Elena Portell Buj

The European Public Health Microbiology Training Programme (EUPHEM), Cohort 2023
Sciensano, Belgium

Background

The ECDC Fellowship Programme is a two-year competency-based training with two paths: the field epidemiology path (EPIET) and the public health microbiology path (EUPHEM). After the two-year training, EPIET and EUPHEM graduates are considered experts in applying epidemiological or microbiological methods to provide evidence to guide public health interventions for communicable disease prevention and control. The Administrative Decisions ECDC/AD/2022/16 Rev.01 and ECDC/AD/2023/06 govern the European Union (EU)-track and Member States (MS)-track, respectively, of the ECDC Fellowship Programme, field epidemiology path (EPIET) and public health microbiology path (EUPHEM), Cohort 2023.

Both curriculum paths provide training and practical experience using the 'learning-by-doing' approach at acknowledged training sites across the European Union/European Economic Area (EU/EEA). This final report describes the experiences and competencies the fellow acquired by working on various projects, activities, theoretical fellowship training modules, other modules or trainings, and international assignments or exchanges during the fellowship.

Pre-fellowship short biography

Elena Portell Buj earned her bachelor's degree in Microbiology from the Universitat Autònoma de Barcelona, Spain. She subsequently completed a master's degree in Clinical Microbiology at the University of Barcelona, where her research focused on evaluating various antibiotics against *Mycobacterium tuberculosis* (*M. tuberculosis*).

In 2021, Elena earned her PhD in Medicine and Translational Research from the Hospital Clínic de Barcelona – University of Barcelona, Spain. Her doctoral thesis focused on the study of different antimicrobial agents targeting *M. tuberculosis* and non-tuberculous mycobacteria (NTM). During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, Elena worked as a public health expert at the Public Health Agency of Catalonia (ASPCAT), Spain. Simultaneously, she pursued a postgraduate degree in Public Health from the Universitat Pompeu Fabra (UPF), Spain. Following the pandemic, Elena worked as a clinical microbiologist at the Hospital Sant Joan de Déu (SJD Barcelona Children's Hospital) in Spain.

Results

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

[1] European Centre for Disease Prevention and Control (ECDC). Manual for the ECDC Fellowship Programme EPIET and EUPHEM paths. Stockholm: ECDC; 2025. Available at: https://www.ecdc.europa.eu/en/publications-data/ecdc-fellowship-programme-manual

Stockholm, 2025

1. Epidemiological investigations

1.1. Outbreak investigations

1.1.1. Outbreak investigation of Salmonella *Enteritidis in a youth organisation in Diepenbeek, Belgium, 2024*

Supervisor: Wesley Mattheus (Sciensano)

Category: Food- and waterborne diseases

Aim: To identify the pathogen and source, avoid further spread, and prevent potential similar outbreaks.

Methods: On 22 October 2024, a possible food-borne outbreak linked to a dinner event at a youth organisation on 19 October in Diepenbeek, Belgium, was reported. A retrospective cohort study was conducted using online questionnaires. Stool and food samples were also collected. A case was defined as an individual who ate food at the event and developed gastrointestinal symptoms within seven days. Food specific attack rates (AR), relative risks (RR), and 95% confidence intervals (95% CI) were calculated for the food items and consumption doses.

Results: The cohort included approximately 340–350 individuals who consumed food at the event. Of these, 219 (95%) responded to the questionnaire. Among respondents, 46 individuals (21%) met the case definition. Tiramisu was the food item most strongly associated with disease (RR=28.00; 95% CI: 6.5–118.4). A positive dose-response relationship was observed for tiramisu consumption.

Salmonella Enteritidis was identified in stool samples (n=2) by agglutination, Luminex® assay, and whole genome sequencing (WGS). *Salmonella* was detected in leftover tiramisu and the eggs used for its preparation using real-time polymerase chain reaction (PCR). WGS sequences from the food samples were identical to those from affected individuals, as confirmed by core genome multilocus sequence typing (cgMLST).

Public health implications/Conclusions: The use of raw eggs in food preparation poses a health risk. Egg retailers should include labelling on egg cartons regarding safe handling practices. Increased inspection of egg-laying farms is recommended, along with improved surveillance systems for monitoring *Salmonella* spp. in eggs.

Role: Elena investigated the outbreak alongside experts from Sciensano and Departement Zorg. She contributed to reviewing the online questionnaire, participated in outbreak investigation team meetings, conducted the data cleaning and analytical epidemiology, and co-authored both the report and the manuscript submitted to *The Flemish Infectious Disease Bulletin* alongside her colleagues.

1.2. Surveillance

1.2.1. Evaluation of the national surveillance system for Yersinia *spp. in Belgium, 2024*

Supervisor: Wesley Mattheus (Sciensano)

Type of project: Evaluating a surveillance system

Aim: To evaluate the national surveillance system for *Yersinia* spp. in Belgium and provide recommendations for improvement.

Methods: Data were collected through direct involvement in the routine surveillance system, meetings with the head of the National Reference Centre (NRC), and the annual *Yersinia* spp. surveillance report. The evaluation criteria included representativeness, timeliness, and completeness of the data.

Results: The surveillance system met its objectives: estimating the burden of yersiniosis, distinguishing between pathogenic and non-pathogenic strains, monitoring trends, and detecting outbreaks/clusters. All findings were regularly shared with the stakeholders and made publicly available in the NRC's annual report. However, several areas for improvement were identified. The lower incidence of yersiniosis reported in Wallonia might be due to under-reporting, the completeness of certain variables was frequently often poor, and there were delays in the reporting of genomic data.

Public health implications/Conclusions: There is a need to raise awareness among regional physicians and clinical laboratories about the importance of participating in disease surveillance. Data providers should be encouraged to submit complete datasets, and faster alternatives for strain sequencing should be explored.

Role: Elena conducted the evaluation and data analysis. She wrote a final report outlining the main findings and recommendations for improvement.

Routine surveillance activities

Integration of next-generation sequencing in the surveillance system for Yersinia *spp., Belgium, 2024*

Supervisor: Wesley Mattheus (Sciensano)

Aim: To integrate next-generation sequencing (NGS) into the routine surveillance system for *Yersinia* spp. at the Belgian NRC.

Methods: A workflow, including wet lab procedures, optimisation, and planning was developed by the NRC staff to incorporate NGS into the surveillance of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. The dry-lab component involved the analysis of genomic surveillance data using existing Galaxy pipelines, including quality control and cgMLST. Genomic indicators were interpreted using BioNumerics to identify potential links with previously detected isolates or known events. Epidemiological and microbiological information were then combined to confirm clusters and outbreaks. As a proof of concept, the European surveillance portal for infectious diseases (EpiPulse) was monitored regularly to detect any events reported on the platform.

Results: In 2024, a total of 506 *Yersinia* spp. strains were sequenced. Biotype 4 was the most frequent (n=439), followed by biotype 2 (n=28). *Yersinia pseudotuberculosis* accounted for 26 strains. Several new and ongoing clusters were identified. During 2024, three EpiPulse alerts were posted by other countries, leading to the following related cases being detected in Belgium:

- 2024-FWD-00007: one case detected in Belgium
- 2024-FWD-00058: five cases detected in Belgium
- 2024-FWD-00114: four cases detected in Belgium.

Public health implications/Conclusions: Genomic surveillance is essential in public health. The systematic collection, analysis, and integration of genomic and epidemiological data allow early detection of health threats. This approach supports timely implementation of preventive or corrective measures, potentially reducing further transmission.

Role: Elena was involved in the dry-lab component, conducted routine surveillance and EpiPulse follow-up. She co-authored both a peer-reviewed journal article and the annual *Yersinia* spp. surveillance report, in collaboration with colleagues from the Human Bacterial Diseases Department at Sciensano.

2. Applied public health microbiology and laboratory investigations (for EUPHEM)

2.1. Association of Yersinia enterocolitica *biotype 1A and gastrointestinal symptoms: an epidemiological and genomic study from 2021 to 2023 in Belgium*

Supervisor: Wesley Mattheus (Sciensano)

Aim: To assess the association between *Yersinia enterocolitica* biotype 1A (BT1A) and gastrointestinal symptoms.

Methods: Clinical laboratories provided *Y. enterocolitica* cultures to the NRC for characterisation. Epidemiological data were collected by online questionnaires to clinicians (n=183). Questions on symptoms, aetiology of gastrointestinal symptoms, hospitalisation, and immunosuppression status were included. WGS using Illumina was performed on 57 of the strains. The dry-lab component involved genotyping which was performed by cgMLST on Galaxy, and screening for virulence genes was done using Bacterial Isolate Genome Sequence Database (BIGSdb). Association between the reported aetiology of gastrointestinal symptoms and genotype was assessed calculating the virulence genes-specific odds ratio (OR) and 95% CI.

Results: Among 183 patients, 99 (53.80%) were female, 60 (32.79%) were ≥ 65 years old and 16 (8.75%) were ≤ 4 years old. Overall, in 103 (56.28%) individuals, BT1A was reported as the aetiological agent of gastrointestinal symptoms; 24 (13.11%) required hospitalisation and 18 (9.84%) were immunosuppressed. In total, 53 strains (92.98%) contained *ystB* and *ymoA* virulence genes. However, these were not significantly associated with being the reported aetiological agent of gastrointestinal symptoms (OR = 4.81, 95% CI: 0.35–267.11, p = 0.19). No association could be determined with a specific cgMLST genotype or virulence gene.

Public health implications/Conclusions: BT1A strains have the potential to cause gastrointestinal symptoms, including in immunocompetent patients. To elucidate the burden of gastrointestinal disease, it is crucial to implement a system for routine identification and characterisation of BT1A.

Role: Elena was involved in the dry-lab component, wrote the EUPHEM study protocol, and performed the data analysis. She also submitted an abstract and presented the study at an international conference (European Scientific Conference on Applied Infectious Disease Epidemiology – ESCAIDE 2024), and wrote a manuscript to be submitted to a peer-reviewed journal.

2.2. Detection of tick-borne encephalitis virus and blood meal sources in Belgian ticks, Belgium, 2024

Supervisor: François Dufrasne (Sciensano)

Aim: To detect tick-borne encephalitis virus (TBEV) in ticks in Belgium and determine their blood meal sources.

Methods: Of the 1 000 tick samples collected in a forest between May and August 2024, a subset was screened individually. After crushing the individual ticks, both DNA and RNA were extracted using spin columns. The extracted DNA was then analysed by quantitative polymerase chain reaction (qPCR) to identify blood meal remnants. Target animals included birds, deer, shrews, squirrels, bank voles, and rabbits. RNA was used to assess the presence of TBEV by qPCR.

Results: A total of 229 individual ticks were screened for TBEV. Among the 187 nymphs tested, six were positive for TBEV. Of the 17 adult females screened, one tested positive, and among the 25 adult males, six were positive. This resulted in a TBEV prevalence in ticks ranging from 3.21% to 24%. For the blood meal analysis (n=229), 43 ticks (18.77%) had fed on birds, 37 (16.15%) on deer, 13 (5.67%) on shrews, 12 (5.24%) on rabbits, 10 (4.36%) on squirrels, and four (1.74%) on bank voles. No animal host could be identified for 132 ticks.

Public health implications/Conclusions: The observed prevalence was higher than that reported in other studies. In Belgium, there is a risk of transmission to humans through tick bites. TBEV has been circulating in the country for years, and ticks in the studied forest frequently fed on birds and deer.

Role: Elena performed the laboratory work and data analysis, submitted an abstract and presented the study at an international conference (European Society of Clinical Microbiology and Infectious Diseases – ESCMID Global 2025), alongside her colleague, Camille Philippe, from the Service of Viral Diseases at Sciensano.

2.3. Detection of azole resistance in Aspergillus fumigatus by high-resolution melting assay, Spain, 2025

Supervisor: Laura Alcázar Fuoli (Spanish National Centre for Microbiology – CNM)

Aim: To detect resistance mechanisms in *Aspergillus fumigatus* (*A. fumigatus*) by high-resolution melting (HRM) analysis.

Methods: 40 well-characterised azole-resistant *A. fumigatus* strains were tested. DNA, previously extracted using a phenol-chloroform method, was quantified with a NanoDrop 8000 spectrophotometer and subsequently adjusted. Reverse transcription polymerase chain reactions (RT-PCRs) were performed to amplify targeted point mutations (M220, G54) in *cyp51A*, as well as the insertion of tandem repeats (TRs) in its promoter region (TR34, TR46). Melting curves were generated by ramping and HRM analysis was performed using LightCycler 480 gene-scanning software in the LightCycler 480 instrument II.

Results: Among the 40 strains tested, 29 harboured TR34, eight had point mutations at M220, and three carried TR46.

Public health implications/Conclusions: The HRM assay allows the identification of single point mutations by analysing DNA sequences without the need for sequencing. This approach helps reduce costs and turnaround time. The results were consistent with previous studies, as the presence of TR34 is the most common resistance mechanism in *A. fumigatus*.

Role: Elena performed the laboratory work and data analysis. She wrote a final report, which was shared with CNM.

2.4. Detection of azole resistance in Candida albicans by Sanger sequencing, Spain, 2025

Supervisor: Laura Alcázar Fuoli (Spanish National Centre for Microbiology – CNM)

Aim: To detect the amino acid substitutions associated with azole resistance in *Candida albicans* (*C. albicans*) by Sanger sequencing.

Methods: Briefly, seven well-characterised azole-resistant strains and one susceptible strain of *C. albicans* were tested. Genomic DNA was extracted using a phenol-chloroform method, quantified with a NanoDrop spectrophotometer and subsequently adjusted. PCRs targeting *Erg11* were then performed. PCR products were analysed using agarose gel electrophoresis. Despite repeating the PCR three separate times, amplification of *Erg11* was not achieved. Different parameters were adjusted in an effort to improve the results, but without success. To confirm the quality and identity of the extracted genomic DNA, the presence of the internal transcribed spacer (ITS) region was assessed using a single-tube PCR assay. The ITS PCR products were analysed via agarose gel electrophoresis.

Results: Sanger sequencing of *Erg11* could not be performed due to unsuccessful amplification. However, the quality and identity of the extracted genomic DNA were confirmed by ITS amplification. The deoxynucleoside triphosphate (DNTPs) used may have been expired, as they were the only reagent not replaced during troubleshooting. Due to time constraints, no further trials could be conducted.

Public health implications/Conclusions: Currently, azoles are the most frequently used antifungals to treat *C. albicans* infections. The emergence of azole resistance, combined with the limited therapeutic options, is of public health concern and underscores the importance of early detection and surveillance.

Role: Elena performed the laboratory work and data analysis. She wrote a final report, which was shared with CNM.

2.5. Establishment of a procedure for the culture of stool samples to detect food-borne pathogens in the National Reference Centre, Belgium, 2025

Supervisor: Wesley Mattheus (Sciensano)

Aim: To establish coproculture at the National Reference Centre (NRC) for food-borne pathogens at Sciensano, to detect pathogens, including *Salmonella* spp., and *Yersinia* spp.

Methods: Laboratory personnel were trained in performing coproculture at a regional hospital. At the NRC, the required equipment and materials were assessed and subsequently ordered. Well-characterised stool samples from a third hospital, along with various faecal matrices, were then processed to standardise the procedure. After standardisation, a comprehensive evaluation was conducted, including assessment of transport media performance, stool conditions upon reception, bacterial recovery rates, result concordance, and feedback from laboratory technicians. Based on this evaluation, necessary improvements were made, and a standard operating procedure (SOP) was developed.

Results: An effective method for culturing stool samples to isolate *Salmonella* spp. and *Yersinia* spp. was established. This enables the NRC to directly isolate these pathogens from stool specimens and subsequently perform their characterisation. An SOP is now available to support routine activities.

Public health implications/Conclusions: The identification and characterisation of food-borne pathogens are essential for public health. As current methods rely on bacterial culture, implementing coproculture at the NRC ensures effective detection and characterisation, especially when this method is not performed in clinical laboratories.

Role: Elena performed laboratory work, reviewed guidelines, assessed required materials, contacted suppliers, and elaborated the SOP.

2.6. Next-generation sequencing for human parainfluenza viruses

Supervisors: François Dufrasne and Sarah Denayer (Sciensano)

Aim: To set up a next-generation sequencing method to detect human parainfluenza viruses (HPIVs).

Methods: A total of 46 HPIV RNA samples (i.e. 12 HPIV1, nine HPIV2, 13 HPIV3, and 12 HPIV4), extracted using the EMAG® nucleic acid extractor, were used for the validation. HPIV sequences were obtained using MinION nanopore sequencing technology. Prior to this, complementary DNA (cDNA) synthesis, PCR primer design, multiplex PCR, and library preparation were performed. After obtaining HPIV sequences, sequence analyses using the Galaxy platform, sequence alignment with BLASTN and/or Clustal Omega, and phylogenetic analyses with Clustal Omega were conducted.

Results: Among the 46 HPIVs sequenced, quality control results were within acceptable limits. Coverage depth was good for HPIV1 and HPIV2. Nonetheless, it was suboptimal for HPIV3 and HPIV4. Coverage for HPIV1 and HPIV2 was optimal, with approximately 99% genome coverage. However, HPIV3 and HPIV4 sequencing yielded suboptimal results, with genome coverage of approximately 85% and 60%, respectively. Repeated MinION sequencing runs did not improve coverage. We hypothesise that the primers used in the multiplex PCR require further optimisation. Reads were successfully mapped to reference nucleotide databases using BLASTN, supporting accurate classification at the type-level. Multiple sequence alignments of the assembled HPIV genomes, performed using Clustal Omega, revealed both conserved regions across all isolates and variable regions. Regarding phylogenetic relatedness, the analysis identified several HPIV clusters, along with additional diverged lineages.

Public health implications/Conclusions: There are significant gaps in the epidemiological and molecular understanding of HPIVs. Therefore, it is key to gather molecular data to guide the enhancement of vaccine development strategies.

Role: Elena performed the laboratory work, data analysis, and produced the final report.

3. Bio-risk management

3.1. Neisseria meningitidis *internal audit, Belgium, 2024*

Supervisor: Wesley Mattheus (Sciensano)

Aim: To evaluate the adequacy and effectiveness of the bio-risk management system, identify opportunities for improvement, and recommend corrective actions at the NRC for *Neisseria meningitidis* (*N. meningitidis*).

Methods: An internal audit was conducted in the laboratory where *N. meningitidis* is handled. Prior to that, literature on *N. meningitidis* and relevant biosecurity guidelines were reviewed, including the World Health Organization (WHO) manual on bio-risk management, reviews on safe laboratory handling of *N. meningitidis*, and materials related to biosafety level-2 (BSL-2) containment measures. Additionally, WHO resources on spill management procedures were examined. The current vaccination schedule in Belgium, particularly recommendations for laboratory personnel (e.g. Conseil Supérieur de la Santé: Vaccination des personnes à risque d'infection au méningocoque) were also reviewed. That schedule was then compared with those of other EU countries, using resources such as the ECDC vaccine scheduler. During the audit, several areas of bio-risk management were carefully assessed (e.g. personnel competency, facilities and equipment, and waste management), and a final report summarising the main findings was produced.

Results: The laboratory was found to be in compliance with bio-risk measures. The internal audit helped identify areas where additional staff training or improved documentation is needed. The presence of a bio-risk management system ensures that corrective actions are implemented promptly when necessary.

Public health implications/Conclusions: Internal audits are valuable for maintaining and improving biosafety, biosecurity, and quality management systems, ensuring the safe handling of specimens, and the quality of laboratory outputs.

Role: Elena organised the internal audit, including the literature review, prepared the necessary templates, conducted the audit, and developed the final report.

3.2. Bio-risk management internal audit, Belgium, 2024

Supervisor: Wesley Mattheus (Sciensano)

Aim: To assess the bio-risk management capacity of BSL-1 and BSL-2 facilities at the NRC for *Neisseria* spp., *Salmonella* spp., *Shigella* spp., *Yersinia* spp., and *Listeria* spp., in accordance with Sciensano guidelines.

Methods: The audit was carried out by auditors from the Department of Quality, Biosafety, and Environment at Sciensano. The first stage involved document control, followed by a tour of the premises during which all aspects of biosafety were assessed, from laboratory protocols to personal protective equipment (PPE). The final stage of the audit consisted of reporting, highlighting good practices as well as any areas requiring improvement.

Results: The existing biosafety measures were deemed optimal, including features such as locked access doors, an emergency decontamination kit, and biological waste management. Where minor adjustments were needed, feedback was provided to the NRC staff. Additionally, Elena learnt the procedures required to conduct audits.

Public Health Implications/Conclusions: Regular auditing processes are essential to maintain sustainable good laboratory practices. A collective commitment from staff at all levels is necessary to ensure efficient biosafety management and to maintain biosafety standards in the laboratory.

Role: Elena observed the internal audit and developed the final report.

3.3. Previous experience handling Mycobacterium tuberculosis *in a biosafety level-3 laboratory*

Supervisors: PhD supervisors (Hospital Clínic de Barcelona)

Aim: To get familiarised with the required biosafety measures for the safe handling of *Mycobacterium tuberculosis*.

Methods: The training focused on safe practices, procedures, and the use of specialised equipment in a BSL-3 laboratory to protect researchers, the public, and the environment.

Results: The combination of training and five years of practical experience ensured the safe handling of the pathogen, thereby minimising the risk of exposure during PhD-related research.

Public health implications/Conclusions: BSL-3 training is essential for laboratory personnel working with hazardous pathogens capable of causing serious or potentially lethal diseases. Proper training helps prevent laboratory-acquired infections and reduces the risk of potential outbreaks. BSL-3 training should be prioritised, and both personnel training and biosafety measures should be regularly reviewed and updated.

Role: Elena completed a specialised BSL-3 training, applied the corresponding biosafety measures, and conducted research under BSL-3 conditions.

4. Quality management

4.1. 2025 AURORAE SARS-CoV-2 and RSV EQA, Belgium, 2025

Supervisor: Sarah Denayer (Sciensano)

Aim: To participate in an external quality assessment (EQA) for SARS-CoV-2 and respiratory syncytial virus (RSV).

Methods: An EQA panel consisting of 10 vials with inactivated virus was received. The samples were reconstituted as indicated by the EQA organisers at Charité – Universitätsmedizin Berlin, Institut Pasteur, and Rijksinstituut voor Volksgezondheid en Milieu (RIVM). RNA extraction was performed using the EMAG® platform. For molecular identification, SARS-CoV-2 quantitative reverse transcription PCR (RT-qPCR), RSV-A/RSV-B quadruplex RT-qPCR and seasonal coronaviruses quadruplex RT-qPCR, and SARS-CoV-2 (E Gene) RT-qPCR were performed. Additionally, NGS of SARS-CoV-2 was performed using MinION sequencing.

Results: Of the 10 EQA samples, four were positive for SARS-CoV-2, two for RSV-A, two for RSV-B, and two were negative for all target viruses. Nonetheless, one of the negative samples was positive for CoV-229E. All SARS-CoV-2 results were consistent and reproducible across experiments. The assays successfully detected varying viral loads for SARS-CoV-2 and RSV-A/RSV-B. No cross-contamination was detected during either extraction or amplification. However, there were some issues with the positive template control ribonuclease P (RNase P), which were expected as EQA samples are often not human specimens. The four SARS-CoV-2-positive samples were planned to be sequenced by NGS. Nevertheless, sequencing data could not be obtained for three of them, as they did not meet the viral load criteria for sequencing (i.e. cycle threshold (Ct) > 25). The remaining sample generated limited sequencing data but was classified as 24A (JN.1) clade of the Omicron variant.

Public health implications/Conclusions: Participation in EQA programmes by all clinical laboratories is essential. By doing so, laboratories validate and verify the reliability of their analyses and analytical methods, and are informed about the weaknesses of their processes.

Role: Elena observed the EQA process, performed laboratory work, and prepared a final report.

4.2. External quality control audit, Belgium, 2024

Supervisor: Wesley Mattheus (Sciensano)

Aim: To participate in an external quality control audit conducted under International Organization for Standardization (ISO 15189) at the NRCs for *Neisseria* spp., *Salmonella* spp., *Shigella* spp., *Yersinia* spp. and *Listeria* spp.

Methods: During the first part of the audit conducted by the Belgian National Accreditation Body (BELAC), a document control was performed. The auditor requested several documents, including those related to sample shipping, annual reports, the risk matrix, and the quality and environmental manual. The second part of the audit involved a tour of the laboratory premises. Several aspects were assessed, such as the laboratory technicians' work plans, and business continuity plan. The audit concluded with a final meeting during which the auditor provided useful feedback. Following the audit, the auditor prepared a final report, which was shared with the head of the NRC.

Results: During the audit, suggestions concerning the clarity of the procedure for the antibiotic sensitivity test, along with a general remark about the information on turnaround time displayed on the website, contributed to improvements in the existing quality management system.

Public health implications/Conclusions: External quality audits are essential for ensuring compliance with specific ISO standards, which help maintain and improve the quality of services as well as the reliability and comparability of laboratory data.

Role: Elena observed the external audit and prepared a final report.

5. Public health microbiology management

5.1. Management of public health microbiology projects

During her fellowship, Elena developed public health management skills by leading and contributing to various projects, organising activities, and collaborating on joint projects. She submitted a funding application for the ESCMID Observership, followed ethical guidelines outlined by ECDC and Sciensano, and in May 2025, conducted a research project with the Spanish Mycology Reference Centre at the Spanish National Centre for Microbiology – CNM.

Elena also strengthened her management skills through outbreak investigations. She collaborated with Sciensano colleagues and public health authorities on the *S.* Enteritidis outbreak investigation, contributing to the final report. Additionally, she communicated with international teams during the *Y. enterocolitica* cross-border outbreak.

In teaching and training activities, Elena enhanced her management and communication skills by coordinating activities, developing materials, and adapting delivery to different audiences. These activities included training on molecular epidemiology at the Institute of Tropical Medicine in Antwerp and *Neisseria meningitidis* bio-risk management at Sciensano in April 2024.

6. Teaching and pedagogy

6.1. Training on molecular epidemiology to master's degree students from the Institute of Tropical Medicine, Belgium, 2024

Supervisor: Wesley Mattheus (Sciensano)

Elena delivered a one-day training on molecular epidemiology to master's degree students at the Institute of Tropical Medicine in Antwerp, Belgium, alongside her supervisor, Wesley Mattheus. The session covered several molecular tools used in outbreak investigations. It also included an overview of Sciensano, the Human and Environmental Risk Assessment (HERA) project, and key activities of the NRCs for tuberculosis, food-borne bacteria, and bacterial meningitis. Elena developed new learning materials for the training, including a PowerPoint presentation, a case study, and interactive quizzes. She also prepared a reflective note to evaluate and improve future sessions.

6.2. Training and assessment of laboratory bio-risk management of Neisseria meningitidis*, Belgium, 2024*

Supervisor: Wesley Mattheus (Sciensano)

Elena delivered a two-hour training on the bio-risk management of *N. meningitidis* to laboratory personnel from Sciensano. The training addressed the hazards related to *N. meningitidis* handling, as well as the measures required to ensure biosecurity and biosafety, including PPE, biosafety equipment and biocontainment. The activity consisted of a presentation, followed by an interactive quiz. Afterwards, there was an opportunity for questions and discussion. Elena developed new learning materials for the training, including a PowerPoint presentation, and an interactive quiz for evaluation. She also prepared a reflective note to evaluate and improve future sessions.

7. Other

7.1. ECDC crowd: The cascade of care for tuberculosis infection, Belgium, 2024

Supervisor: Vanessa Mathys (Sciensano)

Aim: To screen titles and abstracts for a scoping review on the tuberculosis cascade of care.

Methods: The ECDC Crowd is a platform that facilitates evidence review activities by crowdsourcing insights on study design, analysis, and interpretation. The platform provides the training required to participate in a task. After that, participation in the ECDC Crowd was flexible, as the platform allowed participants to engage whenever it suited their schedules. The tasks involved sorting data and reviewing studies to enhance scientific research and collaboration in the tuberculosis cascade of care. The objective was to select studies that followed patients who had been exposed to or tested for tuberculosis through at least two steps of the cascade of care (i.e. identified, evaluated, offered preventive treatment, initiated treatment, or completed treatment). Participants could also add comments explaining their decision to include or exclude an abstract. A session presenting the main results and relevant studies was delivered to the NRC for tuberculosis.

Results: A total of 101 abstracts on tuberculosis were screened. Elena developed a better understanding of crowdsourcing and the literature/scoping and systematic review process. It also enhanced her knowledge and experience in tuberculosis, and improved her communication skills through delivering a session.

Public health implications/Conclusions: It is essential to improve scientific studies and collaboration in the area of public health and infectious diseases. These contributions to public health research can be done through crowdsourcing. Additionally, making research in tuberculosis easier is of public health importance, as it remains the leading cause of death from a single infectious agent.

Role: Elena completed the ECDC Crowd initial training, screened abstracts, and presented the results to the NRC for tuberculosis.

7.2. Rapid literature review: Impact of systematic sexually transmitted infections (STIs) testing among pre-exposure prophylaxis (PrEP) users and/or men who have sex with men (MSM)

Supervisors: Jessika Deblonde and Dominique Van Beckhoven (Sciensano)

Aim: To conduct a rapid literature review on the impact of systematic STI testing among PrEP users and/or MSM.

Methods: A literature search was conducted in PubMed following a defined search strategy, yielding 124 results. Titles and abstracts were screened based on established inclusion criteria, resulting in 82 publications. Of these, 57 were excluded due to poor quality, unsuitable study designs, irrelevant populations, or outdated data. Data from the remaining 25 studies were extracted and summarised in a table. A final discussion with scientists from the Department of Epidemiology of Infectious Diseases (Sciensano) was held to interpret the findings.

Results: There is scarce literature on the topic. Most studies assessed testing frequency or STI incidence but not the impact of systematic testing frequency on STI incidence. In addition, the majority of the literature available showed inconclusive evidence, with some studies presenting contradictory results. Nonetheless, 25 publications were included in the literature review. After a discussion with scientists, five publications were identified as relevant. Authors of these studies will be contacted to explore further insights and possible collaboration.

Public health implications/Conclusions: Further research is needed in order to understand the role of systematic testing on the incidence of STIs among PrEP users and MSM populations. This would allow making more informed decisions regarding PrEP guidelines. This is essential as some decisions result in reducing testing frequency or even eliminating systematic testing. Consequently, this could have an impact on the transmission of STIs not only in these populations but also among the general population.

Role: Elena conducted the literature review, presented the findings to the Department of Epidemiology of Infectious Diseases, and prepared the final report.

7.3. Multidisciplinary training workshop of the Food- and Waterborne Diseases Antimicrobial Resistance - Reference Laboratory Capacity (FWD AMR-RefLabCap) project: an outbreak of Salmonella *spp. in Denmark, Belgium, 2024*

Supervisor: Wesley Mattheus (Sciensano)

Aim: a) To improve the capacity to interpret WGS-data for antimicrobial resistance (AMR) surveillance, outbreak detection and investigation. b) To expand the collaboration between public health epidemiologists and microbiologists in national surveillance and outbreak investigations, and improve the collaboration with the food safety authorities.

Methods: An online meeting was held to introduce the table-top exercise. Following this, two representatives from each country (i.e. a microbiologist and an epidemiologist) collaborated on the exercise. This was followed by analytical support and group discussions involving several countries and a facilitator, allowing for questions and discussion. The process concluded with a final meeting that included relevant oral presentations and a summary of the exercise outcomes.

Results: Within nine days in May 2024, 13 cases of *Salmonella* were detected in four different regions in Denmark. It mainly affected young males and four females (older in age). One case was hospitalised and three cases had a history of travel to the United Kingdom (UK). The outbreak investigation revealed that the source of infection was located in the UK and was introduced in Denmark via travelling. Moreover, the epidemiological link between the cases was a stadium in Liverpool and the consumption of free pork scratchings. This was later confirmed by microbiology results (i.e. AMR profile, WGS and cgMLST).

Public health implications/Conclusions: The importance of cross-country collaboration, timely data, complete questionnaires, and the value of WGS in outbreak detection were highlighted. Notable differences in WGS use across EU NRCs underscored the need for more harmonised surveillance practices.

Role: As a member of the Sciensano participating team, Elena was involved in all training activities and discussions during the exercise. She conducted data analysis and prepared the final EUPHEM report.

7.4. Manuscript reviewing for a scientific journal

Aim: To conduct a peer review of a manuscript submitted to a scientific journal.

Methods: During the review process, several aspects of the manuscript were assessed, including novelty, language and grammar, title, abstract, keywords, introduction, methods, study design, conclusions, statistics, tables and figures, and references. The evaluation followed both the journal's guidelines and the reviewer's own judgement. A final section was provided for the reviewer to include comments to the authors.

Results: After careful assessment, the manuscript was rejected as it did not meet the journal's criteria for publication. However, the review process enhanced Elena's skills in providing assertive and constructive feedback, thereby improving her communication abilities. Additionally, valuable experience was gained in critically evaluating studies conducted by others.

Public health implications/Conclusions: The peer-review process requires voluntary reviewers who are willing to review manuscripts. This process ensures the quality of the studies as they go through the assessment of several experts in the field.

Role: Elena reviewed the manuscript and submitted her review to the journal.

8. Communications related to the EPIET/EUPHEM fellowship

8.1. Manuscripts published in peer-reviewed journals

1. Savin C, Fredriksen N, Calba C, Puzin L, Felten A, Larréché S, et al. Cross-border outbreak of Yersinia enterocolitica bioserotype 2/O:9 infections associated with consumption of French unpasteurised soft goat's milk cheese, 2024. Euro Surveill. 2025;30(26):2500002. Available at: https://doi.org/10.2807/1560-7917.ES.2025.30.26.2500002 [**Elena Portell Buj** has been listed as one of the collaborators].
2. **Portell-Buj E,** Ceyssens P, Van den Bossche A, Vodolazkaia A, Mukovnikova M, Robben L, et al. Association of *Yersinia enterocolitica* biotype 1A and gastrointestinal symptoms [In preparation].

8.2. Other reports

1. Mattheus W, Ceyssens P, **Portell-Buj E**, Van den Bossche A. Nationaal referentie centrum Yersinia: Jaarverslag 2024. Sciensano; 2025. Available at: https://www.sciensano.be/en/nrc-nrl/national-reference-center-nrc-yersinia-enterocolitica-and-yersinia-pseudotuberculosis
2. **Portell-Buj E**, Van Dyck W, Stefani G, Van Cauteren D, Laisnez V, Bynens T, et al. Uitbraak van salmonellose na het spaghetti-diner van een jeugdbeweging. Vlaams Infectieziektebulletin [Submitted].

8.3. Conference presentations

1. **Portell-Buj E**, Ceyssens P, Van den Bossche A, Vodolazkaia A, Mukovnikova M, Robben L, et al. Association of *Yersinia enterocolitica* biotype 1A and gastrointestinal symptoms: an epidemiological and genomic study from 2021 to 2023 in Belgium. Poster presented at: ESCAIDE 2024; Stockholm, Sweden [Presenter: **E. Portell-Buj**]
2. Philippe C, **Portell-Buj E**, Van Gucht S, Dufrasne FE. First detection of tick-borne encephalitis virus in Belgian ticks, May 2024. Oral presentation at: ESCMID Global 2025; Vienna, Austria [Presenters: **E. Portell-Buj** and C. Philippe]

8.4. Other presentations

1. **Portell-Buj E**. Molecular epidemiology. Presented at: Institute of Tropical Medicine, 12 April 2024, Antwerp, Belgium
2. **Portell-Buj E.** Training on laboratory bio-risk management of *Neisseria meningitidis*. Presented at: Sciensano, 22 April 2024, Brussels, Belgium
3. **Portell-Buj E.** ECDC CROWD: The cascade of care for tuberculosis infection. Presented at: Sciensano, 9 July 2024. Brussels, Belgium

9. EPIET/EUPHEM modules attended

- Course in R for Applied Epidemiology, Applied Epi, 19 September–22 September 2023, virtual
- Introductory Course, 25 September–13 October 2023, Spetses, Greece
- Study Protocol and Scientific Writing, 26–27 October and 7–8 November 2023, virtual
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2023, 22–24 November
- 2023, Barcelona, Spain
- Multivariable Analysis, 19–23 February 2024, Berlin, Germany
- Vaccinology, 13–17 March 2024, virtual
- Writing Abstracts for Scientific Conferences, 14 March 2024, virtual
- Rapid Assessment and Survey Methods, 15–19 April 2024, Dublin, Ireland
- Public Health Microbiology II – Biorisk and Quality Management, 21–23 May 2024, virtual
- Public Health Microbiology III – Whole Genome Sequencing, 3–7 June 2024, Vienna, Austria
- Project Review Module 2024, 26–30 August 2024, Lisbon, Portugal
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2024, 20–22 November 2024, Stockholm, Sweden
- Time Series Analysis, 9–13 December 2024, Utrecht, the Netherlands
- Social and Behavioural Sciences, 24–28 March 2025, virtual
- European Society of Clinical Microbiology and Infectious Diseases (ESCMID) 2025, 11–15 April 2025, Vienna, Austria
- Management, Leadership and Communication in Public Health, 1–3 September 2025, Lisbon, Portugal.

10. Other training

- R Online Tutorials, Applied Epi, 17 September 2023, virtual
- 12th European Food- and Waterborne Diseases and Zoonoses Network Meeting, ECDC-EFSA, 2–4 February 2024, virtual
- BioNumerics replacement within ECDC ecosystem, ECDC, 6 November 2023, virtual
- Ninth MD.be Scientific Meeting, MolecularDiagnostics.be, 14 November 2023, Antwerp, Belgium
- Mini-Module on Molecular Epidemiology, EAN, 20–21 November 2023, Barcelona, Spain
- Bacterial Genome Assembly and Quality Control, ECDC, 4–7 December 2023, virtual
- Antimicrobial Resistance Conference, ECDC-Sciensano, 6–8 May 2024, virtual
- Symposium Infectious Diseases 2024, Sciensano, 16 May 2024, virtual
- Outreach for Inclusion Health Settings: Infection Diagnostics for Hard-to-reach Populations, ESCMID, 12 June 2024
- EU Health Task Force, ECDC, 18 June 2024, virtual
- Social and Behavioural Science in Action: Shaping Public Health Strategies for Infectious Disease Prevention, ECDC, 25 September 2024, virtual
- Workshop on *Salmonella* surveillance, United4Surveillance project, 26 September 2024, Brussels, Belgium
- Health Economics, ECDC, 16 October 2024, virtual
- EU Health Task Force, ECDC, 17 October 2024, virtual
- Ethics, ECDC, 6 November 2024, virtual
- Biosafety and Quality Assurance in a Public Health Laboratory, ECDC, 16 December 2024, virtual
- Using Behavioural Science to Counteract Antibiotic Resistance, ECDC, 27 January 2025, virtual
- R data Analysis and Visualisation for Beginners, ECDC, 17–21 February 2025, virtual
- Breaking Barriers for Women: the Role of Behavioural and Structural Factors in HIV Prevention, ECDC, 5 March 2025, virtual
- R4Data Science, Sciensano, 13, 19 and 20 March 2025, Brussels, Belgium
- EpiPulse Cases Training for FWD, ECDC, 7 April 2025, virtual.

11. International assignments

Observership at the Mycology Reference Laboratory, National Centre for Microbiology, Madrid, Spain, 1–31 May 2025

Throughout the entire month of May 2025, Elena completed an observership at the Spanish Mycology Reference Laboratory – National Centre for Microbiology, part of the Instituto de Salud Carlos III (Madrid, Spain). During the first week, she was introduced to the techniques used for the detection and characterisation of fungi, including fungal culture, macroscopic and microscopic identification, minimum inhibitory concentrations (MICs), serology, PCRs, sequencing, and high-performance liquid chromatography (HPLC). Simultaneously, Elena passed the required biosecurity test and reviewed the corresponding SOPs, scientific publications and textbooks. For the remaining three weeks, she conducted her own projects: the detection of azole resistance in *Aspergillus fumigatus* and *Candida albicans*. During the observership, Elena also attended weekly meetings and seminars. She summarised the results obtained and her personal experience in a final report.

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