned to perform the identification of resistance profiles by broth microdilution, and carried out all data analysis, drafted, edited/reviewed and submitted a manuscript for publication in the *Journal of Microorganisms*, Special Edition "*Aspergillus* and Health" (see section 8).

Study 2. *A. fumigatus* azole-resistant strains in Norwegian sawmills: implementation of an algorithm to assess the frequency of resistance

This study focused on evaluating the frequency and mechanisms of resistance in *A. fumigatus* strains in the Norwegian sawmill industry by implementing and using an algorithm to assess the presence of azole-resistant *A. fumigatus* environmental isolates. Norway has a large forest industry in which azole fungicides are frequently used for protection of the timber fields and for processing, preservation and maintenance of wood and equipment. This has a negative impact on the environment and may boost the emergence of azole-resistant fungal strains, thus increasing the risk of occupational health problems for workers in this industry. Since there had been no studies regarding the frequency of azole-resistance in occupational environments in Norway, this study aimed at characterising the viable mycobiota present in environmental samples collected from sawmills in the country, identifying *Fumigati* isolates and resistance genes present in them.

A simple, yet versatile, algorithm to assess viable mycobiota and azole-resistance was applied in this study, which included procedures from sampling to the identification of *A. fumigatus* and its cryptic species and to the identification of resistance by both phenotypic and genotypic methods. This allowed the identification of *A. fumigatus* isolates with reduced susceptibility to azoles, one of which was resistant to two azoles and carried the TR₃₄/L98H mutation in the *cyp51A* gene and its promotor. The study demonstrated the need to monitor the presence of resistant *A. fumigatus* strains in these and other working environments with high fungicide pressure, in order to minimise the risk of human exposure to resistant fungi and contribute to the development and implementation of effective control measures to restrain the emergence of resistance.

The Fellow performed the molecular confirmation of resistance by real-time PCR targeting the TR₃₄/L98H and the TR₄₆/T289A/Y121F mutations and by sequencing and sequence analysis of the *cyp51A* gene and its promotor. He also collaborated in editing and reviewing a manuscript that was published in the *International Journal of Environmental Health Research* (see section 8).

Study 3. Trends in *Aspergillus* epidemiology – perspectives from a National Reference Laboratory Surveillance Programme

This study took a broader approach to the *Aspergillus* epidemiology in Portugal, by looking at laboratory surveillance data collected over 8 years at the National Reference Laboratory for Mycology at INSA. We conducted a retrospective study on the molecular epidemiology of *Aspergillus* isolates obtained from patients with respiratory, subcutaneous or systemic infections, and from environmental samples of different origins, between 2017 and 2018. In addition, the resistance frequency and profiles for the entire collection of *A. fumigatus* isolates of the Laboratory, obtained between 2012 and 2019, was analysed

This study confirmed that *A. fumigatus* sensu stricto continues to be the most frequent species isolated from both clinical (39.7%) and environmental (55.7%) samples, but revealed that cryptic species on clinical specimens are more frequent than reported before. Mixed infections/colonisation were found in 14.7% of patients, showing that therapeutic approaches must take into account the different susceptibilities profiles to antifungal drugs by different species. The overall frequency of resistance was 3% in *A. fumigatus* sensu stricto and 5.1% in *A. fumigatus* sensu lato, consistent with published data in Europe. Sequencing of the *cyp51A* gene revealed the presence of TR₃₄/L98H and F46Y/M172V/N248T/D255E mutations on isolates from 2018/2019 with reduced susceptibility to azoles, but also the presence of the G54R mutation in one isolate from 2015, which suggests that resistant isolates may have emerged in the environment in our country earlier than believed until now. This study demonstrates the value of the *Aspergillus* Surveillance Programme in contributing to guide clinical and public health decision-making.

The Fellow contributed for the molecular confirmation of resistance by real-time PCR targeting the TR₃₄/L98H and the TR₄₆/T289A/Y121F mutations and by sequencing and sequence analysis of the *cyp51A* gene and its promotor. He also contributed in editing and reviewing a manuscript that was submitted for publication in the *Journal of Fungi*, Special Edition "*Aspergillus* Infection" (see section 8).

First National Serological Survey for COVID-19

Supervisors: Ana Paula Rodrigues (Department of Epidemiology, INSA)

The World Health Organisation declared the state of COVID-19 pandemic on 11/03/2020, in the context of the emergence of the infection associated with the novel coronavirus, SARS-CoV-2, in humans in China during December 2019, and its global dissemination during the first quarter of 2020. In Portugal, the first case of COVID-19 was notified on March 2. The daily number of new cases increased to a peak of 1516 cases on April 10. By May 3, at the end of this first wave of the epidemic curve, 25524 cases had been reported. At that time, little information was available that clearly indicated to what extent asymptomatic individuals are able to further spread the virus, or how robust, protective and durable the immune response after infection is, or how it correlates with severity and outcome of the disease. In this context, and similarly to what was done in other countries and following recommendations from the European Centre for Disease Prevention and Control and from the World Health Organisation, INSA sought to develop the first National Serological Survey for COVID-19 (ISN COVID-19) in Portugal, in articulation with the National Health Authorities and in collaboration with the National Association of Clinical Laboratories and with selected hospitals of the National Health Service. This survey aimed at estimating the seroprevalence of SARS-CoV-2 specific antibodies (IgG and/or IgM) in the general population stratified by sex, age group, region, and at determining the fraction of asymptomatic infections in Portugal.

A nationwide cross-sectional seroepidemiological survey was performed with a representative sample of the Portuguese population, consisting of individuals >1 year-of-age, residents in Portugal (including the autonomous archipelagos of Madeira and Açores), who attended at a network of clinical and hospital laboratories after the first wave of the disease in the country for reasons unrelated to COVID-19 and with a medical prescription for a blood test. Sample size was estimated considering an expected seroprevalence of 2.5% for individuals 1-19 years of age, and of 5% for individuals over 19 years, according to data reported in the literature, and stratified by age group,

sex, region, education level, previous contact with a suspected or confirmed case of COVID-19 and presence of symptoms compatible with SARS-CoV-2 infection. Participants were recruited at 96 randomly selected private pathology laboratories and at 18 public hospital laboratories, covering the whole territory, between May 21 and July 8. A blood sample was collected, along with a questionnaire covering sociodemographic, epidemiological and clinical information. Serologic tests for the presence of IgM and/or IgG were conducted at the National Reference Laboratory for Influenza and other Respiratory Viruses of INSA, using commercial ELISA kits. The seroprevalence was estimated as the proportion of individuals who had a positive result for IgM and/or IgG.

A total of 2 301 participants were recruited for the Survey, 1.9% (95%CI: 1.1-3.1) of which were positive for IgM and 1.9% (95%CI: 1.2-3.1) were positive for IgG. The overall seroprevalence of IgM and/or IgG against SARS-CoV-2 was 2.9% (95%CI: 2.0-4.2), which is compatible with a limited spread of the infection in the population during the survey period, and insufficient for the protection of the whole population through herd immunity. No statistically significant associations were found for the distributions by age group or region. On the other hand, a statistically significant association was found for the distribution by sex (the seroprevalence was 2 times higher for man than for women, probably reflecting different occupational exposures), by education level (for unclear reasons, individuals with secondary education had 2-5 times higher seroprevalence estimates than individuals with other educational levels), by contact with a suspect or confirmed COVID-19 case (individuals who reported having a contact had 11 times higher seroprevalence estimates than individuals who did not), and by the presence of symptoms (individuals who reported symptoms compatible with the infection had 3 times higher seroprevalence estimates than individuals who reported not having symptoms). In addition, 44% of individuals not reporting symptoms were seropositive, which could indicate mild or asymptomatic infections, or a limitation of the study due to recall bias. Still, the overall estimated seroprevalence (2.9%) was higher than the attack rate reported for that period, 0.44 cases per 100 000 inhabitants, which is consistent with the knowledge that surveillance systems are less sensitive when it comes to detecting mild or asymptomatic cases.

The Fellow contributed for the selection and recruitment of public hospitals, for the preparation and shipment of materials for recruitment of participants, handled logistic matters for the transportation of specimens from hospitals to the Laboratory, collaborated in the fieldwork monitoring, contributed to data entry and analysis, discussed and interpreted results, reviewed and edited the progress newsletters, the preliminary report, and a manuscript (first submitted to *Eurosurveillance*, but rejected for publication; then submitted to *Acta Médica Portuguesa*, currently under second round of peer review; see section 8).

Training modules

The basic concepts of surveillance and development, validation, evaluation and operability of surveillance systems were provided to the Fellows during the IC2018. This allowed a better and broader understanding of the surveillance systems in place in Portugal relating specifically to *Aspergillus* and COVID-19, and the development of projects that contributed to analysing data generated through those systems and proposing public health recommendations. For this the MVA2018 module was also useful, providing the tools for data analysis and interpretation, and the MLCPH gave insights on how to communicate those findings to different target audiences, from decision-makers to the public.

Educational outcome:

The Fellow had the opportunity to apply the concepts acquired during the training modules. By collaborating in the analysis and interpretation of epidemiological and microbiological surveillance data, the Fellow gained an insight of the different data generated and the types of analysis that are appropriate, and also to develop recommendations based on evidence and how to communicate those recommendations to relevant audiences. The Fellow gained experience in the design, development, and execution of a national serosurvey, and in generating epidemiological information to support decision making on prevention and control strategies in a pandemic scenario.

2. Applied public health microbiology research

Seroprevalence of meningococcal serogroup C bactericidal antibodies in the Portuguese population, a decade after vaccine introduction in the National Immunisation Programme

Supervisors: Maria João Simões (National Reference Laboratory for *Neisseria meningitidis*, INSA)

The incidence of invasive meningococcal disease due to serogroup C (MenC) decreased in Portugal over the past decade, associated with the introduction of the conjugate vaccine (MCC) in the Portuguese market in 2001 and in the National Immunization Plan (NIP) in 2006. However, the literature reports a waning in the antibody levels in the vaccinated population over time which, associated with the different vaccination schemes used and the circulation of *N. meningitidis* serogroup C in the country, could indicate that the Portuguese population may be at increased risk

of infection. To quantify the protected fraction of the population and reflect on the need of a booster dose of the vaccine as to ensure the protection of the whole population through herd immunity, we developed a nationwide study in which we measured serum bactericidal antibody (SBA) activity in a representative sample of the Portuguese population aged 2-64 year of age, ten years after the introduction of the vaccine in the NIP (2015/2016). In the absence of national seroprevalence data for MenC, the study sample was stratified by birth cohort and the sample size was estimated considering expected frequencies of protective SBA titres in similar studies published in the literature. A total of 1500 anonymised serum samples were tested, each corresponding to an individual from which demographic data was available, but not information on vaccination against MenC.

This was the first study on the seroprevalence of meningococcal serogroup C antibodies in Portugal. We showed that only 31% of the population was seroprotected against MenC in 2015/2016. This proportion was particularly low (14%) in young children born between 2012 and 2014 who were vaccinated with a single dose of the MCC vaccine at 12 months of age, but higher in children born between 2006 and 2011 (41%), vaccinated with 2+1 doses at 3, 5 and 15 months of age, and in teenagers/young adults born between 1988 and 2001 (47%), who were vaccinated with a single dose during a catch-up campaign at the ages of 5-18 years. Geometric mean titres were close to, or below, the seroprotective titre (≥ 8).

Three main conclusions were drawn from this study. Firstly, there is a rapid loss of protection with the current vaccination scheme (1 dose at 12 months), demonstrated by small proportion of young children 2-4 years of age who were protected against MenC in 2015/2016. These children were probably already at increased risk of infection at that time, and nowadays, as adolescents, will most probably not be contributing to the protection of the young, through herd immunity. Secondly, adolescents and young adults who in 2015/2016 had a higher proportion of seroprotected individuals within the group, may also currently be at risk of infection, since antibody levels (that were already close to, or below the protective titre) will most probably have waned. Thirdly, the highest protection levels were obtained when the vaccine was administered during adolescence. In summary, we concluded that changes in the vaccination strategy over the past decade in Portugal may have contributed to the low level of seroprotection observed, and the Portuguese population may currently be at increased the risk of infection. We therefore recommended adding, to the existing vaccination strategy, a booster dose of the vaccine during adolescence, and to continue monitoring the efficacy of the prevention strategies in periodical seroprevalence studies.

The Fellow contributed for the study design, provided training on the SBA Assay under BSL3 conditions to a co-author, adapted, implemented and collaborated on the serology tests, analysed the data, produced the draft of the manuscript, reviewed, edited and submitted the final agreed version of the manuscript for publication in *PLoS One* (see section 8). The Fellow also submitted an abstract for presentation at ESCAIDE 2020, which was accepted for poster presentation, and presented the study in a plenary session during the Project Review Module 2020 (see section 8).

Training modules

During the IC2018, Fellows gained insights on the development, writing and presenting study protocols, through a group exercise consisting on developing a study protocol for a hypothetical situation proposed. Here, those insights were put into action and were valuable for the development of the study protocol that was the foundation of the project. The skills on STATA and descriptive univariate analysis acquired during the OIM2018 and the MVA2019 modules were also useful for the data management and analysis. The recommendations proposed based on the data generated were translated into a manuscript and communicated to the National Health Authority using skills and competences gained during the MLCPH2020.

Educational outcome:

The Fellow developed skills in project management by being the lead investigator on a research study. The Fellow gained experience in designing the study, implementing and coordinating activities, training collaborators on specific tasks, writing proposals and procedures and establishing timelines. The Fellow also gained experience in communicating relevant information and recommendations to Health Authorities, decision-makers and other stakeholders.

3. Applied public health microbiology and laboratory investigations

Evaluation of the performance of real-time RT-PCR kits for the laboratory diagnosis of COVID-19

Supervisors: Raquel Guiomar (National Reference Laboratory for Influenza and Other Respiratory Viruses, INSA)

As the reference laboratory for the Portuguese Government, INSA, through its National Reference Laboratory for Influenza and Other Respiratory Viruses and in collaboration with the National Institute for Agrarian and Veterinarian Investigation (INIAV), evaluated the performance of laboratory diagnostics methods based on the detection of SARS-CoV-2 RNA by real-time RT-PCR, in compliance with the guidance from the European Commission.

A total of 8 commercial kits for the diagnosis of COVID-19 by real-time RT-PCR were evaluated. Performance was established by comparing the results of each kit against a control panel consisting of eluates of nucleic acids, extracted from a convenient sample of anonymised clinical specimens collected from patients with a clinical diagnosis of COVID-19 and tested by an in-house real-time RT-PCR adapted from a reference method recommended by the WHO, and dilutions of a SARS-CoV-2 RNA control. Whenever possible, the following parameters were calculated: sensitivity, specificity, efficiency, positive and negative predictive values, and highest dilution detected of the RNA control.

Briefly, of the 8 kits that were evaluated, 3 kits demonstrated a performance that was in accordance of the manufacturer's specifications. For the remaining 5 kits, all produced inconclusive results and 2 produced false-positive results. In respect to the inconclusive results, these were observed in panel samples that, according to the reference method used, were identified as having a low viral load, suggesting sensitivity issues on those kits. Overall, the main variations observed were in terms of the specificity (91% to 100%), positive predictive value (97% to 100%) and efficiency (98% to 100%). The majority of the kits detected the lowest concentration of the control, 10 copies/ μ l. For one of the kits, none of the dilutions produced a conclusive result, which may indicate sensitivity issues that may justify further evaluation by the manufacturer.

Communication of the findings was made through a confidential interim and final reports sent to Infarmed. The individual results of the evaluation for each kit were made available through individual evaluation forms, annexed to that report. The findings were also shared with ECDC through the network of reference laboratories working on COVID-19.

The Fellow collaborated on the study design, established a collaboration protocol with INIAV, was responsible for setting up the control panel, evaluated 3 of the kits, analysed the data from all kits, and produced the individual evaluations forms and the interim and final reports. These documents were classified as confidential and, therefore, not disclosed to public view. A short summary in English of the report was made available in the context of the Fellowship (see section 8)

Training modules

Data management, analysis and reporting skills acquired during the IC2018, OIM2018 and MVA2019, and methods for quality assessment briefly discussed during the MLCPH2020 were applied in this project. Also, team management and communication skills acquired during the MLCPH2020 were useful in the overall management of the project and in communicating the findings to the relevant authorities.

Educational outcome:

The Fellow gained experience in applying principles of microbiology, molecular biology and quality assessment, to provide evidence to support public health decisions. As the operational responsible for the project, the Fellow also gained experience in team management and leadership, organising and distributing the tasks and defining timelines.

4. Biorisk management

The fellow has certified training and several years of experience in working under biosafety level (BSL) 2 and 3 laboratories, and is certified by INSA/WHO for international transport of infectious substances. He provided 7 hours workshop to contribute to the security of health professionals that are frequently exposed to pathogenic microorganisms and/or their vectors.

Workshop on Biosafety, Biosecurity, and use of Protective Personal Equipment

Supervisors: Sofia Núncio (Head of the Emergency Response and Biopreparedness Unit, Department of Infectious Diseases of INSA)

In the course of their activities, public health professionals are frequently exposed to microorganisms of high pathogenic and epidemic potential (such as people or places contaminated with *Bacillus anthracis*, tuberculosis, etc.) or to vectors of zoonotic agents (such as fleas, ticks, rodent faeces, etc.), both in the context of contingency plans or specific surveillance programs. To minimise those risks, some approaches demonstrate to be highly effective, such as compliance with good work practices and the correct choice and use of protective equipment. The workshop

touched upon these approaches, providing or consolidating knowledge in the areas of biosafety and biosecurity, containment levels, risk assessment and risk management, and use of protective personal equipment (PPE). It was addressed to Medical doctors and nurses of the Public Health Unit of ACeS Baixo Vouga that are first-line responders to potential biological health hazards.

The Fellow was responsible for the lectures and practical exercises on the use of PPE (see section 8) and actively collaborated on the final evaluation of the participants.

Training modules

The Biosafety and Quality management module that would have provided competences on this section was cancelled due to the COVID-19 pandemic. The team management, organisational and communication skills acquired during the MLCPH2020 were useful in organising the workshop but also in communicating with the participants.

Educational outcome:

The Fellow had the opportunity to apply and communicate concepts of biosafety and biorisk management applied to field epidemiology, using skills that were acquired or updated during the modules, particularly the MLCPH2020. The Fellow also had the opportunity to improve the knowledge on vectors and vector-borne diseases and the principles of biosafety and risk assessment that apply to them.

5. Quality management

As a governmental institution and in accordance with its mission and competences, INSA is required to comply with the norms and regulations regarding quality, environment, security and health, with the legislation applicable to its activities and with governmental directives. As such, INSA is periodically audited in external quality assessment (EQA) programmes and accredited by the Portuguese Institute of Quality (IPAQ), according to the standards NP EN ISO/IEC 17025, NP EN ISO 15189 and NP EN ISO 9001. The National Reference Laboratories for *Legionella* and for *Neisseria meningitidis* of INSA are evaluated in these periodical EQAs for the laboratory diagnosis of Legionnaire's Disease and Invasive Meningococcal Disease, according to the Portuguese and European standards.

The Fellow was involved in 2 EQA Programmes (ECDC and QCMD) and 2 audits as auditee, one external and one internal, briefly described next.

ECDC External Quality Assessment (EQA) scheme supporting the surveillance of Legionnaires' disease at European level

On November 2019, The European Centre for Disease Prevention and Control (ECDC) coordinated an EQA scheme for "supporting the surveillance of Legionnaire's Disease, to ensure high quality laboratory diagnostic capability, sequence typing and environmental investigation at European level in the European Legionnaire's Disease Surveillance Network (ELDSNet) affiliated laboratories in EU/EEA" (ECDC/NEQAS 4680, Nov2019). The aim of the EQA was to help identify any issues in laboratory processes (pre, post analytical and analytical) which could benefit from improvements to the diagnostic testing capability, by identifying technical gaps or errors and implement the necessary corrective actions. The EQA was set in a simulated scenario of a suspected Legionnaire's Disease "outbreak" that the NRL had to investigate.

The NRL received 10 clinical specimens and relevant clinical and epidemiological information from patients suspected to be associated with the "outbreak". The specimens were analysed according to the routine procedures at the NRL and results were reported to ECDC/NEQAS through an online submission platform. Briefly, the NRL reported that the simulated suspected "outbreak" was due to *Legionella pneumophila* sg1:ST47, with 2 patients involved. The source was traced to a cooling tower nearby the place of residence of the 2 patients.

The results reported for the molecular detection, isolation and genotyping of *Legionella* on respiratory specimens were 100% correct and required no further action. However, the results for urinary antigen detection revealed problems with the method that was being used at the NRL, which required further action. The Fellow reported the incident to the heads of the relevant departments, evaluated the published literature on available methods and endeavoured to replace the faulty method. Method was replaced and will be evaluated in an upcoming EQA.

The activity demonstrated not only the ability of the NRL for *Legionella* to perform EQAs at European level with a high degree of competence, but also the ability to overcome and resolve errors detected during the process, thus maintaining the compliance with the quality requirements of INSA. The NRL maintained the status of reference laboratory for the ELDSNet.

The Fellow was responsible for all aspects relating to this activity.

QCMD 2020 Legionella pneumophila DNA (2019 and 2020) EQA Programme

The NRL for *Legionella* undergoes every year an external quality assessment (EQA) by the QCMD-Quality Control for Molecular Diagnostics, for the molecular detection of *Legionella pneumophila* in clinical specimens. This involves verifying that the in-house method in place at the NRL meets European standards for detection and that the method can be used for the intended purpose.

The NRL participated in the 2019 and 2020 EQAs, receiving 10 blind samples per EQA simulating respiratory clinical specimens, for diagnosis Legionnaire's Disease. Routine methodologies were used and results reported through the QCMD platform. The results reported for the detection of *Legionella pneumophila* were 100% correct. In addition, results reported for the identification of the serogroup of *L. pneumophila* and for the detection of other *Legionella* species, which were not graded in the context of the EQAs, were also 100% correct. In summary, the reported results show that the methods in place at the NRL are working appropriately and are adequate for their purpose.

The Fellow was responsible for all activities included in these two EQAs. The Fellow demonstrated the competency in executing molecular diagnostics methods and integrating multi-laboratory external quality assessments, and comply with the quality requirements of INSA.

Laboratory Audits: National Reference Laboratories for Legionella (external audit) and for Neisseria meningitidis (internal audit)

The NRL for *Legionella* undergoes an external audit by IPAQ every year, for the detection and identification of *Legionella* in clinical specimens by Real-Time PCR. This involves verifying all procedures from the time the specimen arrives at the NRL until the results are sent to the health unit who requested them, and the specimen is stored in the biobank.

Before each external audit by IPAQ, the NRL for *Neisseria meningitidis* undergoes an internal audit by a collaborative institution, for the detection of *N. meningitidis* and identification of the serogroup (A, B, C, W135, Y) in clinical specimens by Real-Time PCR, which is done in order to identify and timely correct any existing problems in the Quality System of the lab. This involves verifying all procedures from the time the specimen arrives at the NRL until the results are sent to the health unit who requested them, and the specimen is stored in the biobank.

Both NRLs were audited in 2019. No non-conformities or improvement opportunities were identified, demonstrating that the Quality System of the Laboratories is working under the Quality System requirements of INSA and IPAQ and that no corrective measures were necessary.

The Fellow participated in the role of auditee, being able to demonstrate all processes and records requested by the audit teams.

Training modules

The Biosafety and Quality management module that would provide competences on this section was cancelled due to the COVID-19 pandemic. Organisational and communication skills acquired during the MLCPH2020 were useful in organising the task and communicating the findings to the audit teams. Also during the MLCPH there was an opportunity to briefly discuss the principles of quality assessment, which were useful during the EQAs.

Educational outcome:

The Fellow had the opportunity to apply and communicate concepts of quality assurance applied to microbiology and laboratory diagnosis and outbreak investigation.

6. Teaching and pedagogy

Implementing laboratory capacity for detection of the novel coronavirus (SARS-CoV-2) in Guinea-Bissau (international mission)

Supervisors: Jorge Machado (Head of the Department of Infectious Diseases of INSA) and Raquel Guiomar (Head of the National Reference Laboratory for Influenza and other Respiratory Viruses of INSA)

In the context of the worldwide preparedness and response efforts to control to the current pandemic associated with the novel coronavirus, SARS-CoV-2, in February 2020 the Ministry of Health of Guinea-Bissau requested the Portuguese Government for collaboration with the National Laboratory for Public Health (LNSP) in Bissau to

implement laboratory diagnosis of the novel coronavirus, in the eventuality of the epidemic spreading to the country. This would involve assessing the conditions of the molecular laboratory at the LNSP, implementing a diagnostic method, training the staff and establishing communication links between the LNSP, Guinean Health Authorities and international partners, including the WHO Regional Office for Africa. As a reference laboratory, INSA was nominated by the Portuguese Government to respond to the request, and immediately prepared to deploy a staff member to the LNSP in Bissau. Having participated in previous intergovernmental missions to Guinea-Bissau, namely in the context of preparedness and emergency response to the 2014 Ebola outbreak in western Africa, and having a good knowledge of the situation both in the field and at the LNSP, the Fellow was invited to carry out the mission on behalf of INSA.

Departure to Bissau was initially planned to take place on February 27. However, on the day of departure, the deployment was postponed by the Guinean Health Minister and by the President of INSA due to the increased instability of the political scenario in Guinea-Bissau, which brought forward safety concerns. On March 11, it was possible to begin communications by messaging and video conference with the LNSP. Through online support and training, the diagnosis was successfully implemented on March 24, just in time to confirm the first COVID-19 cases in the country, which contributed to the adoption of prevention and control measures by the health authorities.

Since the implementation of the SARS-CoV-2 laboratory diagnosis, the communication channels have remained open, allowing continued online support and technical guidance. Extra training sessions have been provided, covering topics from the fundamentals of molecular biology to the use of personal protective equipment, management and communication. More recently, the Fellow was involved in articulating with the Portuguese Government in organising a humanitarian flight to Guinea-Bissau, which included shipment of laboratory materials and consumables donated by INSA to the LNSP, and with the *Comunidade de Países de Língua Portuguesa* (Community of Portuguese-Speaking Countries; CPLP) in developing a comprehensive training programme to be administered during a two-year period to health professionals of the CPLP.

Due to the still unstable political scenario in the region, no other plans were considered for travelling to Bissau.

The Fellow was responsible for the logistics and developing the training materials necessary for the implementation of the diagnostic method (including biosecurity and biosafety regulations and procedures, use of personal protective equipment, collection, conservation, transport and inactivation of specimens, and SARS-CoV-2 RNA extraction, purification and detection). The Fellow was also asked to organise the communication of results to health authorities and the public, to organise the articulation of the laboratory response with other authorities in Guinea-Bissau, and to implement an EQA scheme for monitoring of the diagnostic capability for SAR-CoV-2 at the LNSP.

15th Congress of The European Meningococcal and Haemophilus Disease Society

Supervisors: Maria João Simões (National Reference Laboratory for *Neisseria meningitidis*, INSA)

The European Meningococcal Disease Society (EMGM Society, www.emgm.eu) organises a scientific meeting every two years, hosted by one of its members/countries. In 2019, the 15th EMGM congress was organised by the Portuguese team from the National Reference Laboratory for *Neisseria meningitidis* and the National Reference Laboratory for *Haemophilus influenzae* of INSA, and took place from 27th to 30th May in Lisboa, welcoming over 200 participants from 35 countries around the globe.

The scientific programme included keynote lectures and oral and poster presentations, covering topics such as epidemiology and surveillance, clinical aspects of disease, neonatal infections, antibiotic resistance, WGS and metagenomics, vaccine development and implementation, carriage dynamics, public health threats, prevention and control measures and public health management, selected from over 150 abstracts received. For facilitating networking, 2 social events were planned complemented by discussion times during coffee breaks and poster sessions. Also, a Facebook page was created to share the content of the meeting.

The fellow was actively involved in all aspects of the organisation and scientific content, including abstract review and selection, defining the programme, organising meetings and facilitating networking. Participation in the event was not only a good learning opportunity on Meningococcal and Haemophilus Disease, but also an opportunity to be involved in the planning of such an event, identifying and prioritising scientific content of interest to the audience, creating an opportunity for learning and sharing knowledge among the participants, contributing to work discussions and decisions, using or self-improving communication and organisational skills within different teams, and to actively contribute to the accomplishing the objectives of the meeting.

Biosecurity, risk assessment and management

The Fellow collaborated on the preparation of lectures and exercises on biosafety, biosecurity and containment levels, and vectors and vector-borne diseases, and actively participated in a simulation exercise on risk assessment and management, in the context of the Workshop on Biosafety, Biosecurity, and use of Protective Personal Equipment.

Workshop Legionella species: detection, identification and quantification

Supervisors: Raquel Rodrigues (Head of the Water Microbiology Laboratory, Department of Environmental Health of INSA)

Although being an environmental microorganism, *Legionella* has become frequently associated with infection in humans in the last decades, as a result of more frequent accidental exposures to contaminated man-made environments that facilitate the growth and spread of the bacteria.

The lack of guidelines and standards has been a subject of debate in Portugal, particularly since the large outbreak of Legionnaire's Disease in the Lisboa Region in 2014, one of the biggest worldwide to date. INSA has been collaborating with other public health and environmental authorities and with the national government, providing training to laboratory technicians and other environmental health professionals on *Legionella* and Legionnaire's Disease. This 2-day workshop gave the opportunity to those professionals to become familiar with the concept of Legionellosis (from the clinical to the laboratory diagnosis and to epidemiological surveillance, from outbreak investigation to applied investigation) and with the most recent molecular biology techniques for the detection, identification and quantification of *Legionella* in the environment, hoping to contribute to the effectiveness of the surveillance of *Legionella* in environmental (domestic, recreational and industrial) water systems.

The Fellow was responsible for the lectures and exercises relating to Legionellosis, from the clinical to the laboratory diagnosis and to epidemiological surveillance, from outbreak investigation to applied investigation.

Whole-Genome Sequencing: contribution to the investigation of Legionnaire's Disease outbreaks

Presentation delivered to EUPHEM Fellows during the Outbreak Investigation Module that took place in Berlin, Germany, in December 2018. The presentation consisted on how whole-genome sequencing contributed to the investigation of the second largest outbreak of Legionnaire's Disease that occurred in Lisboa, Portugal, in 2014, as a method that provides a higher resolution power for source attribution and an in-depth investigation of outbreak-related strains.

Problem-based learning exercise (EUPHEM): "The importance of antimicrobial resistance as a public health threat"

During the Introductory Course of the ECDC Fellowship Programme in Greece in 2018, a problem-based learning exercise was proposed to the EUPHEM Fellows. Under a given scenario, the Fellows would have to develop a strategy to address a specific problem. In the scenario, the Fellows would have to give a lecture to master students that addressed, but was not only limited to, mechanisms behind resistance development in *Enterobacteriaceae*, in particular carbapenem resistance.

The Fellow engaged in independent study and group discussions to learn and discuss on the subject, and collaborated with the other Fellows on a group presentation that supported the lecture, which was given to a group of Programme Coordinators who played the role of the master students. The lecture included topics such as the importance of AMR in PH, carbapenem resistance mechanisms (this topic of the responsibility of the Fellow), diagnostics and typing, and clinical studies on carbapenem resistance mechanisms, and a final evaluation of the acquired knowledge by the participants.

This exercise was not only an opportunity to learn and discuss about the importance of antimicrobial resistance, but also a mechanism of learning or consolidating knowledge on how to identify training needs on a given scenario, topic or target audience, and come up with a solution working as a "multidisciplinary team" made of individuals with diverse backgrounds and experiences.

Training modules

During the IC2018, Fellows were given basic communication skills that were further explored during the MLCPH2020. The Project Review Modules (PRM 2019 and 2020) also contributed to fine tuning those skills, contributing to a more effective teaching experience.

Educational outcome:

Teaching has been a part of the Fellows professional responsibilities, as he not only collaborates in the training of other health professionals in several areas in the context of his activities at INSA, but has also taught for many years at University level. These activities were additional opportunities to both share the knowledge accumulated through his academic and professional life, and through the Fellowship, but also to learn and grow from new experiences, subjects and target audiences, thus hoping to become a more proficient communicator.

7. Public health microbiology management

Public health microbiology management (PHMM) was a key component of all projects and activities during the fellowship. This included working, negotiating and developing collaboration protocols with multidisciplinary teams, and communicating to different stakeholders, always in full compliance with ethics, integrity and security matters, particularly those related to information on human subjects while investigating outbreaks and whenever the information was not anonymised.

The Fellow collaborated with different National Reference Laboratories (including in the areas of invasive bacterial diseases, vaccine preventable diseases, tuberculosis, viral hepatitis, aspergilosis and COVID-19) and with the Epidemiology Department (such as during the ISN COVID-19) within the National Institute of Health. There was also the opportunity to work with Local and Regional Health Authorities (such as during the Measles outbreak investigation), with other Portuguese Institutions (such as Infarmed or INIAV), or with international partners in the context of Cooperation (between the Portuguese Government and the Government of Guinea-Bissau and the National Institute of Public Health of Guinea-Bissau). Within those activities, the Fellow collaborated and worked with specialists of different areas, from microbiology to epidemiology, emergency and public health, physicians and statisticians, engaging in fruitful discussions and negotiations, which were an invaluable opportunity for professional growth.

Training modules

The IC2018 and the MLCPH2020 were an opportunity to develop management skills that could be applied to different areas related to public health microbiology management, such as team leading and effective communication of findings to different stakeholders.

Educational outcome:

The Fellow adapted to working in different scenarios and with different multidisciplinary teams, being better acquainted with time management, communication and organisational skills, engaging in discussions, providing and accepting feedback, and communicating findings to different target audiences, both in the scientific community and to the public.

8. Communication

Publications

Paulo Gonçalves, Emma Saéz-López, Sofia Carneiro and Maria João Simões. Seroprevalence of meningococcal serogroup C bactericidal antibodies in the Portuguese population, a decade after vaccine introduction in the National Immunisation Programme. Submitted to PLoS ONE.

Paulo Gonçalves, Aryse Melo, Marta Dias, Beatriz Almeida, Liliana Caetano, Cristina Veríssimo, Carla Viegas and Raquel Sabino. Azole-resistant *Aspergillus fumigatus* harboring the TR₃₄/L98H mutation: First report in Portugal in environmental samples. Submitted to the special edition "Aspergillus and Health" of the Journal of Microorganisms.

Irina Kislaya, **Paulo Gonçalves**, Marta Barreto, Rita Sousa, Ana Cristina Garcia, Rita Matos, Raquel Guiomar, Ana Paula Rodrigues, on behalf of ISNCOVID-19 Group. Prevalence of SARS-CoV-2 infection in Portugal: results of the first National Seroepidemiological Survey (ISN COVID-19). Submitted to *Acta Médica Portuguesa*.

Raquel Sabino, **Paulo Gonçalves**, Aryse Martins Melo, Daniela Simões, Mariana Oliveira, Mariana Francisco, Carla Viegas, Dinah Carvalho, Carlos Martins, Teresa Ferreira, Cristina Toscano, Helena Simões and Cristina Veríssimo. Trends on *Aspergillus* epidemiology – perspectives from a National Reference Laboratory Surveillance program. Submitted to the special edition “*Aspergillus* infection” of the Journal of Fungi.

Carla Viegas, Beatriz Almeida, Liliana Aranha Caetano, Anani Afanou, Anne Straumfors, Cristina Veríssimo, **Paulo Gonçalves** and Raquel Sabino. Algorithm to assess the presence of *Aspergillus fumigatus* resistant strains: The case of Norwegian sawmills. International Journal of Environmental Health Research, DOI:10.1080/09603123.2020.1810210.

Reports

Paulo Gonçalves, Dulce Lourenço, José Monteiro-Júnior, Marina Soares, Manuel Jacinto, Madalena Mourata, Paula Palminha and Carlos Orta Gomes, on behalf of the outbreak investigation teams. Two simultaneous outbreaks of measles in Portugal were linked to travelling to two European countries, October to December 2018. INSA, May 2019.

Paulo Gonçalves and Vasco Peixoto, on behalf of the Department of Public Health of the Regional Health Administration of Lisboa and Vale do Tejo. *Relatório Preliminar do Surto de Sarampo Região de Lisboa e Vale do Tejo, Outubro a Dezembro de 2018* (Preliminary report of the Measles outbreak in the Lisboa and Vale do Tejo region, October to December 2018). DSP ARSLVT, January 2019.

Paulo Gonçalves. Implementing laboratory capacity for detection of the novel coronavirus (SARS-CoV-2) in Guinea-Bissau. Project report. INSA, April 2020.

Paulo Gonçalves, Rita Sousa, Margarida Henriques, Miguel Fevereiro, Raquel Guiomar. *Avaliação de kits comerciais de PCR em tempo-real para o diagnóstico de COVID-19 solicitada pelo Infarmed, Relatório Final* (Evaluation of the performance of commercial real-time PCR kits for the diagnosis of COVID-19, requested by Infarmed, Short Report extracted from the original confidential report). INSA, May 2020.

National Serological Survey ISN COVID-19 Team. *Inquérito Serológico Nacional COVID-19, Relatório de Apresentação dos Resultados Preliminares do Primeiro Inquérito Serológico Nacional COVID-19* (Preliminary report of the National Serological Survey for COVID-19). INSA, July 2020.

Conference presentations

Paulo Gonçalves, Emma Saéz-López, Sofia Carneiro and Maria João Simões. Seroprevalence of meningococcal serogroup C bactericidal antibodies in the Portuguese population, a decade after vaccine introduction in the National Immunisation Programme. Abstract submitted to ESCAIDE 2020, accepted for poster presentation.

Paulo Gonçalves, Emma Saéz-López, Sofia Carneiro and Maria João Simões. Seroprevalence of meningococcal serogroup C bactericidal antibodies in the Portuguese population, a decade after vaccine introduction in the National Immunisation Programme. Oral presentation at Project Review Module 2020.

Paulo Gonçalves, Dulce Lourenço 2, José Monteiro-Júnior, Marina Soares, Manuel Jacinto, Madalena Mourata, Vasco Peixoto, Paula Palminha, Carlos Gomes 9. Two simultaneous outbreaks of measles in Portugal were linked to travelling to two European countries, October to December 2018. Abstract submitted to ESCAIDE 2019; not accepted.

Other

15th EMGM Congress. Activity report, programme book and book of abstracts.

Seroprevalence of meningococcal serogroup C bactericidal antibodies in the Portuguese population, a decade after vaccine introduction in the National Immunisation Programme. Study Protocol.

Workshop on Biosafety, Biosecurity. Reflective note and lectures.

Workshop *Legionella* species: detection, identification and quantification. Reflective note and lecture.

Whole-Genome Sequencing: contribution to the investigation of Legionnaire’s Disease outbreaks. Oral presentation at OIM2018. Lecture

The importance of antimicrobial resistance as a public health threat. Oral presentation at IC2018. Reflective note and lecture.

ECDC External Quality Assessment (EQA) scheme supporting the surveillance of Legionnaires’ Disease at European level. Activity report.

QCMD 2020 *Legionella pneumophila* DNA (2019 and 2020) EQA Programme. Activity reports.

Laboratory Audits: National Reference Laboratories for *Legionella* (external audit) and for *Neisseria meningitidis* (internal audit). Activity reports.

Training modules

The IC2018 and the MLCPH2020 were an opportunity to develop effective communications skills that could be applied to the different project outputs, in scientific manuscripts, oral and poster presentations, in team or group discussions and to different audiences, including decision-makers, the scientific community, the public or the media.

Educational outcome:

The Fellow had the opportunity to develop competences for communicating effectively to the scientific community, to different stakeholders, to the media and to the public, by scientific writing (manuscripts, reports) and presentations (oral and poster), and to learn on the different topics covered.

9. EPIET/EUPHEM modules attended

Introductory Course, Spetses, Greece, September-October 2018 (3 weeks)

Outbreak Investigation, Robert Koch-Institut, Berlin, Germany, December 2018 (one week)

ESCAIDE 2018, Malta, November 2018 (one week)

Multivariable Analysis, Instituto de Salud Carlos III, Madrid, Spain, March 2019 (one week)

Project Review 2019, Institut postgraduálního vzdělávání ve zdravotnictví (IPVZ), Prague, Czech Republic, August 2019 (one week)

ESCAIDE 2019, Stockholm, Sweden, November 2019 (one week)

Management, Leadership, and Communication in Public Health, ECDC, Stockholm, Sweden, February 2020 (one week)

Vaccinology, online webinar, June 2020 (included the Vaccinology MOOC by Institute Pasteur) (one week)

Project Review 2020, online webinar, August 2020 (one week)

10. Other training

I-MOVE+: meeting of the accompanying group of the Portuguese project: Work Package 3 (Antipneumococcal vaccine) and Work Package 2 (Anti-influenza vaccine). National Institute of Health, Lisboa, Portugal, 17/09/2018.

2nd Workshop on Sexually transmitted infections (STIs) in different medical specialities. National Institute of Health, Lisboa, Portugal, 23/10/2018.

Seminar Emergency situations in containment areas. National Institute of Health, Lisboa, Portugal, 22/10/2018.

EPIDEMEX 2018: Simulation exercise on emergency response and global health security. Portuguese Army in conjunction with the National Health Authority and the World Health Organisation, 13/11/2018.

14th Workshop on PPCIRA (programme for prevention and control of infections and antibiotic resistance). National Institute of Health, Lisboa, Portugal, 19/11/2018.

Annual Meeting of the European Legionnaire's Disease Surveillance Network (ELDSNet). Athens, Greece, 09-10 September 2019.

Annual Meeting of the European Study Group on *Legionella* Infections 2019. Athens, Greece, 10-12 September 2019

Discussion

Coordinator's conclusions

The EUPHEM programme exposes fellows to diverse and multidisciplinary public health experiences and activities, thus enabling them to work across different disciplines. This report summarises all activities and projects conducted by Paulo Gonçalves during his two-year EUPHEM fellowship (cohort 2018) as a member state track fellow at the National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal. The EUPHEM Member State track pathway is a unique and represents an excellent opportunity for all Member States to train their own scientists and medical specialists as public health microbiologists and thus strengthen communicable disease surveillance through integrated public health microbiology and epidemiology networks. This has been successfully demonstrated by Paulo who enthusiastically embraced the fellowship showing his value despite the challenging situation coming from being a Member State track in the crisis provoked by the Covid-19 pandemic. His professional attitude and great dedication were instrumental in overcoming the challenges and producing an outstanding portfolio. He covered all the core competencies moving from his specific background on Legionella to many other pathogens including other bacteria, fungi and viruses including SarsCoV2. In fact, he was involved in extended outbreak investigations to surveillance and lab investigation but also in response to Covid-19 pandemic. His contribution through teaching has been invaluable both in supporting his country MoH and in providing cascading expertise. The contribution made by Paulo indicates the importance of developing a future critical mass of highly skilled field public health microbiologists within Member States to contribute towards national preparedness. The EUPHEM Coordinator Team concludes that the fellow has succeeded in performing all his tasks with a high professional attitude and remarkable commitment. Paulo has been an intelligent, dedicated person and a fellow with whom was a pleasure to work with: I wish him every success as a public health microbiologist as he totally deserve it.

Supervisor's conclusions

Paulo Gonçalves was the first Member State-track EUPHEM fellow to be trained at the National Institute of Health Dr. Ricardo Jorge. He has shown a remarkable capacity to comply with the challenges of a demanding training programme, combining modules, wet-lab research projects, epidemiological investigations and working hard to maintain his previous responsibilities at the Institute. He approached his tasks with great enthusiasm and scientific rigour that led to interesting discussions with his colleagues and supervisors, thus creating a mutual learning experience, in the spirit of the EUPHEM programme. He also demonstrates his skills to work in high-pressure in adverse conditions and emergency situations. I have no doubt that Paulo will continue to do an excellent work and contribute as a public health microbiology specialist at national and international level.

Personal conclusions of fellow

The EUPHEM Programme gave me the opportunity to broaden and deepen my knowledge and experience, gained over the 16 years of my career at INSA, through a learning-by doing process in different areas related to public health microbiology and epidemiology. By combining a common set of core competences and skills, delivered through specific modules, to an individually-tailored set of projects, the Programme contributes to establishing a network of specialists at European level that speak the same language and work together towards common goals.

Although at a first glance, working as an MS-Track Fellow within my home institution, may seem to be an advantage for the success of the Fellowship, this may not always be the case. Of course there is the advantage of already knowing the Institution, its organisation, the people and the activities developed there, but this can also be a disadvantage, as dissociating yourself from such "normal" activities is not always straightforward. There is a temptation to get involved in parallel activities that may even seem like good project opportunities, but fail due to constraints associated with the normal functioning of the institution or with its relationships with other institutions and stakeholders. This was particularly difficult over this past year, while the world is facing one of the most difficult periods of its modern history with the COVID-19 pandemic, and there was a temptation to help and get distracted. However, I must emphasise the efforts endeavoured by my site supervisors and coordinators in overcoming these difficulties, which were fundamental in meeting the criteria for graduation. Despite a few limitations, I am very grateful to INSA and to my colleagues for allowing me to carryout projects in different areas, some of which completely new to me, in a welcoming atmosphere.

The Fellowship has given me the opportunity to learn the importance of self-organisation and self-management, of working with, and learning from, multidisciplinary teams and, most importantly, of contributing to building links between microbiology and epidemiology in public health. The EUPHEM modules gave me a set of tools that will be very useful in my future career. Furthermore, it expanded my personal and professional network in Europe, which I think it is one of the most positive aspects of the programme.

The knowledge I have accumulated, as well as the relationships I have created, both within the Institute and with external players, will most certainly make me a better professional, and I am grateful to INSA and to ECDC for this opportunity. These will certainly be two years of my professional, and personal, life to remember.

Acknowledgements of fellow

Firstly and foremost, I would like to acknowledge my site supervisors, Rita de Sousa and Maria João Simões, for believing in my capabilities and always be present and available to push me forward, even during personally difficult times, and to my Frontline Coordinator, Loredana Ingrosso, for her mentoring and counselling, her outside perspective and kind engagement and support in all my activities and, in particular, for her efforts in keeping me on track. My deepest and personal gratitude to the three of you.

Secondly, I would like to thank the EPIET/EUPHEM Programme for this unique opportunity, and to the National Institute of Health, my second home, for continuing investing in my professional growth.

None of the projects I was involved in would have been possible without my project supervisors, Ana Paula Rodrigues, Carlos Orta Gomes, Cristina Veríssimo, Jorge Machado, Maria João Simões, Paula Palminha, Raquel Guiomar, Raquel Rodrigues, Raquel Sabino, Rita Macedo, Sofia Nuncio, and of my colleagues, who received me with open arms and were always available to teach me and guide me through the projects. Thank you.

I would also like to thank Aftab Jasir, for her scientific input and also for her caring for all the Fellows, and to all the facilitators and Scientific Coordinators for creating friendly environments for learning during the modules.

Finally, to my family and friends, for putting up with my late hours and often unavailability, I can only hope to start repaying your patience soon. Thank you.