



EUPHEM REPORT

Summary of work activities

Lorenzo Subissi
European Public Health Microbiology Training
Programme (EUPHEM), 2017 cohort

Background

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

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

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

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

The views expressed in this publication do not necessarily reflect the views of the European Centre for Disease Prevention and Control (ECDC).

Stockholm, August 2019

© European Centre for Disease Prevention and Control, 2015. Reproduction is authorised, provided the source is acknowledged.

This report summarises the work activities undertaken by Lorenzo Subissi, cohort 2017 of the European Public Health Microbiology Training Programme (EUPHEM) at Sciensano, the Belgian Public Health Institute.

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

Biochemist by background, Lorenzo has a PhD in Infectious Diseases from Aix-Marseille University, during which he contributed to the discovery and characterization of the first RNA correction mechanism in an RNA virus (SARS-Coronavirus), and a Master in Epidemiology from the London School of Hygiene and Tropical Medicine. Before joining the ECDC fellowship, he worked in outbreak response and lead epidemiological studies on Emerging viral diseases (Ebola in Guinea, Zika in French overseas territories, and Yellow fever in Angola).

Methods

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

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

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

Results

The objectives of these core competency domains were achieved partly through projects or activities (on-job services) and partly through participation in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the EUPHEM scientific guide¹.

1. Epidemiological investigations

1.1. Outbreak investigations

Necrotising cellulitis outbreak on the islands of São Tomé and Príncipe.

Supervisor: Vilfrido Santana (WHO Country office, São Tomé and Príncipe)

Since week-38 of 2016, the Ministry of Health of the Democratic Republic of São Tomé and Príncipe reported an increasing number of cases of necrotizing cellulitis. An international outbreak investigation team was set up and in March 2017, the final case definition was adopted. The peak was registered in week-50 of 2016 and number of cases went down at week 11 of 2017. From week 12 of 2017 weekly cases (suspected and confirmed) have oscillated between 15 and 40, with few weeks that have registered less than 10 new cases, suggesting endemicity of the disease until end of 2018. Since beginning of 2019, the disease reached background monthly incidence. Laboratory analyses (21 patients) identified co-infections with *S. pyogenes* and *S. aureus* in over 50% of the patients. The strains of *S. pyogenes* and *S. aureus* (a mix of methicillin resistant and susceptible strains and some strains positive for PVL toxin) were however very diverse. During his deployment, the fellow's main findings were that the case definition often included simple cellulitis cases, which lead to a likely overestimation of the overall number of necrotizing cases. The fellow, together with the National Microbiology focal point, performed basic microbiology on samples of new cases and their contacts. The fellow performed time series analysis that found an association of climatic factors such as precipitation and relative humidity with the monthly increase of the number of cases. These climatic factors may have increased environmental survival and the risk of community-based transmission of some bacteria; investigations to identify the actual causative agent are still ongoing.

Shiga-toxin producing E.coli (STEC) serogroup O103 outbreak in a private child care

Supervisor: Steven Van Gucht (Sciensano)

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

On June 29th, 2018, a 2-years-old boy was diagnosed with Shiga toxin (Stx)-1 and Stx-2a positive STEC serotype O103 after an episode of gastrointestinal illness. Two weeks later, a total of 12 cases were diagnosed with STEC serogroup O103. Of the cases, 10 were children: 8 were between 1 and 2 years old and attending the child care and 3 were older siblings aged 4 to 5 years old. One case was a grandparent. A questionnaire on food was administered to the child care manager. Vegetables from the private garden hosted by the child care – all grown organic – were found to be the likely source of infection, however none of those tested positive. The fellow participated in meetings to assess the situation and suggest control measures with stakeholders such as regional outbreak investigation team and the Belgian food safety authority. The latter did the inspection of the child care and recommended closure and thoroughly cleaning of the whole environment. Food safety recommendations were left to the managers of the child care.

Chikungunya virus outbreak in the Democratic Republic of the Congo

Supervisor: Anja De Weggheleire (Institute of Tropical Medicine, ITM Antwerp)

From November 2018 onwards, the Congolese National Institute for Biomedical Research (INRB) received notification from Mont Ngafula health zone of suspected patients with Chikungunya fever. Early January 2019, INRB asked for support to the ITM Antwerp to set up diagnostic capacity to detect Chikungunya virus.

As of May 21st, 2019, the INRB, in collaboration with ITM Antwerp, confirmed Chikungunya virus infection in 382 patients (311 by RT-PCR and 71 by ELISA IgM detection) from 20 health zones in the provinces of Kinshasa and Congo Central. These data are of difficult interpretation because Chikungunya fever is not among disease with mandatory notifications, and samples tested are a mixture of samples from passive and active surveillance. Because of the limited laboratory capacity and the extent of the outbreak, adapting the clinical case definition to the Congolese context, where malaria is hyperendemic, was considered among the priorities. The fellow worked with INRB and ITM clinicians and epidemiologists to develop the protocol, including SOPs, for a study aiming at evaluating the clinical case definition of Chikungunya fever in DRC.

Salmonella enterica (serovar Typhimurium) outbreak affecting over 50 schools linked to a single caterer

Supervisor: Steven Van Gucht (Sciensano)

On May 22nd 2018, the Flemish outbreak control team was notified of 15 gastroenteritis cases in two schools. By May 25th, cases were reported in 30 schools, all supplied by caterer A, that distributed ~10,000 meals daily among 83 schools.

The fellow was part of the team that investigated the extent and source of this outbreak to limit further spread. His work focused on calculating school attack rates and mapping the itineraries of the caterer's car drivers that provided meals to the schools during the period under investigation.

In total, there were 546 cases (399 *Salmonella enterica* serovar Typhimurium confirmed and 147 probable) in 51/83 schools over 21 days. School attack rates were as high as 25%, with a 1.3% increase in AR per 10km from the caterer's location ($p=0.001$). Higher attack rates among distant schools suggested that transport of unrefrigerated food may contributed to the outbreak's extent. Food distributions of the caterer were halted between 25 May and 9 June.

Training modules

1. Introductory Course, Spetses, Greece, September - October 2017 – This three-week course consisted of lectures on epidemiology and microbiology, interactive case studies and developing a research protocol.
2. Outbreak Investigation module, Berlin, Germany, December 2017 – This module focused on the 10-steps of an outbreak investigation. Fellows were trained in the basics of STATA, phylogenetic analysis, and epidemiological analyses using lectures and case-studies.
3. Management, Leadership and Communication in Public Health, Stockholm, Sweden, February 2018 – This module focused on personality traits relevant to professional development and management tasks. Included were exercises on project management, risk assessment, and communication with different authorities in the field.
4. Rapid Assessment and Survey methods module, Athens, Greece, May 2018 - This course focused on field surveys and investigations. Subjects that were covered were risk assessment methodology, sampling strategies, data collection using mobile tools, mapping using Geographic Information Systems.

Educational outcome:

The fellow was involved in all steps of an outbreak investigation including epidemiological and microbiological analyses during four national and international outbreaks. He gained experience in community-acquired, food-borne and mosquito-borne outbreak investigations from the building of the outbreak response team to the implementation of control measures.

1.2. Surveillance

Surveillance of non-tuberculous mycobacteria: a 10 year perspective

Supervisor: Pieter-Jan Ceysens

In 2007, a new federal legislation in Belgium prohibited non-biosafety level 3 laboratories to process culture tubes suspected of containing mycobacteria. This project aimed to present mycobacterial surveillance/diagnosis data from the Belgian National Reference Centre for mycobacteria (NRC) from 2007 to 2016.

This was an observational study investigated the numbers of analyses at the NRC and false positive cultures (interpreted as containing mycobacteria at referring clinical laboratories, but with no mycobacterial DNA detected by PCR in the NRC). We reviewed mycobacterial species identified and assessed trends over time of proportions of nontuberculous mycobacteria (NTM) vs *Mycobacterium tuberculosis* complex (MTBc), and false positive cultures vs NTM.

From 2007 to 2016, analyses requests to the NRC doubled from 12.6 to 25.3 per 100,000 inhabitants. A small but significant increase occurred in NTM vs MTBc proportions, from 57.9% (587/1,014) to 60.3% (867/1,437) ($p < 0.001$). Although NTM infection notification is not mandatory in Belgium, we annually received up to 8.6 NTM per 100,000 inhabitants. *M. avium* predominated (ca 20% of NTM cultures), but *M. intracellulare* culture numbers rose significantly, from 13.0% (74/587) of NTM cultures in 2007 to 21.0% (178/867) in 2016 (RR: 1.05; 95% CI: 1.03-1.07). The number of false positive cultures also increased, reaching 43.3% (1,097/2,534) of all samples in 2016.

We recommended inclusion of NTM in sentinel programmes. The large increase of false positive cultures is hypothesised to result from processing issues prior to arrival at the NRC, highlighting the importance of sample decontamination/transport and equipment calibration in peripheral laboratories.

Epidemiology and severity of non-influenza respiratory viruses during flu seasons 2015/2016 to 2017/2018

Supervisors: Cyril Barbezange and Isabelle Thomas

We described non-influenza virus detection rates and their association with severity in patients with severe acute respiratory infection (SARI).

The Belgian SARI sentinel surveillance relies on data from six hospitals. SARI was defined as an infection requiring hospitalization with onset of fever and cough or dyspnea within the previous 10 days. We tested samples taken for the seasons 2015-2016 until 2017-2018 by multiplex RT-qPCRs for the detection of influenza viruses and 8 non-influenza respiratory viruses. We described detection rates and the affected population (N=3,200). We modelled the relationship with severity by age group using logistic regression adjusting for clustering within hospitals. Twenty-eight per cent of the samples were negative for all viruses tested (n=892), 39% were positive for influenza only (n=1245) and 22% were positive for one single non-influenza respiratory virus (n=701), including human metapneumovirus (HMPV) (5.9%), respiratory syncytial virus (RSV) (4.7%), and picornaviruses (4.8%). We detected 11.3% co-infections (2 to 5 respiratory viruses) (n=362).

Complications (defined as death, admission to intensive care unit, development of acute respiratory distress syndrome, need of invasive mechanical ventilation, or need of extracorporeal membrane oxygenation) were reported in 15% of adults (292/2010, including 165 deaths) and 2.8% children (33/1190, including 2 deaths), respectively. Viral pneumonia was reported in 10.6% of adults and 6.8% of children, respectively.

Among adults, multivariable analysis identified a higher risk of viral pneumonia with HMPV infection versus influenza infection (OR 3.4, 95% CI 2.2-5.5).

One third of the patients was positive for non-influenza viruses. The SARI surveillance also showed evidence of higher morbidity in adults associated to HMPV suggesting that early testing for non-influenza viruses could be beneficial for clinical management of SARI patients.

Use of severe acute respiratory surveillance to cover Respiratory Syncytial Virus (RSV) complete season: A pilot study in Belgium

Supervisors: Cyril Barbezange and Isabelle Thomas

The virological surveillance of SARI patients is ongoing since the 2009 pandemics and tests a subset of samples from SARI patients during every influenza season, typically from December to April, in 6 hospitals in Belgium. RSV season is earlier than influenza, typically from October to December. WHO recommended to assess the feasibility and relevance of extending virological surveillance of SARI patients to capture the RSV season, including its severity. We therefore performed a pilot study to capture the whole RSV season. For the influenza season 2018/2019, we started the virological surveillance of Severe acute respiratory infection (SARI) patients in a subset of sentinel hospitals (3/6) as early as October 2016.

From the epidemiological week 40 to week 53, we received a total of 457 samples eligible for the SARI case definition, a median of 48 samples per week (Interquartile range 45-50).

The epidemiological curve revealed a peak in the detection of RSV at sampling week 49 (early December), with 60 RSV-positive samples. RSV-B was predominantly circulating (234/293), with RSV-A being found in 22% of RSV-positive samples (65/293). RSV-positive co-infections were almost exclusively detected in children (40/82 in the 0-6

months old, 19/82 in the 6-11 months old, 19/82 in 1-14 years old, and only 4/82 among adults). Fifty-one percent of RSV mono-infections were detected in children 0-6 months old (109/211). In total, 59 patients (16 children and 43 adults) had complications, of whom 15 died (all adults >45 y/o). These numbers were too low to test an association of RSV with severity indicators.

In summary, by anticipating the SARI surveillance from October we were able to nicely capture the complete epidemiological curve of RSV season. However, the only partial use of the sentinel network in place did not allow us to draw any conclusion on the possibility to detect RSV severity through SARI surveillance. We therefore recommend to extend this work to the rest of the sentinel hospital to be able to assess whether RSV severity can be quantified by SARI surveillance

Training modules

1. Introductory Course, Spetses, Greece, September - October 2017 – This three-week course consisted of lectures on epidemiology and microbiology, interactive case studies and developing a research protocol.
2. Multivariable Analyses module, Nicosia, Cyprus, April 2018 - A course focusing on applied statistics for epidemiological analyses introducing multivariable analysis, stratified analyses, interaction of variables, building regression models using STATA.

Educational outcome:

The fellow gained experience in a spectrum of methodologies used to analyse surveillance data, including using different regression models and multivariable analyses.

2. Applied public health microbiology research

Towards the switch to NGS for routine surveillance of *Mycobacterium tuberculosis*

Supervisor: Pieter-Jan Ceysens

Mycobacterium tuberculosis (MTB) remains a leading cause of morbidity and mortality worldwide. In 2015, WHO estimated that MTB caused 9 million new active infections and 1.5 million deaths worldwide and over 300,000 new TB cases and 32,000 deaths in Europe only, mostly in Eastern and central European countries (WHO, Global tuberculosis report, 2015). Belgium is a low MTB incidence country, with approximately 10 new cases per 100,000 inhabitants per year. Understanding the transmission routes of the disease is a key factor for its control and for the implementation of efficient interventions. For many years, reference labs have been performing *Mycobacterial interspersed repetitive-unit-variable-number tandem-repeat* (MIRU-VNTR) typing, a well-described method for lineage identification and transmission tracking. The fellow supported in the successful implementation of NGS for Mycobacteria surveillance by working in close collaboration with the bioinformatics team in charge of pipeline development, by testing the different versions of the pipeline for activities such as speciation, prediction of antimicrobial resistance pattern and clustering of strains. This new way of doing TB surveillance will directly improve the tracking of (inter)national transmission routes of TB lineages, prediction of emerging resistances and communication among TB reference labs.

Multi-site case control study to identify risk factors for infection with Hepatitis E virus

Supervisor: Steve Van Gucht

In Europe, Hepatitis E virus is thought to be transmitted through undercooked pork meat and pork products. European countries such as Italy, France, the UK and the Netherlands have identified food items at high risk of Hepatitis E virus (HEV) infection. Food items responsible of HEV in Belgium are however unknown. The fellow has designed a case control study aiming at identifying food items at high-risk for HEV. He wrote the study protocol, analysis plan and designed the patients' questionnaire and created the online platform within the Sciensano server (LIMEsurvey). He managed the project from the beginning to the approval by ethics committee and the study will be started by the end of 2019.

Training modules

1. Introductory Course, Spetses, Greece, September - October 2017 – This three-week course consisted of lectures on epidemiology and microbiology, interactive case studies and developing a research protocol.

2. Multivariable Analyses module, Nicosia, Cyprus, April 2018 - A course focusing on applied statistics for epidemiological analyses introducing multivariable analysis, stratified analyses, interaction of variables, building regression models using STATA.
3. Management, Leadership and Communication in Public Health, Stockholm, Sweden, February 2018 – This module focused on personality traits relevant to professional development and management tasks. Included were exercises on project management, risk assessment, and communication with different authorities in the field.

Educational outcome:

The fellow gained knowledge on all steps of PHM research project, from planning, study designing to publishing the results in international peer-reviewed journals. This included meetings with collaborators from academia, clinics, and other public health institutes and presentation of results to scientific conferences.

4. Applied public health microbiology and laboratory investigations

Serodiagnosis of pertussis to distinguish recently vaccinated from infected individuals

Supervisor: Isabelle Desombere

Measuring IgG antibody titers to pertussis toxin (PT) is the most specific and sensitive method to serodiagnose a *Bordetella pertussis* infection. However, this does not distinguish infection from recent vaccination, as anti-PT IgG are stimulated by the acellular pertussis vaccine. We aim to identify additional *B. pertussis*-specific serological markers that can discriminate between infection and recent vaccination, to improve pertussis serodiagnosis in symptomatic subjects with a recent vaccination history and elevated anti-PT IgG titers or for use in seroprevalence studies.

The utility of measuring IgA directed to vaccine antigen PT, and IgG directed to non-vaccine antigens (Fim2/3, LPS, ACT and CatACT) was evaluated comparing subjects groups with known vaccination and infection status, aged 10-89 years (n=390). Serological responses were monitored using commercial ELISAs (PT, Fim2/3, LPS, ACT) or an in house immunoblot assay (CatACT). Comparing pertussis-infected symptomatic subjects (n=140) with recently vaccinated, non-infected subjects (n=100), revealed the optimal cut-off, accuracy, sensitivity and specificity for each single parameter.

For diagnosis of pertussis infection, the measurement of anti-PT IgA (cut-off 15 IU/mL) and anti-ACT IgG (cut-off 15 U/mL) resulted in accuracies of 95% and 87.5%, sensitivities of 92.9% and 83.6% and specificities of 98% and 93%, respectively. Screening using anti-PT IgA testing followed by confirmation with anti-ACT IgG testing increased the specificity to 100% (95% CI 96.3-100%) at the cost of sensitivity (77.1%, 95% CI 69.5-83.3%). The use of both Anti-PT IgA and anti-ACT IgG markers can discriminate between recent vaccination and infection. When such distinction is needed, the use of these markers can be recommended in the context of both routine pertussis serodiagnostics and pertussis seroprevalence studies.

Training modules

1. Introductory Course, Spetses, Greece, September - October 2017 – This three-week course consisted of lectures on epidemiology and microbiology, interactive case studies and developing a research protocol.
2. Outbreak Investigation module, Berlin, Germany, December 2017 – This module focused on the 10-steps of an outbreak investigation. Fellows were trained in the basics of STATA, phylogenetic analysis, and epidemiological analyses using lectures and case-studies.

Educational outcome:

The fellow gained deep understanding of the preparation of clinical samples for sequencing. He got heavily exposed to the bioinformatics behind next generation sequencing and to the sequence analysis and interpretation. Because he was based at the NRC for Salmonella and *Neisseria meningitidis*, in addition to the NRC mycobacteria, he got exposed in molecular typing techniques still in use such as PFGE, MLVA (for Salmonella) and MIRU (for mycobacteria), and how to interpret them. The fellow also gained experience in immunology and serodiagnostics.

4. Biorisk management

BSL-3 training for work with *Mycobacterium tuberculosis*

Supervisor: Pieter-Jan Ceyskens

The training consisted of a two-day theoretical training which includes biosafety and biosecurity practice and legislation in Belgium. The fellow got experience with personal protective equipment, decontamination and waste control strategies, and national regulations, and sent infectious substances to other reference Institutes.

Training modules

1. Bio-risk & Quality Management 2018, Stockholm, Sweden, February 2018 – During this one week module the fellow gained knowledge on bio-risk and control management, included identification and mitigation of bio-risks, biological sample triple packaging and shipment, methodologies for quantitative and qualitative test controls including WHO guidelines on biosafety management in laboratories, and a visit to a BSL-4 facility.

Educational outcome:

The fellow undertook BSL-3 training and gained understanding of bio-risk management principles, including use of personal protection equipment (PPE) and decontamination processes. The fellow received the certificate for safe packaging of infectious substances.

5. Quality management

External Quality Assessment (EQA) for a multiplex RT-PCR detecting of 16 respiratory virus groups

Real Time MULTIPLEX RESPI tests are used for the detection of various respiratory viruses in nasopharyngeal swabs or other specimens, performed in samples from suspected patients or in culture supernatants. The National Influenza Centre (NIC) has developed a MULTIPLEX RESPI real time consisting of 4 quadriplex PCR for the detection of the following viruses: RSV A, RSV B, PIV 1, 2, 3, 4, HRV / ENV, hMPV, HPeV, HBoV, ADV, CoOC43, CONL63, Co229E, (MERS-CoV), EV-D68. Four different fluorophores are used: FAM, HEX, ROX and Cy5.

The fellow followed the EQA process needed for accreditation of this technique. The EQA expert has brought the attention of the NIC onto few type B non-conformities that have to be addressed in the 3 months following the EQA. In particular, action points following EQA were: the in-house adaptations to the original protocol will be fully described in detail; the experiment to validate detection limits will be done in triplicates; for ct values between 38-40, 'weakly positive' instead of 'positive' will be reported to the hospitals that sent the sample; preparation of the internal controls will be detailed, from the arrival to the lab of each batch until its use; ringtests for 2019 were actually planned but not reported in the ringtest table: they will be added to the ringtest table; the date of preparation and use of each primer and each pre-mix will be noted; Each technician will have to participate to a ringtest once a year. These actions can be reported electronically and do not need the in-site visit of an EQA expert. To conclude, once action points are implemented, we can be confident that this test detects the different respiratory viruses in respiratory samples in a reproducible and sensitive manner and can therefore be accredited.

Training modules

Bio-risk & Quality Management 2018, Stockholm, Sweden, February 2018 – During this one week module the fellow gained knowledge on bio-risk and control management, included identification and mitigation of bio-risks, biological sample triple packaging and shipment, methodologies for quantitative and qualitative test controls including WHO guidelines on biosafety management in laboratories, and a visit to a BSL-4 facility.

Educational outcome:

The fellow was also involved in validation and implementation of novel diagnostic assays and followed an EQA from the beginning to the end of the process.

6. Teaching and pedagogy

Epidemiology course for Sciensano employers (1 week)

The fellow contributed in the planning and preparation of teaching materials for a one-week course on basic epidemiology to Sciensano employers. The fellow obtained experience with giving lectures, moderating case studies, and planning and organizing a course. The lectures covered frequency measures, study designs, measures

of impact, causality, bias, confounding, effect modification, matching, alternative study designs, validity, standardisation and Kaplan Meier survival analysis.

Lecture for the PhD students of the MARIE CURIE Initial Training Network 'ANTIVIRALS'

The fellow was invited to give a 2h lecture on the basics of epidemiology of emerging viral diseases to PhD students of the Marie Curie Initial Training Network ANTIVIRALS, a H2020 funded European programme aiming at discovering new antivirals against RNA viruses.

Practical course on mapping using qGIS

The fellow organized and lead three sessions on the use of qGIS for mapping to different departments of Sciensano (Viral Diseases, Bacterial Diseases, and Food-Borne Outbreaks department) and for local epidemiologists at the INRB (in French).

Hands-on qGIS practical at the epidemiology department, Sao Tome and Principe (6h)

The fellow has organized and led three sessions (2h each) in Portuguese with local epidemiologists (10-15 participants in each session) to teach them how to prepare an incidence map at the province level and the municipality level ('localidades').

Level 3 Accompanist for WHO Survey Scholar course: 'Vaccine coverage cluster surveys: analysis and interpretation'

The fellow provided online support to the participants of WHO Survey scholar course (6 weeks, 2,5 h a week) on newly updated (2016) methodology for vaccine coverage cluster surveys.

Educational outcome:

The fellow gained experience in the identification of training and educational needs for different audiences, from students, senior microbiologists, to public health professionals and epidemiologists. He was involved in the preparation and delivery of teaching materials, modification and moderation of case studies, and practical sessions for the use of specific software (eg qGIS and Epidata).

7. Public health microbiology management

Submission of a multi-centre study protocol to ethics committees of 5 hospitals

Supervisor: Steven Van Gucht

The fellow has designed, written and submitted a study protocol and all additional documents (eg Informed consent, questionnaire...) to the Ethics committees of the 5 centres participating to the study. He dealt with confidentiality issues according to the newly implemented GDPR rules in place since May, 25th 2018. The fellow presented the study in all 5 hospitals before submitting to each ethics committee. Then, he has addressed all concerns of all 5 participating centres until the protocol was approved.

Communication with different actors during the necrotising cellulitis outbreak in Sao Tomé

During his mission in Sao Tomé, the fellow had meetings with the Minister of Health, the WHO country representative and engaged in scientific discussions with world-renown *S. aureus* and *S. pyogenes* experts in the UK and Australia. He presented his most important findings and left short middle and long-term recommendations to the team of the epidemiology department and the Director of Health.

Communication to high authorities from ECDC on the state of the art of the implementation of Next generation sequencing for surveillance in Belgium

The fellow presented to the highest authorities of ECDC, including the Director the latest advances on the implementation of next-generation sequencing for surveillance and outbreak investigation activities for a list of priority pathogens in Belgium. The presentation was followed by a questions session.

Training modules

Management, Leadership and Communication in Public Health, Stockholm, Sweden, February 2018 – This module focused on personality traits relevant to professional development and management tasks. Included were exercises on project management, risk assessment, and communication with different authorities in the field.

Educational outcome:

The fellow learned to deal with the bureaucracy behind study protocol submissions, insurance preparations and the delays that may occur during such processes. He also got exposed to the new, more stringent GDPR rules in place since mid-2018. The fellow interacted with highest authorities such as a Minister of Health, a WHO country representative and the ECDC director.

8. Communication

Publications

1. **Subissi L.**, Keita M., Mesfin S., Rezza G., Diallo B., Van Gucht S., Musa E.O., Yoti Z., Keita S., Djingarey M.H., Diallo A.B. Fall I.S. Ebola re-emergence caused by persistently infected survivors. *The Journal of Infectious Diseases*. Nov 22;218(suppl_5):S287-S291
2. Soetaert K, **Subissi L.**, et al., Strong increase in the true and false positive mycobacterial samples sent to the National Reference Centre for characterization, Belgium, 2007-2016. *Euro Surveillance*, 2019 Mar;24(11). doi: 10.2807/1560-7917.ES.2019.24.11.1800205
3. Suin V., Klamer S., et al. **Subissi L.** Van Gucht S.. Epidemiology and subtype dynamics of hepatitis E virus in Belgium, 2010-2017. *Euro Surveillance*, 2019 Mar;24(10). doi: 10.2807/1560-7917.ES.2019.24.10.1800141
4. **Subissi L.** et al, Subtype-specific differences in the risk of hospitalization among patients infected with Hepatitis E virus genotype 3. *Epidemiology and Infection*. n 147, e224, 1–4. <https://doi.org/10.1017/S0950268819001122>
5. **Subissi L** et al. Anti-PT IgA and anti-ACT IgG as valuable tools for the serodiagnosis of *Bordetella pertussis* infection in recently vaccinated subjects. *Clin Microbiol Infect*. 2019 Oct 11. pii: S1198-743X(19)30528-2. doi: 10.1016/j.cmi.2019.10.001
6. **Subissi L.** et al. Capturing Respiratory Syncytial Virus season using influenza surveillance data: a pilot study in Belgium, 2018/2019. Under review
7. **Subissi L** et al Necrotising cellulitis outbreak on the islands of Sao Tomé and Príncipe, 2016-2018, In preparation
8. **Subissi L.** et al. Non-influenza viruses associated with Severe Acute Respiratory Infections during influenza seasons 2015/2016 to 2017/2018 in Belgium. In preparation

Other Publications

9. Keita *et al.* **Subissi L.** Subsequent mortality in survivors of Ebola virus disease in Guinea: a nationwide retrospective cohort study. *Lancet Infect Dis*. 2019 Nov;19(11):1202-1208. doi: 10.1016/S1473-3099(19)30313-5. Epub 2019 Sep 4.

Conference presentations

1. Oral presentation: Zika virus in Pregnancy: a retrospective case control study, French Polynesia, 2013-2016, ESCAIDE, Malta, 2018
2. Poster presentation: Emergence of Hepatitis E virus in Belgium, 2010-2016, ESCAIDE, Malta, 2018
3. Oral presentation: Preliminary descriptive analysis of the HEVnet database; 2nd HEVnet meeting, Amsterdam, 2019
4. Oral presentation: Anti-PT IgA and anti-ACT IgG are valuable tools for the serodiagnosis of *Bordetella pertussis* infection in recently vaccinated subjects, ESCAIDE, Stockholm, 2019
5. Oral presentation: Non-influenza viruses associated with Severe Acute Respiratory Infections during influenza seasons 2015/2016 to 2017/2018, Belgium., ESCAIDE, Stockholm, 2019
6. Oral presentation: High subsequent mortality of Ebola virus disease survivors in Guinea: a nationwide retrospective cohort study., ESCAIDE, Stockholm, 2019

Training modules

Management, Leadership and Communication in Public Health, Stockholm, Sweden, February 2018 – This module focused on personality traits relevant to professional development and management tasks. Included were exercises on project management, risk assessment, and communication with different authorities in the field.

9. EPIET/EUPHEM modules attended

1. EPIET/EUPHEM introductory course, Spetses, Greece (three weeks)
2. Outbreak investigation, Robert Koch Institute, Berlin, Germany (one week)
3. Multivariable analysis module, Nicosia, Cyprus (one week)
4. Rapid risk assessment and survey methods, Athens, Greece (one week)
5. Project review module, Lisbon, Portugal (one week)
6. Initial management in public health microbiology module, ECDC, Stockholm, Sweden (one week)
7. Bio-risk and quality management module, ECDC, Stockholm, Sweden (one week)

10. Other training

1. MOOC Vaccinology, Institute Pasteur Paris
2. MOOC Resistance of antimicrobial agents, Institute Pasteur Paris
3. MOOC Vaccine coverage WHO scholar A1-A2-A3 modules
4. Qiagen course on CLC workbench workshop (2 days)
5. United Nations Department of Safety and Security online training Basic security in the field and Advanced security in the field (USDSS) (two days, certification)
6. BSL-3 training (one week)

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 report summarises all activities and projects conducted by Lorenzo Subissi during his two-year EUPHEM fellowship (cohort 2017) as an EU track fellow at the Sciensano, Brussels, Belgium. Lorenzo is the first appointed EU track EUPHEM fellow in Brussels. The portfolio includes laboratory and epidemiological projects covering viral and bacterial pathogens across a variety of disease programmes, such as vector-borne diseases, sexually-transmitted diseases, food and waterborne diseases, respiratory tract infections or vaccine-preventable disease. The projects here described are in line with the 'learning by doing' approach of the EUPHEM programme and fulfilled the core competency domains described for professionals in their mid-career and beyond. 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 use of whole genome to identify and investigation *Mycobacterium tuberculosis* transmission chains, outbreak investigations at national (*E. coli* O103 and *Salmonella* Typhimurium) and international level (Chikungunya) or surveillance investigations (non-tuberculosis mycobacteria, non-influenza viruses, Respiratory Syncytial Virus). The projects have been nicely selected to cover not only important international and national public health topics such as Chikungunya infection, pertussis, tuberculosis, non-influenza virus infections among others but also a very broad panel of microorganisms and involved different professional groups, such as physicians, laboratory technicians, epidemiologists, statisticians, government officials, public health officers and logisticians, strengthening the fellow's ability to work in a multidisciplinary team and to adapt to different environments and contexts. Lorenzo has been active in contributing to training of others during his fellowship with the development of new training materials as well as direct training and facilitation activities which highlights the contribution that fellows can make to capacity building beyond the programme. All projects had a clear outcome, with results communicated in scientific journals and at conferences and the activities were complimented by nine training modules providing theoretical knowledge. The contributions made by Lorenzo indicate the importance of developing and maintaining a critical mass of highly skilled field public health microbiologists within Member States to contribute towards national preparedness as well as being available for responses in the interest of the EU. The EUPHEM Coordinator Team concludes that the fellow has succeeded in performing all his tasks to a very high standard and has conducted himself in a highly professional and effective manner throughout. We wish the fellow every success in his future career.

Supervisor's conclusions

During his 2-year fellowship, Lorenzo has developed a good overview and understanding of public health microbiology. He managed to integrate well in very diverse teams and labs and worked on a variety of topics ranging from immunology, bacteriology (including AMR), virology to epidemiology. He proved to have excellent creative writing and analytical skills. Within this 2-year period, he managed to publish several papers on a diverse set of topics. We are truly grateful that we could increase our publication output thanks to Lorenzo's assistance and dedication, which made it a win-win situation for Sciensano and Lorenzo. I also found that the projects performed by Lorenzo facilitated the collaboration within the institute between different microbiology labs as well as between our microbiology and epidemiology departments. In general, the fellowship has helped to expand our network of peers and collaboration with other European public health institutes.

On a personal note, Lorenzo is one of the most highly motivated public health researchers I have encountered in my career. Lorenzo lives and breathes (international) public health and I hope he will be able to further pursue a bright career in international public health.

Personal conclusions of fellow

The EUPHEM fellowship has allowed me to get a broad exposure to the fields of outbreak investigation, surveillance and applied research for an impressive spectrum of disease groups. It was a great opportunity to expand my professional network of colleagues with very different backgrounds but with whom I share a common goal: decrease the burden of infectious diseases in Europe and globally. I believe people with microbiology and epidemiology background are of great importance now that epidemiology also relies on next-generation molecular data for interpretation of public health events. Therefore, I believe the ECDC fellowship, which, since the creation of the EUPHEM path a decade ago, fosters the collaboration between epidemiologists and microbiologists, is paramount for the future of Public Health.

Acknowledgements of fellow

I would like to first thank my host site supervisor Steven Van Gucht for his scientific support, unconditional support as well as his commitment to the research of EUPHEM project opportunities as well as to the EUPHEM programme. I would also like to thank Pieter-Jan Ceysens and Isabelle Desombere for their close supervision during the fellowship. Thanks Silvia Herrera-Leon and Aftab Jasir for their constant and constructive supervision from ECDC. Thanks to all other ECDC EPIET and EUPHEM coordinators for their commitment to high-quality supervision during modules and thanks to the ECDC fellowship office for the constant administrative support. I would also like to thank all colleagues with whom I collaborated in Sciensano and during my international missions in Sao Tomé and Príncipe and in the Democratic Republic of the Congo. Finally, I would like to thank all the fellows from Cohort 2017 for all constructive exchanges during modules, it was a pleasure to meet you all and I am confident that our bond will last very long.