

The main title of the report, "Summary of work activities", in a bold, white, sans-serif font, set against a blue background.

Constantine Michael Vassalos

European Public Health Microbiology Training Programme (EUPHEM), 2015 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 Constantine Michael Vassalos, cohort 2015 of the European Public Health Microbiology Training Programme (EUPHEM) at the National School of Public Health, Hellenic Pasteur Institute, "Sotiria" Chest Diseases Hospital, Hellenic Central Public Health Laboratory and the Hellenic Center for Disease Control and Prevention comprising the EUPHEM Consortium.

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

Constantine Michael Vassalos (hereafter referred as Constantine) is a Medical Doctor who has been working as Microbiology Laboratory Consultant for the national health system in Greece since 2011. The fellow completed his PhD in Microbiology from the School of Medicine of the University of Ioannina, Greece in 2009. He received a Master of Science's degree in Public Health from the National School of Public Health, Athens, Greece in 2001. He obtained a Master of Science in Health Management from the International Telematic Non State University Uninettuno, Rome, Italy in 2015. Constantine has been a Fellow of the Faculty of Travel Medicine of the Royal College of Physicians and Surgeons of Glasgow, Scotland since 2010. He has been Deputy Head of Hospital Infection Committee at hospital since 2012 and responsible for training residents in air quality and environmental microbiology. Constantine joined the EUPHEM training programme (Member State track) as a means to acquire new knowledge on public health microbiology (human, animal/food, water and environmental microbiology) and epidemiology, to obtain an inside experience in outbreak investigations and surveillance activities, to broaden his experience in teaching, and to deepen his knowledge on public health management that would enable him to combine his clinical career with public health.

## 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 guide<sup>1</sup>.

### 1. Epidemiological investigations

#### 1.1. Outbreak investigations

##### ***A. Invasive meningococcal disease outbreak investigation in a Roma population in the greater Athens area, Greece, March 2016/2015***

Supervisors: Georgina Tzanakaki, Konstantinos Kesanopoulos, Theano Georgakopoulou

On 27 and 28 March 2016, a cluster of three invasive meningococcal disease cases occurred in a Roma population camp in the Greater Athens Area (Menidi, Attica). The patients were infants aged 16, 17 and 21 months old. In two the outcome was fatal. Initially, blood samples from cases were sent to the National Meningitis Reference Laboratory and *Neisseriameningitidis* was identified within 4 hours. Twenty four hours later, the available meningococcal isolates were sent to the laboratory for further characterization. All three isolates were identified as *N. meningitidis* serogroup B and were identical by variable number tandem repeat analysis, confirming the presence of a single clone causing the outbreak. The isolates were further characterized as B: P1.18-1,3 : F1-5; for PorA and fetA respectively. Whole genome sequencing revealed that, only one peptide variant of the three included in the Men4CB vaccine was present, while meningococcal antigen typing system analysis revealed that, two of three antigens were expressed. An outbreak investigation team from the Hellenic Centre for Disease Control and Prevention was immediately deployed to the Roma camp in order to proceed with the epidemiological investigation, contact tracing and chemoprophylaxis administration to all close contacts. During the field investigation, 128

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

contacts were recorded (71 children and 57 adults) and chemoprophylaxis was administered. There was no decision for vaccination since the initial PorA sequencing results showed that the PorA VR-1 and 2 (P1.18-1,3) were not included in the Bexsero vaccine. No further invasive meningococcal disease cases were reported after chemoprophylaxis was given.

The fellow, in joint with Kyriaki Tryfinopoulou, EUPHEM fellow, 2014 cohort, had the opportunity to be familiarised with laboratory methods used during a laboratory outbreak investigation of invasive meningococcal disease and introduced to the utility of advanced laboratory methods for investigating an invasive meningococcal disease outbreak; and wrote a report (see 8.B3).

### ***B. A severe gastroenteritis outbreak of *Salmonella enterica* serovar Enteritidis PT8, with PFGE profile XbaI.0024 and MLVA profile 2-9-7-3-2 following a christening reception, Greece, June 2016***

Supervisors: Kassiani Mellou, Georgia Mandilara, Theano Georgakopoulou

On 19<sup>th</sup> June 2016, a *Salmonella* outbreak (n=56) occurred after a christening reception in Central Greece, mainly affecting previously healthy adults; one related death caused media attention. Patients suffered from profuse diarrhoea, fever, and frequent vomiting episodes requiring prolonged hospitalisation and sick leave from work, with a 54% hospital admission rate. The majority of cases experienced serious illness within less than 12 hours of attending the party. An outbreak investigation was conducted to identify the source(s) of infection and contributing factors to the disease severity. From the retrospective cohort study, the cheesy penne pasta was the most likely vehicle of infection (relative risk 7.8; 95% confidence interval 3.6–16.8), explaining 79% of the cases. Seven isolates were sent for further typing, and were identified as *S. enterica* ser. Enteritidis; two *S. enterica* ser. Enteritidis isolates were also typed as phage-type PT8, pulsed-field gel electrophoresis type XbaI.0024, multiple locus variable-number tandem repeat analysis-type 2-9-7-3-2. The strain did not share the single-nucleotide polymorphism address of the concurrent European *S. enterica* ser. Enteritidis PT8 outbreak clusters. Following five consecutive years with no documented *S. enterica* ser. Enteritidis outbreaks in Greece, this outbreak, likely associated with a virulent *S. enterica* ser. Enteritidis strain, prompted actions towards the enhancement of the national *Salmonella* molecular surveillance and control programmes including the intensification of training of food-handlers' for preventing similar outbreaks in the future. Advanced molecular techniques were useful in distinguishing unrelated outbreak strains.

The fellow participated in the different stages of the outbreak investigation, developed the questionnaires, including a questionnaire on disease severity, and conducted telephone interviews with patients. He framed case definitions, entered data, and performed descriptive and analytic statistical analyses with the use of Stata. The fellow participated in a kick-off meeting with veterinarians, which was organised by the Hellenic Centre for Disease Control and Prevention in order for a multidisciplinary coordinated follow up to get started in the country, as indicated by the One Health approach. He was actively involved in the formulation of conclusions and public health recommendations and he wrote the final outbreak report. Constantine prepared an abstract accepted as a poster presentation at European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2017), Stockholm in November 2017 (see 8.D4), and co-first authored a scientific article submitted to "Epidemiology and Infection" (see 8.A2).

### ***Training modules***

The EPIET/EUPHEM introductory course exposed fellows to the basic concepts of logistical and analytical approach to outbreak investigation in several lectures and case studies and introduced them to the ten steps of an outbreak investigation. The outbreak investigation module provided participants essential skills and competencies in practical aspects of data entry and management, questionnaire and trained them in how to perform analytical studies for an outbreak investigation, including descriptive, cohort and case control studies and stratified analyses using Stata. The multivariable analysis module trained fellows in identifying the relevant variables to control for confounding and effect modification, building an optimal model using linear, logistic, Poisson and Cox regression in Stata; and showed them the usefulness of this methodology in its potential application in an outbreak investigation. The training modules taught participants how to make recommendations, best communicate the results, and write outbreak reports and scientific manuscripts. (see 8.A2).

### **Educational outcome:**

The fellow participated in multidisciplinary outbreak control teams, and had hands-on involvement in outbreak investigations; case definitions, case finding, questionnaire design, data collection, data analysis; generating and testing appropriate hypotheses; laboratory results interpretation; efficiently communicating results to multidisciplinary team; and developing recommendations. He understood the need for integration of microbiological and epidemiological knowledge to investigate outbreaks; the importance of a cooperative teamwork among laboratories, local and central authorities; and the advantages of international collaboration. In addition to outbreak report writing, he prepared an abstract and wrote a final manuscript as co-first author.

## 1.2. Surveillance

### ***A. Latent tuberculosis infection surveillance among adults in the Athens Urban Area, 2014–2015***

Supervisors: Simona Karabela, Dimitrios Papaventsis, Evangelos Vogiatzakis

About 5-15% of cases with latent tuberculosis infection (LTBI) will develop active disease at some point of their life. In low-incidence countries, LTBI screening and treatment ability to reduce the risk of developing active tuberculosis has been debated. In Athens Urban Area (AUA), Greece, hit hard by the recent economic crisis, core densities are considerably higher than in London, the European capital of tuberculosis. Immigrants are estimated at 17% of locals. This project used records of the National Reference Laboratory for Mycobacteria in order to estimate LTBI prevalence in AUA's adult population in 2014–2015 and describe the characteristics of 4,072 persons tested for LTBI and 1,073/4,072 LTBI cases. Descriptive analysis and comparisons were performed. An average prevalence of LTBI was estimated at 15.3/100,000 population per year. A four-time higher prevalence was estimated in AUA's foreign-born population. Of foreign-born individuals tested for LTBI, 25% originated from high-tuberculosis-burden countries and 14% came from high-multidrug-resistant tuberculosis burden. Half of people tested for LTBI consisted of elderly Greeks, having immunosuppression or receiving immune-suppressants. Earlier arrivals, now established in AUA, got tested for LTBI when predisposing factors were present as they could easily access the healthcare system. In contrast, recent arrivals mostly presented at hospitals in case of heavy pathologies. Greek LTBI-cases were almost twice more likely to have known factors than foreign-born. That 55% of Greek LTBI-cases reported no known factors may be well attributed to unnoticed social determinants. Half of foreign-born persons not reporting known factors may correspond to opportunistic testing for LTBI, justified by 70% positive LTBI results. In 2015, contact tracing was intensified (increase by 58%) in AUA's Greeks heavily affected by the ongoing economic recession and marginalised for the fear of tuberculosis re-emergence. In 2015, more targeted contact investigations conducted in AUA's immigrants yielded in identifying about 30% more LTBI-cases of LTBI, who might otherwise remained overlooked. Possible instances of transmission might occur between distinct population-groups as the recent AUA transformation through migration had not led to ghetto formation. Taking into account that 1) Greece is a low-tuberculosis incidence country with high levels of immigration from high incidence countries, particularly in AUA, and that 2) the ongoing economic recession still hits AUA especially hard, LTBI screening programmes would be considered for implementation in AUA, while at the same time their cost-effectiveness and public health impact could be further investigated.

The fellow was the primary investigator and was actively involved in all stages of this project. After data clearance, he analysed the data, interpreted the first results extracted from this project, and formulated recommendations on latent tuberculosis infection screening to be included in the country's future guidelines on tuberculosis. Constantine presented parts of the project as a poster presentation at the section "Migrants (immigrants, refugees)" of the 15th Conference of International Society of Travel Medicine (CISTM15), Barcelona in May 2017 (see 8.D2). The fellow as principal author further summarised data from this project, delivered a report as draft for a manuscript for publication in a peer-reviewed journal (see 8.A4).

### ***B. Seasonal influenza surveillance in southern Greece – Season 2015–2016***

Supervisors: Athanasios Kossyvakis, Andreas F. Mentis

The National Influenza Reference Laboratory for Southern Greece is specialised on monitoring the influenza viruses in circulation by performing two laboratory-based activities, one at the antigenic level (haemagglutination inhibition assay) and a second at the genetic level (haemagglutinin and neuraminidase gene sequencing). Representative viruses are sent to the World Health Organization–Collaborating Centre for Reference and Research on Influenza in London, United Kingdom, for advanced antigenic and genetic analysis. The National Influenza Reference Laboratory for Southern Greece is working in close collaboration with the Hellenic Centre for Disease Control and Prevention on the influenza surveillance project. Clinical samples from influenza-like illness cases, collected by 71 sentinel general practitioners weekly during the winter influenza surveillance period, are sent to the National Influenza Reference Laboratory for Southern Greece. Clinical samples are also obtained from patients in intensive care units and other hospitalised patients (non-sentinel). In the 2015–2016 winter period (weeks 40/2015–6/2016), a total of 2,209 respiratory specimens, mainly nasopharyngeal swabs and bronchoalveolar lavage, were tested at the National Influenza Reference Laboratory for Southern Greece. Of these, 1,118 (50.6%) specimens were found positive for influenza virus by real-time reverse transcription PCR. A(H1N1)pdm09 subtype accounted for the vast majority of detected viruses (1,104 in total) in southern Greece. Since week 1/2016, epidemiological data and PCR detections indicated accelerating epidemics of seasonal influenza. A(H1N1)pdm09 subtype was associated with a considerable number of intensive care unit and fatal cases. The greatest proportion of A(H1N1)pdm09 cases were young (<10 years) and adults within 30–69 age group; antigenic and genetic characterisation of a representative subset of viruses revealed California/like antigenic similarity and clustering within the newly designated subgroup 6B.1, respectively. Only a small number of Victoria lineage B viruses (13 in total) and a single H3N2 virus, member of the 3C.2a genetic subgroup, were detected till week 6/2016. Characterisation of the latter virus by haemagglutination inhibition assay continued to be problematic. Data from

the National Influenza Reference Laboratory for Southern Greece together with data from other European countries were used in order to develop vaccine for the next northern influenza season 2016–2017. The fellow became acquainted with influenza surveillance systems at national and international level. He understood the importance of the weekly communication of influenza surveillance data to the Greek health authorities. The fellow contributed to the international influenza surveillance by submitting epidemiological and typing data to The European Surveillance System (TESSy), and sequences to Global Initiative on Sharing All Influenza (GISAID) database. As a co-author, Constantine wrote, edited and reviewed the official report on the country's seasonal influenza surveillance for the 2015–2016 influenza season (see 8.B1), which was uploaded to the World Health Organization (WHO)'s Global Influenza Surveillance and Response System (GISRS) platform as part of the annual WHO Consultation of the Composition of the Influenza Virus Vaccines for the Northern Hemisphere 2016–2017.

### ***C. Effectiveness of trivalent inactivated influenza vaccine for northern hemisphere during the 2010–2016 post-pandemic seasons: a meta-analysis of test-negative studies***

Supervisor: Athanasios Kossovakis, Elina Horefti, Andreas F. Mentis

The main public health strategy for containing influenza is annual vaccination. Every year, WHO recommends a trivalent inactivated influenza vaccine (TIV) for the northern hemisphere (NH) that differs from that for the southern hemisphere. A meta-analysis of test-negative-design TND-studies published after the 2009–2010 pandemic was conducted to assess NH-TIV effectiveness by type/subtype and age-group in order to offer recommendations on improving NH-TIV protectiveness. Medline was searched for TND-TIVE-studies published from May 2011 to December 2016. Inclusion criteria: influenza confirmed by reverse-transcription-PCR; NH-TIVE reported at type/subtype-level, by age-group, for  $\geq 1$  NH-season. Pooled NH-TIVE (as 1–odds ratio) and 95% confidence intervals (CI), were calculated using random-effects model. The elderly were less protected by NH-TIV; and were offered no protection for A(H3N2) across seasons. In the 6-year post-pandemic period NH-TIV appeared to protect one out of two people but more evidence is needed to confirm TIV-protective benefit in children. Optimisation of influenza vaccination for the elderly remained a major task for public health stakeholders. Increasing availability of vaccines containing both B lineages would improve protection, while A(H3N2) needs new methods of vaccine manufacturing.

The fellow was the main investigator. He was actively involved in all stages of the meta-analysis study of published studies containing surveillance data on influenza vaccine effectiveness: from searching the database for TND-studies, reviewing the abstracts, selecting the studies for inclusion, analysing the data by the use of Stata, interpreting the results of vaccine effectiveness assessment, to providing recommendations on vaccine effectiveness improvement. An abstract prepared by Constantine was accepted as a poster presentation at European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2017), Stockholm in November 2017 (see 8.D3). The fellow is the main author of a manuscript currently written based on the findings for submission to "Vaccine" (see 8.A3).

### ***Training modules***

The EPIET/EUPHEM introductory course exposed participants with the main concepts of surveillance, including how to develop, validate, evaluate and operate a surveillance system. The rapid assessment and survey methods module showed sampling methods adapted to study populations and how to contribute to the multidisciplinary response to complex emergency situations and apply their epidemiological skills to serve diseases surveillance. In addition, online vaccinology course organised by Pasteur Institute in Paris introduced concepts such as vaccine coverage, vaccine efficacy and vaccine effectiveness.

### **Educational outcome:**

The fellow learned by doing several crucial aspects of surveillance, such as the need to integrate microbiological and epidemiological data in disease surveillance, the analysis of combined syndromic and laboratory surveillance data, the laboratory-based surveillance, the operation of microbiological support for surveillance systems, and participation in disease-specific networks at national and European level. He also formulated public health recommendations, prepared abstracts, delivered reports and currently writes scientific articles.

## **2. Applied public health microbiology research**

### ***A. Antimicrobial susceptibility patterns of *Neisseria meningitidis* serogroup B strains isolated from carriers in Greece, 2014–2015***

Supervisors: Georgina Tzanakaki, Athanasia Xirogianni, Konstantinos Kesanopoulos Kesanopoulos

*Neisseria meningitidis* can cause invasive meningococcal disease that, if left untreated, is often fatal. In Greece,

overall meningococcal carriage rate was 13% in 2015. *N. meningitidis* serogroup B (MenB) was responsible for 68% of meningococcal infections reported from 27 European countries in 2012. In Greece, MenB accounted for 80% of circulating meningococcal isolates and 39% of carrier meningococcal isolates in 2015. Given that genetic exchange could transfer antibiotic resistance from carrier to patient strains and that Greece currently has one of the highest levels of antimicrobial consumption in Europe in the community. The aim of this pilot study was to describe the current antimicrobial susceptibility of carrier MenB isolates in the country and correlate their phenotypic antimicrobial susceptibility patterns with genotypes. A total of 71 carrier MenB isolates, previously collected in 2014–2015, were studied. A commercially available E-test was carried out, in order to determine the MICs for penicillin, rifampicin and ciprofloxacin. The MIC values of the MenB carriage isolates (phenotypes) were compared to antibiotic susceptibility, predicted from previous studies, retrieving genotypic information from the PubMLST *Neisseria* database. In line with MenB been the most prevalent serogroup among Greek meningococcal carriage isolates in 2014–2015, carrier MenB isolates showed a high rate (60%) of reduced susceptibility to penicillin in the present pilot study, very reminiscent of that (61%) reported in Greek meningococcal isolates in 2007. In addition, as was the case for *N. meningitidis* isolates from Greece in 2007, *penA14* allele was the known altered *penA* allele predominantly harboured by the study carrier MenB isolates. Unique *penA* alleles, not previously recorded but found to be harboured in MenB carriage isolates mainly showing reduced susceptibility or resistance to penicillin in the pilot study, need to be further investigated. Among study carrier MenB isolates, the rate of rifampicin-resistance was reduced to zero in 2014–2015, compared to 6% of rifampicin-resistant meningococcal isolates from Greek carriers reported in 1992. The rate of MenB carriage isolate resistance to ciprofloxacin seemed to remain very low in Greece. That there appeared to be no increase in MenB resistance against ciprofloxacin in the country over a 23-year time span (1992–2015) was indicative of the importance of the ongoing quinolone restriction in the community (enhanced by law in 2003) in infection control.

The fellow was involved in all stages of the research pilot study, from planning to result drafting.

### **B. Training modules**

The EPIET/EUPHEM introductory course familiarised fellows with the development and presentation of research study protocols. The initial management in public health microbiology module focused on other laboratory aspects of research, such as stress management, time management and team working.

#### **Educational outcome:**

The fellow conducted all stages of a research project in public health microbiology, from study design, public health problem identification, revising literature, designing detailed protocol for its execution, applying relevant laboratory methods, obtaining ethical approval, retrieving data from publicly accessible database and drafting results; and identified the use and limitation of diagnostic and typing methods and their interpretation in molecular epidemiology

## **3. Applied public health microbiology and laboratory investigations**

### **A. Assessment of a commercially available multiplex real-time PCR kit against direct immunofluorescence and nested PCRs for the detection of *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica* in sewage**

Supervisors: Gregory Spanakos, Evdokia Vassalou

The major waterborne protozoan diseases are those caused by *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica*. Sewage is important to the dispersion of intestinal protozoa. The effluent of treatment plants is discharged to water bodies or used for irrigation and can cause an additional public health risk if inadequately treated. Currently, no reference methods or regulations exist for the detection of protozoans in environmental samples at national, EU and international level. The aim of this project was to study the performance of a commercial multiplex real-time PCR kit – applied in fecal samples – for the detection of intestinal protozoa in sewage. The multiplex real-time PCR was assessed against direct immunofluorescence assay; and separate, nested PCRs for the detection of *G. lamblia*, *Cryptosporidium* spp., and *E. histolytica*. Multiplex real-time PCR proved to be highly specific, enabling the detection of *E. histolytica* and a subset of *Cryptosporidium* spp. including those mainly responsible for human infections. Multiplex real-time PCR was also highly sensitive, finding 10 times more samples contaminated with *G. lamblia* than direct immunofluorescence assay. Compared with nested PCR for *G. lamblia*, multiplex real-time PCR was highly accurate. At a cutoff cycle threshold value of 37.6, multiplex real-time PCR showed high sensitivity and specificity in detecting *G. lamblia*, while reaching substantial agreement with nested PCR. Despite variable sensitivity by target DNA, the high specificity of multiplex real-time PCR made the test a suitable alternative for fast, simultaneous screening for intestinal protozoa of public health

importance, revealing co-contamination in five sewage samples. The high throughput capacity of multiplex real-time PCR may facilitate informed decision-making for taking appropriate public health measures and drawing up a sewage monitoring plan to detect protozoa that might be adopted in sewage regulation for treatment and reuse in order to minimise the public health risk posed by intestinal protozoan parasites in sewage reclamation.

The fellow was the principal investigator actively participating in all stages of the project from planning, performing laboratory work, performing data analysis, interpreting the results to writing a final manuscript as first author. The results of this project were presented as an oral presentation at the 13<sup>th</sup> IWA Specialized Conference on Small Water and Wastewater Systems (SWWS2016), Athens in September 2016 (see 8.D1). The manuscript has been accepted for publication in "Desalination and Water Treatment" (see 8.A1).

### ***Training modules***

The EPIET/EUPHEM introductory course, the initial management in public health microbiology module and the multivariable analysis module provided the fellow with knowledge necessary for understanding and applying the role and responsibilities of effective management within a public health environment relating to a variety of situations and circumstances, communicating efficiently and writing scientific articles, and learning how to use Stata for regression analysis.

#### **Educational outcome:**

The fellow has deepened his knowledge in public health microbiology spanning, among others, the field of environmental microbiology. He applied concepts of parasitology to the public health microbiology disciplines; identified the use and limitation of laboratory methods and their interpretation; assessed laboratory methods to improve public health monitoring; understood attributes of laboratory method validation; acquired experience in writing project proposal and team working; prepared a scientific presentation at a conference; and wrote a scientific article.

## **4. Biorisk management**

### ***A. Biorisk assessments at the National Reference Laboratory for Malaria***

Supervisors: Kyriaki Tryfinopoulou, Eleni Patsoula, Evdokia Vassalou

The National Reference Laboratory for Malaria was going through external accreditation process according to ISO 15189:2012 Medical Laboratories – Requirements for Quality and Competence by the Hellenic Accreditation Body. Within the frame of the Reference Laboratory Biorisk management, the fellow conducted Biorisk assessments for the different procedures in use at the National Reference Laboratory for Malaria (microscopy, rapid diagnostic testing, and molecular diagnosis) using the BIORAM Biosafety and Biosecurity software.

### ***Training modules***

The Biorisk and Quality management module provided training on techniques for Biorisk assessment and mitigation, including WHO recommendations on laboratory Biorisk management. The module was dedicated to the international regulations for the transportation of dangerous goods and WHO certification was acquired on completion. A visit to the biosafety level-4 laboratory at the ECDC as part of this module illustrated all aspects of biosafety management learned during the module. Post module assignments contributed to consolidation of acquired knowledge.

#### **Educational outcome:**

The fellow understood of the importance of Biorisk management, and the requirements necessary to control risks associated with the handling, storage and disposal of biological agents and toxins in laboratories and facilities. He had the opportunity to conduct Biorisk assessments in real life and as simulation exercises. He also practised safe laboratory procedures, decontamination and experienced different personal protective equipment when working on practical parts of his various projects

## **5. Quality management**

### ***A. External Quality Assessment at the National Meningitis Reference Laboratory***

Supervisors: Georgina Tzanakaki, Athanasia Xirogianni

In order to maintain its accreditation according to ISO 15189:2012, the National Meningitis Reference Laboratory (NMRL) annually participates in European external quality assessment (EQA) schemes for the microorganisms, *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*, through the Invasive Bacterial

Disease Laboratory Network that is funded by the ECDC. However, in 2015, due to lack of funding, no European EQA scheme was organised. Since no established EQA scheme was available, the NMRL considered inter-laboratory comparison as an alternate EQA mechanism in order to meet the EQA requirements addressed by ISO 15189:2012. The Polish National Reference Centre for Bacterial Meningitis and the Laboratory of Clinical Microbiology at the University General Hospital 'Atticon' of the National Kapodistrian University of Athens provided the NMRL external samples for the multiple detection and identification of bacterial agents causing meningitis (*N. meningitidis*, *H. influenzae* type b, *S. pneumoniae*, *L. monocytogenes*, *S. aureus*, *P. aeruginosa*, *Streptococcus* spp. and non-type b *H. influenzae*). The Polish National Reference Centre for Bacterial Meningitis sent the NMRL 16 DNA samples derived from clinical specimens containing unidentified microorganisms. The Laboratory of Clinical Microbiology at the University General Hospital 'Atticon' of the National Kapodistrian University of Athens sent the NMRL 13 unidentified reference strains (cultures). The EQA referred to in house methods with two multiple PCR techniques (PCR-Q1 and PCR-Q2 protocols) for the direct identification of the microorganisms causing bacterial meningitis namely *N. meningitidis*, *H. influenzae* type b, *S. pneumoniae*, *L. monocytogenes* (PCR-Q1), and *S. aureus*, *P. aeruginosa*, *Streptococcus* spp., non-type b *H. influenzae* (PCR-Q2). Although there was a 100% concordance with the intended results for the identification of *H. influenzae*, there was a 93.7% agreement in its serotyping since in only one sample *H. influenzae* serotyped was typed as *H. Influenza* serotype b. The fellow became familiar with all stages of an EQA, from the application of external quality standards, assessment and technical report writing (see 8.B1).

## **B. Internal audit of the National Reference Laboratory for Mycobacteria, 2016**

Supervisor: Dimitrios Papaventsis

Internal audit of the National Reference Laboratory for Mycobacteria was performed as an assignment after the Biorisk and quality management module. The National Reference Laboratory for Mycobacteria is accredited according to ISO 15189:2012 Medical Laboratories – Requirements for Quality and Competence by the Hellenic Accreditation Body. The audit inspected process management, quality control indicators, and documentation. The referral laboratory completed their technical documentation records and kept records of nonconformities.

The fellow completed the audit assignment by interviewing laboratory personnel, going through protocols and methods descriptions and workflows in the laboratories.

### **Training modules**

In the Biorisk and Quality management module the fellows were familiarised with all aspects of laboratory quality management, including EQA and accreditation procedures.

#### **Educational outcome:**

The fellow became acquainted with the quality management in different laboratories; learning about accreditation processes and required laboratory standards. Constantine participated in an EQA, analysed the results and wrote a technical report, performed an internal audit as a simulation exercise highlighting challenges and importance of good practices assurance and detection of targets to be developed, and thus gained insight into how standards are maintained.

## **6. Teaching and paedagogy**

### **A. Lectures in Master of Public Health at the National School of Public Health**

1. One-hour lecture on "Sexually transmitted infections and travellers" 20.01.2016
2. Two-hour lecture on "Sexually transmitted infections according to travel destination – laboratory approach and management" 23.03.2017
3. One-hour lecture on "Traveller's diarrhoea and returning international traveller – laboratory contribution in epidemiology techniques" 20.01.2016
4. Two-hour lecture on "Packaging and shipping of infectious substances" 17.02.2016
5. One-hour lecture on "Intestinal parasites – stains and culture techniques" 09.03.2016
6. One-hour lecture on "Air microbiology, indoor air quality and hospital indoor quality techniques" 18.02.2016

### **B. Lecture to the laboratory staff of the National Influenza Reference Laboratory for Southern Greece, Hellenic Pasteur Institute Health**

1. Two-hour lecture on "Types of epidemiological studies and sample size estimation" 22.03.2016

### C. Case study on *Plasmodium vivax* malaria outbreak in Greece, 2011

Supervisor: Evdokia Vassalou

The case study was built around the 2011 *Plasmodium vivax* malaria outbreak occurred in Evrótas municipality, southern Greece gaining great attention at national, EU and international level. Malaria is a vector (mosquito)-borne disease. Greece was historically malaria-endemic country achieving malaria-free status in 1974. The country remains a malaria-receptive area (local mosquitoes are at risk of becoming infected). Recent immigration influx from malaria-endemic countries added to the country's vulnerability of malaria re-establishment. The learning objectives of the case study were to present the steps applied in malaria outbreak investigations in order for the participants to understand the reason to perform a malaria outbreak investigation; understand the specificities of a malaria outbreak investigation; use genotyping to challenge initial working hypotheses; and identify strategies to control a malaria outbreak. Background information on parasite, mode of transmission and vector and vector-borne disease terminology were offered. Scenario was provided; questions (plus answers) were formulated; and exercises were organised to practise and consolidate the newly-acquired knowledge (see 8.C1).

#### Educational outcome:

The fellow learned and gained experience on planning and delivering lectures, including defining learning objectives and preparing lecture material and exercises to multidisciplinary audiences. Constantine developed a case study on a malaria outbreak, providing background information, questions, answers and exercises as a means for better understanding the characteristics of a vector-borne outbreak investigation.

## 7. Public health microbiology management

### A. Public health microbiology management components as part of regular projects

Public health microbiology management was an integral component of all projects and activities through the fellowship. This included laboratory management, ethical and integrity considerations, team working, research collaboration, quality management knowledge, time management, and working in a multidisciplinary teams (microbiologists, biologists, physicians, veterinarians, laboratory technicians, epidemiologists). The fellow's communication output in terms of manuscripts, reports and presentations are listed in section 8. For numerous of projects the fellow communicated with different supervisors. The physical movement between departments within the consortium of the Greek EUPHEM training site gave the fellow insight to different leading strategies. The presentation on "laboratory preparedness for emerging and re-emerging pathogens in Greece" at ECDC (see 8.E1) was a great challenge. Constantine had the opportunity to encounter, tough to some degree, questions from the ECDC higher authorities and defended Greece's laboratory capacity for (re-)emerging public health treats based on strengths, weaknesses, opportunities and threats analysis.

#### Training modules

In one-week module on initial management in public health microbiology focused on understanding principles, roles and responsibilities in public health management. Topics included how to apply different management styles, team building and team work, tasks delegation, provision of structured feedback and strategies for stress management.

#### Educational outcome:

The fellow gained experience in working in a multidisciplinary public health team; exercised communication skills with different audiences, higher authorities, public and media through simulation exercises. Constantine understood the need of team management and close collaborations in planning, scheduling and organising projects; realised the importance of laboratory database management ensuring security and integrity; and understood the role and responsibilities to be an inspiring leader and an effective manager within public health environment.

## 8. Communication

### Publications

1. Vassalos CM, Charlett A, Vassalou E, Mpimpa A, Dounias G, Tzanakaki G, Mavridou A, Spanakos G. Assessment of a commercially available multiplex real-time PCR kit against direct immunofluorescence and nested PCRs for the detection of *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica* in sewage. *Desalination and Water Treatment* doi: 10.5004/dwt.2017.20803.

2. Mandilara G\*, Vassalos CM\*, Chrisostomou A, Karadimas K, Mathioudaki E, Georgakopoulou T, Tsiodras S, Mellou K. A severe gastroenteritis outbreak of *Salmonella* Enteritidis PT8, with PFGE profile XbaI.0024 and MLVA profile 2-9-7-3-2 following a christening reception in Greece, 2016. *Epidemiology and Infection* [Submitted]. \*Equally contributing first co-author.
3. Vassalos CM, Kossyvakis A, Koutelekos IG, Manetos P, Pogka V, Horefti E, Mentis AF. Effectiveness of trivalent inactivated influenza vaccine for northern hemisphere during the 2010-2016 post-pandemic seasons: a meta-analysis of test-negative studies. [In preparation].
4. Vassalos CM, Papaventsis D, Koutelekos IG, Panagi M, Vassalou E, Vogiatzakis E, Karabela S. Latent tuberculosis infection surveillance among adults in the Athens Urban Area, Greece, 2014-2015. [In preparation].

## Reports

1. Kossyvakis A, Pogka V, Vassalos CM, Kalliaropoulos A, Workneh I, Horefti E, Emmanouil M, Mentis AF. Summary of influenza activity in Southern Greece during the 2015-2016 winter period (weeks 40/2015 - 6/2016). [Annual report to World Health Organization (WHO)'s Global Influenza Surveillance and Response System (GISRS)].
2. External quality assessment at the National Meningitis Reference Laboratory. [Technical report].
3. Report on invasive meningococcal disease outbreak in a Roma camp, Menidi, Attica, March 2016.

## Teaching material

1. Vassalos CM, Vassalou E. Case study on *Plasmodium vivax* malaria outbreak in Greece, 2011. [Authors gave permission in order for the case study to be used as teaching material by the EUPHEM training programme].

## Conference presentations

1. Vassalos CM, Charlett A, Vassalou E, Mpimpa A, Dounias G, Tzanakaki G, Mavridou A, Spanakos G. Assessment of multiplex real-time PCR against direct immunofluorescence and nested PCRs for the detection of *Giardia*, *Cryptosporidium* and *Entamoeba histolytica* in sewage. 13<sup>th</sup> IWA Specialized Conference on Small Water and Wastewater Systems (SWWS2016), Athens, Greece, 14<sup>th</sup>–16<sup>th</sup> September 2016. [Oral presentation, 16.09.2016].
2. Vassalos CM, Papaventsis D, Koutelekos IG, Panagi M, Vassalou E, Vogiatzakis E, Karabela S. Tuberculosis prevalence in established and recent immigrants in Greece, 2014–2015. 15<sup>th</sup> Conference of International Society of Travel Medicine (CISTM15), Barcelona, Spain, 14<sup>th</sup>–18<sup>th</sup> May 2017. [Poster presentation].
3. Vassalos CM, Kossyvakis A, Manetos P, Koutelekos IG, Pogka V, Horefti E, Mentis AF. Effectiveness of trivalent inactivated influenza vaccine for northern hemisphere during the 2010-2016 post-pandemic seasons: a meta-analysis of test-negative studies. European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2017), Stockholm, Sweden, 6<sup>th</sup>–8<sup>th</sup> November 2017. [Accepted as poster presentation].
4. Vassalos CM, Mandilara G, Karadimas K, Chrisostomou A, Georgakopoulou T, Tsiodras S, Mellou K, Vatopoulos A. An outbreak of *Salmonella* Enteritidis phage type 8, MLVA type 2-9-7-3-2, following a christening reception in Central Greece, June 2016. European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE 2017), Stockholm, Sweden, 6<sup>th</sup>–8<sup>th</sup> November 2017. [Accepted as poster presentation].

## Other presentations

1. Vassalos CM. Laboratory preparedness for emerging and re-emerging pathogens in Greece. Oral presentation to ECDC Director, Chief Microbiologist and Chief Scientist during the IPHMM, ECDC, Stockholm, 12.02.2016

## 9. EPIET/EUPHEM modules attended

1. EPIET/EUPHEM introductory course, Anargýrios Korgialénios School of Spetses, Spetses, Greece [three weeks, 29.09–16.10.2015]
2. Bioinformatics and phylogeny module, European Centre for Disease Prevention and Control, Stockholm, Sweden [3 days, 16–18.11.2015]
3. Outbreak investigation module, Robert Koch Institute, Berlin, Germany [5 days, 07–11.2015]
4. Biorisk and quality management module, European Centre for Disease Prevention and Control, Stockholm, Sweden [5 days, 01–05.02.2016]
5. Initial management in public health microbiology, European Centre for Disease Prevention and Control, Stockholm, Sweden [5 days, 08–12.02.2016]
6. Multivariable analysis, Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH, Vienna, Austria [5 days, 14–18.02.2016]
7. Rapid assessment module, National School of Public Health, Athens, Greece [6 days, 20–25.06.2016]
8. Project review module, Instituto de Higiene e Medicina Tropical, Lisbon, Portugal [5 days, 22–26.08.2016]

9. Project review module, Instituto de Higiene e Medicina Tropical, Lisbon, Portugal, 2017 [5 days, 28.08–01.09.2017]

## 10. Other training

1. Internship in Parasitology at the Parasitology and Tropical Disease Laboratory, National School of Public Health, Athens, Greece [2 weeks, 22.10–02.11.2015]
2. Training in Quality Assessment at the National Meningitis Reference Laboratory, Athens, Greece [2 days, 15–16.12.2015]
3. Internship at the National Influenza Reference Laboratory for Southern Greece, Hellenic Pasteur Institute [2 weeks, 18–29.01.2016]
4. Vaccinology massive open online course, Institut Pasteur and Conservatoire National des Arts et Métiers, Paris, France. [Broadcasted from 05.12.2016].
5. Seminar on Biostatistics with the use of R, Hellenic Pasteur Institute, Athens [24 hours, 28.04–01.06.2017]

### Certifications of achievement:

1. Certificate of International Transport of Infectious Substances, World Health Organization, 05.02.2016
2. Basic Security In The Field II, United Nations Department of Safety and Security, 17.04.2016
3. Advanced Security In The Field, United Nations Department of Safety and Security, 20.04.2016

## Discussion

### Coordinator's conclusions

One of the main goals of the EUPHEM programme is to expose the fellows to different public health experiences and activities, thus enabling them to work across various disciplines. This portfolio includes laboratory and epidemiological projects covering bacterial, parasitic and viral pathogens across a variety of disease programmes, such as vector-borne diseases, food and waterborne diseases, respiratory tract infections and vaccine-preventable disease. All projects here described were in line with the 'learning by doing' and 'on-the-job' training service approach of the EUPHEM programme and followed the core competency domains described professionals in mid-career and above. Outbreak, surveillance and laboratory activities contributed to the understanding of important public health issues.

During the two-year fellowship, the fellow, supervisors and training site have demonstrated the capability of addressing communicable disease threats in a structured joint approach between public health microbiology and epidemiology such as the national salmonellosis outbreak investigation or the meta-analysis of test-negative studies on the effectiveness of trivalent inactivated influenza vaccine for northern hemisphere during the 2010–2016 post-pandemic seasons. Projects involved different professional groups, such as physicians, laboratory technicians, epidemiologists, and statisticians or government officials, strengthening the fellow's ability to work in a multidisciplinary team. Those activities were complimented by nine training modules providing theoretical knowledge. Projects had a clear educational outcome, with results communicated in scientific journals and at conferences. The coordinator team concludes that the fellow has succeeded in performing all his tasks and wish him all the best for his future career as a public health microbiologist.

### Supervisor's conclusions

Dr Constantine Michael Vassalos was the second MS-EUPHEM fellow trained among the Greek EUPHEM Consortium. This two years training programme has turned out to be very successful for both the fellow and the training sites as well as an added value for the country as the fellow –through the training modules/projects– contributed in very important Public Health issues. It also gave the opportunity to continue building bridges and strengthening the existing collaborations between the Consortium members in the field of Public Health, i.e. various disciplines of microbiology, virology, epidemiology and clinical medicine.

As Constantine's background was in clinical microbiology, the training gave him a great opportunity to be involved in other aspects of Microbiology especially in such a very interesting and excited field of the Public Health Microbiology through various projects and activities. During the two-year fellowship, Constantine developed both personally and professionally and gained new skills through his involvement in a variety of public health activities both in microbiology and epidemiology. Those activities were organised trying to obtain a good outcome for both, the fellow and the training sites. The projects allow him to gain confidence in the fields of public health microbiology that were not in his background and at the same time allow local supervisors to carry out projects

that would otherwise have been hard to achieve. The Consortium and the supervision team wishes him every success for the future.

## Personal conclusions of fellow

The EUPHEM training is an excellent concept and a very well organised educational programme. The EUPHEM fellowship has offered me a unique opportunity to work on various projects within diverse areas of public health microbiology requiring different methods, many of which were unfamiliar to me from my working experience at clinical settings. A variety of modules, 'learning-by-doing' approach, and continuous interaction with epidemiologists strengthened the connection between the discipline of Public Health Microbiology and that of Epidemiology. The milieu within the Greek site's EUPHEM consortium encouraged reflection and scientific growth based on teambuilding. Local supervisors provided me with all the guidance, opportunity and support needed to complete my fellowship within two years. The fellowship has undoubtedly broaden my perspectives on public health microbiology and instilled confidence in my competencies for the years to come. It is my firm belief that a public health microbiologist combining effective practices on both public health and clinical level can do best service as an intermediary among health services, public health authorities and national/regional health authorities. In this endeavor, I am blessed with a very supportive Europe- and nation-wide network of epidemiologists and public health microbiologists.

## Acknowledgements of fellow

First and foremost, I would like to especially express my gratitude to my main supervisor Dr Georgina Tzanakaki for her strong commitment to the EUPHEM fellowship and coordination of the Greek site's EUPHEM consortium; and for her supervision, encouragement, always finding the right balance between guiding throughout my fellowship and giving me freedom to pursue my own ideas. Her unwavering support every time I needed it cannot be valued enough. Thank you for believing in me! I would like also to acknowledge Professor Alkis Vatopoulos, my co-supervisor, for his mentoring in public health culture and support during stressful times. As the second EUPHEM fellow from Greece, I would like to also thank Kyriaki Tryfinopoulou, the first Greek EUPHEM fellow of 2014 cohort (and co-supervisor in the second year of my fellowship) for her enthusiasm, understanding and engagement in growing the country's EUPHEM family year by year. Many warm thanks also go to local supervisors, namely, in alphabetical order, Theano Georgakopoulou, Elina Horefti, Simona Karabela, Konstantinos Kesanopoulos, Athanasios Kossyvakis, Georgia Mandilara, Kassiani Mellou, Andreas F. Mentis, Dimitrios Papaventsis, Eleni Patsoula, Gregory Spanakos, Evdokia Vassalou, Evangelos Vogiatzakis, Athanasia Xirogianni, for supervising me on the projects, sharing their knowledge, being always available and looking after me. I also would like to thank the staff-members of the labs which have helped me in the rotations. I wish to thank the ECDC training section, the fellowship programme office and all persons contributing to the organisation of modules, training and fellowship during these two years. I will forever be deeply grateful for the kind and invaluable mentoring from my EUPHEM coordinators Aftab Jasir (chief coordinator), Silvia Herrera-León (front-line coordinator), and Androulla Efstratiou (second-line coordinator in the first year of my fellowship) for their guidance, patience and understanding during all my projects and work, keeping me on track and being supportive. A special recognition must go to Aftab for her warm and open-hearted welcome of me to the EUPHEM-family, excellent coordination, always encouraging and supporting my works all through my EUPHEM fellowship; once again, thank you so much for everything you have done for me and my EUPHEM co-fellows! I am also thankful to my EUPHEM and EPIET co-fellows –a network for life beyond compare–, especially Maria Tseroni, the Greek EPIET fellow-Member State track of the 2015 cohort, for their friendship, fantastic team-spirit and amazing company. Last but not least, my warm gratitude belongs to my 'comrade-in-arms', John, for his support in the dark and difficult times; and to my partner for her reminding me of real life. Being forever in-debt, I would like to thank my dear parents for their fantastic commitment, inspiration and love throughout this fellowship. My adolescent son is hugely and warningly thanked for his understanding and endurance of my absence during the two years of my fellowship. Having you as my dearest son, full of energy, intellectual curiosity and spirit, has been the biggest and longest challenge I have ever encountered.