Carmen Espinosa Gongora

The European Public Health Microbiology Training Programme (EUPHEM), Cohort 2022
Public Health Agency of Sweden, Sweden

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/2023/23 and ECDC/AD/2023/06 govern the EU-track and MS-track, respectively, of the ECDC Fellowship Programme, field epidemiology path (EPIET) and public health microbiology path (EUPHEM).

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

Carmen Espinosa Gongora is a veterinarian with a PhD in One Health Antimicrobial Resistance and 15 years of experience. Prior to joining the EUPHEM fellowship, her research focused on combating antimicrobial-resistant pathogens in livestock, domestic animals, and wildlife, and preventing zoonotic transmission. In her previous academic roles at the University of Copenhagen and the Technical University of Denmark, she addressed veterinary and public health threats and generated evidence to support decision-making by national authorities. Her research also investigated how improving farming practices translates into disease prevention and sustainable management to combat antimicrobial resistance. Additionally, she explored the digestive and respiratory microbiomes in health and disease, as well as the mechanisms of microbe-host co-evolution. She has been a project manager for large multi-partner consortia of EU Horizon 2020 projects and coordinator of the pan-European ESCMID (European Society of Clinical Microbiology and Infectious Diseases) study PREPARE-VET aimed at raising awareness of veterinary antimicrobial stewardship and enhancing veterinary curricula across Europe.

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). European public health training programme. Stockholm: ECDC; 2020. Available from: https://www.ecdc.europa.eu/en/publications-data/ecdc-fellowship-programme-manual-cohort-2021

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1. Epidemiological investigations

1.1. Outbreak investigations

National outbreak of cryptosporidiosis in Sweden, 2022

Supervisors: Moa Rehn and Emma Löf

Category: Food- and waterborne diseases

Aim: To determine the extent of an outbreak of cryptosporidiosis affecting multiple regions in Sweden, for the identification of the source of infection, and application of mitigation measures.

Methods: A case-control study was performed by distributing a trawling questionnaire to all confirmed and suspect cases and unmatched controls invited through the Swedish panel of controls. Possible sources of infection were investigated by univariate and multivariable analysis using logistic regression in R. Microbiological investigations of stool samples included *gp60* subtyping and multilocus variable-number tandem repeat analysis (MLVA).

Results: A total of 107 cases of cryptosporidiosis were identified in weeks 38 to 42, 2022 in 15/21 regions in Sweden. Response to the questionnaire were collected from 64 confirmed cases and 449 controls. Cases had a median age of 40 years and 63% were women. The most frequently reported symptoms were diarrhoea (n=107), stomach pain (n=97), and nausea (n=90). Multivariable analysis identified mixed salad as the most probable source of infection (OR=15, 95% CI= 6.4–35.1). Microbiological analysis of 15 stool samples identified a common *C. parvum* subtype (IIaA15G2R1) with a single MLVA profile in all the outbreak isolates analysed. Due to the short shelf life of mixed salad, mitigation measures were not applicable. Intermediate steps in the food chain, such as packaging, could not be ruled out as possible sources of contamination.

Public health implications: The investigation raised awareness of potential intermediate sources of contamination, such as packaging plants, and of the importance of timely typing methods with appropriate discriminatory power to characterise *C. parvum* subtypes.

Role: The investigation was led by the project supervisors. Carmen contributed with an R Markdown script which was able to generate outbreak reports in several formats (word, pdf, html) from the collected raw data. She also participated in meetings within and outside the Public Health Agency of Sweden (PHAS), including the Swedish National Food Agency and representatives of the regional health institutes, where she took part in discussions about hypothesis generation and approaches for epidemiological analysis. She was actively involved in the selection of samples for typing, and participated in the analysis of the MLVA results.

Severe influenza B cases among adolescents in Sweden, March 2023

Supervisors: Emmi Andersson, Tove Samuelsson, Oskar Karlsson Lindsjö, Moa Rehn, AnnaSara Carnahan

Category: Respiratory diseases

Aim: To investigate a potential outbreak of influenza B with severe complications (myocarditis, encephalitis/meningitis/meningoencephalitis) following the identification of three epidemiologically linked cases in adolescents attending a school in Örebro county, and assess the presence of any associated viral factors.

Methods: An investigation was prompted aiming to determine the extent of the outbreak, review the literature to catalogue previously described genetic markers of severe influenza symptoms, and perform whole genome sequencing (WGS) of influenza B isolates from season 2022–2023, including severe and non-severe cases, to explore genetic clustering and markers linked with severe outcomes. The review covered articles indexed in PubMed from 2013–2023, using keywords and medical subject headings (MeSH) terms related to virulence and target symptoms.

Results: 20 severe cases of influenza B, all B/Victoria (V1A.3a.2), were identified. The literature review led to screening of 140 abstracts and 50 full-text reviews. Severe outcomes were mainly associated with genetic markers in the haemagglutinin segment, followed by RNA polymerase, and neuraminidase genes. Most severity markers were reported in avian influenza A and H1 subtypes, with only 10/50 studies addressing influenza B specifically. Neurological symptoms and unspecific pathogenicity markers were common in influenza A reports, while myocarditis was rare. Influenza B studies either lacked follow-up viral genome analysis for the target complications or reported unspecific pathogenicity. The investigation by WGS analysis did not support clustering of the severe cases, which distributed across tree branches along with the uncomplicated cases. None of the reviewed markers were found in the outbreak strains.

Public health implications: This investigation highlighted the limited knowledge on influenza B virulence and the need for influenza preparedness, and supported a WGS approach in public health.

Role: Carmen attended internal meetings, followed up on the reported cases, and participated in the discussions of the investigations which were undertaken. She performed the literature review of the virulence factors previously associated with severity in influenza infections, with the aim to survey the associated influenza B strains sequenced from the severe cases. She contributed to PHAS' yearly national influenza report, including the results of the literature review. The fellow also prepared a proposal to perform a research study on the virulence of influenza B using WGS,

which could be used to initiate follow-up studies. This project was also presented at the 2024 Nordic mini-module in Helsinki.

Outbreak of norovirus in Örebro, Sweden, 2024

Supervisor: Moa Rehn

Category: Food- and waterborne diseases

Aim: To identify the source of infection and apply mitigation measures to a norovirus outbreak at a combined kindergarten and school in Örebro, Sweden.

Methods: A retrospective cohort study in the affected kindergarten/school was performed by distributing a web-based questionnaire through the school management. Potential sources of exposure were investigated by univariate, stratified, and multivariate analysis, using logistic regression. Samples were collected from affected individuals, available food, and surfaces at the school canteen.

Results: 106 questionnaire responseswere collected, revealing 44 cases. Risk of illness was higher in children in school year 3 or younger (RR 2.3, 95% CI 1.16–4.57, p= 0.008), and individuals having lunch at the school canteen on Monday 6 November (RR 3.3, 95% CI 0.90–12, p= 0.032). The epidemiological study could not identify a single food item as the source of infection. Milk was consumed on Monday by more than half of the cases, but was only significantly associated with being a case within the older age groups (classes 4–6) (RR 8.5, 95% CI 2–13.6, p= 0.0185), where it explained 5/7 cases. Norovirus was confirmed in 3/4 stool samples, but not in vomit, food, or environmental samples (n=13). As norovirus can easily spread through direct or indirect contact and survive on contaminated surfaces, the presence of one or more unidentified sources was likely.

Public health implications: Future investigations should consider the multiple transmission pathways of norovirus, including contaminated food, infected individuals, and contaminated surfaces, as these factors often contribute to outbreaks involving various sources.

Role: Carmen participated in all discussions during the investigation, attended meetings with representatives of the school and the regional infectious disease department, performed the analytical epidemiology, contributed to the formulation of the conclusions of the investigation, and wrote the report in collaboration with current fellows, Anna Ohlson and Hilde De Clerck at PHAS.

1.2. Surveillance

Evaluation of the national surveillance system for rotavirus in Sweden, 2022

Supervisors: Helene Englund, Klas Straat, Lena Dillner, and Emmi Andersson

Aim: To evaluate whether the current goals defined in the rotavirus surveillance system are met, and extend the system's outputs in support of a potential need of action or decision making at PHAS.

Methods: The achievement of goals in the current rotavirus surveillance system were examined based on: i) five interviews with those responsible for national rotavirus surveillance, ii) European rotavirus surveillance network (EuroRotaNet) guidelines, iii) rotavirus surveillance system in Finland, and iv) ECDC documentation.

Results: The key finding was the deficient submission of samples to PHAS, which failed to represent the yearly cases, geographical regions, or age groups, and might be biased by infection severity. Additionally, the following were recommended:

a. Specify which rotavirus knowledge is needed to simplify data collection (e.g. monitoring pathogenicity associated with reassortment events).

b. Adjust the sequencing sample size based on objectives (e.g. mapping, detecting rare/new genotypes), and set criteria for sample selection when submissions exceed this size.

c. Encourage sample submission through active communication and feedback to laboratories.

d. Use a genotyping method that accurately identifies vaccine strains.

e. Rephrase the objective, 'To avoid reporting of recently vaccinated individuals shedding weakened virus' to 'To include genotyping information in the reporting system that would allow discerning cases caused by vaccine strains'.

Public health implications: Achievement of the goals described in the Swedish rotavirus surveillance protocol is essential to generate the knowledge that enables informed decisions to be made on disease prevention and management, and will allow evaluating the health and economic impact of the vaccination programme.

Role: Carmen revised the current national rotavirus surveillance system documentation, interviewed those responsible for it, shared experiences with those responsible for the national surveillance system in Finland, and mapped the guidelines, objectives and recommendations of ECDC and EuroRotaNet. She wrote an internal report with a detailed assessment and recommendations for improvement of the system.

Introduction and assessment of early warning systems for detection of emergent SARS-CoV-2 variants using wastewater genomic surveillance in Sweden, 2023

Supervisors: Elin Movert, Emmi Andersson, Moa Rehn

Aim: To assess the specificity of mutations of SARS-CoV-2 lineage BA.2.86 compared to other lineages in order to increase the sensitivity of its detection in wastewater samples compared to the current algorithm (the Freyja tool), and to describe the introduction and spread of BA.2.86 in Sweden using the data generated alongside patient surveillance data.

Methods: Sensitivity and specificity of BA.2.86-defining mutations (from GitHub cov-lineages/pango-designation issue #2183 and UShER/Cov2Tree) was explored in the GISAID (the Global Initiative on Sharing All Influenza Data) database. 144 wastewater samples from 18 Swedish water facilities were collected from weeks 31–38, 2023, and sequenced using Illumina MiSeq and Ion Torrent platforms. Mutations were identified with iVar, and lineages were assigned using Freyja and our specific markers. Patient data were collected from the Swedish COVID-19 surveillance system.

Results: The Freyja tool is ideal for reporting SARS-CoV-2 variants in wastewater, although its algorithm requires specific criteria for lineage designation. Sensitivity for detecting BA.2.86 increased with our marker specificity assessment. Sequencing wastewater and assessing marker specificity can serve as a surveillance tool for early detection and monitoring of emerging SARS-CoV-2 variants. Integrating surveillance and research data on the virulence and immune evasion characteristics of new variants can provide robust evidence to guide public health actions. However, effective genomic wastewater surveillance depends on the global sharing of high-quality genomes of emerging SARS-CoV-2 variants.

Public health implications: Our study advanced wastewater-based SARS-CoV-2 early warning systems at PHAS and improved our understanding of wastewater's potential for monitoring SARS-CoV-2 and potentially other pathogens. This sustainable approach offers long-term benefits for surveillance.

Role: Carmen was actively involved in study design, choice of methodology, and data analysis, and wrote the original manuscript published in the scientific journal, *Eurosurveillance* (see section 7.1).

Integration of respiratory syncytial virus and other respiratory pathogens in the Swedish primary care sentinel surveillance system, season 2022–2023.

Supervisors: Emmi Andersson, Neus Latorre Margalef, AnnaSara Carnahan.

Aim: To explore the potential of a broad PCR respiratory panel for integrated surveillance, and assess the contribution of the sentinel system to respiratory syncytial virus (RSV) surveillance and early warning.

Methods: All sentinel samples from season 2022–2023 were retrospectively tested for 20 respiratory pathogens using the multiplex QiaStat Respiratory Panel (Qiagen). We analysed panel results, demographics, influenza-like illness (ILI)/acute respiratory infection (ARI) symptoms, and the effect of time from symptom onset to sample collection on negative results using logistic regression, and compared the earliest RSV detection with voluntary laboratory-based surveillance estimates.

Results: Sentinel samples (n=314) predominantly represented individuals aged 30–60 (n=147) and over 60 (n=100), with minimal representation of 0–4-year-olds (n=8). Most individuals (255/314) presented with ILI, and the majority (170/314) tested negative for all pathogens. The panel provided 57 additional diagnoses. RSV positives (22/314) were mostly over 60 years old (15/22), with high positivity in 0–4-year-olds (3/8). The voluntary lab surveillance was able to detect RSV earlier. Negative samples were collected 3.7 days later than positive samples on average. Integrated surveillance requires revising syndromic algorithms, potentially increasing the ARI ratio, and improving representability. Our findings support setting a maximum time since onset for patient inclusion to improve monitoring efficacy.

Public health implications: An integrated respiratory surveillance system may be critical to identify at-risk populations and develop strategies to prevent future outbreaks. Integrating RSV responds to ECDC's call to report RSV detection via TESSy starting from 2022, and opens the discussion on the need for RSV genomic surveillance to monitor population immunity and identify emerging strains.

Role: Carmen performed the data analysis, interpreted results, and formulated conclusions. She wrote an internal report for PHAS, and presented the results at ESCMID Global 2024.

Managing zoonotic antimicrobial resistance surveillance data for enhancement of the national reporting strategy: project proposal

Supervisors: Maria Egervärn, Nadja Karamehmedovic, Hanna Billström, Tomas Söderblom, and Lina Thebo

Aim: To facilitate the management of non-typhoidal *Salmonella* spp. and *Campylobacter jejuni* phenotypic and genotypic antimicrobial resistance (AMR) data by: i) reviewing the current guidelines for reporting to the FWD-Net database and GLASS, ii) comparing the information contained in the two datasets (phenotypic and genotypic) and their AMR estimates, and iii) generating recommendations for PHAS' contribution to international AMR reporting.

Methods: Protocols and available documentation for collecting and reporting AMR data for *Salmonella* and *Campylobacter* from the FWD-Net and GLASS were reviewed. This included mapping of the database's preferences with regards to phenotypic or genotypic AMR data. Reporting strategies from other countries will be retrieved from past reports. Using this protocol, resistance estimates from PHAS' phenotypic and genotypic data will be calculated and compared, highlighting the factors influencing differences between estimates.

Results: During the preparation of the PPF, the available documentation for AMR reporting to FWD-Net and GLASS was reviewed.

Public health implications: This study will improve the data management of non-typhoidal *Salmonella* and *Campylobacter jejuni* AMR results generated from the phenotypic and genotypic methods available at PHAS. Importantly, the study will generate information to support PHAS in making an informed decision on their AMR reporting strategies at national and international levels, leading to a more effective approach to controlling AMR in zoonotic pathogens.

Role: Carmen held several meetings with the two units at PHAS responsible for generating or compiling the phenotypic and genotypic AMR data, investigated the available documentation for reporting to ECDC and WHO, and prepared a PPF. Depending on data availability, this PPF will be passed on to future fellows to carry on these tasks.

2. Applied public health microbiology research and laboratory investigations

Assessment of mpox-specific neutralising antibodies following the Modified Vaccinia Ankara-Bavarian Nordic intradermal vaccination scheme, Sweden, 2022–2024

Supervisor: Klara Sondén

Aim: To assess the dynamics of mpox virus-specific neutralising antibodies following the Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN) intradermal vaccination scheme.

Methods: Individuals without previous mpox infection seeking vaccination were invited to enrol. Serum samples were collected before dose 1 and before dose 2, and 28 days and three months after dose 2. Controls included samples from 20 anonymised donors and 19 previously mpox-infected individuals. Neutralising antibody titres were obtained using the cytopathic effect test with VERO cells and live virus. Titres were compared over time using paired Wilcoxon tests and linear regression, exploring the role of smallpox vaccination and HIV status.

Results: A cohort of 97 individuals were enrolled, resulting in 321 samples. All identified as men who have sex with men (MSM). 23 individuals were smallpox-vaccinated, 11 of whom received a single vaccine dose. 17 were HIV-positive with CD4 counts >500 cells/mm3 and receiving antiretroviral therapy. One month after full vaccination, 50% of the smallpox-unvaccinated group had detectable neutralising antibodies (median titre 10) whereas 94% of smallpox-vaccinated individuals had immunity (median titre 30). Immunity waned significantly after three months, raising questions about the vaccine's long-term protection, and prompting interest in booster dose studies.

Public health implications: The results of this study are highly relevant to formulate public health actions with regards to the need and timing of mpox booster vaccinations. This benefits the MSM population in particular, due the increased risk of infection, but is also translatable to the general population.

Role: Carmen performed neutralisation assays at the high security laboratory, performed the data analysis, contributed to the information presented as press releases and at ESCMID Global 2024, and wrote the manuscript.

Retrospective investigation of historic Cryptosporidium parvum *outbreaks in Sweden using Multilocus Variable Number Tandem Repeats analysis compared to* gp60 *subtyping*

Supervisors: Ioana Bujila and Caroline Rönnberg

Aim: To import a new multilocus variable number tandem repeats analysis (MLVA) method for typing of *Cryptosporidium parvum* (*C. parvum*) during epidemiological investigations in Sweden. Additionally, to compare the result of historic *C. parvum* outbreak investigations in Sweden based on *gp60* subtyping with MLVA.

Methods: The *C. parvum* MLVA scheme by Robinson et al. (2022) was used to type 114 samples from 11 historic outbreaks in Sweden between 2016 and 2022. Samples included outbreak cases, associated sporadic cases, and suspected sources such as samples from calves and foods. The Applied Biosystems Genetic Analyzer 3500 and Bionumerics were used for fragment and data analysis. MLVA results were compared to *gp60* subtyping in terms of discriminatory power and effectiveness in outbreak investigations.

Results: MLVA was superior in detecting genetic diversity, as seen in six outbreaks displaying multiple MLVA profiles among samples with the same *gp60* subtype. Additionally, in five outbreaks, MLVA suggested the presence of additional outbreak cases, previously considered sporadic. Notably, MLVA discerned all calf samples from outbreak cases, ruling out calves as the source of the outbreak where they were initially suspected. MLVA also improved cost-effectiveness and turnaround time compared to *gp60* subtyping (results available within a working day instead of two). However, MLVA limitations included reduced typeability (81%), specific equipment requirements, and lack of a suitable open-source software for data analysis.

Public health implications: The findings suggest that MLVA is a useful, cost-effective, and rapid tool for *C. parvum* outbreak investigations. The higher discriminatory power facilitated our understanding of outbreaks and improved source attribution.

Role: Carmen helped gather all necessary materials and performed the laboratory analysis. She set up the Bionumerics MLVA database and analysed fragment analysis data to generate MLVA profiles. She generated a detailed protocol for the implementation of the method at PHAS. She performed MLVA data analysis and wrote two abstracts for international conferences (ESCAIDE and ESCMID Global), and presented this work at ESCMID Global 2024 as a flash oral presentation. This work was also presented at the 2023 Project Review Module in Lisbon.

Performance of an in-house broth microdilution assay for routine minimum inhibitory concentration determination of antimicrobials against the Mycobacterium tuberculosis *complex*

Supervisors: Mikael Mansjö, Jim Werngren and Ramona Groenheit

Aim: To qualify an in-house broth microdilution (BMD) assay for phenotypic drug susceptibility testing (DST) of *Mycobacterium tuberculosis* complex (MTBC) strains for implementation in a routine DST workflow.

Methods: A BMD assay was developed based on the EUCAST reference protocol including of 14 anti-tuberculous drugs. The agreement between minimum inhibitory concentration (MIC) and genotypic drug susceptibility testing (gDST) results was assessed using a panel of 40 MTBC strains with various drug resistance profiles by calculating diagnostic accuracy metrics and discussing the distribution of mutation across MIC values.

Results: Test accuracy metrics showed high specificity and positive predictive values for 13 out of 14 drugs, highlighting the assay's ability to identify and accurately classify susceptible genotypes. However, five drugs showed slightly lower sensitivity and negative predictive values, suggesting a small chance of misclassifying strains with resistance mutations as susceptible. Most discrepancies were attributed to mutations conferring MICs near the applied critical concentration (CC) or due to hetero-resistance. Our study provided valuable insights into the role of Lineage 1 in increasing pretomanid MIC, allowed discussion on the CC of ethambutol and para-aminosalicylic acid, and helped interpret the impact of specific mutations on MIC.

Public health implications: The BMD assay, now integrated with gDST, will be part of Sweden's MTBC diagnostic protocol, enhancing treatment decisions through rapid sequencing and accurate susceptibility testing, and improving understanding of resistance mutations. Importantly, the assay can be adjusted to new drug recommendations and concentrations, tailored to local needs.

Role: Carmen performed data analysis (diagnostic accuracy metrics, distribution of mutations across MIC values), and wrote the manuscript submitted to the journal, *Antimicrobial Agents and Chemotherapy*.

3. Biorisk management

Biosafety risk assessment: MDR-TB biorisk scenario

Supervisors: Module facilitators

Aim: To perform the biosafety risk assessment of working with a fictional pathogen (multidrug-resistant Titan blue - MDR-TB)

Methods: A biosafety and biosecurity risk assessment was performed for culturing MDR-TB for antibiotic susceptibility testing using the BioRAM Lite form. The risk was assessed for the individual performing the test in the laboratory and for the community, including humans and animals. Each question of the BioRAM Lite form was assigned a value from 0 to 4.

Results: Based on the estimated values for likelihood of exposure and consequence of infection, the risk of MDR-TB infection via inhalation was considered high for individuals in the laboratory and moderate for individuals in the community. Results also indicated a moderate risk of MDR-TB via ingestion in the individual performing the tests, and low for the community. Similarly, the risk for animals was considered high via inhalation, and moderate via ingestion. The risk via percutaneous exposure or direct contact was considered very low.

Public health implications: Biosafety risk assessments prevent infections, ensure containment, and facilitate adherence to safety standards.

Role: Carmen participated actively in the module and within the group work, and performed this assignment as a post-module activity.

Biosafety level-3 training and activity

Supervisor: Klara Sondén

Aim: To obtain clearance for Biosafety level-3 (BSL-3) laboratory work and implement biorisk management rules to control exposure to live mpox virus during the performance of neutralisation assays.

Methods: Accessing the BSL-3 facilities involved a security and psychological assessment, a safety protection training, and revision of vaccination needs. Safety protection training included access to the BSL-3 building, safety instructions, measures in the event of incidents, spills and incidents, emergency alarm procedures, and use of the lifeline system.

The neutralisation assays were conducted using the cytopathic effect (CPE) test inside class II biosafety cabinets. Briefly, Vero cells were grown in minimum essential medium containing antimicrobials. Mpox virus clade IIB was propagated on Vero cells and titrated. Heat-inactivated serum samples were diluted in duplicate two-fold dilution series and mixed with equal volumes of titrated Mpox virus. The serum-virus solutions were incubated for one hour and transferred to the plates containing confluent Vero cells. After incubation for five days, the cells were inspected for CPE using optical microscopy. Samples were considered neutralising if a >50% reduction of CPE was observed in comparison to the controls.

Results: Carmen successfully trained and thereby obtained access to the BSL-3 laboratory. She was able to apply her training using a laboratory protocol appropriate for BSL-3 and familiarised herself with preventive and containment measures. Her BSL-3 work produced the results of her research project, with important implications for mpox vaccination regimens.

Public health implications: BSL-3 training ensured the safe handling of mpox virus, allowing the fellow to generate valuable data for public health interventions.

Role: The fellow underwent tests and interviews for security and psychological clearance, studied the relevant documentation for the safety protection training, and received relevant vaccinations. She actively performed neutralisation assays manipulating live mpox virus while applying the appropriate safety measures.

4. Quality management

Laboratory audit: molecular diagnosis of mpox, Sweden, 2023

Supervisor: Klara Sondén

Aim: To ensure compliance with standards, accuracy, reliability, and consistency of mpox molecular diagnostic tests and results.

Methods: Using the Biorisk and Quality Management module template, information on process and quality control, and documentation was collected. This included 40 questions on facilities, quality management and assurance, specimen management, and analytical processes. Information pertaining to documentation included 78 questions about document quality and control, technical records, non-conformity control, equipment logbooks, standard operating procedures, and documentation about biosafety, personnel, transport, and surveillance and outbreak response.

Results: The mpox molecular diagnosis lab has a well-established system for process management, quality control, and documentation. Samples are submitted according to the guidelines shared with healthcare centres, received in the designated areas, and handled as per standard operating procedures, which are recorded in the internal digital system. A system for audits, and internal and external quality assurance are in place. Access to the lab once inside the building is possible, but only authorised persons can enter the building via double identification by card. Improving the national digital system for the reporting of test results (*SmiLab*), to start allowing data retrieval by location, patient name, or test result would facilitate data collection.

Public health implications: Quality-assured laboratory molecular detection of mpox ensures accurate and reliable diagnostics, allowing outbreak detection and control, and informed public health decisions.

Role: Carmen collected the responses from persons responsible for mpox molecular diagnostics, quality personnel at PHAS, and relevant documentation, including standard operating procedures and laboratory reports.

External Quality Assessment of the Microbiology Department at the Public Health Agency of Sweden by the Swedish Board for Accreditation and Conformity Assessment, 2024

Supervisor: Emmi Andersson

Aim: The aim of the External Quality Assessment (EQA) was to renew the accreditation of the microbiology laboratory at PHAS. Accreditation means formal recognition of the competence to perform specific microbiological analyses.

Methods: The Swedish Board for Accreditation and Conformity Assessment (SWEDAC) examined whether PHAS meets the requirements of the Standard ISO 15189 for medical laboratories, and maintain the status as an accredited laboratory. At the day of the assessment, SWEDAC informed each unit about the topics that would be audited. Subsequently, quality representatives in each unit collected the relevant documentation. Through the day, the assessors asked detailed questions about the selected topics, revising laboratory methods, reporting systems, personnel in charge, and management. At the end of the day, SWEDAC gathered all units again to briefly give an overview of the deviations and propose dates to present the mitigation actions.

Results: Fourteen deviations were identified. Continued accreditation was recommended on the condition that corrective measures and supporting documentation were reported to SWEDAC within 25 June 2024 so that they can be approved. Accreditation was confirmed after all actions were delivered and approved within this date.

Public health implications: Accreditation ensures accurate and reliable results, and appropriate data sharing and reporting, ultimately preserving public health.

Role: Carmen participated in the opening and closing meetings, and followed the assessment of the virology laboratory, with the opportunity to enquire and access the results.

5. Public health microbiology management

Organisation of EAN molecular epidemiology mini-module, Barcelona, 20–21 November 2023

The EPIET alumni network (EAN) called for experts to help develop the content and deliver a mini-module on molecular epidemiology. From May to November, the organising team defined the objectives, mapped teaching materials, proposed sessions, and assigned responsibilities. EAN members' learning needs were enquired in a survey. The responsibilities of Carmen included drafting the programme and organising the session, 'Monitoring antimicrobial resistance using WGS'. Registrations were closed in October. Logistics and materials were finalised in early November, and a post-module evaluation was circulated.

Assessment of national preparedness for zoonotic influenza, Sweden, 2023

A project was formulated to assess Sweden's national preparedness for zoonotic influenza within the EU project's United4Surveillance framework. Objectives included conducting a stakeholder analysis, improving inter-agency communication, clarifying responsibilities, performing an influenza H5N1 risk assessment, and documenting zoonotic influenza preparedness in other Nordic countries. Carmen attended United4Surveillance meetings and began mapping national stakeholders. The project was presented at the 2023 Nordic mini-module in Copenhagen. The project was put on hold due to difficulties in performing the stakeholder analysis in the intended format.

Collaborations with stakeholders involved in PHAS activities

Carmen has collaborated with relevant stakeholders outside of PHAS on several projects:

- *C. parvum* outbreak investigation: Liaised with the national reference laboratory for *Cryptosporidium* in Wales to import the MLVA scheme, and with the Swedish Veterinary Association for calf samples analysis.
- Norovirus outbreak: Coordinated with the regional health authority in Örebro County to manage the investigation, understand the settings, and communicate with the affected school.

- Rotavirus surveillance: Consulted with the Finnish Institute for Health and Welfare (THL – Finland) on their national surveillance methods to improve the Swedish system.
- Mpox immunity investigation: Worked with the Venhälsan clinic at Södersjukhuset on enrolment, data sharing, and discussions of results and next steps.

6. Teaching and pedagogy

Surveillance and outbreak investigation trainings for future public health professionals, Sweden, 2022–2023

Carmen delivered surveillance and outbreak investigation trainings to:

- medical doctors from the public health residency program (2.5 hours).
- students from the Infectious Diseases Master's programme at Södertörn University (6 hours).
- veterinary students from the public health module at the Swedish University of Agricultural Sciences (3.5 hours).

The material was developed alongside EPIET fellow, Hilde De Clerck, by adapting previous materials into a session that promoted active learning. The course evaluation indicated general satisfaction, though one student consistently gave low scores without clear explanation.

Member of the assessment committee at PhD defence, Spain, 2022

Carmen was a member of a PhD assessment committee on 19 December 2022 at Universidad CEU Cardenal Herrera in Valencia, Spain. She reviewed the thesis, titled, 'Bacteriophage Application for *Salmonella* Control in Poultry and its Applications on Their Microbiota and Metabolome', prepared a preliminary recommendation report, formulated discussion topics, and engaged in a scientific debate with the candidate during her defence.

Monitoring antimicrobial resistance using WGS: Case study II - transmission of cephalosporin resistance between farm animals, chicken meat, and humans. EAN mini-module in molecular epidemiology, Spain, 2023

Carmen adapted a laboratory exercise provided as empiric teaching in a PhD course which she was responsible for in her previous role as assistant professor at the University of Copenhagen. The previous empiric teaching exercise was adapted to a case study where students received the results and could focus on understanding the different steps of the microbiological investigation and formulating conclusions. The data provided within the case study were based on results by de Been et al (2014).

7. Communications related to the EPIET/EUPHEM fellowship

7.1. Manuscripts published in peer-reviewed journals

Espinosa-Gongora C, Berg C, Rehn M, Varg JE, Dillner L, Latorre-Margalef N, et al. Early detection of the emerging SARS-CoV-2 BA.2.86 lineage through integrated genomic surveillance of wastewater and COVID-19 cases in Sweden, weeks 31 to 38 2023. Euro Surveill. 2023;28(46):2300595. Available at: https://doi.org/10.2807/1560-7917.ES.2023.28.46.2300595

Mansjö M, **Espinosa-Gongora C**, Samanci I, Groenheit R, Werngren J. Performance of an in-house broth microdilution assay for routine minimum inhibitory concentration determination of 14 anti-tuberculous drugs against the *Mycobacterium tuberculosis* complex based on the EUCAST reference protocol. 2024. Under review in Antimicrob Agents Chemother.

Espinosa-Gongora C, Christ W, Mayola-Danés N, Eichler-Jonsson C, Filén F, Storgärd E, et al. Mpox-specific neutralizing antibody titre analysis following intradermal MVA-BN vaccination in Sweden. In preparation.

7.2. Other reports

Influenza in Sweden – Season 2022–2023. The Public Health Agency of Sweden. Article: 23162. Stockholm; 2023. Available at: https://www.folkhalsomyndigheten.se/publikationer-och-material/publikationsarkiv/i/influenza-in-sweden-season-2022-2023/?pub=126761

7.3. Conference presentations

Espinosa-Gongora C, Robinson G, Risby H, Troell K, Rönnberg C, Bujila I. Retrospective investigation of historic *Cryptosporidium parvum* outbreaks in Sweden using Multilocus Variable Number Tandem Repeats analysis (MLVA)

compared to *gp60* subtyping (oral - flash presentation of poster). Presented at ESCMID Global; 30 April 2024; Barcelona, Spain.

Espinosa-Gongora C, Carnahan A, Latorre-Margalef N, Zanetti S, Samuelsson Hagey T, Öckinger JB, Dillner L, Rehn M, Andersson E. Beyond influenza and SARS-CoV-2: advancing respiratory sentinel surveillance in Sweden (poster presentation). Presented at ESCMID Global; 27 April 2024; Barcelona, Spain.

Sondén K, Christ W, Mayola Danés N, Eichler Jonsson C, Westergren V, Storgärd E, Filén F, Klingström J, Johansen K, **Espinosa-Gongora C**, Ekström AM. Immune response to MPXV wanes rapidly after intradermal vaccination with MVA-BN (poster presentation). Presented at ESCMID Global; 30 April 2024; Barcelona, Spain.

Espinosa-Gongora C, Carnahan A, Latorre-Margalef N, Zanetti S, Samuelsson Hagey T, Öckinger JB, Dillner L, Rehn M, Andersson E. Preparing for the 2023-2024 season: Exploring the introduction of testing for respiratory syncytial virus (RSV) in the Swedish sentinel surveillance system. Submitted to ESCAIDE 2023.

Espinosa-Gongora C, Robinson G, Risby H, Vainio A, Troell K, Rönnberg C, Bujila I. Usefulness of a Multilocus Variable Number Tandem Repeats (VNTR) analysis (MLVA) scheme for *Cryptosporidium parvum* epidemiological investigations in Sweden. Submitted to ESCAIDE 2023.

7.4. Other presentations

Espinosa-Gongora C. Projects and outputs of the EUPHEM fellowship at PHAS 2022-2024. Folkhälsoforum; 2 September 2024; Solna, Sweden.

Studies highlight waning antibodies after mpox vaccination. Center for Infectious Disease Research and Policy (CIDRAP) News; 1 April 2024. Available at: https://www.cidrap.umn.edu/mpox/studies-highlight-waning-antibodies-after-mpox-vaccination

Swedish study indicates a significant decline of neutralising antibodies to monkeypox virus already during the first month after vaccination. European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Reports and Proceedings; 30 March 2024. Available at: https://www.eurekalert.org/news-releases/1039386

Espinosa-Gongora C. Influenza B virulence. Nordic Project Review Mini-module. Finnish Institute for Health and Welfare; 29 February 2024; Helsinki, Finland.

Espinosa-Gongora C. Usefulness of a new MLVA scheme for *Cryptosporidium parvum* epidemiological investigations in Sweden. Presented at Mikrobiologiskt Forum, PHAS; 8 December 2023.

Espinosa-Gongora C. Early detection of SARS-CoV-2 variant BA.2.86 in Sweden: genomic surveillance of wastewater and cases. Presented at: Kliniska mikrobiologiska laboratorier (KML) digital forum; 15 November 2023, virtual.

Espinosa-Gongora C. Circulation of respiratory pathogens: results from a retrospective analysis using a multi-pathogen panel on primary care sentinel samples. Presented at: Influenza Day, PHAS; 25 September 2023, Solna, Sweden.

Espinosa-Gongora C. Usefulness of a new MLVA scheme for *Cryptosporidium parvum* epidemiological investigations in Sweden. Project Review Module. Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA); 30 August 2023; Lisbon, Portugal.

Espinosa-Gongora C. Training the next generation of veterinary prescribers (oral presentation). Presented at workshop: Antimicrobial resistance (AMR) - human behaviour, policy and communication; 2 June 2023; London, United Kingdom.

Espinosa-Gongora C. Zoonotic influenza preparedness. Nordic Project Review Mini-module. Statens Serum Institute; 13 March 2023; Copenhagen, Denmark.

5. EPIET/EUPHEM modules attended

- Introductory Course, 26 September–14 October 2022, Spetses, Greece
- Outbreak Investigation, 5–9 December 2022, Berlin, Germany
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2022, 23–25 November 2022, Stockholm, Sweden
- Nordic Mini Project Review Module, 15–15 March 2023, Copenhagen, Denmark
- Biorisk and Quality Management, 16–17 March 2023, virtual
- Multivariable Analysis, 22–26 May 2023, Frankfurt, Germany

- Rapid Assessment and Survey Methods, 19–23 June 2023, Stockholm, Sweden
- Project Review Module 2023, 28 August–1 September 2023, Lisbon, Portugal
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2023, 22–24 November 2023, Barcelona, Spain
- Time Series Analysis, 11–15 December 2023, Rome, Italy
- Nordic Mini Project Review Module 2024, 29 February–1 March 2024, Helsinki, Finland
- Vaccinology, 4–8 March 2024, virtual
- Qualitative Research – 19 and 22 March 2024, virtual
- European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2024, 27–30 April 2024, Barcelona, Spain
- Public health microbiology III – Whole Genome Sequencing & Bioinformatics, 3–7 June 2024, Vienna, Austria
- Management, Leadership and Communication in Public Health, 24–28 June 2024, Stockholm, Sweden
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2024, 20–22 November 2024, Stockholm, Sweden

6. Other training

- The Epidemic Intelligence Online Mass Gathering Training. ECDC Epidemic Intelligence. Online. 15–19 July 2024.
- EVA E-learning course on Epidemic Intelligence. Online.
- EVA E-learning course on Rapid Risk Assessment. Online.
- WHO Introduction to Epidemic Intelligence from Open Sources (EIOS). Online.
- Biosafety level 2 (BSL2) incident training, 30 May 2024, PHAS, Solna, Sweden.
- United Nations Department of Safety and Security. BSAFE. 29 June 2023. Online.

7. International assignments

- Two-week stay to support the epidemic intelligence activities during mass gathering events. ECDC, 22–30 July 2024, Stockholm, Sweden.
- Six-week stay as Temporary Adviser at the WHO Regional Office for Europe. My role was to contribute to the analysis of AMR data from The Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) network, and writing of the corresponding report that will be published by WHO. WHO Europe, 1 August–11 September, 2024, Copenhagen, Denmark.

8. Other activities

- Lab visit to the EUCAST development laboratory (EDL) for bacteria. Växjö, Sweden, 22 August 2024.
- Supporting MS-track EUPHEM fellow, Ioana Bujila with her project on typing of *Shigella* / EIEC by calculating and comparing the sensitivity and specificity of WGS and laboratory methods.

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