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One Health surveillance in practice: Experiences of integration among human health, animal health, environmental health, and food safety sectors

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Published in
Frontiers in Public Health
Frontiers in Medicine

ISSN 1664-8714
ISBN 978-2-8325-4590-4
DOI 10.3389/978-2-8325-4590-4

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One Health surveillance in practice: Experiences of integration among human health, animal health, environmental health, and food safety sectors

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Citation

Prada, J., Benedetti, G., Scheutz, F., Hendriksen, R., Boisen, N., eds. (2024). *One Health surveillance in practice: Experiences of integration among human health, animal health, environmental health, and food safety sectors*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4590-4

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frontiers | Frontiers in Public Health

TYPE Editorial
PUBLISHED 01 March 2024
DOI 10.3389/fpubh.2024.1384988

OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 11 February 2024
ACCEPTED 16 February 2024
PUBLISHED 01 March 2024

CITATION
Benedetti G, Boisen N and Prada JM (2024) Editorial: One Health surveillance in practice: experiences of integration among human health, animal health, environmental health, and food safety sectors.
Front. Public Health 12:1384988.
doi: 10.3389/fpubh.2024.1384988

Editorial: One Health surveillance in practice: experiences of integration among human health, animal health, environmental health, and food safety sectors

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KEYWORDS

One Health, surveillance, human health, animal health, environmental health, food safety

Editorial on the Research Topic
One Health surveillance in practice: experiences of integration among human health, animal health, environmental health, and food safety sectors

Recognizing that human and animal health are interconnected brings along the challenge of integrating their respective health systems, including routine disease surveillance, outbreak management, and emergency preparedness. However, approaches in these different sectors are still unaligned in many ways, including their respective agendas, both at country and supranational levels. Since the early 2000s, the World Health Organization (WHO), the Food and Agricultural Organization of the United Nations (FAO), and the World Organization of Animal Health (WOAH) paved the road of multi-sectorial One Health (OH) approaches and collaborations, leading to the publication of the Tripartite Zoonoses Guide. Recently, the "One Health European Joint Programme" fostered cooperation in OH practice within and across European countries (1). Furthermore, various scientific networks and consortia have been set up to bring together professionals and experiences from different sectors.

Integration is key to the OH agenda, and to the challenge of preparedness and response to endemic diseases and other emerging threats. This Research Topic gathered first-hand, successful, and inspirational experiences about the integration of approaches, procedures and methodologies for OH surveillance across the human health, animal health, environmental health, and food safety sectors, at the local, national, or supranational levels.

To integrate existing surveillance systems effectively, the OH-EpiCap tool plays an important role by offering a semi-quantitative evaluation of "One-Healthiness". Tegegne et al. developed this tool to strengthen OH surveillance systems, focusing on assessing their organization, operations and outputs. The tool is applicable to any disease surveillance system of OH relevance. The OH-EpiCap tool necessitates stakeholders' recognition of the importance of assessing their systems. Further, Moura, Collineau et al. measured the

perceptions of users of the OH-EpiCap tool when applied to various national antimicrobial resistance (AMR) surveillance systems. They described the OH-EpiCap functionality, emphasizing its user-friendly application, comprehensive coverage of previously overlooked elements (such as the impact of integrated surveillance), and its focus on the governance of OH surveillance. The application of the OH-EpiCap tool was further explored with the Danish Integrated Antimicrobial Resistance Monitoring and Research Program for AMR and antimicrobial use in animals and humans (Moura, Høg et al.).

Introducing the OH perspective to disease surveillance entails recognizing how changing existing systems might affect stakeholders. Hence, the importance of collaboration among the participating actors. This was the focus of a qualitative evaluation of collaborations among AMR surveillance programmes in France by Bourély et al.. The study found that collaborations were mainly created through good personal relations between individuals in different sectors/areas of the programmes, who worked together due to personal interest, rather than due to the structure of the system. On the other hand, the mapping of the stakeholders and processes to integrate OH surveillance is a valuable exercise. Through questionnaires, data mapping and case studies, Amato et al. illustrated the feasibility of mapping OH-relevant foodborne pathogen surveillance across human health, animal health, and food safety sectors in European countries. This adaptable methodology facilitates integration and underscores the need to transcend silo thinking for OH success, stressing the need for broader collaboration. Avila et al. further showed the importance of a holistic approach to disease surveillance, in a multidisciplinary and inclusive OH perspective by evaluating the presence of *Toxocara* spp. in public squares and parks in San Juan province, Argentina. Identifying zoonotic parasites with infection potential for humans in urban areas underscores the necessity of integrating expertise among different sectors. Addressing public health threat at the human-veterinary interface through "collaboration, communication, and coordination", for positive health outcome in both humans and animals is key. This study by Le Bouquin et al. presents a routine surveillance system that brings together the human and veterinary sectors for the emerging zoonoses botulism in France, expanding the focus from farmed animals, which are usually under the OH spotlight, to wild animals and the entire ecosystem.

To design, implement, and evaluate integrated surveillance systems, Rivers et al. developed a framework focusing on output-based standards, with an emphasis on zoonotic threats, following the case of *Echinococcus multilocularis* in Great Britain. Defining objectives for such a system is important, and depends on the hazard—whether it is an endemic disease or a potential new introduction. Additionally, quantifying and communicating uncertainty, especially to non-technical audiences, can be challenging.

An understanding of laboratory methods is important to successful integration of surveillance systems. However, existing schemes often target single sectors, while cross-sectoral panels are key to OH. Tast Lahti et al. evaluated European laboratories' cross-sectoral proficiency for foodborne pathogens *Campylobacter* spp., *Salmonella* spp. and *Yersinia enterocolitica*, informing future proficiency tests and external quality assessments in OH. Takeaways emphasized the critical importance of well-defined targets and robust characterization methods for effective pathogen detection. These schemes foster international collaborations, which are pivotal to outbreak investigations and standardization. Future assessments should integrate genomic analysis to advance foodborne zoonosis methodologies.

Setting up an information system for genomic surveillance is challenging, as highlighted by Knijn et al. in their development and application of the IRIDA-ARIES infrastructure in Italy, but leads to better standardization processes of routine surveillance data. Imagining a OH-relevant surveillance system in Europe that is aligned with European agencies requirements is a step toward creating findable, accessible, interoperable, and reusable data (2). Indeed, the availability of diagnostics to identify hazards of interest is also important for any sustainable programme. In recent years, molecular techniques have been used more extensively to allow a better understanding of new, emerging threats. In this context, Cherchame et al. evaluated Salmonella enterica serovars and enhanced the open-access databases with 73 new genomes, including more reference genomes, which improves bioinformatics surveillance.

Finally, this Research Topic gathered the experiences from a multi-country OH foodborne outbreak simulation exercise as part of the One Health European Joint Programme (Alves et al.). The exercise put the functional response to a threat of OH relevance into practice across different sectors and across multiple countries in Europe. It focused on the countries' capacities and capabilities for outbreak preparedness, management and response and it remains a rich collection of pitfalls and opportunities that can serve as a benchmark for the participating countries and as an inspirational guide for others.

In the post-SARS-CoV-2 pandemic world, preparedness has become the new buzzword of public health practice and OH a never-failing "buzz-adjective". Given its key role, it is high time for OH surveillance to become practice and routine.

Author contributions

GB: Writing—original draft, Writing—review & editing. NB: Writing—original draft, Writing—review & editing. JP: Writing—original draft, Writing—review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 773830: One Health European Joint Programme.

Acknowledgments

The authors of this editorial wish to thank all the editors who took part in the Research Topic "One Health Surveillance in Practice: Experiences of Integration among Human Health, Animal

Health, Environmental Health, and Food Safety Sectors". Special thanks goes to Rene Hendriksen and Flemming Scheutz for their contributions to the realization of this Research Topic.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 08 September 2022
DOI 10.3389/fpubh.2022.963188

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases – Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

RECEIVED 07 June 2022
ACCEPTED 02 August 2022
PUBLISHED 08 September 2022

CITATION
Cherchame E, Ilango G, Noël V and Cadel-Six S (2022) Polyphyly in widespread *Salmonella enterica* serovars and using genomic proximity to choose the best reference genome for bioinformatics analyses. *Front. Public Health* 10:963188. doi: 10.3389/fpubh.2022.963188

Polyphyly in widespread *Salmonella enterica* serovars and using genomic proximity to choose the best reference genome for bioinformatics analyses

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Salmonella is the most common cause of gastroenteritis in the world. Over the past 5 years, whole-genome analysis has led to the high-resolution characterization of clinical and foodborne *Salmonella* responsible for typhoid fever, foodborne illness or contamination of the agro-food chain. Whole-genome analyses are simplified by the availability of high-quality, complete genomes for mapping analysis and for calculating the pairwise distance between genomes, but unfortunately some difficulties may still remain. For some serovars, the complete genome is not available, or some serovars are polyphyletic and knowing the serovar alone is not sufficient for choosing the most appropriate reference genome. For these serovars, it is essential to identify the genetically closest complete genome to be able to carry out precise genome analyses. In this study, we explored the genomic proximity of 650 genomes of the 58 *Salmonella enterica* subsp. *enterica* serovars most frequently isolated in humans and from the food chain in the United States (US) and in Europe (EU), with a special focus on France. For each serovar, to take into account their genomic diversity, we included all the multilocus sequence type (MLST) profiles represented in EnteroBase with 10 or more genomes (on 19 July 2021). A phylogenetic analysis using both core- and pan-genome approaches was carried out to identify the genomic proximity of all the *Salmonella* studied and 20 polyphyletic serovars that have not yet been described in the literature. This study determined the genetic proximity between all 58 serovars studied and revealed polyphyletic serovars, their genomic lineages and MLST profiles. Finally, we enhanced the open-access databases with 73 new genomes and produced a list of high-quality complete reference genomes for 48 *S. enterica* subsp. *enterica* serovars among the most isolated in the US, EU, and France.

KEYWORDS

***Salmonella enterica*, genomic proximity, polyphyletic serovars, MLST profile, cgMLST, pan-phylogenetic analysis, reference complete genomes**

Introduction

For routine disease surveillance activities and outbreak investigations, the use of whole-genome sequencing (WGS) to identify and subtype foodborne bacterial pathogens has replaced traditional slide agglutination methods; likewise, to cluster and associate epidemiological strains, core genome multilocus sequence type (cgMLST) and single nucleotide polymorphism (SNP) analyses have replaced pulsed-field gel electrophoresis (PFGE) and multiple loci VNTR (MLVA) analyses. To meet the needs of real-time surveillance and ensure public health and economic benefits, the analysis of the complete genome is now routine for many reference laboratories around the world. cgMLST and SNP analyses are fast and several user-friendly tools exist for investigations of outbreak clusters (1–4). Nevertheless, although SNP phylogenetic core-genome analysis enables more detailed clustering between strains and better calculation of genomic distances between genomes, it requires complete genomes for processing the obtained data (3, 5). To ensure good-quality, complete reference genomes, which is essential for these epidemiological association analyses, we recently developed an open-source tool (SalmoDEST) that can download well-characterized good-quality, complete reference genomes from the open-access GenBank database (6). This tool can extract complete *Salmonella* genomes with a coverage higher than 50x and genome length over 4 Mb; it verifies the serovar to which genome belongs and identifies the corresponding MLST profile (6).

Nevertheless, although the number of compete genomes deposited in the open-access databases increases every year, a complete reference genome is still not available for several *Salmonella* serovars. The choice of a good reference genome is critical to ensure the sensitivity of the analyses performed when analyzing closely related genomes (5, 7). Selecting a reference genome close to the strains under study increases the fraction of the genome on which SNP variants can be screened for, thereby increasing method sensitivity. For instance, we have shown that the use of the reference genome Typhimurium LT2 led to an 11% loss of core genome information (89% of breadth coverage) in the SNP phylogenetic investigation of the *Salmonella* Wellikade outbreak occurred in 2016 in France (5). However, choosing the *S.* Gaminara strain SA20063285 reference genome provided 92% breadth coverage, corresponding to a loss of only 8% of core-genome information (5). When the complete genome is not available for the serovar studied, we proposed an operating protocol (5) that can be used in any laboratory involved in surveillance activities, outbreak management and emergency preparedness (5). The protocol identifies the closest complete genome to use for SNP phylogenetic analysis among the ones available in the EnteroBase *Salmonella* database (1, 8). We indicate how to query EnteroBase by searching for the closest hierarchical cluster (HC) 2,000 profile of the serovar under study and visualize results using the GrapeTree clustering analysis (5, 9).

Finally, when choosing the most suitable complete genome, polyphyletic serovars require special attention. A polyphyletic serovar derives from multiple independent ancestors (1). For example, a study of the phylogeny of the *Salmonella* Derby serovar showed that strains displaying the same antigenic pattern *S.* 1,4,[5],12: f,g: (10, 11) according to the White-Kauffmann-Le Minor scheme (12) — and, consequently, sharing the name *Salmonella* Derby — belonged to at least three distinct genomic lineages (13). A similar situation was reported for *Salmonella* Newport in 2013 (14). For *Salmonella* Derby, the three lineages were fully consistent with thoses identified by MLST analysis and were named according to their ST profile names (ST40, ST71, and ST682). The strains belonging to the ST40 lineage were distinct from those belonging to the ST71 lineage, differing by 26,957 SNPs with a standard deviation (SD) of 1,583. The genomes belonging to the ST682 lineage were the most genetically distant from ST40 and ST71, with an average of 33,961 SNPs and an SD of 4,102 SNPs (13). With such genomic distances between lineages, it seems evident that the choice of the appropriate reference genome for polyphyletic serovars is critical and cannot be based only on serovar name.

With the goal of providing a ready-to-use map of the genomic diversity of the *Salmonella enterica* subsp. *enterica* serovars prevalent in human health, animal health and the food sector, we carried out a phylogenetic study of the most frequently isolated serovars to give an overview of the main polyphyletic serovars and their genomic lineages.

Materials and methods

Selection of serovars and genomes

The serovars analyzed in this study are those identified as being the most frequently isolated in humans and the agri-food chain over a period of 10 years (from 2006 to 2016) in the United States (US), Europe (EU) and France (FR). The list of the most frequently isolated serovars was compiled based on data reported by the CDC, the USDA, the ECDC and the EFSA reports (11, 15–18). For FR, data from the official controls collected by the *Salmonella* Network, part of the Anses Laboratory for Food Safety (LSAl), and reports from the National *Salmonella* Reference Center were taken into account (10, 19). More than 1.5 million reported human cases and, animal and food isolates were compiled in six lists according to serovar prevalence. Three lists (i.e., one list for the US, one for EU and one for FR) were compiled for the serovars isolated from humans and three other lists for those collected from the agri-food sector. The three lists for human cases and the three lists for the agri-food isolates were used separately for the Venn diagram analysis that was carried out using the ggVennDiagram

TABLE 1 List of the 58 *Salmonella* serovars identified as being the most frequently isolated in humans and the agri-food sector over a period of 10 years (from 2006 to 2016) in the United States, Europe and France.

Agona	Derby	Johannesburg[b]	Muenster	Schwarzengrund
Albany	Dublin	Kedougou[c]	Napoli	Senftenberg
Anatum	Enteritidis	Kentucky	Newport	Stanley[c]
Banana[a]	Gallinarum[c]	Kottbus	Ohio	Tennessee
Bareilly[b]	Give	Livingstone	Oranienburg	Thompson
Bovismorbificans	Goldcoast[c]	London	Panama	Typhi
Braenderup	Hadar	Manhattan	Paratyphi B and Java	Typhimurium
Brandenburg	Havana	Mbandaka	Poona	Uganda
Bredeney	Heidelberg	Minnesota	Reading[b]	Veneziana[a]
Cerro	Indiana	Mississippi[b]	Rissen	Virchow
Chester	Infantis	Montevideo	*S.* 1,4,[5],12:i:-	
Coeln	Javiana[b]	Muenchen	Saintpaul	

Forty-seven serovars were selected because they were common to the United States, Europe and France according to the Venn analyses in Figure 1. Eleven other serovars (visualized by gray fill color in the list) were added because they belong to the top 20 serovars from each country but were not common to all countries. a: serovar from to the top 20 in FR; b: serovars from the top 20 in the US; c: serovars from the top 20 in EU.

R package (v.1.2.1) (20). Finally, the serovars selected for this study were chosen according to the following criteria: being common to at least two lists and belonging to the leading 20 serovars of each list (Table 1).

For each of the serovars selected, the most common MLST profiles were identified using the data available in the EnteroBase *Salmonella* database on 19 July 2021. The MLST profiles with 10 or more genomes in the EnteroBase database were selected for this study. For each of these MLST profiles, three good-quality genomes were downloaded. The complete or contig genomes were searched and downloaded using the SalmoDEST tool (6) and manually *via* the GenBank and EnteroBase *Salmonella* databases. Good-quality genome criteria were a length > 4 Mb, coverage > 50x and an analysis of how well genome matched the predicted serovar using SeqSero2 (21). When available, genomes from the Anses LSAl collection were selected and sequenced for this study. One genome of *S.* Javiana was obtained from the strain S11LNR1976 (renamed 2019LSAL01686) from the French National Reference Laboratory Collection (LNR-Anses) in Ploufragan-Plouzané-Niort Laboratory. Three genomes of *S.* Paratyphi B were obtained from the strains CIP 106179, CIP 55.42 and CIP 106950 (renamed 2019LSAL01933, 2019LSAL01934 and 2019LSAL01936, respectively) of the French CIP collection (*Collection de l'Institut Pasteur*, Paris, https://www.pasteur.fr/en/public-health/biobanks-and-collections/collection-institut-pasteur-cip).

Whole-genome sequencing analyses

Sequencing and assembly

Seventy-three genomes from Anses *Salmonella* Network collection were sequenced using the Illumina system producing paired-end reads as described in Cadel-Six et al. (22). The quality control, normalization and assembly were carried out with an in-house workflow called ARtWORK (23). The serovar and the multilocus sequence type (MLST) were attributed using the SeqSero2 (21) and MLSTseeman tools (24).

cgMLST analysis

The core-genome MLST (cgMLST) analysis was carried out with SeqSphere+ (Ridom® GmbH, Münster, Germany) under the EnteroBase cgMLST scheme based on 3002 loci (25).

Pan-genome phylogenetic analysis

The pan-genome kmer phylogenetic analysis was carried out with the QuickPhylo workflow as previously described (26), setting the Mash tool parameter to 1,000 selected kmers of 15 bases (27) and setting the DendroPy tool parameter to the neighbor-joining (NJ) method (28).

Tree annotation

Trees were visualized and annotated using R with the ggtree package (20, 29, 30).

Results

Salmonella serovars and genome selection

Fifty-eight *S. enterica* subsp. *enterica* serovars, the most frequently isolated in human cases and the agri-food sector in the US, EU and FR were selected for this study. The Venn analyses allowed selecting 47 prevalent common serovars in

the US, EU and FR. The Venn diagrams in Figure 1 illustrate the intersections between the 25 and 50 most isolated serovars in humans and the agri-food sector in the US, EU and FR (Figure 1). The distribution of these 47 common serovars is illustrated in Supplementary Figure 1. Eleven other serovars were added because they belong to the leading 20 serovars of each list and were absent from the previous list comprising 47 common serovars. The 58 final serovars retained for this study are showed in Table 1.

For these 58 major *S. enterica* subsp. *enterica* serovars, 639 genomes were collected from GeneBank, EnteroBase, CIP and the Anses *Salmonella* Network databases following the criteria described above. Eleven *Salmonella* samples from the other subspecies were also included. Genome ID, accession number, predicted serovar, MLST profile, genome length and coverage of the 650 genomes retained in this study is reported in Supplementary Table 1. Of the final total set of 650 genomes, 83 were complete genomes and 567 were contigs. Among the complete genomes, 77 belonged to the *S. enterica* subsp. *enterica* and 6 to other subspecies. Of the 567 contig genomes selected, 562 belonged to the subspecies *enterica* and 5 contig genomes belonged to other subspecies (Supplementary Table 1).

Phylogenetic analyses

The total set of 650 genomes was used for the first cgMLST analysis. From this analysis, a subset of 219 genomes were selected for the final phylogenetic cgMLST and pan-genome analyses. To conserve the genomic diversity of the first panel of 650 genomes, one genome per MLST profile was selected, favoring complete genomes when available. The subset of 219 genomes was composed of 74 complete genomes and 145 genomes in contigs. Genomes selected for the second 219 genome subset are shown in bold and gray fill color in the Supplementary Table 1.

cgMLST analysis of the panel of 650 genomes

Among the 650 genomes, the 11 genomes belonging to the other subspecies were used as outgroups. The cgMLST analysis shows that the 639 genomes belonging to *S. enterica* subsp. *enterica* were separated into four groups. Two of these groups, called groups A and B, included 90% of genomes ($n = 580/639$) and 50 of the 58 serovars studied. The 10% of the remaining *S. enterica* subsp. *enterica* genomes clustered into two separate groups, groups C and D (Figure 2).

Group A included all genomes of serovars Anatum, Braenderup, Coeln, Dublin, Enteritidis, Gallinarum, Hadar, Heidelberg, Java, Kottbus, London, Manhattan, Muenchen, Newport, Ohio, Paratyphi B, Saintpaul, Stanley, Thompson, Typhimurium, 4,[5],12:i:-, Uganda and Virchow. Group A also included the genomes belonging to the MLST profiles Bareilly ST203, 362, 464, 909, 1,612, 2,129, 2,270, 2,553, Bovismorbificans ST142, 377, 1,499, Derby ST682, Infantis ST32, 603, 2,283, 2,146, Livingstone ST543, 1,941, 2,247, Reading ST1628 and Schwarzengrund ST2250.

Group B included all genomes of the serovars Agona, Albany, Brandenburg, Bredeney, Chester, Give, Goldcoast, Havana, Javiana, Johannesburg, Kedougou, Kentucky, Mbandaka, Minnesota, Montevideo, Muenster, Oranienburg, Panama, Poona, Rissen, Senftenberg, 1,3,19:z27:- and Tennessee. Along with these last genomes, the Group B included also the genomes belonging to the MLST profiles Banana ST683, 1,035, 4,745, 5,220, Bovismorbificans ST50, Cerro ST1291, Derby ST39, 40, 71, 72, Infantis ST79, Livingstone ST457, 638, Mississippi ST425, Reading ST93, 412 and Schwarzengrund ST96, 322.

Group C included all genomes of serovar Indiana, the genomes of serovar Cerro characterized by the MLST profiles ST367, 1,593, 2,407, Banana ST7024 and Bareilly ST5146.

Finally, Group D included all genomes of serovars Typhi, Veneziana, Napoli and the genomes of the serovar Mississippi characterized by MLST profiles ST448 and 5,834 (Figure 2 and Supplementary Figure 2).

cgMLST and pan-phylogenetic analyses of the subpanel of 219 genomes

Among the subset of 219 genomes, 214 belong to the subspecies *enterica* for the 58 serovars studied. The five genomes belonging to the other subspecies were used as outgroups for the cgMLST and pan-genome phylogenetic analyses. Both analyses revealed four groups in accordance with the results obtained with the first panel described above. Moreover, the comparison between the two phylogenetic approaches (cgMLST and pan-genome kmers) revealed the same composition of serovars and MLST profiles within each of the four groups of trees (Figure 3 and Supplementary Figure 3).

Polyphyletic serovars

Both cgMLST and pangenome kmers analyses revealed 25 polyphyletic serovars within the 58 serovars studied (Table 2). Of these 25 polyphyletic serovars, only the serovar Banana was scattered across four branches that distinguish four independent genomic lineages (three lineages in Group B and one in Group C). In our panel, we found seven serovars scattered across three lineages: Bareilly, Derby, Kottbus, Newport, Oranienburg, Reading and Saintpaul, with Reading shared between groups A and B, and Bareilly shared between groups A and C. Finally, the last 17 polyphyletic serovars were characterized by two lineages as shown in Table 2 and Figures 2, 3. Of these 17 serovars, 6 presented lineages in different phylogenetic groups: the serovars Bovismorbificans, Cerro, Infantis, Livingstone, Mississippi and Schwarzengrund. The serovars Bovismorbificans, Infantis,

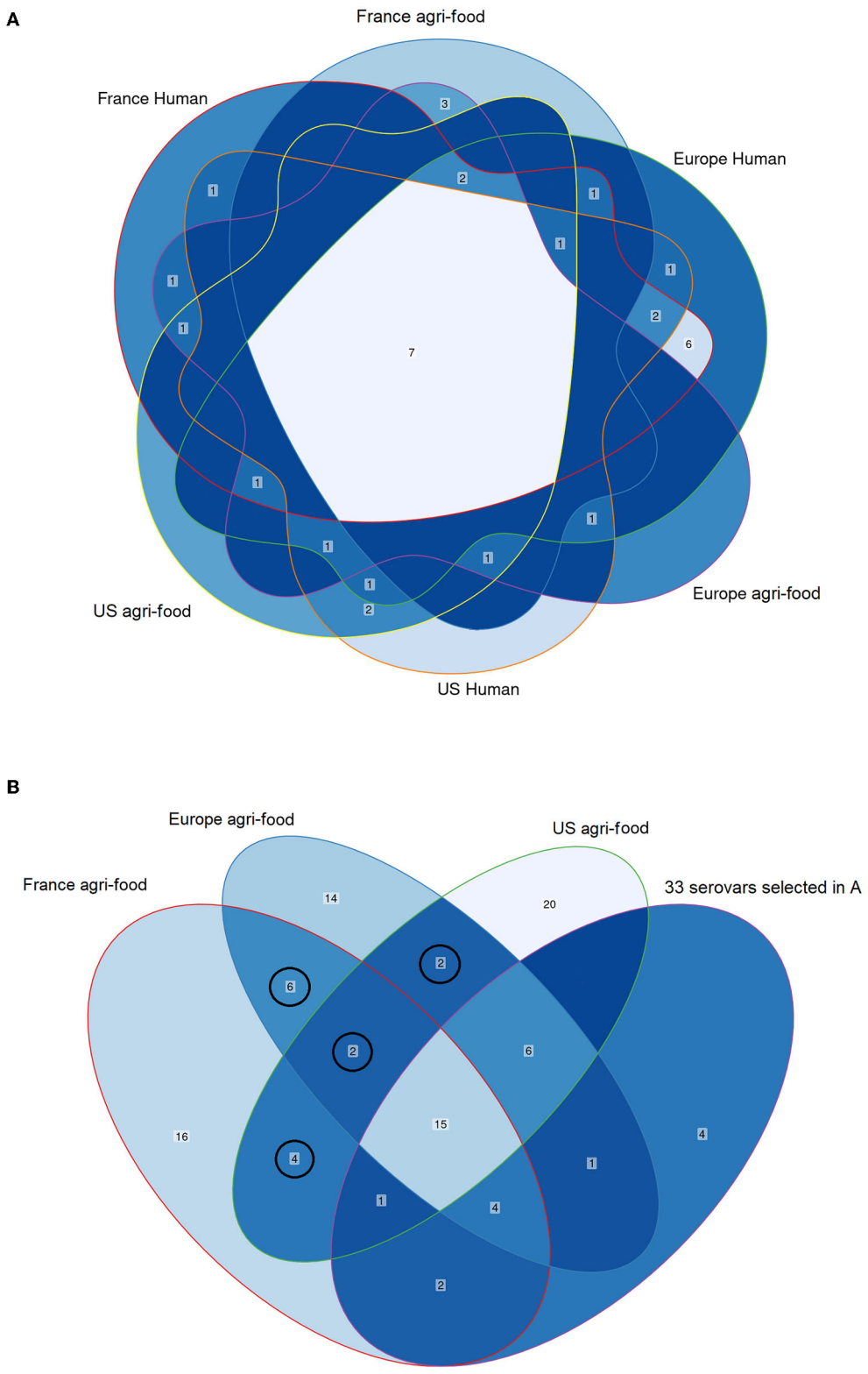

FIGURE 1
Venn diagrams illustrating the intersection between the most frequently isolated serovars in the United States (US), Europe (EU) and France (FR) from human cases and from the agri-food sector. Venn analysis was carried out in two steps showed in **(A,B)**. **(A)** Intersections between the top 25 serovars in human cases and the agri-food sector. The leading 25 human US, EU and FR serovars are included in the orange-, green- and red-outlined areas, respectively. The leading 25 agri-food sector US, EU and FR serovars are included in the yellow-, purple- and blue-outlined areas, respectively. The logical relation between the top 25 serovars in human cases and agri-food sector revealed 33 common serovars. The numbers within the intersections correspond to the common serovars. **(B)** Intersections between the leading 50 US, EU and FR agri-food sector
(Continued)

FIGURE 1 (Continued)
serovars with the 33 serovars previously selected in **(A)**. The top 50 US, EU and FR agri-food sector serovars are included in the green-, blue- and red-shaded areas, respectively. The previous 33 selected serovars in **(A)** are included in the purple-shaded area. The logical relation between these four groups revealed 14 other common serovars. The new 14 common serovars are surrounded by black circles. The numbers within the intersections correspond to common serovars. The resulting 47 common serovars obtained by Venn analyses are showed in the Supplementary Figure 1.

Livingstone and Schwarzengrund were shared between Groups A and B, Cerro was shared by groups B and C and Mississippi was shared by groups B and D.

The MLST profiles characterizing the different lineages of the polyphyletic serovars are compiled in the Table 2. The only difference between the cgMLST and pan-genome kmer analyses involved the genomes of serovar Saintpaul belonging to the MLST profile ST1934 that was observed in two different lineages (lineages II and III). All other data on the distribution of the MLST profiles among the different genomic lineages were concordant between the two types of phylogenetic analyses.

Description of genomic proximity between serovars

Nine subgroups of related serovars were observed in the cgMLST and pan-genome kmer analyses. In Group D, we found a subgroup — which we called "section Typhi" — comprising the genomes of serovar Typhi ST1 and 2, the genomes of the lineage Mississippi I (ST448, 5834), the genome of serovars Napoli and Veneziana (Table 3). Within Group A, we observed five subgroups, called sections "Livingstone," "Enteritidis," "Typhimurium," "Newport" and "Muenchen." The "section Livingstone" (section A1 in Figure 2) comprises the genomes of the lineage Livingstone I (ST543, 1941, 2247) and the genomes of the serovar Ohio. The "section Enteritidis" (section A5 in Figure 2), comprises the genomes of serovars Enteritidis (ST11, 183), Gallinarum (ST78, 331), Dublin (ST10, 73, 4406) and Berta (ST435). The "section Typhimurium" (section A4 in Figure 2), is composed of the serovars Typhimurium (ST19, 34, 36), Heidelberg (ST15), Coeln (ST1995, 2015), the genomes of the lineage Saintpaul I (ST49, 27, 50, 680, 3,602, 1,934) and the genomes of the lineage Reading I (ST1628). The "section Newport" (section A11 in Figure 2) is composed of serovar Kottbus ST212, 808, the genomes of the lineage Newport I (ST31, 45, 47, 132, 157, 614) and Newport II (ST156, 166). Finally, "section Muenchen", (section A10 in Figure 2) composed of the genomes of the lineage Muenchen I (ST82, 112, 1,606, 2,769), Muenchen II (ST83) and the lineage Manhattan I (ST18, 44, 2,200). In Group B, there are two subgroups that we called sections "Montevideo" and "Bredeney." The "section Montevideo" (section B9 in Figure 2) is composed of the genomes of the lineage Montevideo I (ST4, 195) and the genomes of the lineage Oranienburg I (ST179, 864, 1392, 1512). The "section Bredeney" (section B14 in Figure 2) is composed of the genomes of the lineage Bredeney I (ST241, 306, 505, 897), the genomes of the lineage Bredeney II (ST505), the genomes of the lineage Give II (ST654) and the genomes of the lineage Schwarzengrund II (ST96, 322) (Figures 2, 3). Within Group C, we observed the genomes of the lineages Banana I (ST7024), Bareilly I (ST5146) and Cerro I (ST367, 1,593, 2,407) with the genomes of the serovar Indiana.

Interestingly, our analyses reveal that the polyphyletic serovars Banana, Bovismorbificans, Derby, Newport, Muenchen and Reading arose independently on divergent branches of the tree strongly associated with genomes of other serovars (Table 3). For example, the lineage Banana I (ST7024) arose in Group C and is associated with the genomes of the lineage Bareilly I (ST5146), the lineage Cerro I (ST367, 1,593, 2,407) and the genomes of serovar Indiana (ST17, 2040). The lineages Banana II (ST4745), III (ST1035) and IV (ST683, 5,220) arose in Group B. Nevertheless, the lineage Banana II is associated with the lineages Derby II (ST39, 40) and Livingstone II (ST457, 638). The lineage Banana III is associated with the serovar Tennessee (ST319, 1565) and the lineage Rissen II (ST469) and the lineage Banana IV is associated with the lineage Derby III (ST71, 72). On the other hand, contrary to the lineages Derby II and III, the lineage Derby I (ST682) arose in Group A and is associated with serovar London (ST155).

Reference genome panel available in public databases

Given the selected set of high-quality complete genomes and the phylogenetic analyses carried out, we compiled a list of reference genomes with metadata and associated quality data (Supplementary Table 2). We selected 83 complete genomes from the initial set of 650 genomes, with 1 *S. enterica* subsp. *salamae*, 2 *S. enterica* subsp. *arizonae*, 1 *S. enterica* subsp. *diarizonae*, 2 *S. enterica* subsp. *houtenae* and 77 *S. enterica* subsp. enterica, representing 48 serovars and 71 MLST profiles.

Discussion

SNP phylogenetic analysis is the most suitable approach to use in investigations of outbreaks with the goal of clustering epidemiologically related strains and calculating pairwise distance between genomes of the same serovar. However, when analyzing genomic diversity between different serovars,

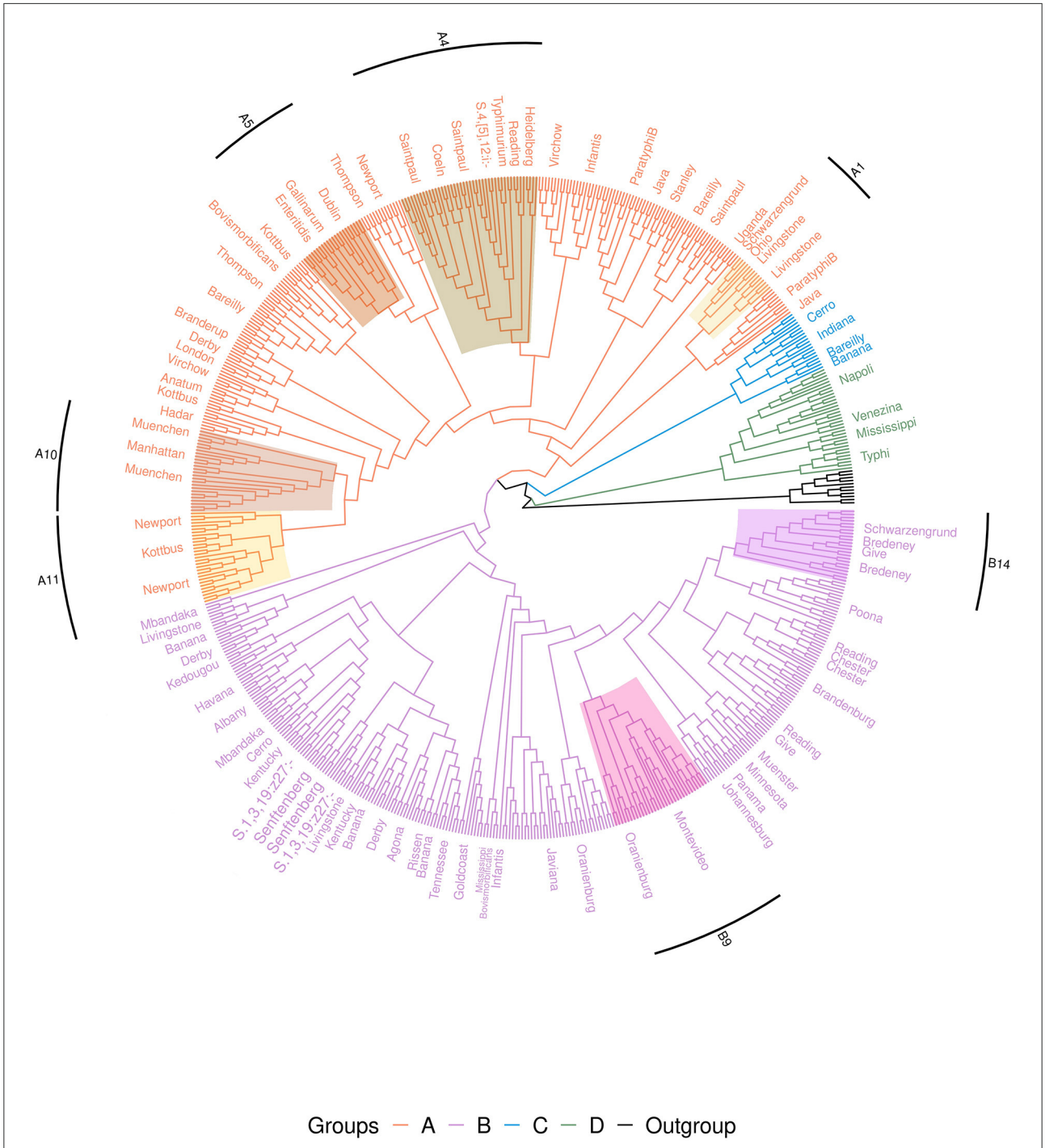

FIGURE 2
Phylogenetic cgMLST distance tree of the 650 genome set of *Salmonella*. The tree is rooted on the *Salmonella entrerica* subsp. *arizonae*, *diarizonae*, *houtenae*, *indica* and *salamae* genomes. For better visualization of the groups, the tree is shown without branch lengths. Branches are colored to distinguish the four groups. Due to the high number of strains in the tree, strain labels are not shown and serovars are indicated along corresponding branches. Strongly supported subgroups are shaded in different colors (for the description of these subgroups see also Figure 3 and Table 3).

the cgMLST and the pan-genome kmer analyses are more appropriate approaches. The cgMLST predictions based on the 3,002 gene scheme are extremely stable (1). In a comparison of more than 100 000 *Salmonella* genomes, the cgMLST scheme can predict serovars with fewer errors than the slide agglutination reference method (1, 12). Moreover, the cgMLST

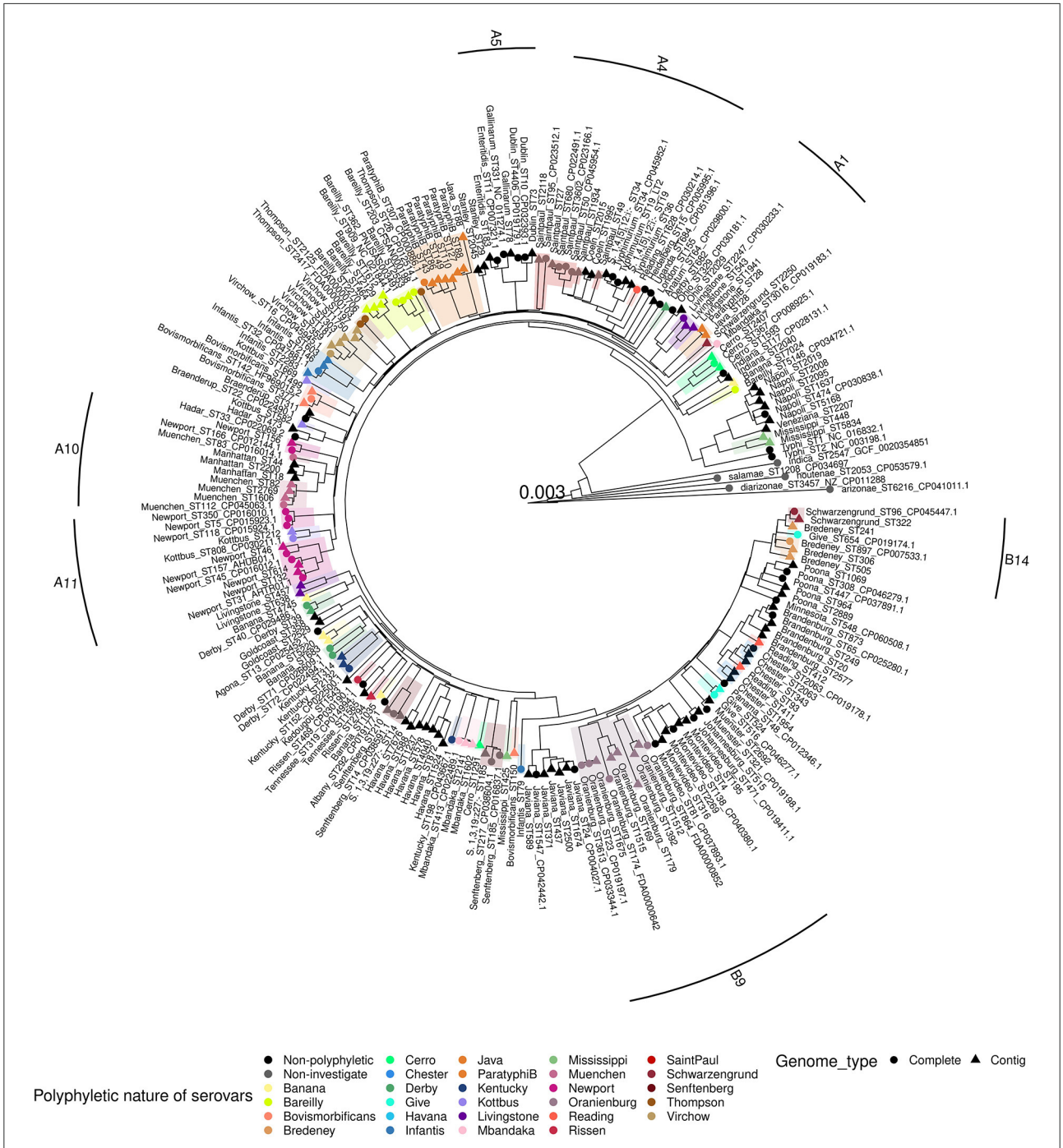

FIGURE 3
Phylogenetic pan-genome kmer distance tree of the 219 genome subset of *Salmonella*. The tree is rooted on the *Salmonella entrerica* subsp. *arizonae, diarizonae, houtenae, indica* and *salamae* genomes. The tree is shown with branch lengths. Polyphyletic serovars are shaded in different colors. Complete genomes are indicated with circles and contigs with triangles. Labels contain the serovar and the MLST profile of each strain. The strongly supported subgroups listed in the Table 3 are highlighted in the margin of the tree. For example, A1 corresponds to the subgroup called "section Livingstone" in Table 3.

method is more widely employed than SNP analysis with large genome panels because it is computationally less demanding (1). Owing to these advantages, the hierarchical clustering of cgMLST sequence types was also chosen in EnteroBase as the method of choice to map new bacterial strains to predefined population structures at multiple levels of resolution (9). On the other hand, accessory genes contribute to ecological specialization and the pattern of horizontal gene transfer

TABLE 2 Polyphyletic and monophyletic serovars studied.

Serovar and lineage L1 (MLST profile)	L2	L3	L4	Serovar and lineage L1 (MLST profile)	
Banana	ST7024	ST4745	ST683, 5,220	ST1035	Brandenburg	ST20, 65, 249, 873, 2,577
Bareilly	ST5146	ST464, 1,612, 2,129, 2,270	ST203, 362, 909, 2,553		Coeln	ST1995, 2,015
Derby	ST682	ST39, 40	ST71, 72		Dublin	ST10, 73, 4,406
Kottbus	ST1669	ST582	ST212, 808		Enteritidis	ST11, 183
Newport	ST5, 118, 350	ST156, 166	ST31, 45, 46, 132, 157, 614		Gallinarum	ST78, 331
Oranienburg	ST23, 169, 174, 1,515, 1,675, 3,613	ST179, 1,392, 1,512	ST864		Goldcoast	ST358
Reading	ST1628	ST412	ST93		Hadar	ST33, 473
Saintpaul[a]	ST95, 2118	ST49	ST27, 50, 680, 1,934, 3,602		Havana	ST578, 588, 872, 1,237, 1,524, 4,040, 7,676
Bovismorbificans	ST142, 377, 1,499	ST150			Heidelberg	ST15
Bredeney	ST214, 306, 897	ST505			Indiana	ST17, 2,040
Cerro	ST367, 1,593, 2,407	ST1291			Javiana	ST24, 371, 437, 589, 1,547, 1,674, 2,500
Chester	ST411, 1,954	ST343, 2,063			Johannesburg	ST471, 515
Give	ST516, 524	ST654			Kedougou	ST1543
Infantis	ST32, 603, 2,146, 2,283	ST79			London	ST155
Kentucky	ST152, 314, 2,132	ST198			Manhattan	ST18, 44, 2,200
Livingstone	ST543, 1,941, 2,247	ST457, 638			Minnesota	ST548
Mbandaka	ST3016	ST413, 1,602, 2,141			Montevideo	ST4, 81, 138, 195, 316, 699, 2,269
Mississippi	ST448, 5,834	ST425			Muenster	ST321, 2,692
Muenchen	ST83	ST82, 112, 1,606, 2,769			Napoli	ST474, 1,637, 2,019, 2,008, 2,095, 5,168
Paratyphi B[b]	ST28	ST43, 86, 88, 110, 127, 149, 307			Ohio	ST329, 2,029
Rissen	ST2794	ST469			Panama	ST48
Schwarzengrund	ST2250	ST96, 322			Poona	ST308, 447, 964, 1,069, 2,889
Senftenberg[c]	ST14, 210	ST185, 217			Stanley	ST29, 2,045
Thompson	ST26	ST2125, 2,417			Tennessee	ST319, 1,565
Virchow	ST16, 181, 303, 359	ST197, 1,750			Typhi	ST1, 2
Agona	ST13				Typhimurium[d]	ST19, 34, 36
Albany	ST292				Uganda	ST684
Anatum	ST64				Veneziana	ST2207
Braenderup	ST22, 311					

The polyphyletic and monophyletic serovars identified in the pan-genome phylogenetic analysis (kmer approach) are indicated with the corresponding MLST profiles. The polyphyletic serovars are indicated in bold. a: The difference between kmer and cgMLST phylogenetic approaches involves the Saintpaul MLST profile ST1934, which in the cgMLST tree is associated with ST49; b: the MLST profiles ST28, 43, 86, 88, 110, 127, 149 and 307 comprise the genomes of serovars Paratyphi B and Java; c: the MLST profiles ST14 and 185 comprise the genomes of serovars Senftenberg and S. 1,3,19:z27:-; d: the MLST profiles ST19 and ST34 comprise the genomes of serovar Typimurium and S. 4,[5],12:i:-.

among phylogroups can provide important complementary information (31), so that analysis of the pan-genome can lead a better picture of microbial organism proximity (32). Moreover, the pan-genome analysis of thousands of prokaryote samples is possible on a standard desktop without compromising the accuracy of results (33). Last, but not least, neither cgMLST nor kmer pan-genome analyses need a reference genome. This is a crucial point when analyzing the diversity of *Salmonella* genomes represented by a large number of different subspecies and serovars as in this study.

For this study, the 58 most frequently isolated serovars in France, EU and the US with their major sequence-type profiles

TABLE 3 Identified subgroups.

Genotype	
Section	**MLST profile**
Livingstone A1	Livingstone ST543, 1,941, 2,247
	Ohio ST329, 2,029
Typhimurium A4	Saintpaul ST27, 49, 50, 680, 1,934, 3,602
	Coeln ST1995, 2,015
	Typhimurium ST19, 34, 36
	Reading ST1628
	Heidelberg ST15
Enteritidis A5	Enteritidis ST11, 183
	Gallinarum ST78, 331
	Dublin ST10, 73, 4,406
	Berta ST435
Muenchen A10	Muenchen ST82, 83, 112, 1,606, 2,769
	Manhattan ST18, 44, 2,200
Newport A11	Newport ST31,45,46,132, 157,614
	Kottbus ST212, 808
Montevideo B9	Oranienburg ST179, ST864, 1,392, 1,512
	Montevideo ST4, 81, 138, 195, 316, 2,269
Bredeney B14	Schwarzengrund ST96, 322
	Give ST654
	Bredeney ST241, 306, 897, 505
Indiana C	Banana ST7024
	Bareilly ST5146
	Indiana ST17, 2,040
	Cerro ST367, 1,593, 2,407
Typhi D	Typhi ST1, 2
	Mississippi ST448, 5,834
	Veneziana ST2207
	Napoli ST474, 1,637, 2,008, 2,019, 2,095, 5,168

The subgroups called "section Livingstone, Typhimurium, Enteritidis, Muenchen and Newport" belong to Group A. The subgroup referred to as "sections Montevideo or Bredeney" belong to Group B. The subgroup "section Indiana" belongs to Group C and "section Typhy" to Group D.

were selected with a view to human health, animal health and the agri-food safety sector at the national and international levels. From the first set of 650 genomes analyzed for these 58 prevalent serovars, the final taxon sampling genomes was composed of five outgroups and 214 ingroup *S. enterica* subsp. *enterica* strains. The cgMLST and pan-genome kmer phylogenetic analyses both uncovered a deep split that delineates four sister groups within *S. enterica* subsp. *enterica*, including the two previously partially described groups (14, 25, 31). Although many of the relationships reconstructed in this study are consistent with previous reports, our taxon dataset provides a more thorough interpretation of polyphyletic serovars than any other study. The large selection of serovars and sequence-type profiles included allowed deeply appreciating the relationship between serovars, their genomic lineages and MLST profiles.

For each serovar, we included a larger selection of sequence-type profiles than previously. This large diversity gave a good overview of the complexity of the genetic diversity in *S. enterica* subsp. *enterica* and identified 25 polyphyletic serovars, 17 of which have never been described before, such as Banana, Bareilly, Kottbus and Reading for which we identified three distinct lineages, with the exception of Banana characterized by four lineages. Finally, among the 25 polyphyletic serovars identified, one serovar was characterized by four distinct lineage, seven by three and 17 by two distinct lineages. All of these serovars, such as Newport and Derby (13, 34), likely derive from multiple independent ancestors during the evolutionary history of *Salmonella*. Interestingly, *via* the whole-genome comparisons, we demonstrated for Derby (4, 13), as previously shown for Newport (35), that heterogeneity between lineages mostly occurs in the prophage regions and that lineage-specific characteristics are also present in the *Salmonella* pathogenicity islands and fimbrial operons. Further analyses are needed to investigate the other polyphyletic serovars identified in this study.

Although 25 polyphyletic serovars have been identified in our taxon dataset, there are probably more. For example, the serovars Agona, Havana and Montevideo were not identified as polyphyletic in our study, but have been described as such previously (14, 25, 36). The MLST profiles selection parameters applied in this study (i.e., for each serovar, we included the MLST profiles with 10 or more genomes in the EnteroBase database on 19 July 2021) did not make it possible to highlight the polyphyly of these three serovars. Furthermore, the number of distinct lineages for a polyphyletic serovar also depends on the taxon dataset selected. For example, we previously described four distinct lineages for the serovar Derby (37). In the dataset selected for the study of the diversity of the serovar Derby in France, the genomes belonging to ST39, even if most closely related to ST40 genomes (i.e., with an average of 3,962 SNPs and an SD of 20 SNPs), were identified as an independent lineage.

The influence of the dataset on the results was also observed on the genomic groups identified. In our panel, we underlined strongly supported subgroups that confirm previous observations (14, 25, 31, 34, 38). We called these subgroups "sections" to echo previous descriptions. For example, in this study, "section Typhimurium" comprises the genomes of *Salmonella* Typhimurium, Heidelberg and Saintpaul (14) and encompasses the genomes of *Salmonella* Typhimurium ST19, 34, 36, Heidelberg ST15, Saintpaul ST27, 49, 50, 680, 1,934, 3,602 as well as Coeln ST1995, 2,015 and Reading ST1628. When describing genomic serovar associations, the ST profiles must also be mentioned. For example, in the "section Typhimurium," only the genomes of serovar Reading ST1628 belong to this section, because this serovar is polyphyletic and the two other Reading lineages ST412 and 93 are genetically distant.

This study confirms that, since the advent of WGS and advances in knowledge on the genomic diversity in *S. enterica* subsp. *enterica*, it is no longer possible to cite a serovar without referring at least to its ST group (1, 8, 38). The serovar-based nomenclature is still useful to maintain a link with data collected in the past and to continue to ensure smooth communication at the international level with testing laboratories and countries that have limited access to molecular techniques. Furthermore, European regulations regarding zoonoses stipulate that the serovar name is the mandatory reference nomenclature for *Salmonella*. Nevertheless, MLST profile information should be added to the serovar whenever possible. When the genome is available, the information regarding the MLST profile is easily accessible on open-access bioinformatics platforms such as EnteroBase or *via* the Center for Genomic Epidemiology (CGE) website (http://www.genomicepidemiology.org/) (39). For some laboratories that do not have the resources to perform WGS, it is still possible to determine the MLST profile with a PCR thermocycler and small first-generation sequencers. These labs can also send the PCR amplicons to sequencing companies for a lower cost than for a WGS system.

Furthermore, to avoid creating a two-tier system, it is the role of reference laboratories to provide genomic phylogenetic analyses, such as the analyses carried out in this study, to allow other laboratories to locate their strains on the trees.

Associating the historical name of the serovar with its MLST profile is an essential step toward the future of the nomenclature of *Salmonella*, which should have the advantage of clearly identifying the genomic lineage to which it belongs and indicating possible close links with other serovars (8, 9, 40). This association will also make it possible to obtain a more precise vision of the prevalence of certain serovars (and their genomic lineages) in various sectors, e.g., are all three Reading serovars prevalent in the poultry sector or is it only one of its three genomic lineages adapted to this sector?

In this study, we also provide a list of complete genomes that can be used as references and point out the absence of complete genomes for the following serovars: Banana, Kedougou, Mississippi and Veneziana. We also note the absence of complete genomes for the following lineages: Bareilly L2, Bovismorbificans L2, Chester L1, Infantis L2, ParatyphiB L1, Reading L2 and L3, Rissen L1, Saintpaul L2 and Schwarzengrund L1. We are currently sequencing short and long reads of these serovars to provide high-quality reference genomes for them.

Finally, in response to future outbreak situations or One Health surveillance of prevalent *Salmonella* and other emerging serovars, our study opens the way to a better understanding of the genomic diversity of *S. enterica* subsp. *enterica* and sheds light on the prevalent polyphyletic serovars at the national and international levels.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

Conceptualization and supervision: SC-S. Methodology, writing – original draft preparation, writing – review & editing, and visualization: EC and SC-S. Formal analysis: GI and EC. Data curation: EC, VN, and SC-S. All authors read and approved the final manuscript.

Funding

This work was supported by the project Cross-sectoral framework for quality Assurance Resources for countries in the European Union (CARE), which has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 773830.

Acknowledgments

We thank the ANSES *Salmonella* Network team of the LSAl-Anses, Laetitia Bonifait of the LNR-Anses and Dominique Clermont of the CIP (Institut Pasteur) for providing isolates for sequencing. We would like to thank Corinne Danan for the suggestions given during the proofreading of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.963188/full#supplementary-material

SUPPLEMENTARY TABLE 1
Accession number, taxonomic affiliation, MLST profiles and genomics data for the panel of 650 *Salmonella* strains analyzed. Genomes selected for the second 219 genome subset are shown in bold and gray fill color.

SUPPLEMENTARY TABLE 2
List of reference genomes with metadata and associated quality data for the *Salmonella* serovars identified as being the most frequently isolated in humans and the food sector over a period of 10 years (from 2006 to 2016) in the United States, Europe and France. Complete genomes selected for the second 219 genome subset are shown in bold and gray fill color.

SUPPLEMENTARY FIGURE 1
Venn diagrams illustrating the 47 *Salmonella* serovars identified to be common to the United States, Europe and France by logical relation analyses showed in Figure 1. The serovars are illustrated separately for agri-food isolates and human cases. To these 47 serovars, eleven others were added because they belong to the top 20 serovars from each country but were not common to all countries. The final list of 58 serovars retained for this study is showed in Table 1.

SUPPLEMENTARY FIGURE 2
Phylogenetic cgMLST distance tree of the 650 genome set of *Salmonella*. The tree is rooted on the *Salmonella entrerica* subsp. *arizonae*, *diarizonae*, *houtenae*, *indica* and *salamae* genomes. The tree is shown with branch lengths. Branches are shaded with different colors to distinguish the five groups identified.

SUPPLEMENTARY FIGURE 3
Phylogenetic cgMLST distance tree of the 219 genome subset of *Salmonella*. The tree is rooted on the *Salmonella entrerica* subsp. *arizonae*, *diarizonae*, *houtenae*, *indica* and *salamae* genomes. The tree is shown with branch lengths. Polyphyletic serovars are shaded in different colors. Complete genomes are highlighted with circles and contigs with triangles. Labels contain the serovar and the MLST profile of each strain.

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 24 November 2022
DOI 10.3389/fpubh.2022.1003917

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention,
a section of the journal
Frontiers in Public Health

RECEIVED 26 July 2022
ACCEPTED 08 November 2022
PUBLISHED 24 November 2022

CITATION
Le Bouquin S, Lucas C, Souillard R, Le Maréchal C, Petit K, Kooh P, Jourdan-Da Silva N, Meurens F, Guillier L and Mazuet C (2022) Human and animal botulism surveillance in France from 2008 to 2019.
Front. Public Health 10:1003917.
doi: 10.3389/fpubh.2022.1003917

Human and animal botulism surveillance in France from 2008 to 2019

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Botulism is a human and animal neurological disease caused by the action of bacterial neurotoxins (botulinum toxins) produced by bacteria from the genus *Clostridium*. This disease induces flaccid paralysis that can result in respiratory paralysis and heart failure. Due to its serious potential impact on public health, botulism is a closely monitored notifiable disease in France through a case-based passive surveillance system. In humans, this disease is rare, with an average of 10 outbreaks reported each year, mainly due to the consumption of contaminated foods. Type B and to a lesser extend type A are responsible for the majority of cases of foodborne botulism. Each year, an average of 30 outbreaks are recorded on poultry farms, about 20 cases in wild birds and about 10 outbreaks in cattle, involving a large number of animals. Mosaic forms C/D and D/C in birds and cattle, respectively, are the predominant types in animals in France. Types C and D have also been observed to a lesser extent in animals. With the exception of botulinum toxin E, which was exceptionally detected throughout the period in wild birds, the types of botulism found in animal outbreaks are different from those identified in human outbreaks over the last ten years in France and no human botulism outbreaks investigated have been linked to animal botulism. In line with the One Health concept, we present the first integrative approach to the routine surveillance of botulism in humans and animals in France.

KEYWORDS

botulism, poultry, wild bird, One Health, surveillance, bovine

Introduction

Botulism is a neurological disease common to humans and animals, caused by the action of botulinum toxins (BoNT) produced by bacteria from the genus *Clostridium*. There are seven BoNTs described historically, identified from A to G. Human botulism is mainly associated with toxins A, B, E and F (1) and animal botulism with toxins C, D and the mosaic forms C/D and D/C (1, 2). BoNT are recombinant BoNT types. BoNT C/D is composed of the light chain of BoNT C and the heavy chain of BoNT D and BoNT D/C is composed of the light chain BoNT D and the heavy chain of BoNT C (3). Botulism occurs on all continents and is variable in incidence. In all species, the disease presents with flaccid paralysis, including respiratory and heart failures (1). Animal botulism affects many species, mainly birds and cattle in France (4), but also fur animals (i.e., minks or foxes) in northern European countries (5, 6) and horses in the United States (7). Based on the current knowledge available, intoxication is the main mode of contamination of cattle at the origin of clinical signs. It is therefore the ingestion of preformed toxins in food, water or any contaminated substance that is currently considered the cause of botulism. Avian botulism is the result of consumption of *Clostridium botulinum* spores. It is assumed that toxin production occurs *in vivo*. Ingested spores germinate, proliferate and produce toxin primarily in the cecum. Absorption of toxin formed in the digestive tract is responsible for the symptoms (8). In humans, it is a rare disease. Five types of botulism are typically described in humans, depending on the mode of contamination and exposure to the toxin: foodborne botulism, intestinal botulism, wound botulism, iatrogenic botulism and inhalational botulism (9, 10). Foodborne botulism and infant intestinal botulism are the two most common forms observed (11).

Animal botulism is considered an emerging problem in Europe (12). At the European level, botulism is monitored through the surveillance of zoonoses and zoonotic agents and the protection of workers (exposure to biological agents at work). In France, the regulatory framework requires mandatory official notification, both in humans and in animals, regardless of the species affected. Human botulism has been monitored by the French health authorities since the establishment of the National Reference Center for Anaerobic Bacteria and Botulism (NRC, *Institut Pasteur de Paris*) in 1978 and reporting the disease to *Santé Publique France* (SPF) has been compulsory since 1986. Any suspicion of human botulism requires notification to the regional health agency (ARS) and its biological confirmation by the NRC. In animals, botulism has been regulated since 2006, first in poultry and then in wild birds and cattle. Until then, it was classified as a first category health hazard for all susceptible species (13). With the promulgation of the Animal Health Law at European level in 2016 (14), the status of this disease has changed, because it does not appear as such in the list of diseases transmissible to animals or humans that must be subject to fixed prevention and control measures. A National Reference Laboratory for avian botulism was designated in France in 2011 (NRL, ANSES Ploufragan-Plouzané-Niort Laboratory).

Case reports of human and animal botulism are regularly published, but studies compiling surveillance data on botulism are scarce, particularly with respect to animal botulism.

Here, we present the results of human and animal botulism surveillance based on SPF data as well as NRC and NRL biological investigations. First, annual variability in the occurence of botulism is discussed, followed by a description of the outbreaks observed.

Materials and methods

Definitions

Before analyzing the surveillance data, it is important to note the differences in definition between the terms "case" and "outbreak" of botulism in human and animal health. In human health, a case of botulism refers to a single individual, whereas an outbreak of botulism refers to one or more individuals infected from a single source. In animal health, the terms case and outbreak refer to two different animal populations, regardless of the number of animals involved: the term case is only used for infections in wildlife, and the term outbreak is used for infections in domestic animals.

The incidence rate defined as the number of cases per 100,000 habitants was used in the following analysis for human botulism considering a French population of 65,9 millions over the 2008–2018 period according to Insee data (15).

This terminology will be used throughout the article.

Data availability and study periods

Historically, the NRC diagnosed botulism in both humans and animals. In response to the sharp increase in the number of outbreaks reported on poultry farms in the late 2000s (16), an NRL for avian botulism was created at the ANSES Ploufragan Laboratory (Brittany, France) in 2011. Since then, some of the animal diagnoses have been carried out there, first on poultry and now also on wild birds. In 2017, the NRL also started to diagnose outbreaks in cattle. Here, this summary presents the results of human botulism surveillance based on epidemiological data from Santé Publique France (SPF, the French Public Health Agency) and the NRC's biological investigations, and those of animal botulism based on confirmed cases transmitted by the two reference laboratories, the NRC and the NRL. All reports of human botulism are recorded by the French health authority through SPF and human cases are confirmed by the NRC. These data concern metropolitan

France and overseas. However, suspicions of animal botulism are not always confirmed or even tested, in particular those involving wild birds. Our analysis covers the period since 1987 with a focus on 2008–2018 for human botulism (17–19) and the period since 2005 with a focus on 2009–2019 for animal botulism. It was not possible to study exactly the same period in humans and animals. Nevertheless, the period considered for both covers a decade. A complementary analysis was carried out using NRL data to provide a more detailed description of the characteristics of the disease and its occurrence in animals since 2013.

Diagnostic methods

Given that the symptoms are usually very typical, a presumptive diagnosis can be made on the basis of clinical findings alone, regardless of the species. However, several diseases are included in the differential diagnosis, and laboratory investigations are requested for the definitive diagnosis. In humans, the confirmatory diagnosis is based on the detection and identification of BoNT in serum and stool and/or the detection of the neurotoxigenic bacterium *C. botulinum* and some strains of *Clostridium baratii* and *butyricum* in stool or gastric contents. The bacterium and its toxin can also be tested for in suspect foods (20). The gold standard for the diagnosis of botulism is the mouse bioassay (21). Alternative methods such as Endopep-MS (22) have been developed, but are not currently used in France for the diagnosis of human botulism.

As in humans, clinical signs of animal botulism are evocative but not specific and are part of a differential diagnosis. Laboratory analyses are required to confirm the diagnosis established on clinical signs. There is no standard for the diagnosis of animal botulism and several laboratory methods are used. As in humans, the aim is to detect either the BoNT or BoNT-producing clostridia (23). Detection of BoNT-producing clostridia, often conducted using polymerase chain reaction (PCR) tools, could be questioned as this bacteria is ubiquitous. Based on the low prevalence of samples collected from asymptomatic animals and providing positive PCR results (24–26) compared to the high prevalence detected in animals with signs of paralysis (27), detection of BoNT-producing clostridia appears to be a valuable diagnostic strategy (23). Before 2010, diagnosis of animal botulism in France generally involved detecting BoNT in serum using the mouse bioassay (28), method that has been considered as the gold standard for laboratory confirmation of botulism for a long time. However, this bioassay does not discriminate between mosaic forms and non-mosaic forms. Today, the approach commonly used in France to confirm animal botulism is the detection of *C. botulinum* in biological samples such as feces, digestive contents as well as organs using PCR after an enrichment step in anaerobic broth (2, 29, 30). This choice has been made on the basis of the efficiency of this approach (user-friendly, time-saving, cost, ethical aspects) for detecting BoNT-producing clostridia in animals with clinical signs.

Statistical methods

The variability of the number of human botulism cases was analyzed using the R incidence package (31). The log-linear regression model of the package was used. The fitted model is of the form $\log(y) = r \times t + b$ where y is the incidence, t is the number of year since the first year of the analysis, and b is the intercept. The value of the parameter r characterizing the annual growth rate and its 95% confidence interval was determined using the fit() function of this package.

The results and graphs for animal botulism were produced in R (32), R-4.1.1 version using the ggplot2 package (33). The networkD3 package (34) was used for preparing Sankey diagrams.

Food description

Foods involved in human botulism outbreaks were described with Foodex2 terminology (20). The foods at the origin of the outbreaks were described with term and facet as detailed as possible. FoodEx2 was also used to defined groups of food and the production method (see Supplementary material 1).

Results

Occurence of human and animal botulism cases and outbreaks in France

Human botulism

Figure 1 shows the number of cases of human botulism observed since the establishment of an official surveillance system in France. The number of outbreaks appears to have decreased significantly during the 1987–2018 period (Figures 1A,C). The annual number of cases and outbreaks of foodborne human botulism in France has remained stable over the last 10 years (Figures 1B,D) with an incidence rate of 0.02 per 100,000 population. The annual number of outbreaks ranged from 3 to 13 outbreaks (average of 7.5 outbreaks) and for the number of cases per year from 4 to 25 (average of 14.5 cases). Of the 100 outbreaks of human botulism during the 2008–2018 period, 82 (89.8% of cases) were foodborne, 17 (9.6% of cases) were cases of infant intestinal botulism and 1 (0.6% of cases) were a wound botulism case observed in 2008 following an open leg fracture in a road traffic accident. No cases of infectious botulism in adults (intestinal colonization) were observed. The

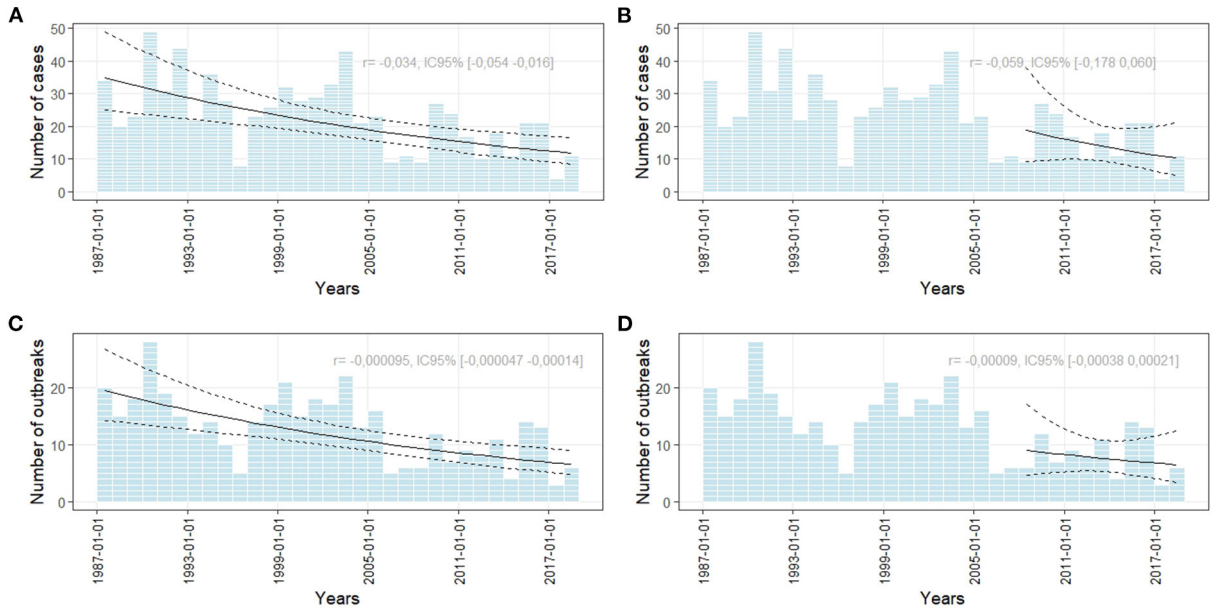

FIGURE 1
Number of foodborne human botulism outbreaks and cases based on NRC data. The curves represent a trend analysis over the period 1987–2018 **(A,C)** and over the period 2008–2018 [panel **(B,D)**]. *r* represents the growth rate of the log-linear model used for assessing the growth or decline of the number of cases or oubreaks.

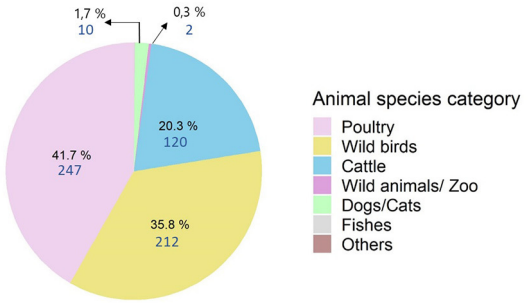

FIGURE 2
Distribution of animal botulism outbreaks from 2009 to 2019 by species (n = 592).

82 foodborne botulism outbreaks represented a total of 159 cases. The maximum number of people involved in a single outbreak was six.

Animal botulism

For the 2009–2019 period, 592 outbreaks of animal botulism were observed (Figure 2). Botulism was mainly detected in poultry (n = 247 or 41.7%), wild birds (n = 212, 35.8%) and cattle (n = 120, 20.3%). There were also a few outbreaks in dogs/cats between 2010 and 2015 (n = 10), fish in 2014 (n = 1) and wild/zoo animals in 2009 and 2011 (n = 2). Only the three major animal categories (poultry, wild birds and cattle) were analyzed in this study.

The annual average number of outbreaks in poultry farms recorded between 2005 and 2011was 53.0 (SD = 21.3), with a sharp increase in 2007 when a peak of 95 outbreaks was observed (Figure 3). The origin of this peak has never been identified. Since 2011, this number has decreased to an average of 17.4 (SD = 3.8) outbreaks per year. Each year, an average of 21.7 (SD = 11.0) cases are recorded in wild birds and 10.9 (SD = 5.0) outbreaks in cattle. However, this number fluctuates from year to year.

Description of botulism cases and outbreaks

In humans

Over the period 2008–2018, type B was responsible for 53 (64%) outbreaks and 106 (67%) cases of foodborne botulism and type A for 15 (18%) outbreaks and 30 (19%) cases (Figure 4). Types E (two outbreaks) and F (two outbreaks) were responsible for four outbreaks involving four and five cases, respectively. Finally, for 10 outbreaks (14 cases) it was not possible to determine the BoNT type involved in the outbreaks or the cases (due to missing, insufficient or delayed biological samples, or unidentified or unavailable food).

Due to the unavailability of food for analysis, identification of contaminated food was only possible in 41 (50%) outbreaks

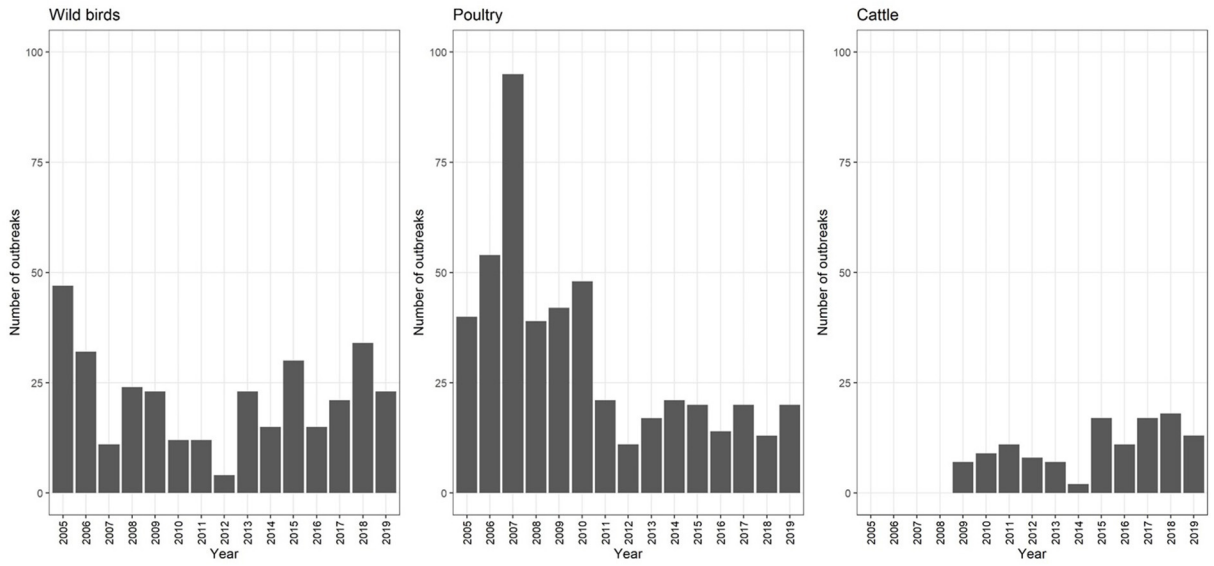

FIGURE 3
Evolution of the number of botulism cases in wild birds (2005–2019), outbreaks in poultry (2005–2019) and cattle (2009–2019) (n = 592).

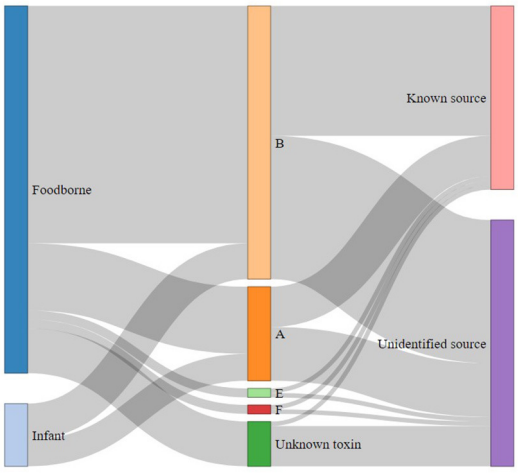

FIGURE 4
Distribution of human outbreaks (n = 82) and infant botulism outbreaks (n = 14) according to botulinum toxin type and case origin identification over the period 2008–2018 in France.

(Figure 5). Considering that cases of infectious botulism (intestinal colonization) are rare, those outbreaks are considered to be foodborne even if the food at the origin to BoNT has not been identified. The most common types of food involved in human botulism outbreaks were canned foods and homemade products. The two main food sources were raw ham (n = 17) and canned vegetables (n = 12). Three composite foods, i.e., smoked fish, salted fish and minced meat, were also the source of botulism outbreaks. For each outbreak with identified food, a detailed description on the foods according to FoodEx2 classification (35), together with the toxin type and the number of cases per outbreak is provided in Supplementary Table 1.

Of the 14 reported cases of infant botulism, 6 were of type A and 8 of type B. All the putative food samples possibly involved and analyzed during the investigations were negative and the origin of these cases remains unexplained.

In poultry

From 2009 to 2019, the most common BoNT type in poultry was BoNT C/D (n = 112, 48.7%), like in wild birds. BoNT D (n = 45, 19.6%) and D/C (n = 27, 11.7%) were also frequently detected (Figure 6). No BoNT E was recorded in France during the study period.

Based on the data available from the NRC and the NRL, the species most affected by botulism were turkeys (n = 41 outbreaks, 51%), followed by birds of the genus *Gallus* (laying hens and broilers) (n = 28 outbreaks, 35%). BoNT D/C was more frequently encountered in turkeys than in other species. Among the 49 occurrences with known toxin types, BoNT D/C represented 31% of the outbreaks (n = 15). In *Gallus* the majority of the 33 occurrences were due to BoNT C/D (n = 28, 85%). For guinea fowl, BoNT C/D was the most common (n = 7, 50%). Only three occurrences were observed over the period in ducks, two of which were associated with BoNT C/D.

Of the 64 outbreaks for which information is available, the majority of cases occurred at the end of the breeding period, regardless of the species. The median age of onset of the disease in turkeys (n = 37), broilers (n = 19) and guinea fowl (n = 6) was 88, 43 and 47 days, respectively. Few data are available on

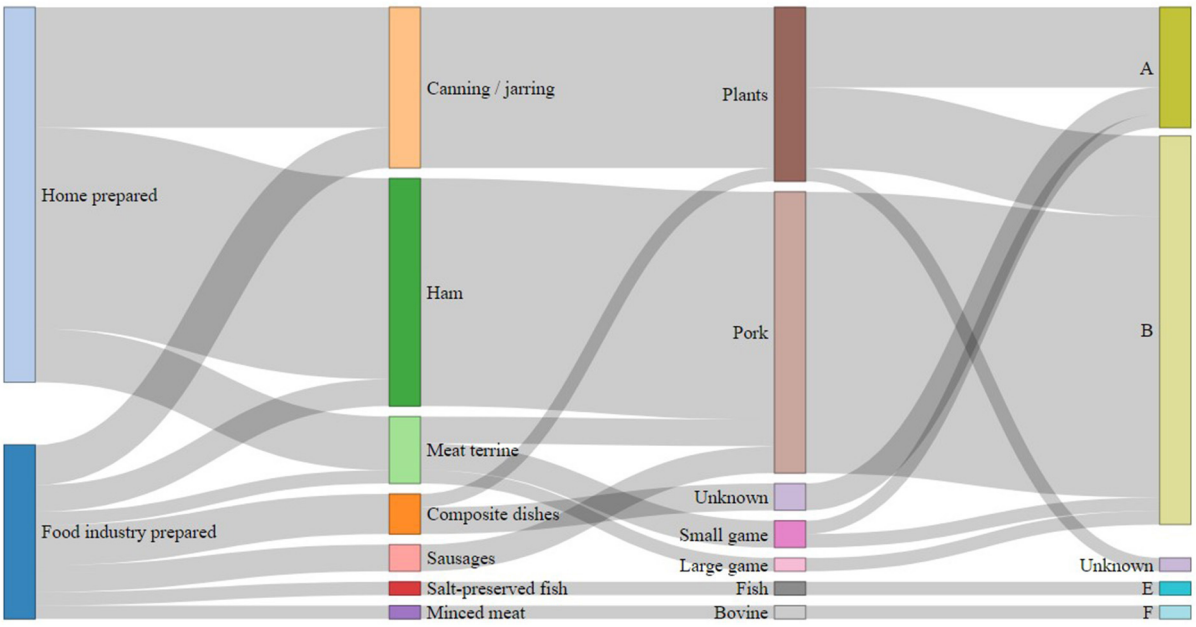

FIGURE 5
Distribution of foodborne botulism outbreaks with identification of the food source ($n = 41$) in France between 2008 and 2018 according to the type of preparation, nature of the food, origin of the food and type of botulinum toxin.

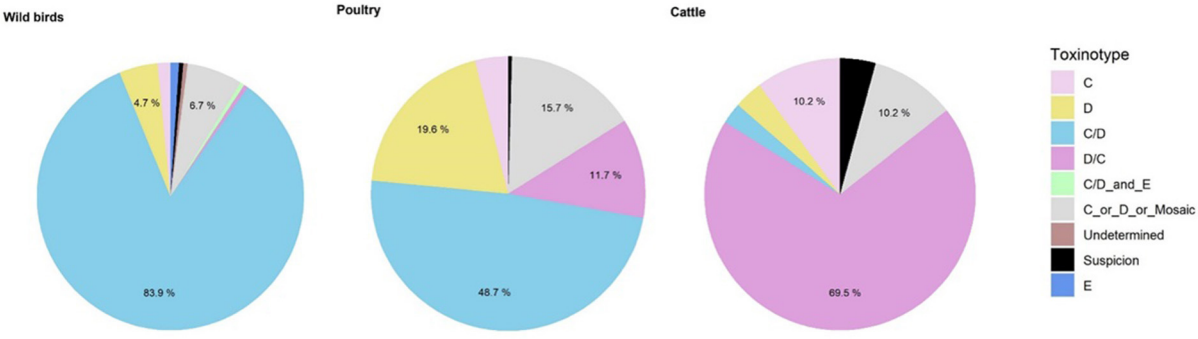

FIGURE 6
Distribution of botulism toxins from 2010 to 2019 for wild birds ($n = 193$), poultry ($n = 231$) and cattle ($n = 118$).

the poultry production stages. Of the few data available ($n = 44$), BoNT C, D and C/D were observed in the meat stage for all species. Only one C/D outbreak was observed on a breeding farm. Of the 14 outbreaks that occurred between 2013 and 2019 for which this information is available, half involved certification label or organic poultry, the other seven involved standard or certified poultry.

Most of the outbreaks occurred in Brittany ($n = 32$, 42%). Of the 91 outbreaks studied since 2013, almost half were observed in the third quarter of the year ($n = 43$, 47.3%), with a large number observed in the fourth quarter ($n = 24$, 26.4%).

In wild birds

Since the development of laboratory techniques to distinguish mosaic forms and their implementation for routine analysis (2010), botulism type C/D has been the most common ($n = 162$, 83.9%) (Figure 6). Three outbreaks involving *C. botulinum* BoNT E were detected in 2018 in wild birds [mute swan (*Cygnus olor*), mallard duck (*Anas platyrhynchos*) and stork (*Ciconia ciconia*)], always associated with *C. botulinum* C/D.

The bird species most affected by botulism are those belonging to the family *Anatidae* (geese, swans, ducks, etc.) ($n = 71$ outbreaks, 87%). Among the 74 occurrences of toxin

types found in *Anatidae*, BoNT C/D was the most frequent (n = 71, 96%). Botulism outbreaks in other species of wild birds were less common (n = 11, 13%) (*Laridae*: seagulls, gulls, etc. and *Rallidae*: rails, coots, etc.). BoNT C/D was again the most common toxin type in these species.

The cases were distributed across the whole country and were more frequently observed during the third quarter of the year (i.e., July, August, or September) (n = 52, 80%). A smaller proportion of outbreaks was observed in the fourth quarter (n = 13, 20%). Only one case was recorded in the first quarter of the year.

In cattle

For cattle, BoNT D/C was the most prevalent toxin type (n = 82, 69.5%) followed by BoNT C (n = 12, 10.2%) (Figure 6). No BoNT D outbreaks have been confirmed on cattle farms in France in recent years. Regarding the seasonality of bovine botulism outbreaks, the 36 outbreaks observed appear to be spread over the first (n = 8, 22%), second (n = 14, 39%) and third quarters (n = 9, 25%). A few outbreaks were also observed in the fourth quarter (n = 5, 14%), with no evidence of a seasonal effect.

Most outbreaks occurred in Brittany (n = 20, 56%). The median age of onset of the disease was 27 months in affected cattle.

Discussion

The analysis of surveillance data made it possible to assess human and animal botulism in France. Our study confirms that the disease is present in many species, being rare in humans with an occurence of 10 persons affected per year, and much more common in animal species, essentially in birds (wild and poultry) and cattle, which are the two most affected categories of animals. Each year, on average, 10 outbreaks are recorded in the bovine sector, 30 in poultry sector and 20 cases in wild birds, each of which can affect several thousand birds (30).

At the European level, human data come from systems equivalent to the French mandatory reporting system (36, 37). These surveillance systems can be considered as exhaustive for the detection of severe forms of botulism. The number of confirmed cases over the 2011–2018 period was relatively stable with ~100 cases reported per year. The incidence rate in Europe is around 0.02 cases per 100,000 inhabitants, similar to the incidence rate in France (36). The countries with the highest number of confirmed cases are Italy, the United Kingdom, Poland, Romania and France. In Italy, 466 cases of botulism were identified from 1986 to 2015: 93% were foodborne botulism, infant botulism accounted for only 6% of cases and wound botulism for 1% (38). In Turkey between 1983 and 2017, 95 cases of botulism were identified, and the food category primarily responsible for the cases was home-canned vegetables (39). In Ukraine, between 1955 and 2018, 8614 cases of botulism were reported (40).

Infant botulism is the most common form of botulism in the United States and has accounted for 80% of reported cases of childhood botulism worldwide since this form of the disease was first recognized in 1976 (41). It has an average annual incidence rate of 2.1 cases per 100,000 live births (42). A recent review covering the 1976–2016 period identified 1345 cases (6.5 cases/100,000 live births/year) caused by types A, B, Ba, Bf and F in the state of California (43). The average annual incidence rate was calculated at 4.3 cases per million live births in Canada during the period 1979–2019 (44).

For animals, few data of surveillance are available at the global level and most of them come from France, where this disease is particularly monitored in animals. Botulism has previously been reported in 264 bird species representing 39 families (30). *Anatidae* is one of the most affected families, at least in France, as highlighted in our study and in at least one other study (16). In poultry, the species affected by botulism outbreaks are broilers, turkeys, pheasants and, to a lesser extent, ducks, laying hens (raised on litter or free-range only), geese, quails and guinea fowl (2, 16, 45–47). For cattle, only case reports are available in the literature and prevalence has not been reported.

Regarding other animal species, few are affected by botulism in France. A few cases were observed in domestic carnivores (cats and dogs) and only one case was reported in fish during this period. Information on the presence in fish is of great importance, because fish may be naturally affected by type E botulism responsible for human botulism. Mortalities due to botulism type E have been described around the world in wild species (e.g., the round goby *Neogobius melanostomas* in the Great Lakes region of North America, the catfish *Ictalurus punctatus* in the Mississippi Delta in the United States) (48–50). Regularly described on aquaculture farms from the 1960s to the 2000s (especially on trout or salmon farms), botulism outbreaks in aquaculture seem to have become rare, due in part to changes in farming and health management practices. The only relatively recent references, apart from those relating to cases of botulism E affecting fish in the Great Lakes region (48), involve botulism in catfish reported from some farms in the southern United States of North America (51, 52).

Our analysis of occurrences during the period studied here shows that the incidence of human botulism has been relatively stable over time. Similarly, animal botulism also appears to experience relative stability, although there are annual variations for which the origin cannot always be identified. Comparison over a longer period is made difficult by the changes in the animal botulism surveillance system in France over time and especially the significant development of diagnostic methods. Before 2010, the BoNT detection method did not allow the identification of mosaic forms, and different analytical methods were used to differentiate between BoNT

types. The characteristics of the tests have also improved, with the optimization of sampling methods (choice of matrices, sampling protocol, transport and storage methods) (2, 46) probably leading to better sensitivity in regards to detection or diagnostic confirmation. Nevertheless, there are still situations, particularly in the bovine sector, where clinical suspicions strongly suggestive of the disease cannot be confirmed by laboratory analyses. For example, sera collected on symptomatic animals are often negative for BoNT using the mouse bioassay (45), probably because BoNT is not circulating any more when the sample is collected. A difference in sensitivity to BoNTs between mice and cattle could also be hypothesized. It has indeed been suggested that cattle are 12.88 times more sensitive to BoNT C than a mouse on a per kilogram weight basis (53), BoNT C has moreover been shown to be the least toxic BoNT types for mice (54). On the contrary, mice are extremely sensitive to BoNT D/C, which harbored the highest toxic activity among tested BoNT types (54). While a difference in sensitivity between mice and cattle may explain the failure of the mouse bioassay to detect BoNT C in serum samples from cattle, this seems unlikely as far as BoNT D/C concerns considering the high sensitivity of mice to this BoNT type. Detection of BoNT-producing clostridia could be sometimes tricky when contamination is low and not homogenous within the matrix. Several matrices (liver, ruminal content, fecal samples…) collected from different symptomatic animals should be analyzed to make sure *C. botulinum* will be detected.

The cases presented in this report correspond to those identified by the NRC and the NRL. In France, any suspicion that is submitted for laboratory diagnosis currently goes through a reference laboratory. The severe forms of human botulism are probably reported exhaustively (mild forms may not be detected in humans, e.g., solely involving digestive discomfort), but it is likely that a certain number of animal botulism suspicions are not reported, and their extent cannot be assessed. This under-reporting is probably limited in the cattle sector. In the poultry sector, because botulism outbreaks occur at the end of the rearing period, we cannot exclude the possibility of flocks being sent to slaughter at the start of an outbreak of botulism. Surveillance of botulism in wild birds, which is based on event-based surveillance, leads to an obvious under-representation of cases, but it is not possible to assess to what extent.

Analysis of the toxin types occurring in France confirmed the predominance of types A and B in human botulism—in both foodborne and infantile cases—and exceptionally type F (55, 56). At the international level, BoNT types that cause human cases are types A and B, followed by E and, occasionally, F. A meta-analysis of outbreaks including 197 outbreaks of foodborne botulism (nearly half of which involved outbreaks in the US) identified BoNT A, B, E, and F as the causative BoNT in 34, 16, 17, and 1% of outbreaks, respectively (57). BoNT B is the most prevalent BoNT in France, like in Poland where type B represented 83% of the cases in 2016 (58). In Italy, from 1986 to 2015, BoNT B was involved in 79.1% of cases (261/330), followed by BoNT A (9.7%, 32/330), with BoNT F, Ab, and Bf, accounting for 0.3 (1/330), 1.5 (5/330), and 0.6% (2/330) of all cases, respectively (38). In Ukraine, BoNT B (59.64%), E (25.47%), and A (7.97%) are the most common, with cases related to BoNT C being very minor (0.56%) and only suspected (40). In North America, foodborne botulism outbreaks originate from vegetables (home-canned), but mostly BoNT E, originating from fish or marine mammals prepared in indigenous communities using traditional methods (e.g., fish fermentation) (42). Similarly, in various Asian countries, outbreaks typically arise from traditional food preparations (59–61).

The C/D mosaic form is the predominant BoNT in birds in France. BoNT C and D are also observed, but to a lesser extent. Other European countries report similar findings on field collections of strains from animal botulism outbreaks (3, 62). Although the majority of bird species are experimentally sensitive to various BoNTs, the only BoNTs naturally involved in outbreaks in birds are BoNTs C, D or their mosaics C/D and D/C, BoNT E and, much more rarely, BoNT A (63). BoNTs C, D or their mosaics C/D and D/C are the most frequent, both in wild and domestic species. BoNT E is less frequently detected, and regularly causes sporadic cases or epizootics in wild fish-eating birds in northern regions, but is rarely the cause of epizootics in farmed species. Type A botulism has only been described a few times in the United States in avifauna including deaths of seagulls in the Klamath River basin in California (63) and it seems to be excessively rare on farms [one outbreak on a broiler farm in the United States, see Graham and Schwarze (64)].

In France, only BoNTs D/C and C have been identified in recent years in bovine botulism outbreaks. In Europe, BoNT D/C is the currently cause of the majority of bovine cases (62, 65). Very rare cases with BoNT A were reported in the middle of the 20th century in France Prévot et al. (66, 67) cited by the French Agency for Food safety and Animal health (68), in zebus (*Bos indicus*) in Brazil (69), in dairy cows in Egypt in 1976 (4) and very recently in the state of New-York in the United States (70). Type B outbreaks have also been described in the literature in dairy herds: in the United States in 1984, 1992 and 2001, in Israel in 2000 (71–74) and in the Netherlands in about 30 dairy herds in 1976 and 1977 in the Netherlands in connection with the incorporation of contaminated brewers' grains in the feed ration (75).

The detailed analysis conducted on NRL data on avian botulism provided interesting details, particularly for poultry farms (species involved, age of onset of cases, dominant toxin types by species, etc.). In our data, there were no differences between males and females in poultry farming. In the literature, males appear to be more affected than females, particularly in turkeys (47, 76, 77). No explanation for this observation has been provided to date. For example, males have a longer rearing period than females, but the impact of this factor on the occurrence of an outbreak of botulism has not been evaluated. Botulism can also occur as a result of stress or a biosecurity

failure at the time of removal of the females. Most of the outbreaks in both cattle and poultry are located in Brittany, in an area with a high density of poultry and dairy farms, and a high number of mixed farming, which may explain frequent cross-contamination and this higher prevalence. It is unlikely that there is detection bias, because the level of disease surveillance is the same throughout the country.

It was not possible to conduct a detailed analysis on cattle due to the lack of previous data, available only since 2017. In any case, this analysis remains difficult to conduct retrospectively. The information available is based almost exclusively on more or less complete information forms accompanying the samples sent to the NRL. A standard information form listing the essential data to be transmitted with the samples would facilitate the monitoring of animal botulism in France.

Addressing the study of pathogens not sector by sector but from a global perspective is the basis of the One Health concept. This approach address a health threat at the human-animal-environment interface based on collaboration, communication, and coordination across all relevant sectors and disciplines with the ultimate goal of achieving optimal health outcomes for both people and animals (78). Botulism is part of the European list B of Annex I of the zoonoses Directive (79), surveillance and study of botulism, BoNTs, and BoNT-producing clostridia logically fall under the One Health concept. If botulism is notifiable for humans in Europe, this is not systematically the case for animals. In France, botulism is a notifiable disease, both in humans and animals, regardless of the species affected, which allows for an overall view. The occurrence of botulism cases and outbreaks is closely monitored through a case-based, passive surveillance system. This is a first step in the application of the One Health approach to disease surveillance by juxtaposing animal and human surveillance. In the majority of cases, surveillance systems continue to be developed and operated within a highly sectoral approach (80). But to be effective, the management of complex health issues should shift from isolated, sectoral and linear, to systemic and transdisciplinary approaches to health (81). Our study has shown that human botulism is mostly due to ham (pig sector) and canned vegetables, indicating the importance of collection of surveillance data from food industry, animal sectors as well as surveillance of this pathogen in the environment. These results show that even if surveillance is implemented for both human and animal health, progress are still needed to improve data collection and surveillance of food, feed sectors and environmental contamination.

With the exception of BoNT E, which was exceptionally detected throughout the study period in wild birds, the types of botulism found in animal outbreaks are different from those identified in human outbreaks over the last 10 years in France and no human botulism outbreaks investigated by SPF and the NRC have been linked to animal botulism. But both human and animals are known to be sensitive to some similar BoNT types. As a result, detecting a BoNT E outbreak in wild birds or in poultry, or a BoNT B outbreak in cattle is crucial to prevent any contamination to humans. Furthermore, there are currently very few cases of type C, D, C/D, D/C in humans. It is important to continue to monitor over time that this is still the case. Early detection of zoonotic pathogens through enhanced laboratory capacity and surveillance at the animal–human interface is a crucial step toward controlling and preventing zoonoses (82).

Given that botulism is ubiquitous in the environment and can cause disease in both humans and animals, it is essential to enhance links between human and animal surveillance systems. Accordingly, in line with the One Health concept, this study presents the first integrative approach to the routine surveillance of botulism in humans and animals in France.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SL, CLM, RS, LG, and CM were responsible for the concept and design of the study, interpretation of results, writing, and critical review of the manuscript. CLM, RS, and LG were responsible for data collection. CL and LG were responsible for statistical analysis. CLM, KP, PK, and FM were responsible for reviewing and editing the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the French Ministry of Agriculture and Food.

Acknowledgments

The authors thank Marianne Chemaly, Sandra Rouxel, Typhaine Poezevara, Amandine Avouac, Emmanuelle Houard, from the National Reference Laboratory for Botulism, ANSES Ploufragan-Plouzané-Niort Laboratory and Laure Diancourt, Julie Germond, Jean Sautereau, from the National Reference Centre for Anaerobic Bacteria and Botulism, Institut Pasteur, Paris. The authors are grateful to the ANSES expert committee panel in charge of assessing animal health and animal welfare risk, and to the working group Botulism, including the authors FM, SL, CLM, CM as well as (in alphabetical order): Catherine

Belloc, Stéphane Bertagnoli, Alain Boissy, Henri-Jean Boulouis, Frédéric Carlin, Eric Collin, Jean-Claude Desfontis, Michel Federighi, Maria-Eleni Filippitzi, Matthieu Fournier, Philippe Fravalo, David Fretin, Jean-Pierre Ganiere, Emmanuelle Gilot-Fromont, Etienne Giraud, Lionel Grisot, Nadia Haddad, Viviane Hénaux, Didier Hilaire, Elsa Jourdain, Sophie Le Poder-Alcon, Monique L'Hostis, Gilles Meyer, Elodie Monchatre-Leroy, Hervé Morvan, Pierre Mormède, Carine Paraud, Ariane Payne, Carole Peroz-Sapede, Claire Ponsart, Claude Saegerman, Gaëlle Simon, Michèle Tremblay, Cédric Woudstra, Jean-Pierre Vaillancourt.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1003917/full#supplementary-material

SUPPLEMENTARY MATERIAL 1
Foods involved in human botulism outbreaks from 2008 to 2018.

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frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 17 February 2023
DOI 10.3389/fmed.2023.1102396

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Pathogenesis and Therapy, a section of the journal Frontiers in Medicine

RECEIVED 18 November 2022
ACCEPTED 02 February 2023
PUBLISHED 17 February 2023

CITATION
Avila HG, Sandon L, Anes PE, Meli SA, Giboin GA, Pérez VM and Periago MV (2023) Environmental *Toxocara* spp. presence in crowded squares and public parks from San Juan Province, Argentina: A call for a "One Health" approach.
Front. Med. 10:1102396.
doi: 10.3389/fmed.2023.1102396

Environmental *Toxocara* spp. presence in crowded squares and public parks from San Juan Province, Argentina: A call for a "One Health" approach

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Introduction: Canine soil-transmitted helminth (cSTH) parasites need specific environmental conditions to complete their life cycle. *Toxocara canis* and *T. cati* are the most important zoonotic cSTH, since they are the causal agents of human toxocariasis. Canine STHs are dispersed in feces from infected domestic and wildlife canines. In this study, the presence of STH in canine feces was evaluated in 34 crowded public parks and squares from San Juan Province (Argentina).

Methods: Fecal samples were collected during different seasons in 2021–2022 and analyzed by standard coprological methods, including Sheather and Willis flotation and Telemann sedimentation. InfoStat 2020, OpenEpi V. 3.01 and R and RStudio® were used for statistical analysis and QGIS 3.16.10 for mapping.

Results: From a total of 1,121 samples collected, 100 (8.9%) were positive for at least one intestinal parasite (IP) and three cSTH species were detected: *Toxocara* spp., *Toxascaris leonina* and *Trichuris vulpis*. The most prevalent cSTH species was *T. vulpis* (64/1121; 0.057%), while the least prevalent was *Toxocara* spp. (19/1121; 0.017%). The detection of *Toxocara* spp. eggs was significantly different depending on the season. The geo-spatial variation of each cSTH per season is described.

Discussion: This is the first study in San Juan Province to identify environmental contamination of cSTHs in public areas. The specific localization of areas with the presence of cSTH eggs could provide information to guide strategies to reduce the cSTH infection burden in dogs and promote serological screening of the human population for *Toxocara* spp. Given the zoonotic nature of *Toxocara* spp. We hope this information will help to reinforce activities of control programs, focusing on the "One Health" approach.

KEYWORDS

***Toxocara canis*, soil transmitted helminths, One Health, spatial epidemiology, San Juan, Argentina**

1. Introduction

The One Health approach recognizes that the health of humans, domestic and wild animals, and the wider environment (including ecosystems) are closely linked and interdependent; the term aims to sustainably balance and optimize the overall health of our planet and its inhabitants (1). The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for clean water, energy and air, safe and nutritious food, taking action on climate change, and contributing to sustainable development (2). The One Health approach supports global health security by collaboration and communication at the human-animal-environment interface to address shared health threats such as zoonotic diseases, and others. The zoonotic parasitic diseases transmitted by dogs´ feces are considered under this approach, since it interferes with animal and human health, and its propagation generally occurs in the environment (3). In addition, the concept of one health contemplates the consequences produced by climate change (1), a determining factor in the transmission of canine soil-transmitted helminths (cSTH).

The high number of free-roaming dogs found in urban areas can serve as a source of pathogens which may be dangerous to humans; dogs can act as definitive hosts for a high number of parasites (3), some of which are considered zoonotic because they can cause disease in humans. *Toxocara canis*, *Toxocara cati*, and *Ancylostoma caninum* are, respectively, the primary species of zoonotic cSTHs. Other species of non-zoonotic cSTHs could also be present, e.g., *Toxascaris leonina*, and *T. vulpis* (4, 5), although they are not dangerous for humans, they do have an effect on animal health. For this reason, epidemiological studies aid in determining the parasitological status of the population, parasite burden and potential risk areas (6).

Toxocariasis is a parasitic disease transmitted usually from dogs and/or cats that are infected with *T. canis* and *T. cati* (3), to humans. Hosts include cats, dogs, foxes, coyotes, and wolves. These hosts harbor the nematodes in their guts, shedding the eggs in their feces. The embryonated eggs remain infectious for years outside the host. In the wild, carnivorous animals such as cats and dogs consume infected meat or simply soil containing the eggs, and the parasite persists in their gut. Additionally, transplacental transmission has been documented in dogs and cats (7, 8).

In general, individuals infected with these species are asymptomatic, but some develop clinical syndromes which include visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis (NT) and covert/common toxocariasis (CT) and can associate with allergic, neurological and/or visual disorders, or cognitive and intellectual deficits in children (9). Recent epidemiological research has estimated that ~1.4 billion people worldwide (10), particularly in subtropical and tropical regions, are infected with, or exposed to *Toxocara* species, indicating that human toxocariasis is a neglected tropical disease (NTD). Diagnosis in humans is based on clinical, epidemiological, and serological data. Indirect IgG ELISA is a widely used serological method for toxocariasis and western blots can be used to confirm positive ELISA findings to reduce false-positive results (11).

Embryonated *Toxocara* spp. eggs in the environment are considered as the most important source of human toxocariasis. These eggs, however, are also a source of infection for definitive and paratenic hosts (12). To become infective, *Toxocara* spp. eggs need specific conditions of temperature and soil (13, 14), which are present in public squares and parks from different tropical and subtropical countries (15). In this study, canine fecal samples from different parks and squares from San Juan Province, Argentina, were analyzed. The samples were collected during the four seasons (autumn, winter, spring, and summer) in each selected area, with the aim to estimate the association between seasons, weather, presence of cSTH eggs and zoonotic risk.

2. Methodology

2.1. Study area

This study was carried out in the urban area of San Juan Capital (−31.54, −68.52), in the homonymous province; the main squares and parks were included. This area encompasses a surface of 239.12 km^2. It has about 450,000 inhabitants with a population density of approximately 1880 inhabitants/km^2. The elevation of the city is ~650 m and it is located in a valley at the eastern border of the Andes Mountain range. According to the Köppen-Geiger climate classification (16, 17), San Juan has an arid climate (BWh/BWk), with low rainfall (<20 mm on any given month), significant diurnal and annual temperature variation (ranging from an average of 32°C in January to 8°C in July), while the average annual temperature is 18°C. The most populated areas were selected for sampling.

2.2. Sample collection and coprological analysis

During 2021 and 2022, fresh canine fecal samples from each square and park were collected during each season: autumn (Epidemiological Week – EW – 21 of 2021/May), winter (EW 32/2021/August), spring (EW 45/2021/November) and summer (EW 8/2022/February). The entire samples were collected in pre-labeled plastic bags and subsequently inactivated at −20°C for 2 weeks. Each sample was homogenized, and 10 grams were used and processed using three different concentration methods; two different flotation techniques, Sheather method (saturated sugar solution, 1.25 specific gravity) and Willis method (saturated NaCl solution, 1.20 specific gravity) as well as a sedimentation technique (Telemann method) (18). The techniques chosen for this study are standard concentration techniques that increase the chances of detecting intestinal parasitic structures, including helminth parasites such as *Toxocara* spp. Each sample was microscopically examined at 100× and 400× magnifications. The identification of *Toxocara* spp. eggs was performed using morphological reference (19). Samples were classified as positive if the presence of eggs was confirmed (20).

2.3. Statistical analysis

This is a descriptive, cross-sectional, and observational study. The aim was exploratory and descriptive, focused on finding possible associations between the presence of cSTH, specifically *Toxocara* spp. and location and characteristics of the squares and the seasons. The

association was examined through χ^2 tests, using InfoStat® V.19 software (21). The parasitic prevalence was calculated and their association with season, department and square/park was analyzed. The Risk Ratio (RR) and Odds Ratio (OR), with 95% Confidence Interval (CI), of statistically significant associations were obtained using OpenEpi V. 3.01 (22).

To explore the distribution characteristics of the most prevalent parasites found, a calculation between observed and expected cases was performed assuming a uniform distribution of positive cases, using R and RStudio®.

2.4. Spatial analysis

Given the low number of *Toxocara* spp. positive cases found during the study, a correlation analysis was performed using only *Toxocara* spp. positive parks ($N = 12$). The correlation between its presence and a composite remote sensed index, which can be identified as a proxy for tree shadow, was analyzed. This new index, specifically created for this study, was named the Tree Magnitude Index (TMI) and it is calculated through the multiplication of the Topographic Index Position (TPI) obtained from a Digital Surface Model (DSM) and the Normalized Difference Vegetation Index (NDVI) obtained from satellite imagery. The TMI was treated both as a response variable and as an explanatory one, using the difference between the observed and expected value of positive cases, assuming a homogeneous distribution. High TPI values suggest surface objects that stand out from their surroundings; high NDVI values suggest vigorous vegetation. As a result, when these two factors are multiplied, high values of TMI would indicate high-rise vegetation, such as trees, whereas low values would indicate low and flat lands with little to no vegetation. TMI might therefore be thought of as a tree magnitude index and as a proxy for tree shadows. Only positive NDVI values were taken into consideration to prevent positive outcomes brought on by both negative indices.

The DSM used had a 5 m resolution, generated from a photogrammetric aerial survey by the Argentinian IGN (23). The bandwidth for TPI was 100 m. NDVI was retrieved from Google Earth Engine (24), and it was computed using Sentinel 2 Level-2A imagery (10 m spatial resolution); the values of the image represent the analysis time-span average. The values of TPI (resampled at 10 m) and NDVI were extracted for each pixel, then the product of the two terms was calculated, and finally, the average value of the multiplication of these two indices was computed for each square and park.

2.5. Weather data analysis

Weather data from the nearest weather station, San Juan Airport (12 km east of the city center) (25), was retrieved to gauge weather conditions during the 4 weeks of analysis. Seven variables were retrieved: mean temperature (daily average temperature), Diurnal Temperature Variation (DTV), accumulated precipitation, air humidity, cloud cover, solar energy, and wind speed. For every variable, a value for each of the 4 weeks of the analysis was quantified. Values were the weekly average of the rolling mean of the previous 21 days, except for precipitation data, which was the weekly mean accumulation of the previous 21 days. This range was selected due to previous reports stating there were no significant differences detected in the viability of eggs until day 21 (20); maximum infectivity of larvae eggs has been reported up to day 30 of incubation (26, 27).

3. Results

3.1. Sample collection and coprological analysis

After less than a year of sampling, 1,121 samples were collected, 271 samples in Autumn, 280 samples in Winter, 342 samples in Spring and 228 samples in the Summer. In 8.9% (100/1121) of them, at least one type of cSTH (*Toxocara* spp., *T. vulpis* and *T. leonina*) was found. *Toxocara* spp. eggs were detected in 0.017% (19/1121) of the samples (Table 1). With respect to the other cSTHs found, the most prevalent species was *Trichuris vulpis* (0.0571%; 64/1121), followed by *Toxascaris leonina* (0.0259%; 29/1121). Twelve samples showed almost one type of co-infection (Table 1). Figure 1 shows the overall number of positive samples of the three STH found per sampling location and the general study area that was included in this study. During the study, 67.6% (23/34) of the squares and parks sampled showed environmental contamination with at least one type of cSTH. The presence of *Toxocara* spp. was detected in 12 of the analyzed squares and parks (35.3%).

TABLE 1 Description of the canine fecal samples collected, prevalence of canine soil-transmitted helminths (cSTH) found in total and per season in San Juan City, San Juan, Argentina (2021–2022).

	Autumn (N=271)	Winter (N=280)	Spring (N=342)	Summer (N=228)	Total (N=1,121)	χ^2; d.f.=3	p
Parasite presence (%)	28 (10.33%)*	32 (11.43%)**	25 (7.31%)	15 (6.58%)	100 (8.9%)	5.46	0.1410
Trichuris vulpis presence (%)	13 (4.80%)	24 (8.57%)	16 (4.68%)	11 (4.82%)	64 (5.7%)	5.69	0.1279
Toxascaris leonina presence (%)	11 (4.06%)	9 (3.21%)	6 (1.75%)	3 (1.32%)	29 (2.6%)	5.17	0.1597
Toxocara spp. presence (%)	12 (4.43%)	3 (1.07%)	3 (0.88%)	1 (0.44%)	19 (1.7%)	16.34	<0.001

*There were eight samples which presented co-infection: three between *T. vulpis* and *T. leonina* and 2 between *T. vulpis* and *Toxocara* spp.
**There were four samples which presented co-infection: three between *Toxocara* spp. and *T. leonina*, 3 between *T. vulpis* and *T. leonina* and 1 between *T. vulpis* and *Toxocara* spp.

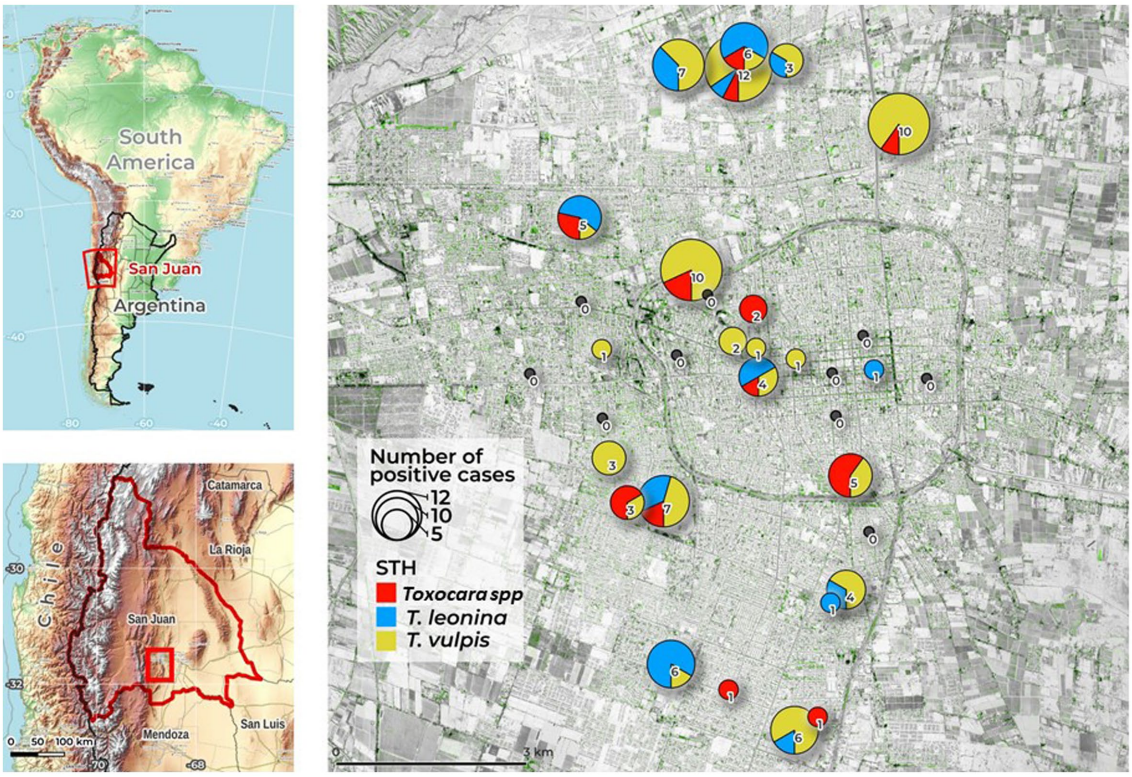

FIGURE 1
Study area of San Juan City (San Juan, Argentina) in the context of South America. Main Map: Cumulative number of canine soil-transmitted helminths (cSTH) found in each sampling location (2021–2022) by species. Map backgrounds: main map from ©2022 Google; inset map from ©OpenStreetMap, ©OpenTopoMap (CC-BY-SA). Map data: August 09, 2022.

FIGURE 2
Mean Tree Magnitude Index (TMI) for four of the 34 sampled squares from San Juan City (San Juan, Argentina).

3.2. Statistical and spatial analysis

The distribution of cSTHs per season was not uniform, being *T. vulpis* the most prevalent in the samples collected in the autumn, winter, and summer, with *T. leonina* being the most prevalent in the spring. The highest prevalence of *Toxocara* spp. was observed in autumn (4.43%; $p < 0.01$); while there was no statistically significant difference in the prevalence of the other cSTH per season (Table 1). Moreover, the risk and odds ratio analysis showed that there is 5 times greater risk of finding *Toxocara* spp. in dog fecal samples in the autumn compared to the other three seasons (RR = 5.38, 95% CI 2.14–13.5; OR = 5.58, 95% CI: 2.17–14.32). Using simple linear regression, the TMI significantly predicted *Toxocara* spp. prevalence ($R^2 = 0.67$, $F(1, 10) = 23.2$, $p < 0.01$), with the following fitted regression model: Δ Observed-Expected Value = 0.54 + 0.81*(TMI). Figure 2 shows the

mean TMI during 2021 in four of the sampled areas. TMI of the entire study area of San Juan City (San Juan, Argentina) during 2021 is shown in Supplementary Figure 1.

Additionally, the observation of the distribution of the three cSTH found in the different sampled areas, shows that they are heterogeneously and not homogeneously distributed (Figure 3A). This figure shows those areas where the presence of the parasites is either higher (red) or lower (green) than expected. The presence of *T. vulpis* (Figure 3B) was detected in 19 out of 32 (59.4%) of the parks and squares sampled; its presence was higher than expected in 5 of them. Although *T. leonina* was detected in 12 of the sites sampled (37.5%), its presence was higher than expected in 6 of these (Figure 3C). Like *T. leonina*, *Toxocara* spp. was found in 12 of the parks and squares samples and its presence was higher than expected in 6 of these sites (Figure 3D). The difference between observed and expected values, assuming a homogeneous distribution, for each sampled area, is presented in Table 2.

3.3. Weather data analysis

As previously stated, given that the detection of *Toxocara* spp. eggs was significantly more frequent during the autumn, the weather data was explored to identify any characteristics that might be driving this

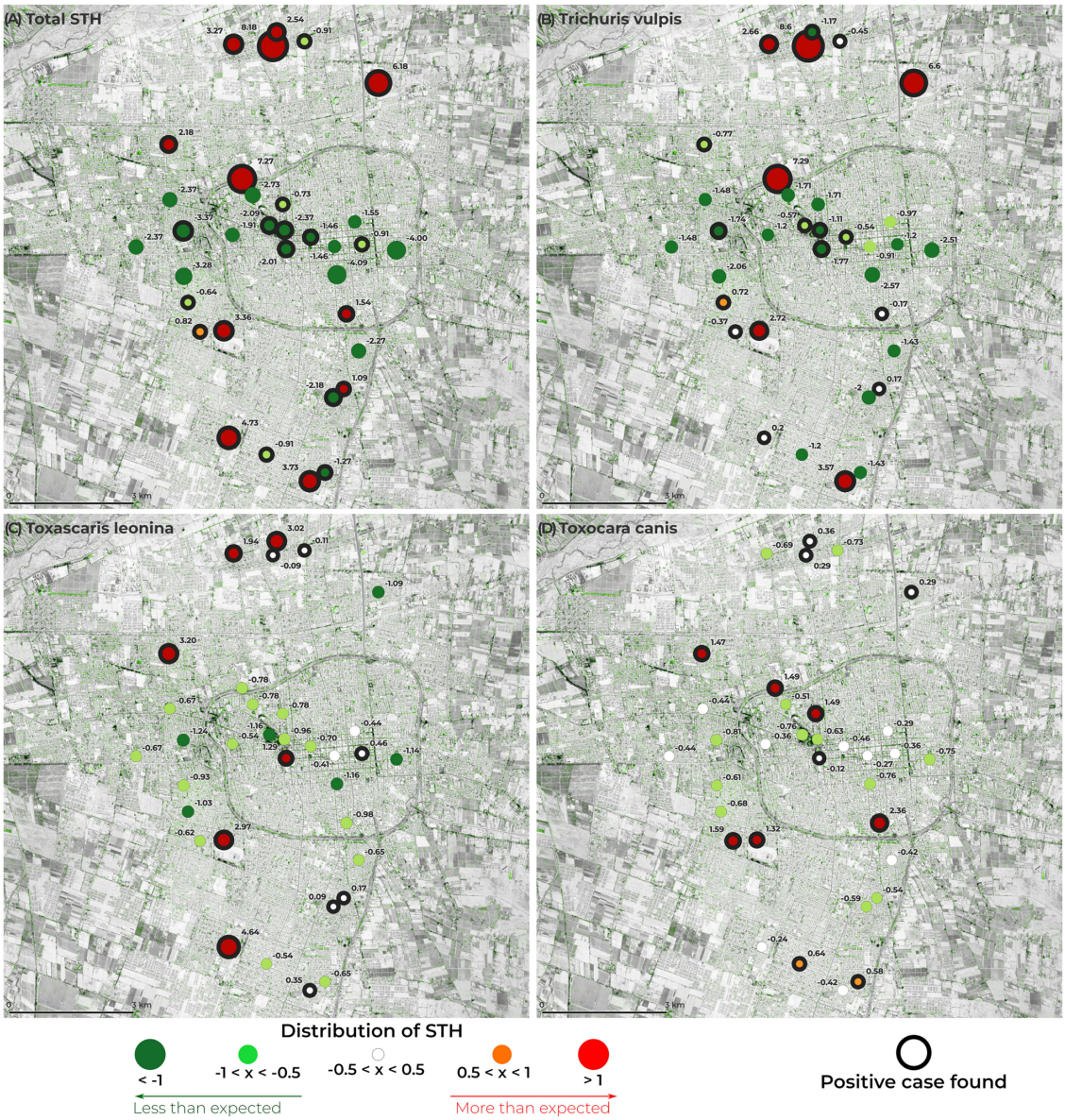

FIGURE 3
Distribution of canine soil-transmitted helminths (STH) in the study area of the city of San Juan (San Juan, Argentina). **(A)** Canine STH as a group. **(B)** *Trichuris vulpis*. **(C)** *Toxascaris leonina*. **(D)** *Toxocara* spp. Circles in red represent positive values where observed cases were higher than expected. Circles in green represent positive values where observed cases were less than the expected. In white, the difference between observed and expected values was not different. The circles with a black border represent those sites where positive samples were detected.

TABLE 2 The difference between observed and expected values, assuming a homogeneous distribution, of positive cases of canine soil-transmitted helminths (cSTHs) in canine environmental feces collected from different squares and parks from San Juan City (San Juan Province, Argentina).

Number	Difference from expected				
	Name	Total cSTH	*Trichuris vulpis*	*Toxascaris leonina*	*Toxocara* spp.
1	Plaza B° Ramos	2.54	−1.17	3.02	0.36
2	Plaza B° Chimbas	8.18	8.6	−0.09	0.29
3	Plaza Dep.Chimbas	−0.91	−0.45	−0.11	−0.73
4	Plaza B° Güemes	3.27	2.66	1.94	−0.69
5	Plaza B° Los Andes	6.18	6.6	−1.09	0.29
6	Plaza Barrio Aramburu	2.18	−0.77	3.2	1.47
7	Plaza Barrio del Carmen	7.27	7.29	−0.78	1.49
8	Plaza Ejército Argentino	−2.73	−1.71	−0.78	−0.51
9	Plaza Cementerio	−0.73	−1.71	−0.78	1.49
10	Parque de Mayo	−2.09	−0.57	−1.16	−0.76
11	Plaza España	−2.37	−1.11	−0.96	−0.63
12	Centro Cívico - Teatro	−2.01	−1.77	1.29	−0.12
13	Plaza Laprida	−1.46	−0.54	−0.7	−0.46
14	Plaza 25 de Mayo	−1.46	−0.91	−0.41	−0.27
15	Plaza Gertrudis Funes	−1.55	−0.97	−0.44	−0.29
16	Plaza Aberastain	−0.91	−1.2	0.46	−0.36
17	Zona Terminal	−4	−2.51	−1.14	−0.75
18	Plaza Hipólito Yrigoyen	−4.09	−2.57	−1.16	−0.76
19	Plaza Italia	−1.91	−1.2	−0.54	−0.36
20	Plaza Manuel Belgrano	−3.37	−1.74	−1.24	−0.81
21	Plaza Desamparados	−2.37	−1.48	−0.67	−0.44
22	Plaza Villa San Roque	−2.37	−1.48	−0.67	−0.44
23	Plaza Barrio Bancario	−3.28	−2.06	−0.93	−0.61
24	Pza. Barrio Foeva	−0.64	0.72	−1.03	−0.68
25	Pza. 2 Jardín Policial	0.82	−0.37	−0.62	1.59
26	Pza. Villa Sta. Anita	3.36	2.72	2.97	1.32
27	Plza Barrio La Estación	4.73	0.2	4.64	−0.24
28	Plaza Centenario	−0.91	−1.2	−0.54	0.64
29	Plaza B° San Ricardo	3.73	3.57	0.35	−0.42
30	Plaza Grillo	−1.27	−1.43	−0.65	0.58
31	Plaza Villa Fleuri	−2.18	−2	0.09	−0.59
32	Plaza Villa Lerga	1.09	0.17	0.17	−0.54
33	Plaza Echegaray	−2.27	−1.43	−0.65	−0.42
34	Plaza Almirante Brown	1.54	−0.17	−0.98	2.36

difference. The analysis of the different climatic variables (Figure 4) showed that for EW 21/2021, the air humidity was notably higher, while the wind speed and solar energy were somewhat lower. These weather features might be involved in the higher prevalence of *Toxocara* spp. eggs observed during the autumn given that high humidity, low wind speed and low solar radiation are a good combination of weather factors for the survival of *Toxocara* spp. eggs in the soil (28–32).

4. Discussion and conclusion

Through the sampling of fecal canine samples collected from 34 of the main urban parks and squares of the City of San Juan (San Juan, Argentina), the presence of different cSTH species was detected, including *Toxocara* spp., which is a zoonotic parasite that poses a risk to humans. The overall prevalence of cSTH found in this study was 8.9%, which is lower than the prevalence reported in other studies

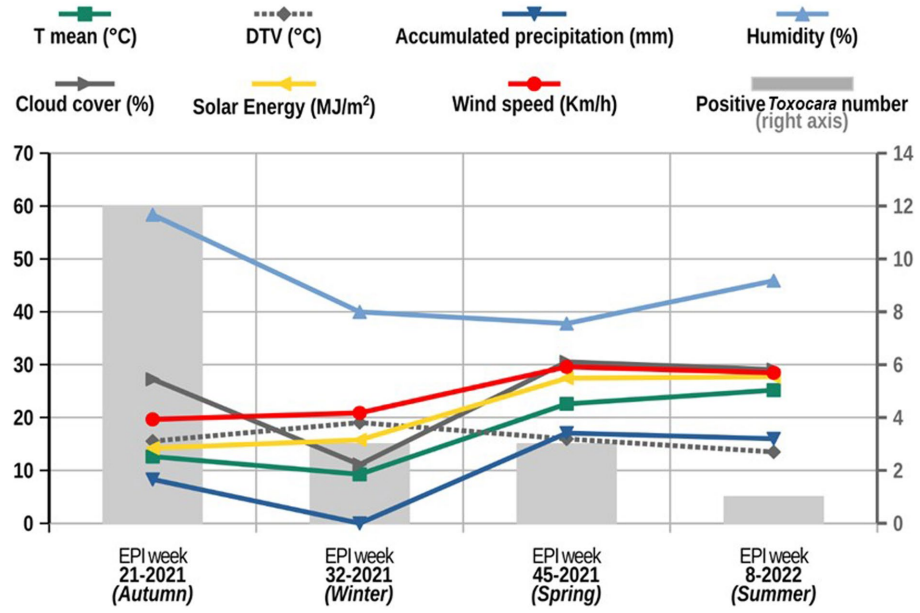

FIGURE 4
Description of the climatic variables retrieved from the weather station from San Juan Airport in San Juan (Argentina) and the number of *Toxocara* spp. cases found during the sample period (2021–2022). Climatic variables retrieved are shown on the left axis, including mean temperature (T mean in °C), Diurnal Temperature Variation (DTV), accumulated precipitation (mm), air humidity (%), cloud cover (%), solar energy (MJ/m^2) and wind speed (km/h). The number of positive cases of *Toxocara* spp. found for each season (autumn, winter, spring and summer) is reflected on the right axis.

from urban areas in Argentina (33–36), and similar to the prevalence reported in Ushuaia (37) (Supplementary Table 1). Herein, the most prevalent cSTH was *T. vulpis*, while in other studies the most prevalent species found was *A. caninum* (33, 35, 38–40). The same pattern was observed in other countries such as Australia and Nigeria, where this hookworm species was the most prevalent (41, 42). In some of these studies, infection by protozoan species was higher than helminth infections (37); in this study, protozoan parasites were not detected, although the modified Ziehl-Neelsen technique (43), which is more sensitive, was not used.

In this study, *T. vulpis* was the most prevalent cSTH, this could be due to the longer survival time of eggs of this species in the soil; this might be increasing the chances for dogs that frequent the parks/squares to become reinfected (44). Moreover, *T. vulpis* has a prepatent period of 3 months, therefore antiparasitic treatment should be routinely repeated at monthly intervals to kill all the worms as they mature and prevent contamination of the environment (3). On the other hand, *Toxocara* spp. and *T. leonina* require only a few weeks to mature (1–2 months), and with a second dose of anthelmintic administered 2 or 3 weeks after the first one, the dogs would be free of all the worms. Considering that the samples analyzed herein are from the environment and that the status of each definitive host is unknown, we could assume that canine deworming is either not being performed or not given in periodic intervals.

Unfortunately, since fecal environmental samples were used, the association with characteristics of the dogs themselves (i.e., age, free-roaming or kept, underlying conditions, among others) (45) and with the conduct of care of the owners (i.e., antiparasitic treatment) (46) could not be considered. Moreover, the setting where this study was conducted was urban and the prevalence and variety of parasites found might be greater in rural areas where there is also exposure to other animals (4, 47, 48).

Additionally, differences in prevalence could also be due to the climatic and soil conditions of San Juan, given that it is an area with very low precipitation and other studies have shown that the average amount of rainfall was found to be strongly associated with the environmental contamination of parks with cSTH (41). Through the analysis of the association between the presence of *Toxocara* spp. and environmental characteristics, in this study, the regression analysis revealed that shadow significantly contributes to the increased prevalence of the parasites as measured by the TMI, as previously observed in other studies (28–32). The presence of trees and their shadows, along with other factors like irrigation and management of the park (not considered in this study), could create an ecological urban niche for the parasites to develop in the soil regardless of the general dry environment of San Juan. The significantly higher prevalence of *Toxocara* spp. observed in the autumn coincides with increased air humidity, lower wind speed and sun radiation, these environmental conditions could potentially facilitate transmission of *Toxocara* spp. eggs. This was confirmed under laboratory conditions in a previous study (32). In addition, other studies have shown that Argentina and Brazil have optimal humidity conditions for the development of *Toxocara* spp. eggs (49–51). Nevertheless, extreme temperatures (high or low) are also important as they can lead to desiccation of eggs and larval stages or arrested development of infective stages in the environment (52).

The cSTH species found herein were not homogeneously spread throughout the city, and there were areas that had conditions that were more appropriate for the transmission from one dog to another. In general, the cSTH detected in this study were found in the areas surrounding the Capital Department. Nonetheless, when analyzing per

species, the prevalence of *Toxocara* spp. was greater than expected within the Capital Department, which is the most densely populated. These areas with the detection of a higher prevalence of *Toxocara* spp. than expected may be used to guide public health measures for screening of antibodies specific for *Toxocara* spp. in humans, especially children, given that Toxocariasis is a silent disease that could be acquired during infancy and have severe consequences (9). Further studies could be conducted to determine the possible risk factors associated with these areas (53). Unfortunately, evaluation of the egg's viability and ability to become infective (54) was not performed, future studies must be conducted to evaluate these, given its implications on the risk to public health.

The regular administration of anthelmintic treatments and the promotion of responsible dog ownership, including picking up dog feces and hand hygiene are important measures which need to be adopted to minimize environmental contamination with *Toxocara* spp. and other STHs (41). Multidisciplinary research, formulated under "one health approaches" can deliver reinforced tools for exploring zoonotic parasites, including cSTHs (46).

Due to the low number of public squares and parks studied herein ($N = 34$), future studies with a higher number of squares and public parks should be conducted to improve the correlations analysis.

This is the first study in San Juan, Argentina to describe the presence of cSTH parasite species in public areas. The specific localization of squares and parks infected with cSTH eggs aim to provide information to design strategies to lower the cSTH infection burden in dogs and to provide information to direct serological screening of the human population, specifically for *Toxocara* spp. Given the zoonotic nature of these cSTHs we hope this information will help to reinforce activities of control programs, focusing on the "One Health" approach.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HA, VP, and MP: conceptualization. MP, HA, LS, PA, and VP: methodology. HA and MP: formal analysis. HA, PA, and SM: investigation. HA, LS, and MP: data curation. GG: statistical analysis. LS: spatial analysis. HA, GG, and LS: writing—original draft preparation. MP: writing—review and editing. VP and MP: supervision. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by Fundación Mundo Sano and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET).

Acknowledgments

The authors want to say thanks to Milagros de la Rosa, Matías de la Rosa, Mg. Daniel Martínez, Mg. Sonia Silva (INTA-San Juan) and student bachelors of Biochemistry from Technological and Chemical Sciences Faculty, Catholic University of Cuyo, for your valuable contribution in this work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1102396/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Mean Tree Magnitude Index (TMI) of the study area of San Juan City (San 473 Juan, Argentina) during 2021. The squares and parks where the samples were collected are 474 marked in red.

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 08 March 2023
DOI 10.3389/fpubh.2023.1129083

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention, a section of the journal Frontiers in Public Health

RECEIVED 21 December 2022
ACCEPTED 16 February 2023
PUBLISHED 08 March 2023

CITATION
Tast Lahti E, Karamehmedovic N, Riedel H, Blom L, Boel J, Delibato E, Denis M, van Essen-Zandbergen A, Garcia-Fernandez A, Hendriksen R, Heydecke A, van Hoek AHAM, Huby T, Kwit R, Lucarelli C, Lundin K, Michelacci V, Owczarek S, Ring I, Sejer Kjeldgaard J, Sjögren I, Skóra M, Torpdahl M, Ugarte-Ruiz M, Veldman K, Ventola E, Zajac M and Jernberg C (2023) One Health surveillance—A cross-sectoral detection, characterization, and notification of foodborne pathogens. *Front. Public Health* 11:1129083. doi: 10.3389/fpubh.2023.1129083

One Health surveillance—A cross-sectoral detection, characterization, and notification of foodborne pathogens

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Introduction: Several Proficiency Test (PT) or External Quality Assessment (EQA) schemes are currently available for assessing the ability of laboratories to detect and characterize enteropathogenic bacteria, but they are usually targeting one sector, covering either public health, food safety or animal health. In addition to sector-specific PTs/EQAs for detection, cross-sectoral panels would be useful for assessment of the capacity to detect and characterize foodborne pathogens in a One Health (OH) perspective and further improving food safety and interpretation of cross-sectoral surveillance data. The aims of the study were to assess the cross-sectoral capability of European public health, animal health and food safety laboratories to detect, characterize and notify findings of the foodborne pathogens *Campylobacter* spp., *Salmonella* spp. and *Yersinia enterocolitica*, and to develop recommendations for future cross-sectoral PTs and EQAs within OH. The PT/EQA scheme developed within this study consisted of a test panel of five samples, designed to represent a theoretical outbreak scenario.

Methods: A total of 15 laboratories from animal health, public health and food safety sectors were enrolled in eight countries: Denmark, France, Italy, the Netherlands, Poland, Spain, Sweden, and the United Kingdom. The laboratories analyzed the samples according to the methods used in the laboratory and

reported the target organisms at species level, and if applicable, serovar for *Salmonella* and bioserotype for *Yersinia*.

Results: All 15 laboratories analyzed the samples for *Salmonella*, 13 for *Campylobacter* and 11 for *Yersinia*. Analytical errors were predominately false negative results. One sample (*S.* Stockholm and *Y. enterocolitica* O:3/BT4) with lower concentrations of target organisms was especially challenging, resulting in six out of seven false negative results. These findings were associated with laboratories using smaller sample sizes and not using enrichment methods. Detection of *Salmonella* was most commonly mandatory to notify within the three sectors in the eight countries participating in the pilot whereas findings of Campylobacter and *Y. enterocolitica* were notifiable from human samples, but less commonly from animal and food samples.

Discussion: The results of the pilot PT/EQA conducted in this study confirmed the possibility to apply a cross-sectoral approach for assessment of the joint OH capacity to detect and characterize foodborne pathogens.

KEYWORDS

One Health surveillance, External Quality Assessment, proficiency tests, detection and characterization, notification, foodborne pathogens

1. Introduction

One Health (OH) is a concept often defined as an integrated, unifying approach to sustainably balance and optimize the health of people, animals and ecosystems (1, 2). OH recognizes the health of humans, domestic and wild animals, plants and the wider environment are closely linked and interdependent. This OH approach, therefore, calls for collaboration, coordination, communication and capacity building across disciplines, sectors, organizations and national borders in support of complex health challenges (2). Although OH is not a new concept, it was in 2008 adopted as a joint strategy of the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the World Organization for Animal Health (WOAH, then OIE) (3). To address the European challenges of foodborne zoonoses (FBZ), antimicrobial resistance (AMR) and emerging threats (ET), a 5-year One Health European Joint Programme (OHEJP) was established in 2018 as a partnership between 37 partners across 19 countries in Europe (3). The main focus of the OHEJP is to enhance harmonization of methodologies, databases and procedures for the assessment and management of FBZ, AMR, and ET across Europe. Surveillance of zoonoses and investigations of foodborne and zoonotic outbreaks are examples of OH activities requiring correct diagnostics and sensitive and specific analytical methods across sectors and disciplines.

National, regional, and local authorities, physicians, veterinarians, food business operators and laboratories within animal health, food safety, and public health sectors may have different approaches on when and how to analyze a sample from animals, food, or humans for enteropathogenic *Campylobacter*, *Salmonella* and/or *Yersinia*. The samples may, for instance, originate from official control or surveillance programmes on animal health or food safety, or from Hazard Analysis Critical Control Point (HACCP) samplings at food companies or be taken from patients in hospitals or from outpatients for determination of an illness or be part of an outbreak investigation (4, 5). The protocols for testing these pathogens may vary, for instance, between sectors, countries, regions, or sample types. In addition, after the laboratory analyses, the findings of the pathogens may have a different legal status regarding whether the finding is mandatory to notify or not to a corresponding authority (4). Thus, these variabilities have an impact on the possibilities to detect, investigate and contain clusters and outbreaks and thus impose control or preventive measures.

Also, collection and interpretation of data across sectors and countries can be challenging in a OH perspective. Thereby, the context of the data collected and reported needs to be known to correctly evaluate the results. Other tools to improve the comparability of data between the sectors and countries are, to a certain degree, to harmonize laboratory methods and/or testing the capacity for detection and characterization of the relevant pathogens independently of the laboratory methods used.

According to Zoonoses Directive 2003/99, all Member States in the European Union (EU) shall collect relevant data on zoonoses and zoonotic agents in primary production and/or at other stages in the food chain. *Campylobacter* and *Salmonella* are among the zoonotic agents to be included in monitoring, whereas *Yersinia* is to be covered according to the epidemiological situation. Also, Member States shall investigate foodborne outbreaks. Data collected within the monitoring programmes and investigations of foodborne outbreaks shall be reported to the European Food Safety Authority (EFSA) but data from other samplings may not be collected. However, as only part of the monitoring is harmonized, results from the national monitoring programmes are difficult to compare (4).

On EU level within the public health sector, notifications of campylobacteriosis, salmonellosis and yersiniosis are mandatory in most Member States (4). In some countries, notifications can also be based on a voluntary system. The EU case definitions https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:

32018D0945&from=EN#page=12 for the diseases are updated regularly taking into account, e.g., developments of diagnostic techniques. The case definitions on national level and the capacity of detecting a case can, however, differ between countries, for reasons which could often be attributed to other factors rather than the diagnostic capacity of the laboratories. The number of reported cases generally underestimates the true number or cases (6, 7). Underestimation may occur when asymptomatic cases or cases with mild symptoms do not seek health care, medical care does not test cases or not notify them (8).

Proficiency testing (PT) is, according to ISO 17043:2010, defined as an evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons. The PT schemes can, for instance, be qualitative, quantitative, or sequential in nature. The term External Quality Assessment (EQA) is more often used in the medical field as a synonym for PTs, but EQAs can also be designed to provide insight into the complete path of workflow of the laboratory, and not just the testing processes. A common feature in EQA programmes is education of participants. Some EQA programmes are compulsory, either required by an accrediting body or by law whereas others are voluntary, and the quality manager may choose to voluntarily participate in an EQA programme (https://www.who.int/publications/m/item/overview-of-external-quality-assessment-eqa). Participation in PT or EQA schemes is pivotal for assessment of the performance of the laboratory and identification of potential problems.

There are national, EU-wide, and international sector-specific PT and EQA schemes designed in a quality-assured manner for assessing the ability to detect, identify and characterize enteropathogenic bacteria, especially for *Campylobacter* and *Salmonella* and to a certain extent for *Yersinia* (9, 10). The EU Reference Laboratories (EURL) of food, feed and animal health appointed by the European Commission are obliged to annually organize PTs to the National Reference Laboratories of Member States (10, 11). Likewise, EQAs are routinely organized for the national public health laboratories on characterization but not on detection of these pathogens (10, 12, 13). PTs/EQAs are also offered by national and international commercial quality assurance panel providers. Metagenomics-based cross-sectoral or sector-specific PTs involving viruses (14–16), parasites (17), and recently also bacteria have been organized (18). However, joint cross-sectoral panels for detection of foodborne pathogens from simulated samples are, to the authors knowledge, currently lacking.

The pilot PT/EQA aimed at assessing the cross-sectoral capacity of European laboratories to detect, characterize and notify three defined zoonotic foodborne bacteria and developing recommendations for future cross-sectoral PTs/EQAs. Detection in this study refers to the diagnostic test, i.e., the analysis steps identifying the target pathogen whereas characterization refers to species, (bio)serotype (BT) and sequence type (ST) determination. Notification in this study is defined as reporting of a finding of a pathogen to the responsible authorities. The specific objective was to prepare simulated samples to resemble matrices (samples) analyzed at animal health, food safety and public health laboratories. Public health laboratories in this study refer to clinical microbiological laboratories (primary laboratories) and national public health laboratories. The laboratories were also asked to describe if findings of these pathogens were mandatory notifiable according to their national legislation or guidelines.

2. Materials and methods

2.1. Outline of the PT/EQA

The participants of the pilot PT/EQA were recruited among the partner institutions of the OHEJP CARE "Cross-sectoral framework for Quality Assurance Resources for countries in the European Union" project ($n = 12$) in eight countries including Denmark, France, Italy, the Netherlands, Poland, Spain, Sweden, and the United Kingdom. Additionally, three public health laboratories participated in the pilot from one of the partner countries. Of the 15 participating laboratories, five represented public health, four food safety, two animal health, three both food safety and animal health and one laboratory covered both public health and food safety. These categorisations are based on the information the participants reported.

The participants received a fictive scenario of a foodborne outbreak among persons hunting wild boar and visiting a small-scale abattoir (Supplementary material 1). The dispatched samples were to simulate stool samples from diseased patients, environmental samples from food-producing premises or fecal samples from animals. The participants were assigned to analyze the samples for detection of *Campylobacter*, *Salmonella* and *Yersinia* using the detection and characterization methods and practices applied at the laboratory. They were also requested to identify the target bacteria at a species level and include information of the serovar for *Salmonella* and bioserotype for *Yersinia* if the participants had methods available for these characterisations.

2.2. Production and quality control of the PT/EQA

Each participant received five samples containing 35 mL of matrix simulating a sample and five vials containing freeze-dried bacteria, designated Care 1-5, hereinafter referred to as C1-5 (Table 1). The concentrations of the target bacteria varied between 4.1×10^4 and 3.7×10^5 colony-forming units (cfu). Before analyzing the samples, the vials with freeze-dried bacteria were to be dissolved with 1 mL of sterile diluent and transferred to the matrix.

Yersinia enterocolitica O:3/biotype 4 and biotype 1A are hereinafter abbreviated to O:3/BT4 and BT1A, respectively. Vials C1-4 were freeze-dried in portions of 0.5 mL (19) using Epsilon 1-12 D (Christ, Osterode, Germany). Vials C5 were freeze-dried using an ALPHA 1-4/LD plus (Christ, Osterode, Germany) in portions of 1 mL.

Quality control of C1-C4 was performed on ten randomly selected vials in conjunction with manufacturing or on five vials if the sample mixture was already approved for homogeneity. Homogeneity of a sample mixture was approved if the values obtained for the test of reproducibility (T) and the test index of dispersion between vials (I_2) did not simultaneously exceed 2.6 and 2.0, respectively (20, 21).

TABLE 1 Mean of concentration (m), index of dispersion (I_2) and reproducibility (T) values from the quality control of the target organisms.

Vial[a]	Target organisms	Analysis[b]	Mean[c]	I_2^e	T[f]
C1	*C. coli*	mCCDA, 37°C, 48 h	3.7 x 10^5	8.1	1.9
C2	*S.* Stockholm	BHI agar, 37°C, 24 h	4.1 x 10^{4d}	2.1	1.6
C2	*Y. enterocolitica* O:3/BT4	BHI agar, 37°C, 24 h	9.9 x 10^{4d}	0.8	1.3
C3	*S.* Enteritidis	BHI agar, 37°C, 24 h	6.0 x 10^{4d}	0.6	1.2
C3	*C. jejuni*	mCCDA, 37°C, 48 h	5.6 x 10^4	0.5	1.2
C5	*Y. enterocolitica* BT1A	BHI agar, 37°C, 24 h	1.2 x 10^5	-	-

[a]Five vials of C1, C3 and C4 and ten vials of C2 and C5 were analyzed in duplicate.
[b]mCCDA, Modified Charcoal Cephoperazone Deoxycholate Agar; d BHI, Brain Heart Infusion.
[c]Concentration mean in cfu/mL.
[d]From analysis of a parallel sample mixture.
[e]Index of dispersion.
[f]Test of reproducibility.

The sample labeled "Matrix" represented an environmental sample from an abattoir or a stool sample or a composite environmental sample from wild boars, i.e., all laboratories received the same matrix composition. The matrix was prepared by dissolving 0.5 kg of autoclaved pig manure in 4 L sterilized buffered peptone water (BPW) (Oxoid LP0034, Basingstoke, UK) with NaCl (Merck 6404, Rahway, NJ, USA), mixed by swirling and then stored at +4°C overnight. The following day, the solution was decanted and autoclaved at +134°C for 45 min. The matrix was stored at +4°C until use.

Quality controls of the matrix were performed with cultivation methods and biochemical tests to analyze if *Campylobacter jejuni*, *C. coli*, *Salmonella* spp. or *Y. enterocolitica* were present above the detection limit that could influence the participants' downstream results. In addition, the presence of *Salmonella* spp. was analyzed using MicroSEQTM *Salmonella* detection kit (Thermo Fisher Scientific, Waltham, MA, USA). The cultivation methods and biochemical tests used to examine the matrix were performed according to the following methods from the Nordic Committee on Food Analysis (NMKL No. 119 3rd ed. 2007, NMKL No. 71 5th ed. 1999, NMKL No. 117 3rd ed. 1996). *C. jejuni*, *C. coli*, *Salmonella* spp. or *Y. enterocolitica* were not present above the detection limit in the matrix. The matrix was also tested on the BD MAX™ System (BD Diagnostics, Hunt Valley, MD, USA), a fully automated extraction and real-time PCR machine, using the BD MAX™ Enteric Bacterial Panel and BD MAX™ Extended Enteric Bacterial Panel at a public health laboratory. The BD MAX™ System was not able to detect *Campylobacter, Salmonella* or *Yersinia* spp. in the matrix.

2.3. Methods for characterization of the target organisms by the PT/EQA providers

All target organisms were characterized using whole genome sequencing (WGS). Automated nucleic acid extraction and purification were performed with PSS MagLEAD 12 gC (Precision System Science Co., Ltd, Chiba, Japan) and DNA concentration (ng/μL) was quantified with Qubit® 2.0 Fluorometer (Thermo Fisher Scientific). The Ion Xpress™ Plus Fragment Library Kit for AB Library Builder™ System (Thermo Fisher Scientific) was used for library preparation and Ion S5 XL system (Thermo Fisher Scientific) for sequencing. An additional sequencing of the samples was performed using Illumina NovaSeq 6000 (Illumina, Inc., San Diego, CA, USA), and carried out at the Clinical Genomics, Science for Life Laboratory, Stockholm, Sweden.

Quality trimming and assembly of the genome were performed with CLC Assembly Cell software (version 5.2.0.; Qiagen, Denmark) using the settings (clc_quality_trim –c-25 and clc_assembler -v -q -o). Species was identified by BLAST toward an in-house database with reference sequences (22) and sequence type (ST) was determined using the Multi Locus Sequence Typing (MLST) scheme from PubMLST for *Campylobacter* (23), the MLST scheme from Enterobase for *Salmonella* (24–26) and the Enterobase McNally MLST scheme for *Yersinia* (26).

In silico serovar prediction for *Salmonella* was performed with an in-house database of STs and corresponding serovars in combination with SeqSero (27).

2.4. Distribution of the PT/EQA

The participating laboratories were informed on 5 January 2021 *via* email about the anticipated number of samples and approximate time point (month) for the PT/EQA.

Samples were dispatched under refrigeration by a courier in accordance with the International Air Transport Association (IATA) packing instructions 650 for UN3373, on 12 April 2021. All 15 participants received five vials, five matrix samples, a temperature logging device, instructions, and a material safety data sheet.

2.5. Questionnaire

Instructions and a personal link for reporting were sent by email to the contact person(s) at each laboratory. The laboratories were instructed to initiate the analyses the same week the PT/EQA was received. The participants were requested to report their results *via* a web-based questionnaire at the latest on 31 May 2021. In

addition to questions on the results of the pilot PT/EQA, the web-based questionnaire included questions on the laboratory methods applied, on notification practices as well as the type of samples the laboratories usually receive (Supplementary material 2).

3. Results

3.1. Quality control

Sequencing using the IonTorrent and Illumina platforms yielded the same result except for one sample (Table 2). Analysis of the Illumina sequence data of *Y. enterocolitica* O:3/BT4 of C2 showed that the virulence factors YadA (*Yersinia* adhesin A), VirF, and the Yops (*Yersinia* outer proteins) were missing, while being present in the Ion Torrent sequence data, suggesting that the *Yersinia* ~70-kb virulence plasmid (pYV), encoding the virulence factors, may have been lost. The genome size for the Illumina sequence data showed a smaller genome compared to the Ion Torrent genome size, indicating a plasmid loss. The extracted DNA for the Illumina sequencing were from an additional cultivation cycling.

3.2. Arrival of the PT/EQA and start of the analysis

The participants received the pilot PT/EQA on 13 April (13 participants) and 14 April 2021 (2 participants).

The analyses were initiated on 13 April ($n = 4$), 14 April ($n = 3$), 14 and 15 April ($n = 1$), 19 April ($n = 4$), 13 May ($n = 1$), and 15 May ($n = 1$), 2021. One participant initiated the analysis of *Salmonella* on 19 April, the analysis of *Campylobacter* and that of *Yersinia* on 3 May 2021. After arrival, the package was stored at refrigerator temperature (+3–+8°C) at 11 laboratories, in a freezer (−20°C) at two laboratories and at room temperature (+20–+22°C) at two laboratories. The laboratories that stored the package at room temperature initiated the analysis upon arrival.

3.3. Detection and characterization of *Campylobacter* spp.

Of the 15 participating laboratories, 13 analyzed the samples for *Campylobacter*. Eight participants performed enrichment prior to plating onto a selective medium. There were some differences between the laboratories whether one or two selective media were used for detection (Appendix Table 1), and whether one or several methods, biochemical tests, Matrix-Assisted Laser Desorption/Ionization- time-of-flight mass spectrometry (MALDI-TOF), microscopy, PCR and WGS were used for species identification (Appendix Table 2). In total, five laboratories used PCR for detection and characterization of *Campylobacter* and one public health laboratory used the commercial real-time PCR system BD MAX™ (BD Molecular Diagnostics). The amount of the sample used for detection varied between 10 µL and 10 mL, the public health laboratories used smaller sample sizes (Appendix Table 1).

Campylobacter spp. were present in two vials, *C. coli* in C1 and *C. jejuni* in C3. Of the laboratories testing for *Campylobacter*, all 13 correctly detected the target organism in C1 (Table 3). Eleven laboratories reported the result at species level (*C. coli*), one at genus level and one as either *C. jejuni* or *C. coli*. Twelve of the laboratories testing for *Campylobacter* reported a correct detection result for C3. Ten laboratories reported the result at species level (*C. jejuni*), one at genus level and one as either *C. jejuni* or *C. coli*.

One false negative result was reported for sample C3 and one false positive result of *Campylobacter* spp. for sample C5. Two different laboratories reported these results and the laboratory reporting the false negative result for sample C3 correctly detected *Campylobacter* in sample C1.

3.4. Detection and characterization of *Salmonella* spp.

All fifteen participating laboratories analyzed the samples for *Salmonella*. Nine laboratories performed both pre-enrichment and enrichment prior to plating onto selective media (Appendix Table 3). Five of the six laboratories not performing pre-enrichment belonged to the public health sector and two of them did not use any enrichment methods. The amount of the sample used for detection varied between 10 µL and 25 mL, the public health laboratories using smaller sample sizes.

Species identification was performed using one or several methods: biochemical tests, MALDI-TOF, PCR and WGS (Appendix Table 4). Two public health laboratories used PCR for detection of *Salmonella*, one of them the commercial real-time PCR system BD MAX™.

Most laboratories performing serotyping of *Salmonella* used conventional slide agglutination according to the White-Kauffmann-Le Minor scheme. Three laboratories used WGS for species identification, *in silico* serovar and ST determination, either as a primary method or in addition to the other methods.

Salmonella spp. was present in two vials, *Salmonella* Stockholm in C2 and *Salmonella* Enteritidis in C3. Two public health laboratories reported false negative results for *S.* Stockholm and were the only laboratories that did not use enrichment methods. The other laboratories detected *Salmonella* and nine of them reported serovar Stockholm. All laboratories detected *Salmonella* in sample C3 and ten laboratories reported serovar Enteritidis (Table 3). One false positive result for *Salmonella* was reported for sample C4.

3.5. Detection and characterization of *Y. enterocolitica*

Of the 15 participating laboratories, eleven analyzed the samples for *Yersinia* spp. Six laboratories used enrichment methods prior to plating onto a selective medium (Appendix Table 5). The laboratories not using any enrichment methods were from the public health sector. The amount of the sample used for detection varied between 10 µL and 25 mL, the public health laboratories using smaller sample sizes.

TABLE 2 Microorganisms present in the vials. Target organisms are characterized with whole genome sequencing and indicated in bold font.

Vial	Microorganisms	Reference[a]	Sequence type (ST)
C1	***Campylobacter coli***, *Citrobacter freundii*, *Escherichia coli* O157 (*stx* neg) and *Listeria monocytogenes*	CCUG 45147	ST860
C2	***Salmonella* Stockholm**, ***Yersinia enterocolitica* O:3/BT4**, *Escherichia coli* and *Klebsiella rhizophila*	SLV-390, CCUG 45643	ST3214, ST276
C3	***Salmonella* Enteritidis *Campylobacter jejuni*** *Escherichia coli* and *Staphylococcus saprophyticus*	SLV-436, SLV-540	ST11, ST21
C4	*Micrococcus* sp., *Klebsiella oxytoca*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Candida* spp. and *Clostridium perfringens*		
C5	***Yersinia enterocolitica* BT1A**	CCUG 46850	ST147

[a]Culture collection. CCUG, Culture Collection University of Gothenburg, Sweden; SLV, Swedish Food Agency.

Species and bioserotype identifications were performed by one or several methods: biochemical tests, MALDI-TOF, PCR and WGS (Appendix Table 6). Three public health laboratories used PCR for detection of *Y. enterocolitica*, one of them used real-time PCR and one the commercial real-time PCR system BD MAX™.

The target organism *Y. enterocolitica* was present in two vials: O:3/BT4 in C2 and BT1A in C5 (Table 2). Seven of the eleven participating laboratories correctly identified *Y. enterocolitica* in sample C2. Four of the laboratories reported the results at a bioserotype or serotype level, correctly assigning O:3/BT4 or O:3 (Table 3). False negative results were reported by four public health laboratories not using enrichment methods in their routine methodology.

All eleven laboratories testing for *Yersinia* spp. identified *Y. enterocolitica* in sample C5, however, one of the laboratories obtained deviating results, reporting both *Y. enterocolitica* and *Campylobacter* spp. in the sample. Five of the eleven laboratories correctly reported BT1A.

3.6. Accreditation status of the participating laboratories

Of the 15 participants, all, except one, were accredited or quality assured for detection of *Salmonella*, eleven for detection of *Campylobacter* and seven for *Yersinia*. Five of the six public health laboratories were accredited or quality assured for detection of all the three target pathogens. Of the 11 laboratories accredited or quality assured for detection of *Campylobacter*, five covered public health, three food safety, two animal health and one both animal health and food safety. Five of the six laboratories accredited or quality assured for detection of *Yersinia* covered public health and one food safety. No animal health laboratory was accredited or quality assured for detection of *Yersinia*.

3.7. Notification of *Campylobacter* spp., *Salmonella* spp. and *Y. enterocolitica*

Notifications of findings of *Salmonella* in human samples were mandatory in six countries (Denmark, Italy, Poland, Spain, Sweden, and the UK) and in two countries (France and the Netherlands) notifications were based on a voluntary system (Table 4). Notification of *Salmonella* in food samples was mandatory in seven countries whereas conditional in animal samples in five of the eight countries. Notifications could depend on the serovar or whether the sampling was performed within official monitoring programmes. Notifications of findings of *Campylobacter* from human samples were mandatory for five countries (Denmark, Poland, Spain, Sweden, and the UK) and in three countries (France, Italy, and the Netherlands) notifications were based on a voluntary system. Notification in animal and food samples could depend on the animal species and/or matrix or whether the sampling was performed within official monitoring programmes. Detection of *Yersinia* was rarely notifiable in animal and food samples. In human samples notifications of yersiniosis were mandatory in five countries (Denmark, Poland, Spain, Sweden, and the UK), notifications based on a voluntary system in two countries (France and Italy), whereas the Netherlands has no surveillance system in place for yersiniosis. In two countries BT1A of *Y. enterocolitica* was excluded from the case definition. Two of the public health laboratories indicated that no pathogenic *Yersinia* was detected in sample C5, which was correct according to the notification criteria for these participants, since the target bacterium was *Y. enterocolitica* BT1A.

3.8. Detection or characterization of *Campylobacter* spp., *Salmonella* spp. and *Y. enterocolitica* from routine samples

Of the participants, all but two replied that they routinely received samples for detection of *Salmonella,* 11 for testing of *Campylobacter* and five for *Yersinia* (Table 5). Twelve participants received isolates of *Salmonella* for further characterization, eight for *Campylobacter* and six for *Yersinia*. The four laboratories not analyzing *Y. enterocolitica* routinely belonged to the food or animal health sector.

4. Discussion

This pilot PT/EQA is, to the authors' knowledge, the first cross-sectoral PT/EQA organized on detection and characterization of bacterial foodborne pathogens in matrices simulating samples analyzed within public health, animal health and food safety. The aim of this pilot was to assess the joint capacity to detect and characterize the target pathogens by using not specific predefined

TABLE 3 Results of the PT/EQA reported by the participants.

Lab code	Vial							False negative results	False positive results
	C1	C2	C2	C3	C3	C4	C5		
L1	*C. coli*	*S.* Stockholm	*Y. enterocolitica* O:3/BT4	*S.* Enteritidis	*C. jejuni*	No target microbes	*Y. enterocolitica* BT1A	0	0
L2	*C. coli*	*S.* Stockholm	*Y. enterocolitica*	*S.* Enteritidis	*C. jejuni*	ND	*Y. enterocolitica*	0	0
L3	*C. coli*	*S.* Stockholm	*Y. enterocolitica* O:3/BT4	*S.* Enteritidis	ND	ND	*Y. enterocolitica* BT1A	1	0
L4	*C. coli*	*S.* Stockholm	ND	*S.* Enteritidis	*C. jejuni*	ND	*Y. enterocolitica* BT1A	1	0
L5	*C. coli*	*S.* Stockholm	NA	*S.* Enteritidis	*C. jejuni*	ND	NA	0	0
L6	*C. coli*	*S.* Stockholm	*Y. enterocolitica* O:3	*Salmonella* spp.	*C. jejuni*	ND	*Y. enterocolitica* non-pathogenic	0	0
L7	*C. coli*	ND	ND	*S.* Enteritidis	*C. jejuni*	ND	*Y. enterocolitica* BT1A	2	0
L8	*C. coli/jejuni*	*Salmonella* spp.	ND	*S.* Enteritidis	*C. coli/jejuni*	ND	*Y. enterocolitica* non-pathogenic	1	0
L9	NA	*S.* Stockholm	NA	*S.* Enteritidis	NA	ND	NA	0	0
L10	*C. coli*	*S.* Stockholm	*Y. enterocolitica* BT4	*S.* Enteritidis	*C. jejuni*	ND	*Y. enterocolitica* BT1A	0	0
L11	*C. coli*	*Salmonella* spp.	*Y. enterocolitica*	*Salmonella* spp.	*C. jejuni*	ND	*Y. enterocolitica*	0	0
L12	*C. coli*	*Salmonella* spp.	*Y. enterocolitica*	*Salmonella* spp.	*C. jejuni*	ND	*Y. enterocolitica* non-pathogenic	0	0
L13	*Campylobacter* spp.	ND	ND	*Salmonella* spp.	*Campylobacter* sp.	ND	*Y. enterocolitica Campylobacter* spp.	2	1
L14	*C. coli*	*Salmonella* spp.	NA	*Salmonella* spp.	*C. jejuni*	*Salmonella* spp.	NA	0	1
L15	NA	*S.* Stockholm	NA	*S.* Enteritidis	NA	ND	NA	0	0

NA, not analyzed; ND, not detected.

10.3389/fpubh.2023.1129083

10.3389/fpubh.2023.1129083

TABLE 4 Notification status of the findings of *Campylobacter*, *Salmonella*, and *Yersinia enterocolitica* within animal health, food safety and public health of the participating countries.

Country	*Campylobacter* spp.			*Salmonella enterica spp.*			*Yersinia enterocolitica*		
	Animals	Foods	Humans	Animals	Foods	Humans	Animals	Foods	Humans
Denmark	No	No	Mandatory	Yes	Yes	Mandatory	No	No	Mandatory
France	No	No	Voluntary	Conditional[c]	Yes	Voluntary	No	No	Voluntary
Italy	Conditional[a]	Conditional[a]	Voluntary	Yes	Yes	Mandatory	Conditional[a]	Conditional[a]	Voluntary
Netherlands	No	No	Voluntary	Conditional[d]	Yes	Voluntary	No	No	No
Poland	No	Yes	Mandatory	Conditional[a]	Yes	Mandatory	No	No	Mandatory
Spain	Conditional[a]	Conditional[a]	Mandatory	Conditional[a]	Conditional[a]	Mandatory	Conditional[a]	Conditional[a]	Mandatory
Sweden	Conditional[b]	No	Mandatory	Yes	Yes	Mandatory	No	No	Mandatory
UK	No	No	Mandatory	Conditional[e]	Yes	Mandatory	No	No	Mandatory

[a]Notifiable if the sampling was performed within official monitoring programmes.
[b]Only findings in poultry are notifiable.
[c]Mandatory notification of serovars Typhimurium (and the monophasic variant), Enteritidis, Infantis, Virchow, Hadar.
[d]Mandatory notification of serovars Typhimurium (and the monophasic variant) and Enteritidis.
[e]Mandatory notification if detected from livestock.

methods but by using the methods available at the laboratories, i.e., simulate the conditions of investigations of foodborne outbreaks. Molecular methods are more commonly used at primary and reference laboratories and WGS has become an important tool for typing. Genomic data enables more reliable and precise information on source attribution.

All the participants, except one, used accredited or quality assured methods for detection and characterization. Most of the participants detected the target pathogens *Campylobacter, Salmonella* and *Y. enterocolitica* in the samples C1, C2, C3 and C5 of this PT/EQA. Regarding deviating results, most of the reported false negatives, six out of seven, were reported for sample C2 including the target bacteria *Salmonella* Stockholm and *Y. enterocolitica* O:3/BT4. These were concentrated to public health laboratories not using enrichment methods as part of their routine methodology in addition to using smaller sample sizes. The absence of enrichment, a smaller sample size and a lower concentration of target organisms in this sample may explain the observed challenges in detection, especially in a complex background flora as feces. For detection of *Salmonella* in stool samples, enrichment culture was significantly more sensitive than PCR using BD MAX (28). Thus, enrichment could be recommended unless a PCR method is shown as sensitive as the culture method.

The concentration of target bacteria in the vials used in the PT/EQA varied between 4.1 x 10^4 and 3.7 x 10^5 cfu/mL. When analyzing food samples or animal samples for asymptomatic carriers for *Campylobacter*, *Salmonella* or enteropathogenic *Yersinia* the aim is to detect low levels of these bacteria. On the contrary, when clinical samples are analyzed, the detection limit does not need to be as low, due to the higher number of pathogens. Thus, for detection in animal and food matrices by using enrichment methods, the pilot PT/EQA was probably not challenging whereas for public health laboratories not applying an enrichment step, the levels of 10^4 cfu/mL could be close to the detection limit. However, detection of *Campylobacter* at the same levels was not problematic.

Moreover, two false positive results were reported by different laboratories, one for *Salmonella* and one for *Campylobacter*. These results might have been a result of cross-contamination at the laboratory or a mistake in the reporting phase.

Especially on the public health side, more and more laboratories are changing from culture-based detection methods to PCR-based. Using PCR or other molecular-based methods, test results can be available already after 2–3 h if an enrichment is not applied whereas the culture-based methods can take from one up to several days. Many laboratories do not necessarily proceed further after the PCR step and isolation attempts may be performed only when testing for antimicrobial resistance is needed for treatment, for typing in outbreak investigations, or for targeted surveillance. The PCR results are often enough for notification as a criterium of a laboratory confirmed case, as the EU case definitions show. For detection and characterization of these pathogens from food and animal matrices, according to the EU Control Regulation 2017/625, the use of standard methods is preferable. Alternative methods, such as PCR, are allowed if they are validated against the standard method according to ISO 16140-6:2019.

Three of the public health laboratories used multiplex PCR as the primary detection method, either a commercial system or an in-house method. The BD MAX™ system for enteric pathogens was used by one laboratory without performing any enrichment of the samples. In a study using spiked samples, BD MAX™ system demonstrated 100% sensitivity for *C. jejuni* and *Salmonella* spp. tested at the following concentrations of bacteria in a sample (artificially produced by mixing stool samples with bacteria): 10^7 cfu/mL, 10^6 cfu/mL and 10^5 cfu/mL (29). At 10^4 cfu/mL the sensitivity of BD MAX™ was 100% for *C. jejuni* but only 69% for *Salmonella* spp. and 44% at 10^3 cfu/mL, which might explain the difficulties with detecting *Salmonella* spp., but not *Campylobacter* spp. in the pilot PT/EQA.

A poor performance of *Y. enterocolitica* detection and lack of non-*Y. enterocolitica* detection was demonstrated by assessing four commercially available real-time PCR systems, including the BD MAX™ system (30). The poor agreement observed in the study

TABLE 5 Detection and characterization of *Campylobacter*, *Salmonella*, and *Yersinia enterocolitica* from primary samples or isolates within animal health, food safety and public health of the participating laboratories.

Lab code	*Campylobacter*		*Salmonella*		*Yersinia*		Sector
	Detection	Characterization	Detection	Characterization	Detection	Characterization	
L1	Yes	Yes	Yes	Yes	No	No	F + V
L2	No	No	Yes	Yes	No	No	F + V
L3	No	Yes	No	Yes	No	Yes	F + P
L4	Yes	Yes	Yes	Yes	Yes	Yes	P
L5	Yes	No	Yes	Yes	No	No	V
L6	Yes	Yes	Yes	Yes	Yes	Yes	F
L7	No	Yes	No	Yes	No	Yes	P
L8	Yes	No	Yes	No	Yes	No	P
L9	Yes	Yes	Yes	Yes	No	No	F + V
L10	Yes	No	Yes	Yes	No	No	F
L11	Yes	No	Yes	No	No	No	V
L12	Yes	No	Yes	No	Yes	Yes	P
L13	Yes	Yes	Yes	Yes	Yes	Yes	P
L14	Yes	Yes	Yes	Yes	No	No	F
L15	No	No	Yes	Yes	No	No	F

F, food safety sector; P, public health sector; V, animal health sector.

10.3389/fpubh.2023.1129083

of the four PCR systems for detection of *Y. enterocolitica* might be explained by known heterogeneity between strains and different choices of chromosomal target genes such as *ail*, for detection of pathogenic *Y. enterocolitica,* and *yst*B, which is also present in most BT1A strains (31, 32). The target gene for *Yersinia* in the BD MAX™ system is *invA* which is also present in non-pathogenic *Yersinia*. Some commercial PCR systems use *ail* as the target gene, which will, with few exceptions, exclude *Y. enterocolitica* BT1A. The *ail* gene is also used as the target gene in the international standard ISO/TS 18867 for the detection of pathogenic *Y. enterocolitica* in the samples of the food chain. On the other hand, different PCR methods for detection of *Salmonella* and *Campylobacter*, in general, do not encounter similar issues related to different target genes.

Analysis of sequence data of *Y. enterocolitica* O:3/BT4 from sample C2 derived from Ion Torrent and Illumina showed that virulence factors involved in the pathogenicity of *Y. enterocolitica*, YadA, VirF, and the Yops, carried on a plasmid, were present in the first sequencing data from Ion Torrent and absent in the later sequencing performed using the Illumina platform. These findings suggest that a spontaneous loss of the pYV plasmid, encoding the virulence factors, may have occurred. The use of plasmid markers alone, may therefore not be sufficient for identification of pathogenic *Y. enterocolitica* in diagnostic settings.

In general, rapid detection or exclusion of bacterial gastrointestinal pathogens in human, food and animal samples is highly requested for the patients, the food industry and the animal keepers. However, bacterial isolates are still required for species determination, subtyping and for susceptibility testing. In future, new molecular techniques like metagenomics, probably minimize the need for cultivation of microorganisms for typing purposes, also for fecal samples.

According to the responses from the PT/EQA participants, the notification practices varied between pathogens, sectors and countries. Notification of all these three pathogens was most common within public health. Findings of *Salmonella* were notifiable across sectors although the notification could be conditional, especially within animal health. Findings of *Campylobacter* in animal or food samples were either not notifiable or conditionally notifiable and findings of *Yersinia* in animal samples were rarely notifiable in any of the countries. In a foodborne outbreak investigation, the findings of these pathogens would nevertheless be reported as part of the outbreak investigation. Due to the differences in legal notification practices, it is specifically challenging to compare and interpret surveillance data between sectors where different criteria are set enabling only specific serovars to be notified or notification is only required within specific animal matrices. However, few studies have investigated the compliance to the notification criteria. A clear variation in incidence and notification of *Campylobacter* and *Salmonella* were seen in a British general practice area (8). Whether there are variations in the compliance to the notification criteria in other regions and other sectors, is unclear.

The matrix in the present panel was similar for all the participants and independent of the sector recipient. This matrix was chosen to enable the same conditions regarding inhibitors, homogenization issues, and concentrations of the target pathogens that could influence the detection for the participants. For further studies to consider in the future, another option could be having different matrices, consisting of the same target pathogens if the sensitivity within the specific matrix would be an important aspect to cover.

The panel was set in an epidemiological context of an outbreak scenario. Cross-sectoral panels put in an outbreak scenario should trigger further discussions between the sectors on differences in methods for detection and typing, and notification rules. For future cross-sectoral panels, the results outcome of the panels, methods used, and notification criteria could be discussion topics for cross-sectoral post PT/EQA workshops. This could in turn increase awareness of cross-sectoral differences which need to be taken into consideration when interpreting surveillance data within OH.

The target foodborne zoonotic organisms for future panels could also have specific resistance profiles, which could be part of the testing capacity. Approaches for phenotypic testing of antimicrobial resistance may vary between sectors. In addition, using WGS for predicting antimicrobial resistance and typing has increased during the last years for e.g., *Salmonella* and *Campylobacter* and could be considered as a characterization option in future schemes. WGS for determination of antimicrobial resistance would primarily be used for surveillance purposes (33) and not for assessing treatment regimens as the phenotypic and genotypic methods do not fully correlate.

In conclusion, this pilot PT/EQA showed that a cross-sectoral approach could be used for assessment of the OH capacity to detect and characterize foodborne pathogens. PTs of the food and animal laboratories are often used to test a specific predefined method whereas the EQA schemes of the public health laboratories are most often used to assess the capacity to correctly detect and characterize independent of the applied methods. Cross-sectoral PT/EQA schemes could result in more general recommendations, e.g., on the target genes for PCRs, or on the characterization methods to apply. Moreover, the organization of such comparative testing schemes stimulates collaboration and discussion across laboratories working in different countries and sectors, setting the ground for further development of methodologies applied to face foodborne zoonosis. Future cross-sectoral PT and EQA schemes should include a genomic aspect, for instance by assessing the performance of the analysis of bioinformatics. The pilot showed that the participating laboratories, however working in different countries and sectors obtained a wide level of agreement even if using different methodologies. This information is currently limited and is pivotal for ensuring comparability of results at the EU level, especially when considering scenarios such as outbreak investigations.

Data availability statement

Original datasets on sequencing data are available in a publicly accessible repository: The original contributions presented in the study are publicly available. This data can be found here: https://www.ebi.ac.uk/ena/browser/home accession number PRJEB57080. The other original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

ET, HR, LB, and CJ designed the study and analyzed the results. ET, NK, HR, LB, and CJ drafted the manuscript. All authors collected data, contributed to the interpretation of the results, contributed to the manuscript, and accepted it.

Funding

This work was supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme and was part of the OH EJP project CARE. Research at the National Veterinary Research Institute (PIWet), Poland, was also partially supported by the Polish Ministry of Education and Science from the funds for science in the years 2018–2022 allocated for the implementation of and co-financed international project.

Acknowledgments

The authors would like to express their gratitude to Ségolène Quesne, Sandra Rouxel and Emmanuelle Houard (Anses, France), Yngve Dahlborg (Gävle Region, Sweden), Wendy van Overbeek and Lucas Wijnands (RIVM, the Netherlands), Jonas Ilbäck och Marianne Törnquist (Swedish Food Agency, Sweden), María García, Estefanía Rivero, and Nisrin Maasoumi (VISAVET-UCM, Spain) for their technical skills in performing the pilot study and other contributions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1129083/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 18 April 2023
DOI 10.3389/fpubh.2023.1129851

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention, a section of the journal Frontiers in Public Health

RECEIVED 22 December 2022
ACCEPTED 30 March 2023
PUBLISHED 18 April 2023

CITATION
Amato L, Benedetti G, Di Giuseppe P, Hénaux V, Lailler R, Nordeng Z, Scharffenberg TAZ, Skjerdal T and Cito F (2023) Mapping food surveillance chains through different sectors. *Front. Public Health* 11:1129851. doi: 10.3389/fpubh.2023.1129851

Mapping food surveillance chains through different sectors

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European countries are investing in strengthening disease surveillance from a One Health (OH) perspective. During the MATRIX project, in the context of the One Health European Joint Programme, existing surveillance chains across the sectors of animal health, food safety, and public health have been investigated through questionnaires. Provided information has then been selected to be displayed in a single slide using an implemented mapping template. Two real-life scenarios are presented as case studies: the surveillance activities in place in France for *Salmonella* in the pork meat food chain, and in Norway for *Listeria monocytogenes* in the dairy food chain. The results collected through the questionnaires and the lessons learnt during the mapping process are reported, to share the advantages and drawbacks of the methodology. Moreover, the presented template could be adjusted and applied to different contexts. Mapping the components of existing disease surveillance systems is a fundamental step in understanding the relationships between its components, and subsequently facilitating their collaboration and integration under a OH approach.

KEYWORDS

One Health, surveillance, food safety, *Salmonella*, *Listeria*, Norway, France

1. Introduction

One Health (OH) defined as "an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems," has become a widely accepted topic in the current debate about disease surveillance, and has a significant impact on the related health agenda (1–3). However, the practical application of the OH approach to real-life, existing surveillance systems is not easy. One Health surveillance (OHS) systems are not developed from scratch and the starting point is usually a combination of different hazard-specific problems, approaches, and objectives across the human, animal, and food safety sectors (4–6). Surveillance systems are complex structures and making the information gathered by a surveillance system useful for the involved stakeholders is not effortless (7–9). The OH approach necessarily adds complexity to existing surveillance systems and their chains of data flow. The

complexity of the OH approach is related to the persistence of silo thinking (10), which, despite being effective and useful in terms of following up on specific actors and topics, complicates collaborations among actors within each segment of the 'farm-to-fork' chain.

European countries have invested in strengthening disease surveillance from a OH perspective with some successful collaborations, such as the Med.Vet.Net Association and the One Health European Joint Programme (OHEJP), which are now paving the way forward (11, 12). The OHEJP is a partnership between 44 European food, veterinary, and medical laboratories and institutes across Europe and the Med.Vet.Net Association (12). Among the many activities, including training opportunities and collaborations with the European intergovernmental agencies European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), the programme supports various research and integrative projects to stimulate the scientific development and integration of surveillance systems in a OH perspective (13).

In MATRIX, one of the OHEJP projects, the aim was to advance the implementation of OHS in practice, by building on existing resources, adding value to them, and creating synergies among sectors. The project created practical solutions for European countries to support and advance the implementation of OHS (14). MATRIX operated with a focus on specific pathogens/hazards (hazard tracks, HT) to ensure that the solutions developed by the project were relevant to their surveillance. The hazards were chosen in 2019, based on the operational priorities of the 19 MATRIX partner institutes across 12 European countries and their OH relevance, namely: *Campylobacter*, *Listeria monocytogenes, Salmonella,* and emerging threats, including Hepatitis E virus.

Prior to the integration of any surveillance system is the understanding of the relationships among its components. Mapping the components of existing disease surveillance systems is a fundamental step to facilitate subsequent integration of them from a OH perspective. As part of the broader objective to identify current examples of best practices and multi-sectorial collaborations across surveillance systems, one of the tasks of MATRIX aimed to map existing surveillance chains across the sectors involved in the surveillance of the project HTs, for at least one country per HT. Since the considered HTs are foodborne pathogens, the investigation followed the 'farm-to-fork' chain approach. The results of this work are detailed in a document published on Zenodo (15), the open repository developed under the European OpenAIRE programme. However, the mapping exercise allowed the identification of both opportunities and challenges of this investigation approach of what is already in place in different countries. In this paper, we therefore will describe our methodological approach, and be presenting two real-life scenarios as case studies.

The two scenarios chosen as case studies are the surveillance of *L. monocytogenes* in dairy products in Norway, and the *Salmonella* surveillance in pig meat in France. The scenarios concern pathogens that are of importance for human health based on the severity (*L. monocytogenes*) or the frequency (*Salmonella*) of the infections.

In 2020 listeriosis was the fifth most reported zoonosis (1,876 cases) in Europe, mainly affecting people over the age of 64 (16). In Norway, the number of annual cases of listeriosis in humans has been increasing gradually. Between 15 and 50 cases have been reported annually during the last decades, including a total of 37 cases in 2020 (17, 18). Given the severe symptoms and fatality rate of listeriosis cases, and a high probability of an increased human burden of disease, *L. monocytogenes* was ranked in the top five groups of biological hazards in a risk ranking and source attribution study carried out by the Norwegian Scientific Committee for Food and Health (19).

In general, the prevalence of *L. monocytogenes* in food is low, but the bacterium can grow rapidly when there are optimum conditions of pH, temperatures between 30 and 37°C, and a water activity of 0.99 (20). The theoretical minimum for growth is in conditions of pH 4.3, water activity of 0.92, and a temperature of −2°C, and both in presence or absence of oxygen (20). The minimum infectious dose is not known, but dose–response models indicate that the marginal probability of developing invasive listeriosis upon ingestion of one cell of *L. monocytogenes* per individual for the general population is 8×10^{-12}, and 3×10^{-9} for extremely susceptible subpopulations (21). Applying this to concentrations of *L. monocytogenes* in food, these numbers fit with the observation that the estimated probability of illness increases at 1,000 cfu/g for the most vulnerable consumers and at 100,000 cfu/g for adults with no underlying illness, provided that the usual portion size is 100 g of food (22). When the growth conditions are good or the shelf life of the food is long, a high concentration of the bacterium can be reached before consumption. Foods with growth potential for *L. monocytogenes* that have a sufficiently long shelf life to exceed the critical concentrations mentioned above are regarded as risk products, unless they are heat-treated or *L. monocytogenes* is killed by other means before consumption. Contaminated, unpasteurised milk and other food ingredients are only some of the possible sources for the introduction of *L. monocytogenes* into dairies (23). *L. monocytogenes* can enter production facilities and remain for an extended time, even decades, contaminating the food at regular or irregular intervals (24). In addition, soft and semi-soft maturing cheeses are both examples of risk products for listeriosis. Outbreaks have been observed with cheeses from both pasteurised and unpasteurised milk: the largest in Norway was related to camembert cheese from a small-scale producer using pasteurised milk (25).

Dairy products are important both economically and culturally in Norway. Norwegian cheeses are, with only a few exceptions, produced and consumed domestically. In 2021, the annual consumption of cheese per person in Norway was 20,35 kg, of which 82% was produced in Norway (26). The import of cheese was about four times higher than the export (27). The variety of products from small-scale producers is large, and includes both pasteurised and unpasteurised products; the majority of dairy products sold are however coming from a few large producers, who produce from pasteurised milk and have extensive internal sampling programmes in place (15).

On the other hand, *Salmonella* is estimated to be responsible for more than 75 million foodborne infections worldwide each year (28). In Europe, salmonellosis was the second most frequent zoonotic disease reported, with more than 91,000 cases reported each year until 2018, representing an economic burden of around 3 billion euros (29). A marked improvement in this epidemiological situation can however be noted in comparison to the 200,000 annual number of human cases reported before 2004. The last Joint European zoonosis report from ECDC-EFSA highlighted decreasing number of human salmonellosis cases and *Salmonella* detection in food and animal sectors from 2016 to 2020. Nevertheless, this may be partly due to underreporting during the COVID-19 pandemic and Britain's EU departure (16).

However, the number of positive sampling units related to the 'pigs' sector was stable in Europe over the same period (2016–2020). Pig meat and products thereof remained the second-largest source of salmonellosis

food-borne outbreaks, with 11 strong-evidence outbreaks in 2020, compared to 37 outbreaks due to eggs and eggs products. Numerous *Salmonella* serovars were detected all along the food chain. Of these, *S.* Typhimurium, monophasic *S.* Typhimurium (1,4,[5],12:i:-) and *S.* Derby belonged to the top five, and were primarily related to pig sources (16). For these reasons above, the second scenario chosen as a case study is the *Salmonella* surveillance in pig meat in France.

In France, 139 among the 1,010 food-borne outbreaks declared in 2020 were attributable to *Salmonella* (120 were confirmed to have the presence of *Salmonella* in food, and 19 cases were suspected) (30). The annual number of illnesses attributable to *Salmonella* is estimated at 183,000, including 4,110 hospitalizations and 67 deaths (31). In France, 13 food-borne outbreaks were identified between 2002 and 2017, associated with products of porcine origin (32).

Contaminated raw animal food products are the main source of human infection. Contamination may occur during the processing stages from improper food handling and/or inadequate hygienic measures. Eating behaviours involving ingesting raw or undercooked products also pose a risk of infection (33). Most (42%) of reported cases of salmonellosis are linked to the consumption of eggs or egg products (34), but products from the pigs and dairy cattle sectors are also recognised as important reservoirs (35).

In pig farming, when an outbreak occurs, symptoms may include diarrhoea and growth delay. In farms with high biosecurity standards, the introduction of breeding animals and feed are considered the major routes for the introduction of *Salmonella*. Contamination of meat products most often occurs during the slaughtering of infected animals, when hygienic practices are lacking. For this reason, active monitoring is in place and is performed by the competent authority. In 2020, French food business operators (FBOs) performed more than 14,000 official controls at slaughterhouses and detected 4.8% (IC 95%: [4.4–5.2]) of pig carcasses contaminated by *Salmonella* (16).

At this stage, however, the integrated surveillance of *Salmonella* in the pig sector does remain needed in France. A shift towards a multi-sectorial approach is currently ongoing with the implementation of a collaborative and multidisciplinary platform dedicated to food chain surveillance (36).

The purpose of the paper is to describe the methodological approach we used to map the components of the existing disease surveillance systems for these two case scenarios, to enable its further application, and to share the lesson learned.

2. Materials and methods

2.1. Online questionnaires

Within the activities of the project MATRIX, a multiple-choice questionnaire was created for each of the four hazards (*Salmonella, Campylobacter, L. monocytogenes,* and Hepatitis E virus), to gather the necessary information for the mapping of the existing food chain surveillance activities from national experts in the field. As an adaptation of the approach from 'farm-to-fork' to 'farm-to-patient', each questionnaire was divided into three different sections: (I) focusing on the animal health aspects (AH), (II) on the food safety aspects (FS), and (III) on public health (PH). In each section, the surveillance was assessed by gathering information on actors, sampling context, collected sample types, laboratory methods for diagnosis, available data sources, and cross-sectoral collaboration in place. To ensure to include all the relevant information, eight experts were consulted during the implementation of the specific questionnaires for each sector.

The draft version was circulated amongst the MATRIX participants for evaluation and implementation. The MATRIX partners were asked to suggest possible contact persons with expertise in the specific field of interest, between project partners and non-partners institutions. The identified experts were individually contacted to verify their interest and availability in taking part in the survey. The final version of the questionnaires was put online on the survey platform Survey Monkey©, for dissemination to the relevant experts previously selected. Given the specificities of the information required, a PDF version of the questionnaires (see Supplementary material, modified with permission from Cito et al., 2022 (15)).

2.2. Mapping template

A questionnaire was considered completed when answers from the three involved sectors (AH, FS, PH) were obtained. Upon the reception of the three compiled sections, a preliminary evaluation of the results was carried out. Where missing or unclear information emerged, we requested clarifications by re-sending the questionnaire to the reference expert (or to a different one). For this reason, the questionnaires were open for completion for a period of about six months.

In order to evaluate and display the collected information, a categorisation was put in place: information was classified as part of 'data', 'metadata', 'events', 'event producing data (EPD)', and/or 'identified data source (IDS)' (15).

The subsequent step was then the identification of the most relevant information, for their graphic representation on a map. Therefore, the information regarding the actors, the sampling context, the collected sample types, the laboratory methods in use in the diagnosis, and the available data sources, for each one of the sections, were highlighted. For the purpose of the task, we designed a template of the mapping and displayed it using MS PowerPoint© (Figure 1).

2.3. The two case studies

One of the main objectives of the MATRIX project was to map the surveillance systems along the food chain. To achieve this objective, we selected a specific food chain to be investigated in detail per each hazard. Combinations that are relevant from the public health point of view were selected, based on a consensus among the MATRIX Consortium on the epidemiological situation in 2020 in Europe.

Concerning *Listeria*, the selected food chain was dairy products, given the epidemiological relevance of these products for the transmission of *L. monocytogenes* to humans. The investigated country was Norway, because of the economic and cultural importance of dairy products (23, 37).

Regarding *Salmonella*, we decided to assess surveillance activities in France in the pork meat food chain to avoid overlapping with the OHEJP project NOVA (38), which investigated the poultry food chain with regard to *Salmonella*

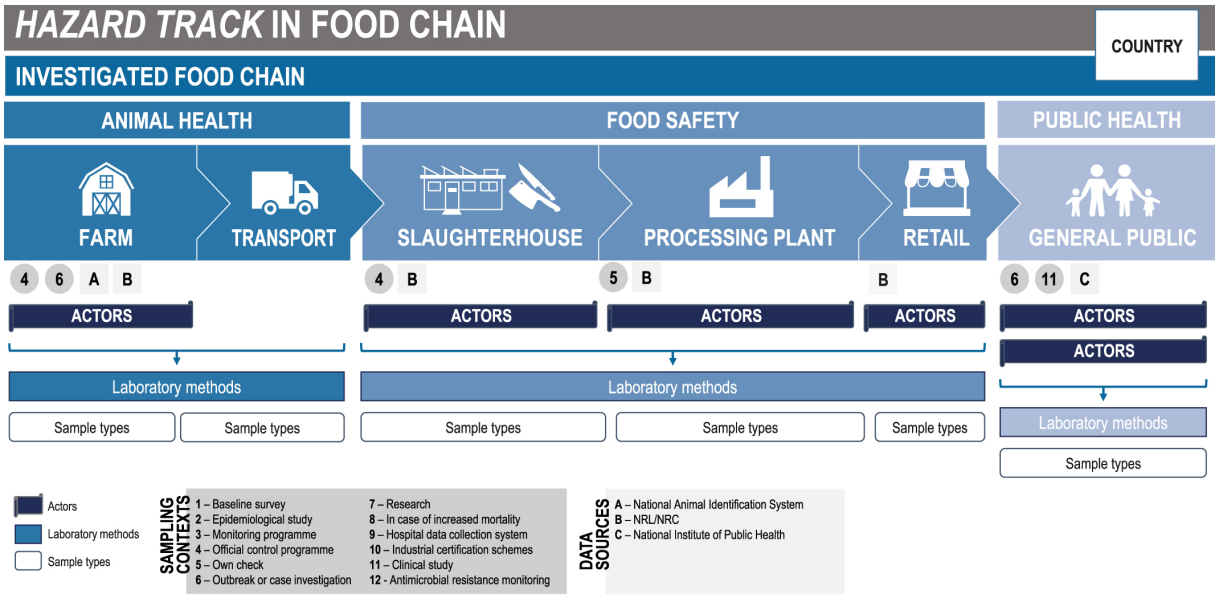

FIGURE 1
Mapping template. Modified with permission from Cito et al., 2022 (15).

surveillance activities. For this reason, some information was already available, while less information existed for the pork meat food chain and the same pathogen.

3. Results

We present below the results collected through the questionnaires on *L. monocytogenes* in dairy products in Norway, and *Salmonella* in the pork meat food chain in France, based on the information provided by the experts involved.

3.1. *Listeria*

In Norway, the national and regional surveillance programmes in place are designed to detect illness cases among humans and animals, and non-compliance to food safety criteria in food, adapted to different production routes (Figure 2).

3.1.1. Animals

Veterinary technicians and/or private veterinarians carry out surveillance activities in the animal sector and perform outbreak investigations in case of increased mortality. Abortions are investigated, and bulk milk and blood from sick animals are collected. The bulk milk is routinely analysed at large-scale dairies, where the focus is on milk quality and production hygiene indicators rather than on *L. monocytogenes* specifically. Neurolisteriosis (meningitis) in animals is not a notifiable disease in Norway: clinical cases are not registered systematically, and clinical suspects are only rarely confirmed by laboratory diagnosis. The few laboratories that are involved in the diagnostics of listeriosis in animals work collaboratively at the national level. Even though laboratory results are not shared automatically, information can be made available upon request. The number of confirmed animal cases per region is reported and shared at the national level (15).

3.1.2. Foods

The sampling plans in the official national programmes are designed to cover imported foods and local small-scale dairy products. Large-scale dairies usually have their own sampling programmes. The surveillance of small-scale producers includes the sampling of summer products. In some programmes, '24 h samples' (which means sampling the day after the start of the maturation process) are implemented in farms and small-scale dairies, as several pathogens can be found at the highest concentration at this stage. This kind of sampling allows for the rapid detection of anomalies and allows for sampling without the loss of the entire cheese.

Sampling is also performed at the retail level, in compliance with the microbial criteria in the food legislation. In addition, metadata like production date, shelf-life date, animal species, whether the product is made of pasteurised or unpasteurised milk, producer, sampling place (address and kind of shop), and sampler can be recorded. For all products, a picture of the product is also collected. Auditors from the official control authorities carry out the sampling and the follow-up of positive samples with the producers.

The National Reference Laboratory for *Listeria* in food, which is represented by the Norwegian Veterinary Institute (NVI), carries out the analysis of *L. monocytogenes* and other microbes. Detection and enumeration of *L. monocytogenes* are always included in the analyses. Whole genome sequencing (WGS) is newly applied, while it was not fully operational at the time at which the questionnaire was available for response. Isolates are stored for further analyses, for instance in case of outbreak investigation or research. Positive results are directly notified to the auditors, to allow rapid outbreak

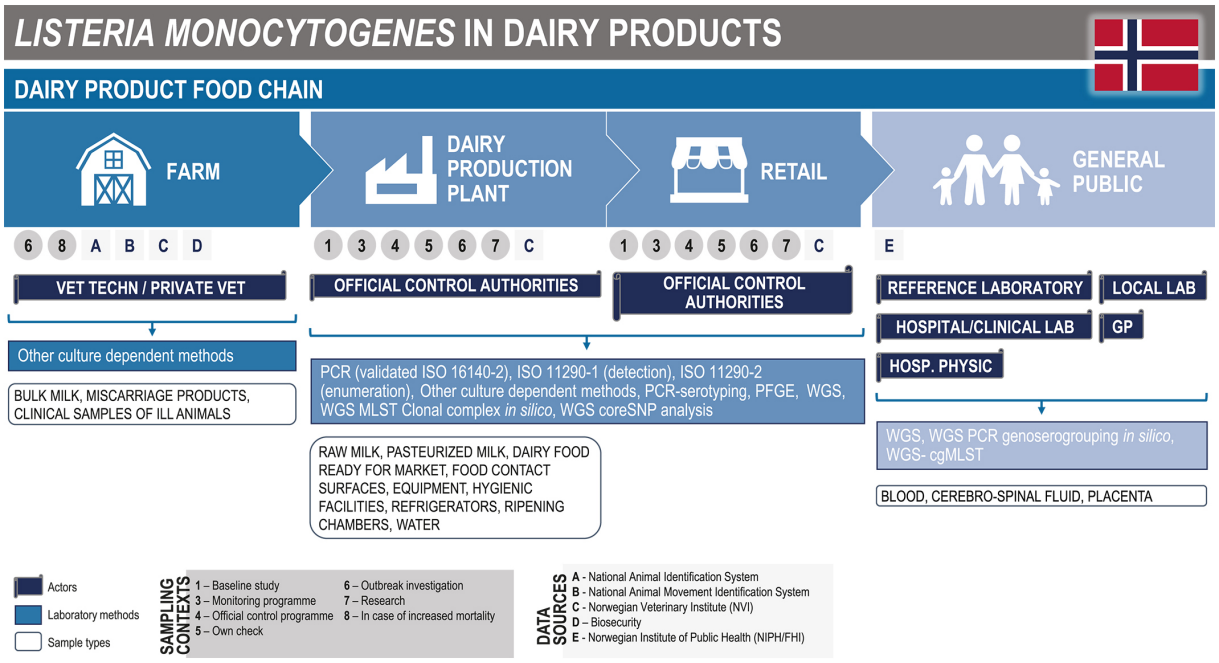

FIGURE 2
Listeria monocytogenes mapping. Modified with permission from Cito et al., 2022 (15).

investigations and direct follow-up in case of non-compliance. In addition, all the results are anonymised, categorised, and presented annually or at the end of the programme. However, the national active surveillance programme for cheese and milk products is adapted intermittently: the focus foods for surveillance are decided every 1–3 years, based on priority lists for hazards and foods of particular concern.

Besides the official surveillance programme, the farmers and dairies have their own-check sampling programmes in place, and hazard analysis and critical control points (HACCP) plans. Sampling in these cases may include the testing of surfaces, equipment, refrigerators, and water.

3.1.3. Humans

Human listeriosis in Norway has been nominatively notifiable in the Norwegian Surveillance System for Communicable Diseases (MSIS) (39) since 1991 (NIPH, 2022). Age, gender, place of residence, and travel history are among the parameters collected. The official number of cases is updated daily (15).

Medical microbiological laboratories in Norway are obligated to send clinical *L. monocytogenes* isolates to the National Reference Laboratory for Enteropathogenic Bacteria at the Norwegian Institute for Public Health (NIPH). WGS is performed routinely for confirmation, surveillance, and outbreak purposes (NIPH, 2022). All listeriosis cases are routinely investigated with a trawling questionnaire. When a WGS cluster is detected, epidemiological parameters as well as information from the trawling questionnaire are considered before the outbreak investigation is initiated.

During an outbreak investigation, the NIPH works in close collaboration with municipality doctors, the Norwegian Food Safety Authority, and the NVI.

3.2. *Salmonella*

In France, the *Salmonella* surveillance is based on a national system composed of approximately fifteen components or networks (36). The system covers the entire food chain and most populations who are more at risk for these pathogens. Surveillance aims at reducing the risk for consumers through earlier detection of contamination by *Salmonella* in the food chain, limiting the economic impact of these contaminations in the production chains, and advancing knowledge.

The French Public Health Institute, named 'Santé publique France' (SpF), defines a foodborne outbreak at the national level as the occurrence of at least two cases of similar symptomatology, generally gastrointestinal, which are attributed to the same food origin. The notification of cases has been mandatory since 1987. A notification can lead to investigations through the whole food chain and within different animal and food production sectors (Figure 3). In the past, the pork food chain has been impacted on several occasions by *Salmonella* contamination (40, 41).

3.2.1. Animals

In the animal sector, many activities for *Salmonella* surveillance are implemented at the farm level in France (Figure 3), which are carried out by official control authorities, laboratories, farmers, the industry, private veterinarians or technicians, and eventually research centers or institutions like universities.

In the framework of monitoring programmes, outbreak investigations, or research projects, these actors collect environmental samples, including fecal material, water, and feed to detect and identify the bacteria by phenotypic or molecular methods. Laboratories implement official methods to serotype all isolates and, among this

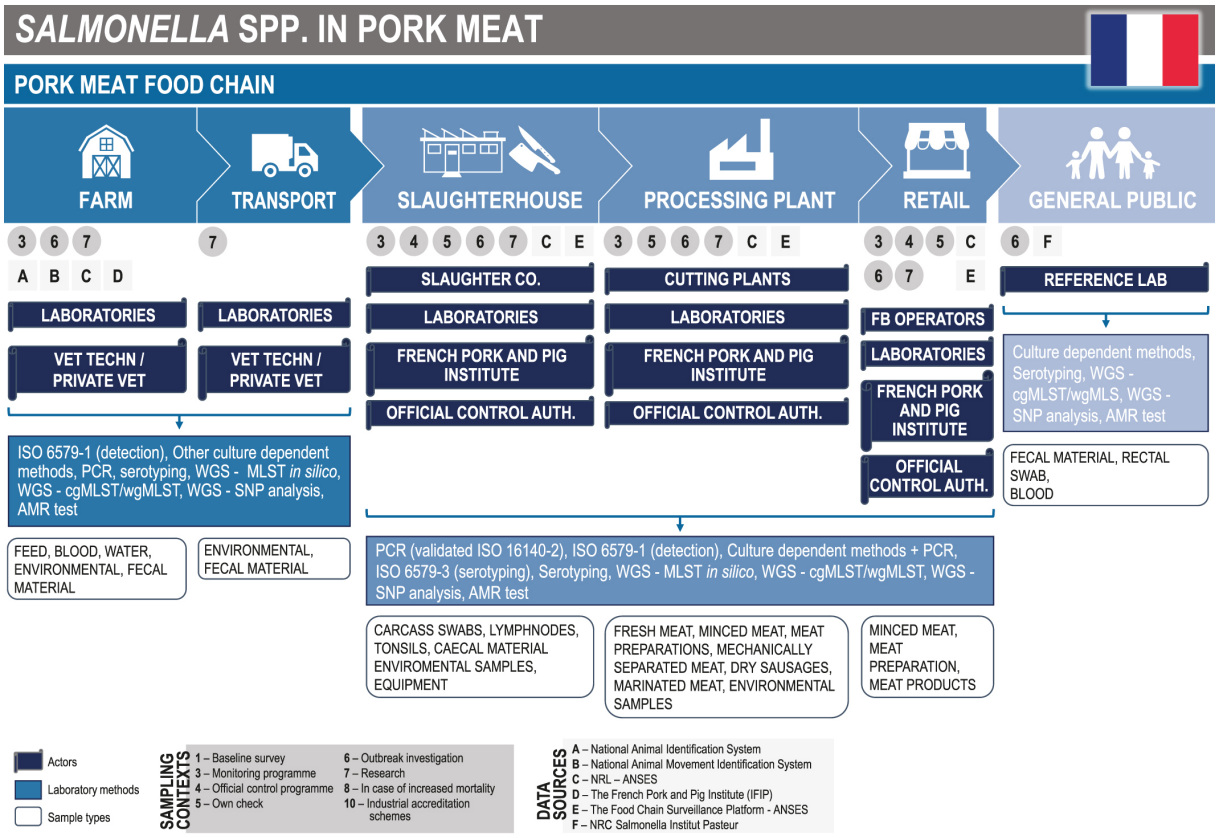

FIGURE 3
Salmonella mapping. Modified with permission from Cito et al., 2022 (15).

panel, only a part of the samples is typed in depth by polymerase chain reaction (PCR), SNPs, or cgMLST. All strains isolated in an outbreak context are sequenced with the technical support of the National Reference Laboratory (represented by the French Agency for Food, Environmental and Occupational Health and Safety - ANSES). These surveillance activities (through research) also concern animal movements.

The monitoring and control of the application of biosecurity measures are particularly important, for both breeding and fattening pig farms. For this reason, additional data including personnel movement, and records of cleaning and sanitation procedures, is collected. The French Pork and Pig Institute (IFIP) stores the collected data at national and regional levels, and shares with other actors information on the coverage of surveillance activities and descriptive epidemiological results.

3.2.2. Foods

For the food sector, official control authorities, the private sector, laboratories, and the IFIP predominantly perform activities at the slaughter and processing plants. Carcass swabs sampled at the slaughterhouses for official control programmes, are collected with other samples retrieved from the environment and equipment during monitoring programmes, own-checks, or outbreak investigations. Information on the activities performed at the retail stage, provided through the questionnaires, included that minced meat and meat preparations/products are subject to monitoring and research activities, outbreak investigations, official control programmes, and own-check.

In France, sampling conducted within established surveillance programmes aims to investigate the exposure to *Salmonella* spp. In addition, sampling is targeted at consumer groups (e.g., vulnerable consumers, and consumers of a high amount of a particular food), and import/export. In case of non-compliance, depending on the results of the risk analysis, additional analyses may be carried out on the relevant products. Routinely, laboratories test samples for *Salmonella* detection by culture-dependent and molecular methods based on PCR. Each isolate is serotyped by the method of reference (ISO 6579-3:2017). WGS is performed to type strains that are suspected to be linked to food-borne outbreaks when epidemiological evidence (descriptive or analytical) is limited. The percentage of typed strains depends on the context but represents only a small fraction of the isolated strains. The overall process of testing and reporting may take months to conclude, even if the testing process is typically quite rapid.

In 2018, the Food Chain Surveillance Platform was created to support surveillance activities and to promote an operational OH approach at the national level. This innovative structure is based on public and private governance. It effectively coordinates notably working groups on *Salmonella* with stakeholders including the IFIP, the *Salmonella* National Reference Laboratory (NRL), and National Reference Center (NRC), which are both hosted by a research unit

from ANSES and Institut Pasteur, respectively, and numerous partners involved in the French *Salmonella* surveillance system (36).

3.2.3. Humans

In France, sporadic cases of salmonellosis are not notifiable diseases. Several actors, from local health authorities to hospitals/clinical/reference/local laboratories, monitor for human salmonellosis. In general, consistent data related to case detection are collected on a routine basis, while additional epidemiological data are collected mainly during outbreak investigations.

A research unit from Institut Pasteur hosts the French mandate of NRC for *Salmonella*. This reference laboratory collects strains and data related to human cases confirmed by contaminated blood or faecal material. NRC shares confidential data related to each case with SpF, including the severity of symptoms, and spatial and temporal data. WGS is systematically performed, and results are centralised. Algorithms using this database produce weekly alerts when clusters based on microbiological data occur, and then the NRC informs SpF of these situations. Currently, there is no automatic tool or shared database in place at the national level to allow prompt interaction between human and non-human sectors. To date, the ability to share data mainly depends on the interpersonal connections between scientists working at the reference laboratories (NRC and NRL).

In conclusion, the collaboration between sectors exists mostly for foodborne outbreak surveillance and investigation. The exchange of information issued from investigation frameworks is in place between the Regional sanitary authorities in charge of human surveillance ('Regional health agency') and of food safety, animal health, and welfare ('Departmental Directorate for Social Cohesion and Population Protection'). Additionally, information is shared with the national competent authorities to implement adjusted control measures. The NRC and NRL have a central position in the framework, managing laboratory networking, developing, and harmonising analytical methods, and interacting with administrative organisations and professional and technical centers (including research).

4. Discussion

4.1. The online questionnaires

The methodological approach adopted during the MATRIX project included the use of online questionnaires to collect information about surveillance in place in European countries. Our approach allowed for a substantial set of information to be obtained, in terms of both quality and quantity.

Although in some cases surveillance activities are regulated by the existing European legislation [i.e., control programmes regarding *Salmonella* (42), official controls under Regulation 2017/625 (43) to verify that food complies with microbiological and process hygiene criteria established by Regulation 2073/2005 (44) or epidemiological surveillance of communicable diseases (45)], in other there is no harmonised surveillance in the European Union. For this reason, the collection of information from the existing European legislation would have represented only a fraction of the overall amount of information gathered by the questionnaires.

The questionnaires mainly asked closed questions with multiple-choice answers and checkboxes. This can potentially lead to biases, defined as a 'deviation of results or inferences from the truth, or processes leading to such a deviation' (46). The biases may particularly result from the design of the questions and questionnaires, and/or from their modalities of administration and completion (46). Semi-directive interviews may have allowed for collecting information that is more comprehensive. However, the conduction of interviews would have been more time-consuming and potentially introduced a greater risk of biases, given the interviewer's subjectivity. Moreover, the use of questionnaires was a good alternative to in-person workshops, which were not feasible during the period of travel restrictions due to the SARS-CoV-2 pandemic. The questionnaire and the subsequent mapping made possible the drawing up of the initial description of the surveillance structure as the starting point for working collectively, and in more detail on each aspect.

When using online questionnaires to collect information, the implementation can be an involved process, and it requires resources with expertise to design, pilot, and put them online. Both compiling and responding to the questionnaires also require deep knowledge of the subject. Therefore, depending on the involved expert in the compilation and response respectively, possible biases may be introduced. In addition, the splitting of the questions according to the three investigated sectors could not be sufficient, because even within the same sector the skills are diversified. As consequence, it could not be expected that each expert had the expertise to cover all aspects included in a single sector questionnaire (i.e., from the surveillance programmes in place, to existing information systems, and to laboratory tests used for diagnosis).

To mitigate these risks, we applied the approach of involving, first, a country expert within the OHEJP MATRIX partner institutes and asking them to share the questionnaires with the appropriate experts, which could belong to different agencies. In this way, we gathered information not only from project partners but also from all three sectors involved in the surveillance of the pathogen under investigation.

4.2. Mapping template

The mapping process could be a key step in initiating collaborative work to set up or improve a surveillance system. It seemed essential to clearly identify the actors involved in the monitoring, their role, and their position in the organisation, before considering implementation or possible adaptations and changes as actions, to achieve pre-established consensual objectives (36).

Although some examples of mapping were already available (47), we designed a new template to display the relevant actors and other data regarding HT-specific surveillance. The key aspect of the mapping is the presentation, with a single figure, of the three investigated sectors, and for each sector the implemented surveillance activities. In this way, a clear visualisation and a quick comparison of the information reported is possible and the One Health approach is represented.

The three involved sectors were animal health, food safety, and public health. Besides the food safety area, the OH approach can be applied to many others, covering complex health issues and requiring close collaboration across sectors, stakeholders, and countries (48). Hence, our template can be applied to several different

contexts, by simply adjusting the underlying structure. Beyond the purpose of the MATRIX project, in which a method to display/map surveillance activities was developed, the same method could be applied to several other scenarios. As a generic approach, the implementation of this template could facilitate also the description of areas within chemical monitoring, for example, using a preliminary adaptation of the questionnaire. Across further applications, the mapping approach could cover a whole production sector, impacted by several contaminants, or a specific contaminant monitored by multiple production sectors.

4.3. The two case studies

In this study, we emphasised the methodology rather than the data collected using the questionnaire. Significantly more data than those shown on the maps were collected. The complete results are enclosed in a specific deliverable of the MATRIX project (15). Here, we presented the application of the mapping of *L. monocytogenes* in Norway and *Salmonella* in France, as they were representative of two situations in which such information was thoroughly reported.

The discussion with the experts on the two case studies highlighted how communication between official partners is generally more efficient when colleagues from different sectors know each other. Direct familiarity and trust can be important added values for successful surveillance and outbreak investigations (49).

The mapping clearly showed that surveillance of the animal and food sectors needs to be specifically designed to catch the production, processing, and use of the food products, by covering features such as seasonality, regional differences within a country, and large versus small-scale productions. The mapping method could be particularly useful in the case of a food category with a domestic market and small-scale producers, to follow up with the producers who do not have the size or economy to carry out many analyses. The additional value of using this approach, besides building connections and trust among authorities and producers, is to identify conditions that could lead to outbreaks, rather than detecting outbreaks when they have already started. The approach of having sampling schemes designed for the detection of risk factors within each sector, and combined with suited characterisation analyses and data sharing with other relevant sectors, can result in cost savings and rapid detection of OH challenges, regardless of the original purpose of the surveillance programme.

For the food health segment, the focus has been placed on the consumers. It is possible to arrange different surveillance programmes for various vulnerable groups, but this aspect is already targeted in passive surveillance systems, when consumers go to the doctor if they are ill. The human health surveillance programme operates in a similar manner, regardless of the food segment covered. The contact between animal, food, and the human sector is likely to be easier for domestically produced and consumed food, as the options for signaling are more between people who know each other and work together on a regular basis, than if animal, food, and human health segments need to be alerted with official channels first.

However, it is critical to define the specific situations under which other sectors should be alerted, and what information (in terms of data and metadata) should be shared among the different identified actors. Generally, the implementation of the OH approach is easier under the circumstance of an outbreak, since all the involved actors have the common goal of identifying the source of the infection and implementing control measures. The same thing does not happen during routine surveillance. Therefore, there is a general need for 'traffic lights' and checkpoints, about what to share, when, and why. While it is true that trust is important for sharing and respecting the rules agreed upon, active communication between sectors is a prerequisite for building trust. Collaborations are established gradually, based on the adhesion of the partners to a common organisation. A mapping stage could therefore be a prerequisite for establishing a shared and integrative vision of the organisation of surveillance activities, as a ground for further collaborative efforts. As an example, an approach to OH surveillance of listeriosis was suggested already in 2001 from France but was not followed up by other countries (50). The current work in France and Norway to improve the efficiency of food hazard surveillance throughout the food chain is highlighting how long, sensitive, but successful, the process is.

However, these food-borne hazards are not solely present within specific countries but are widespread in Europe and beyond. Because animals, food, and people move between countries, establishing links between specific country hazard maps would be useful. Likewise, efforts towards a OHS should be first made at the national level, and at some point linked internationally.

5. Conclusion

During the MATRIX project, we proved that it is possible to map surveillance chains of foodborne pathogens of One Health relevance across the human health, animal health, and food safety sectors in various European countries, and the methodological approach described in this manuscript is replicable in several contexts. Although many efforts are implemented to remove barriers to a better application of the One Health, the importance of shifting from silo thinking should not be underestimated. The methodological approach that we presented can support identifying new opportunities for integrating OHS, while lifting our heads and looking further than we normally do, as it happens during research projects.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon request, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

LA, GB, PG, TSk, and FC: idea and conceptualization. LA, FC, and TSk: methodology. LA, PG, and FC: data curation. LA, GB, VH, RL, ZN, TSc, TSk, and FC: original draft preparation and revision and editing. FC: supervision. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 773830: One Health European Joint Programme.

Acknowledgments

The authors would like to thank the experts, the institutes involved in the OHEJP MATRIX project and the external experts involved in answering the questionnaires who helped the authors' efforts.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article have been modified with permission from Cito et al., 2022 (15), and can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1129851/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Methods
PUBLISHED 20 April 2023
DOI 10.3389/fpubh.2023.1129776

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention, a section of the journal Frontiers in Public Health

RECEIVED 22 December 2022
ACCEPTED 10 March 2023
PUBLISHED 20 April 2023

CITATION
Rivers S, Kochanowski M, Stolarek A, Ziętek-Barszcz A, Horigan V, Kent AJ and Dewar R (2023) A framework for the design, implementation, and evaluation of output-based surveillance systems against zoonotic threats.
Front. Public Health 11:1129776.
doi: 10.3389/fpubh.2023.1129776

A framework for the design, implementation, and evaluation of output-based surveillance systems against zoonotic threats

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Output-based standards set a prescribed target to be achieved by a surveillance system, but they leave the selection of surveillance parameters, such as test type and population to be sampled, to the responsible party in the surveillance area. This allows proportionate legislative surveillance specifications to be imposed over a range of unique geographies. This flexibility makes output-based standards useful in the context of zoonotic threat surveillance, particularly where animal pathogens act as risk indicators for human health or where multiple surveillance streams cover human, animal, and food safety sectors. Yet, these systems are also heavily reliant on the appropriate choice of surveillance options to fit the disease context and the constraints of the organization implementing the surveillance system. Here we describe a framework to assist with designing, implementing, and evaluating output-based surveillance systems showing the effectiveness of a diverse range of activities through a case study example. Despite not all activities being relevant to practitioners in every context, this framework aims to provide a useful toolbox to encourage holistic and stakeholder-focused approaches to the establishment and maintenance of productive output-based surveillance systems.

KEYWORDS

output-based, surveillance, framework, zoonotic, design, implementation, evaluation

1. Introduction

The concept of One Health (OH) promotes the decompartmentalization of human, animal, and environmental health for more efficient and sustainable governance of complex health issues (1). This article details a framework developed as part of the MATRIX project, part of the OH European Joint Programme (OHEJP). The OHEJP is a partnership of 44 food, veterinary and medical laboratories and institutes across Europe and the Med-Vet-Net Association. MATRIX aims to build on existing resources within OH Surveillance by creating synergies along the whole surveillance pathway including the animal health, human health, and food safety sectors. This work aims to describe the design, implementation, and evaluation of surveillance systems against zoonotic threats using output-based standards (OBS).

An OBS does not strictly define the surveillance activity that must take place in a geographical area, e.g., to randomly collect and test X samples per year from Y location. Instead OBS is defined by what the surveillance system must achieve, e.g., to detect a set prevalence of a hazard with a set

confidence level (2). Output-based standards therefore allow for variation in how surveillance is conducted, influenced by a variety of country/region specific factors including hazard prevalence, performance of the tests used and mechanisms of infection. These standards can also enable the comparison of results from different surveillance programs across different geographical contexts (3). Due to this flexibility, and ability to compare surveillance results across countries and sectors, OBS are useful in the OH context where animal pathogens may act as risk indicators for human health. In directing efforts to minimize spread of zoonoses in the animal population with robust surveillance, OBS may help to curtail the spread of disease at the public health level. Surveillance systems implemented using OBS will hereafter be referred to as OBS systems.

The flexibility of OBS systems also necessitates a far more involved decision-making process when designing and evaluating them. While passive surveillance can form part of the implementation of OBS, active surveillance would also be needed to ensure that surveillance is sufficient to detect the design prevalence set out in the OBS. If conducting active surveillance for a pathogen, practitioners implementing OBS have the flexibility but also the responsibility to select the most appropriate host or medium to sample from, the test type to use, and the geographical sampling distribution. They must then calculate the appropriate sample number to meet their OBS, and make sure that each of these decisions works within the practical and budgetary constraints of the existing organizational systems in their surveillance area. Guidance has already been produced for analyzing conventional surveillance systems in tools such as SERVAL (4), RISKSUR (5), EpiTools (6), and OH-EpiCap (7). And while research such as the SOUND control project is developing tools to encourage and aid OBS implementation in Europe (8), there is currently no broadly applicable, practical framework showing how OBS surveillance systems can be designed, implemented, and evaluated. In this paper we provide a framework that aims to describe the surveillance format, provide evidence-based decision-making on the best ways of applying it, and showcase methodologies to evaluate these systems using worked examples.

This framework is aimed at those who are considering OBS as a solution to a surveillance need, whether they are looking to design and implement a system from scratch, replace a conventional surveillance system, or consider potential improvements to an existing OBS system. Not all sections may be relevant to all users. Thus, while a loose sequence exists throughout the framework, most sections can be read out of order or in isolation. Depending upon your starting point, the recommended route through this framework will differ; a diagram showing these routes can be found in Figure 1.

Throughout this framework, we will use the surveillance system for *Echinococcus multilocularis* in Great Britain (GB) as a worked example. We have chosen this pathogen because GB employs OBS for *Echinococcus multilocularis*, it is a zoonotic pathogen with a wide range of stakeholders that illustrate this process well.

2. Methods

2.1. Setting the scope of the framework

The goal of this work under the Matrix project was to develop guidelines for the design, implementation and evaluation of official controls, in this case active surveillance systems, which use OBS. This needed to include methods for:

1. Identifying operational partners and stakeholders
2. Selecting appropriate output-based systems
3. Evaluating output-based methods.

Tools for evaluating surveillance systems have already been produced such as SERVAL (4), RISKSUR (5), EpiTools (6), and OH-EpiCap (7). However, these tools do not cover all essential aspects of OBS. Hence, we wanted to produce a framework that would draw from this past work, but would focus on the practical elements of designing, implementing, and evaluating OBS systems.

2.2. Overarching approach

We sought to establish the essential attributes of OBS systems. For the design section, we developed a series of activities that would support the selection of appropriate design options for each attribute. The implementation section provides activities and general practical advice to assist with the roll-out of the final OBS system design. The evaluation section of the framework includes methods to assess the efficacy of the implemented design against the current context. Applying these methods would provide recommendations for improving existing OBS systems.

2.3. Identification of design attributes

To identify the essential design attributes of OBS systems, we drew from a literature search conducted by Horigan (9) which included a search of Scopus[1] and PubMed[2] using the search string "output or risk and based and surveillance or freedom" in the "title, keyword, or abstract." This provided articles on a range of OBS systems for zoonotic and non-zoonotic hazards.

From these articles, several surveillance attributes were found to be especially important for the success of OBS systems:

1. A strong understanding of the life cycle of the target hazard. Hazard life cycles influence the selection of host species and/or medium tested for the hazard (10–13).
2. An appropriate sample number and distribution. For example, selection of risk-based, random or convenience sampling to provide a statistically robust demonstration of the hazard prevalence (10, 14, 15).
3. A sufficiently cost-effective testing approach. This influences the practical feasibility and sustainability of the system (13, 16, 17).

We then investigated the OBS system for *E. multilocularis* in GB to validate these attributes and gain further insight into these systems. Contact with the Animal and Plant Health Agency (APHA) Parasitology

1 www.scopus.com
2 www.ncbi.nlm.nih.gov/pubmed

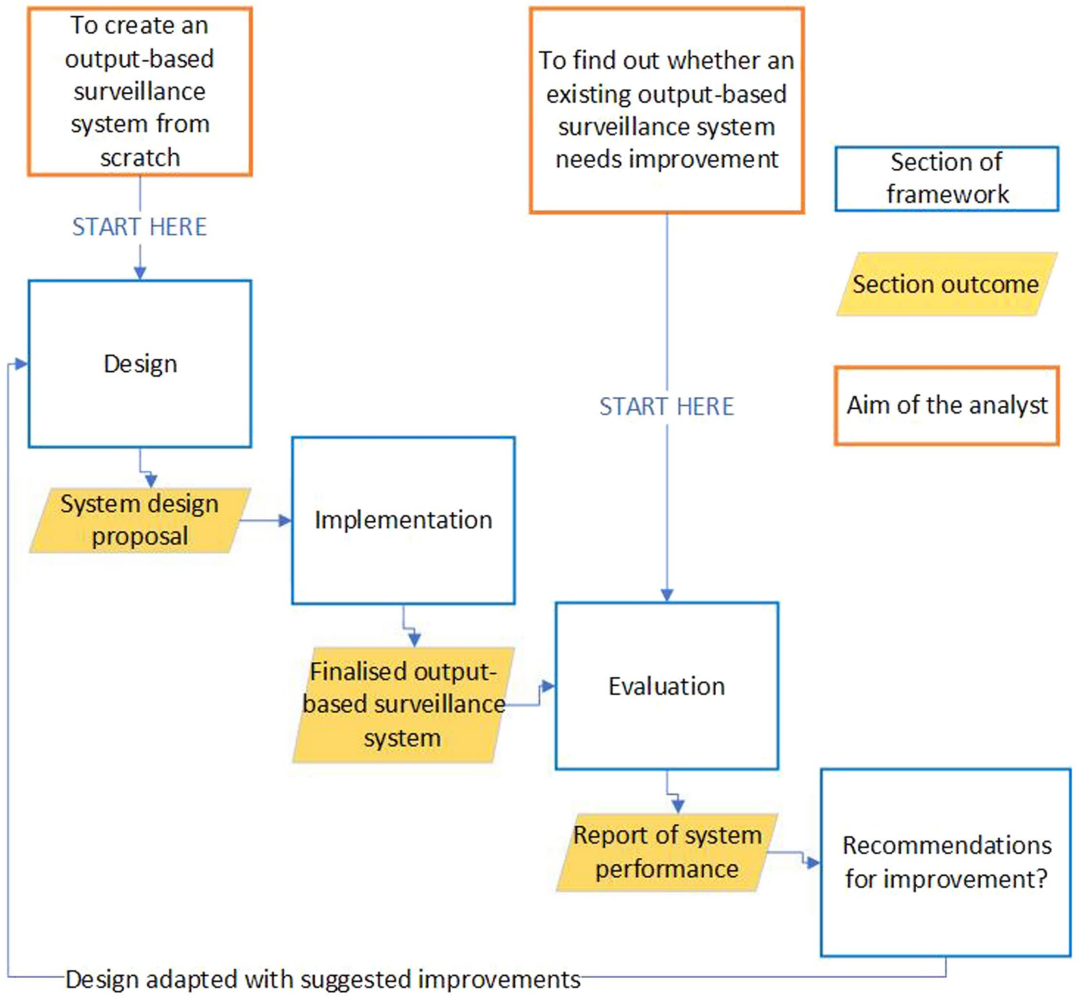

FIGURE 1
Showing the recommended route an analyst should take through this guidance if they either know they want to improve an existing surveillance system, want to design and implement an output-based surveillance system from scratch, or want to assess the performance of an existing OBS system.

discipline lead and laboratory coordinator for *E. multilocularis* surveillance in GB raised three further aspects to consider:

4. The clear definition of OBS system objectives
5. The identification and engagement of key stakeholders within the system
6. The appropriate communication and reporting of results.

2.4. Development of framework activities

In the design section we developed activities to help ensure system designs considered these six identified attributes. These activities were mainly documentation exercises, providing an outline of the information that should be gathered and the design choices that should be made.

In the implementation section we followed systems mapping work conducted in the COHESIVE project, a partner project to MATRIX in the OHEJP. Their approach effectively described the Q fever reporting and testing system in GB (18). Recognizing the practical challenges of implementing OBS systems, we also explored project management techniques applicable to the implementation of large, complex systems, including project left-shift, integrated stakeholder feedback, and operational risk analysis and risk management, drawing practical advice from the field of systems engineering (19).

The evaluation section included activities that would provide recommendations to improve the performance of the OBS system. These were also grounded in the six OBS system attributes listed above and based on a range of previously published work and practical experience. We developed a stakeholder analysis based on work by Mendelow (20), selected because of its inclusion in the COHESIVE project (21). A methodology for cost-effectiveness analysis was also developed based on COHESIVE project outputs (22), using information gathered under a literature review of economic analysis approaches. A bespoke method for a flexibility analysis to assess how easily recommended changes to the system could be implemented was developed based on published research in the systems thinking field

(23). Methodologies were also set out for evaluating the minimum required sample sizes and true prevalence of hazards in host populations using EpiTools (6), based on practical experience from the Polish *E. multilocularis* surveillance system.

3. Framework

3.1. Design of an OBS system

Primarily, design is about selecting the appropriate attributes of a surveillance system to deliver on its defined objectives, this requires information gathering, decision-making, and objective setting. Here we set out methodologies to define the:

- System objectives
- Key stakeholders
- Target hazard and surveillance stream(s)
- Sampling methods
- Testing methods and costs
- Data reporting

3.1.1. System objectives

The objectives describe what the surveillance system aims to achieve from a top-level perspective, for example, to fill a regulatory requirement, to contribute to a national strategy, or to assist with disease or hazard control at the local level. Thus, the objective of an OBS system could be to demonstrate freedom from disease, or to show disease or hazard prevalence in a population with a certain level of confidence. For an OBS system the important attributes which should be considered when setting the objectives are:

- Design Prevalence: This is a fixed prevalence used to determine the hypothesis that disease/hazard is present in a population of interest (24). It can be thought of as the minimum prevalence that you would expect to detect using a given surveillance system.
- Confidence levels: This is the level of certainty that the result is correct. That is, when compared to the true level in the population, the result of surveillance would be 'correct' X% of the time, where X is the confidence level. The range of values for which that remains true (sample prevalence = population prevalence in X% of cases), is known as the confidence interval (25).
- Surveillance streams: these refer to the supply chain of samples from a particular host population or medium (with associated risk level) to the laboratory in which they are tested. A single hazard could have several surveillance streams. For example, the hazard could be tested for in both live animals and bulk milk from those animals, making up two surveillance streams within the one system.
- Probability of introduction: Likelihood of the disease or hazard in question being introduced to at least the number of units (e.g., animals) that would be infected given the design prevalence.

One method of compiling a list of objectives is to use a hierarchy of objectives which divides objectives into three tiers: policy, strategic, and project (26). The policy objective is the overarching reason for implementing this system at the top level such as providing confidence in disease freedom. Below this, the strategic objectives outline what needs to be achieved to attain the policy objective such as testing a specific design prevalence. Below strategic objectives are project objectives. These are the practical constraints and drivers that need to be worked within to achieve the strategic and policy objectives. Objectives in a tier below can be thought of as the 'how' of objectives in the tier above, while objectives in the tier above can be thought of as the 'why' of objectives in the tier below.

The objectives can be defined and validated through communication with the prospective system stakeholders.

Example: Great Britain must demonstrate freedom from *Echinococcus multilocularis* by upholding surveillance in accordance with an output-based scheme prescribed by the European Commission (27). Although GB has left the European Union (EU), this surveillance is still mandated by retained legislation. In this example, the policy objective therefore is to provide evidence of freedom from *Echinococcus multilocularis*. The strategic objectives describe how this OBS system aims to achieve this policy objective by detecting a 1% prevalence in a representative host population with 95% confidence, but also to do so cost-effectively. The project objectives include the sampling from appropriate definitive host(s) across a representative geographic spread, the testing using a test of appropriate sensitivity and specificity, and to do all of these within the budgetary constraints of the project.

3.1.2. Key stakeholders

Stakeholders, defined as "any parties who are affected by or who can affect the surveillance system" (28), have oversight of the surveillance system and are a useful resource for informing design choices to optimize the surveillance system design.

Generally, stakeholders comprise of three distinct groups: first, governance stakeholders with the influence to set the required output of the surveillance system, e.g., a regulatory authority like the European Food Safety Authority (EFSA); second, delivery stakeholders who are actively involved in the delivery of the required outputs, such as the collection of samples, laboratory analysis or planning and strategy roles; and finally, beneficiaries who directly or indirectly benefit from the system running well, and whose wellbeing would be directly or indirectly affected by a change to the surveillance system. The general public, for example, are beneficiaries of surveillance systems involving zoonotic pathogens.

The list of stakeholders should be created based on the available information about the hazard and the objectives of the system. Once a list of stakeholders has been established, a strategy for engagement should be devised. A simple strategy could be to reach out to stakeholders using links within your network. For example, through people in your institution who have worked with them in the past. Once contact with at least one stakeholder has been established, these may then be used to establish contact with other stakeholders in the system. Following initial engagement, stakeholders can be good sources for further information gathering. A structured interview with a pre-planned series of questions is recommended.

Example: In GB, we identified potential stakeholders for the *E. multilocularis* surveillance system using literature research (particularly previous EFSA reports) and known contacts. We then contacted one of our known stakeholders to develop a wider stakeholder list. The final list, per stakeholder group, was as follows:

Governance:

- The World Organisation for Animal Health (WOAH); who record the disease status of *E. multilocularis* following the compilation of GB results.
- The GB Department for Food, Environment, and Rural Affairs (DEFRA); who compile the results.
- Local councils, who play a role in maintaining good education on the disease/hazard and responding to cases.
- The European Free Trade Association (EFTA); who advise on the measures which should be in place to control *E. multilocularis* given a change in GB's status.

Delivery:

- The Animal and Plant Health Agency (APHA), who maintain the surveillance system, collecting samples and running analysis.
- The national reference laboratory (NRL) for Echinococcus
- APHA wildlife management team
- APHA wildlife risk modeling team.
- Veterinary practitioners, who respond to cases in dogs and hold a stake in maintaining their good health.
- UK Health Security Agency (UKHSA), who respond to and detect human cases.
- Hunters and gamekeepers, who provide carcasses from across the country for testing.

Beneficiaries:

- The Wildlife Trust, who support the welfare and environmental influences of surveillance on fox populations and the general ecology. They have a voice in ensuring surveillance does not severely, or unnecessarily, impact the wellbeing of foxes.
- Fera science, a wildlife science advice organization who receive samples from foxes and other wildlife for rodenticide survey, and who could benefit from collection of foxes for this surveillance.
- Science Advice for Scottish Agriculture, who also receive samples from foxes for rodenticide survey.
- Pet owners, who hold a stake in making sure their pets remain healthy, and who are at risk of infection in the event of incursion.
- Media outlets, who have an interest in distributing information on the quality of surveillance and in the event of case detection.
- The general public: good surveillance ensures that any incursion of *E. multilocularis* reaches as few members of the public as possible.

3.1.3. Target hazard and surveillance stream

Knowledge of the hazard both informs the choice of surveillance stream, and heavily impacts the downstream practical decisions around how the system will function. Structured interviews with stakeholders along with literature research can provide knowledge about the target hazard which can be compiled into a profile. Any relevant information can be added to this profile, but it should aim to be a complete overview covering all OH aspects. If the hazard is a zoonotic pathogen, particularly if it is foodborne, this should be flagged at this stage. As with the target hazard, the choice of surveillance stream, including the target host population and/or detection medium (e.g., red fox feces or bulk milk) is key to the system design. Sampling is usually from the population considered most at risk of infection or contamination and therefore the one in which you are most likely to detect a positive case. The choice of population, and the medium from which this population are sampled, has implications on almost all areas of the workflow, including the applicable sampling types and methods, and the geographical area(s) sampled.

Example: In the case of *E. multilocularis*, the red fox is the most relevant to sample in GB as it is a definitive host for the hazard and is also widely abundant. Additionally, sampling individual animals rather than collecting environmental samples or sampling from intermediate hosts is more compatible with the available testing methods for the hazard, which require tissue samples. This also ensures that positive detection relates to one animal, rather than leaving potential for multiple sources of contamination as environmental samples would. It ensures the species and approximate location of death is known.

3.1.4. Sampling methods

The distribution of the target population and the sampling strategy are essential for informing the type of test used, and how the final design proposal will be implemented.

Samples may be taken using a risk-based framework or by taking randomly from the entire population. While convenience sampling could detect a case and thereby rule out disease freedom, it is not recommended for output-based surveillance as it would be unlikely to support representative sampling of the host population to prove disease freedom. Delivery stakeholders can provide the contextual knowledge to inform the type of sampling that is most appropriate and feasible. Additional external information sources such as population surveys could provide further information to support the chosen sampling type.

Regardless of the sampling method chosen, we recommend including all populations that are relevant to the probability of introduction of the pathogen. For farmed or kept animals, this will likely include multiple surveillance streams, for example, sampling from slaughter animals, imported and moved animals. For wild animals, relevant surveillance streams may include samples from trapped or hunted animals, roadkill, resident populations, and transient or migratory populations, particularly where they cross borders.

The sampling methods link closely to the testing method chosen because the number of samples required will vary based on the sensitivity of the test used, and because certain tests will only be compatible with certain sample media (e.g., serum, nasal swab, or feces). In order to confirm the number of samples required, and to validate confidence in the test results, we suggest using a sample size calculator such as EpiTools (29).

Example: Using *E. multilocularis* in GB as an example, the red fox population was 357,000 (30). The egg flotation test can be run on intestinal tissues of fox carcasses with an estimated test sensitivity of 0.78 (31). With these inputs, EpiTools output was a suggested sample size of 383 fox carcasses to detect the hazard at a 1% design prevalence with 95% confidence, given a random sampling distribution.

3.1.5. Testing methods and costs

When choosing a testing method, we suggest engaging stakeholders and reviewing literature for an overview of the tests available. From there, the most appropriate method can be chosen, considering the budget and resources available, the sensitivity and specificity of the testing method, the population available for testing and the specific surveillance scheme chosen.

As part of test selection, understanding the costs of testing helps determine whether surveillance is achievable within the budgetary constraints of your system. This is also a useful precursor to establishing which surveillance streams give the best value for money, as described in the cost-effectiveness analysis guidance in the evaluation section.

Generally, the cost of testing can be broken down into the following:

- Consumables and reagents: This covers any routine consumables costs such as reagents, PPE, laboratory, or field consumables.
- Staff: This covers all costs relating to staff, e.g., cost of staff time for sampling, testing, training and travel.
- Equipment: This covers the cost of all equipment used in the system. This may, for example, include the cost of purchasing and maintaining laboratory equipment.
- Other operational costs: This covers all other costs not accounted for, such as sample transport and equipment maintenance.

Delivery stakeholders may be able to provide detailed cost data, depending on which part of the system they are linked to. For example, laboratory stakeholders may be able to provide the procurement costs of reagents if they are already used for other tests. If further information is needed, an average price per item can be sought through the price lists of online retailers.

Example: For the GB *E. multilocularis,* we used the standard operating procedure (SOP) of the egg flotation method to generate a list of consumables, reagents and equipment which were then assigned hypothetical values detailed in Table 1.

TABLE 1 Hypothetical data showing the cost breakdown per test of the egg flotation test, and the data sources associated with these costs.

Parameter	Value	
Test	Egg flotation	
Species sampled	Fox	
Test sensitivity	0.78	
Test specificity	1	
Parameter	Unit	Cost/Value
Consumables and reagents	Per test	€56.88
Staff time (testing)	Per test	€9.26
Operational costs (excluding testing)	Annual cost	€291,593.12
Equipment	Annual cost	€894.15
Tests required at 1% prevalence	No. of tests	383
Cost of testing at 1% prevalence	Total cost	€165,823.53

3.1.6. Data reporting

The types of data to report will depend on the surveillance program. In general, a system should report the frequency of data collection, the sampling strategy and testing method used, along with sensitivity/specificity, target population, sampling period and volume, methodology for results analysis, and results of testing. Commonly, these data are provided in scientific reports to the governance stakeholders.

Example: The full data reporting for GB *E. multilocularis* can be found in the annual reports produced by EFSA prior to 2021 (32), and are explored in this example.

From the 2019/2020 sampling year, GB reported results for 464 samples taken between March 2019 and January 2020, from locations across GB (31).

The testing was conducted using the egg flotation method (31) with an overview of the methodology provided in the report (32). Random sampling was used, with the sample size calculated by the RIBESS tool (33) based on the test sensitivity, and the estimated population size for detection at 1% prevalence with a 95% confidence interval. EFSA evaluated the data provided to determine whether it fulfilled the legal requirements of the legislation and assigned a disease-free status.

3.2. Implementation of an OBS system

To aid system implementation, it is important to outline how the proposed OBS will function in a way that communicates its vision and purpose to the system stakeholders. The stakeholders can then provide feedback on the proposed system design and suggest improvements to make it more practically or economically viable. Once the design has been agreed, a strategy can be devised for maintaining the continued quality of the system through test validation and accreditation.

3.2.1. System mapping

System mapping provides a flow diagram showing all processes from the point of sample collection to the reporting of results. Visualizing the entire system in this way helps document the sequence of the surveillance system and makes the function of the system easily disseminated.

The simplest method for system mapping is constructing a flow diagram with direct input from your stakeholders (18). This should describe the steps from sample acquisition to result analysis. Most of the system structure will already have been determined in the design process. However, any remaining aspects of the system that are unclear should be highlighted in this flow diagram and clarified by the stakeholders. The diagram should outline which stakeholders will be involved at each step in the process.

The system structure map can also be used to represent any synergistic systems linked to the surveillance, for example, if the same samples could be used for other purposes. This helps document the linkages of the surveillance system with other activities and highlights opportunities to make sampling more practical, cost-effective and mutually beneficial. The surveillance system for *E. multilocularis* in GB, for example, has multiple stakeholders each contributing to, and benefitting from, its various stages (Figure 2).

3.2.2. Project management planning

Effective project management is required to coordinate the implementation of your proposed surveillance design, especially if operating to a deadline. Formal training in this field is highly recommended before undertaking the implementation of any large, complex output-based surveillance systems. However, we suggest drawing ideas from systems engineering practices such as project "left shift." This focusses on shifting project funding and input to the start of a project rather than the end of it. Early investment in a project provides better value for money due to inflation. Also, spending more time on the early planning stages of the project can prevent mistakes that may be challenging or expensive to resolve later in the project (34).

In the implementation of output-based surveillance systems, left-shift means investing heavily in building up the cohesion and experience-base of the delivery stakeholders of the system. These are similarly highlighted as important factors in the RISKSUR framework best practices (35). This could include investment in dedicated training for sample collection, analysis, and result reporting, or a pilot, where a small number of samples are collected and tested to ensure all aspects of the system work well together before scaling up. Outreach could be part of this early investment. For example, allowing laboratory staff time to shadow sample collectors and vice versa. Such activities will greatly improve cohesion along the sample analysis pipeline, allowing stakeholders to form close working relationships, facilitating a faster response to problems and potentially contributing to efficiency gains as stakeholders share experiences with one another.

Verification and validation stages with stakeholders during implementation are also recommended. These stages could test whether each part of the system delivers on the original system objectives and provides value to stakeholders as the systems are being implemented (36). Verification, as with all stages of project management, should be well documented and we recommend having a robust documentation process to make sure plans and activities are transparent to the implementation team and wider stakeholders (37–40).

Another recommendation is to conduct an operational risk analysis. This can identify, assess, and derive actions against issues which can occur during the implementation process. In this risk analysis, the probability of each of these risks occurring and the impact if these risks occur as either Low, Medium, or High. This facilitates decision-making on the proportionate action to take to either avoid these risks, mitigate their impacts, or accept them. We recommend guidance in Lavanya and Malarvizhi (41) or the textbook by the Institution of Civil Engineers (42) for further details on the steps to follow for operational risk analysis. All changes made to avoid a risk must be checked against the prior design stages and documented.

Stakeholders should agree with the outcomes of risk analysis, to any resultant changes to the system design and any accepted risks. Agreeing the final system design and implementation strategy with delivery stakeholders will improve the likelihood of successful implementation (43).

3.3. Evaluation of an OBS system

This section provides a range of evaluation exercises to help direct improvements to existing OBS systems.

3.3.1. Evaluation of system objectives

This evaluation determines whether the system objectives are still relevant and complete. For example, the hazard prevalence may have changed since the implementation of the OBS system, so is the design prevalence for detection still appropriate? A new test may have been developed for the target hazard, so how does this compare with the test currently implemented?

Assessing the suitability of the system objectives requires analysis of current research relevant to the OBS system. This can be conducted through a combination of literature review and stakeholder engagement, to explore the following questions:

- Has the level of detection changed since the first implementation of the surveillance system? Has prevalence of the hazard increased/decreased or changed in its geographical distribution?
- Has new evidence come to light on the dynamics of the hazard under surveillance? For example, have new competent hosts been found?
- Have new tests been developed for the same hazard and host as the original surveillance system? Do these new tests offer improved sensitivity and/or specificity to the current option; do they offer other advantages?
- Have any aspects of the surveillance system been recognized to be operating particularly well? For example, have other groups taken inspiration from the current system and implemented the same methods elsewhere?
- Have any issues or doubts about aspects of the surveillance system been raised? Are any of these corroborated by data?
- Has the political or legislative context of surveillance changed? Has the target hazard or population become higher or lower priority to governing bodies? Is the need for surveillance brought in to question by these changes?

3.3.2. Flexibility analysis

It is expected that every system will undergo changes throughout its lifecycle. A good output-based surveillance system needs to be adaptive to technological, practical, or political changes to continue delivering value for its stakeholders. A flexibility analysis determines how changes to a system could affect its various stakeholders and its ability to deliver on its core objectives.

Determining the flexibility of the system requires systems thinking so we recommend using causal loop diagrams to illustrate links between system components and stakeholders. The system components are any aspect of the system that affect its overall function. The surveillance streams, test type, number of tests, design prevalence, and even the method of result reporting and analysis can all be considered system components. Causal loop diagrams illustrate the dynamics of complex systems by showing the positive or negative relationships system components have on one another and on the stakeholders (23, 44). To produce these diagrams, the first step is to identify which system components affect each stakeholder. For example, sample collectors will be directly impacted if they are asked to collect more samples. The number of samples required is influenced by the sensitivity and specificity of the test chosen, and by the design prevalence and required confidence level set out in the system objectives. Hence, these stakeholders are *linked* to the sampling requirements, the test chosen, the design prevalence, and the required

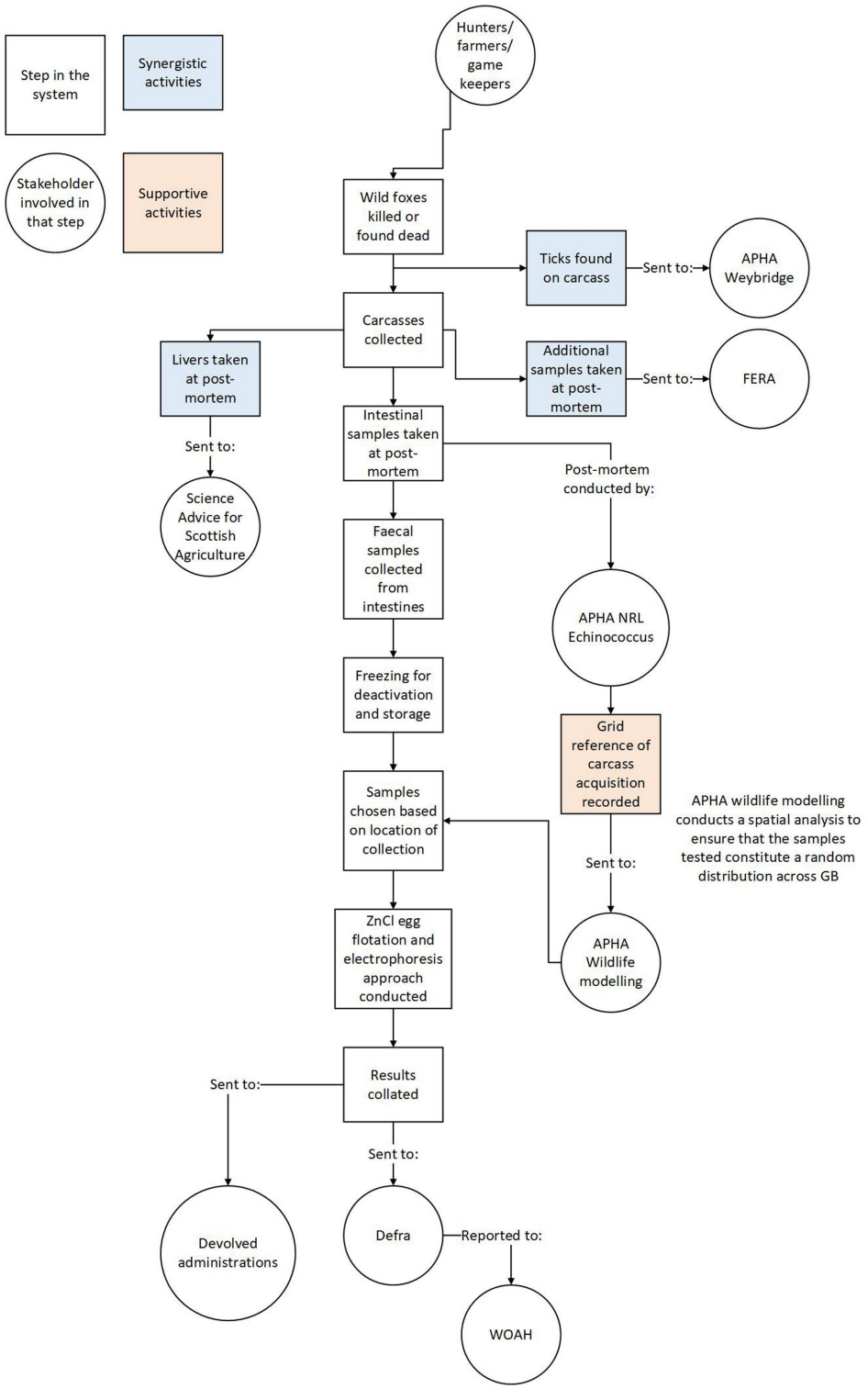

FIGURE 2
Showing the system structure and chronology from carcass collection to result reporting. Rectangles represent steps in the system while circles represent stakeholders involved in relevant steps.

confidence in the results. When a link is demonstrated, it is essential to show whether the relationship is positive or negative. For example, higher test sensitivity has a negative effect on the number of tests required since more sensitive tests are statistically more likely to detect a hazard if it is present. Logically, the number of tests required positively influences the number of samples taken: more tests required means more samples will need to be taken and consequently, these too are linked. While making these links, it is likely that further

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interrelationships between different stakeholders and system components will emerge. Documenting all relevant links will provide a complete picture of the emergent impacts of design decisions on each of the stakeholders.

Once the links between design decisions and stakeholders have been established, engagement of stakeholders is required to determine their tolerance to change. If stakeholders operate under fixed constraints these should be identified and documented. For example, delivery stakeholders may be working within a budgetary range. If they can agree to an increase in sampling rate, what is their maximum sample number? Governance stakeholders may have some tolerance in the design prevalence or testing confidence they expect to see from a surveillance system. What is this tolerance and to what extent could the system adapt before those tolerances are exceeded?

Example: For *E. multilocularis* surveillance in GB, we determined that changing the type of surveillance scheme, for example the test used, would impact the required sample size, and thereby affect both the workload of the delivery stakeholders and the confidence in the test results, altering the outcome for end beneficiaries. By representing the system using a causal loop diagram (Figure 3), we identified 5 distinct interrelationships to be aware of if any changes to the system are considered. These were:

- The chosen surveillance scheme will affect how many carcasses are collected, and where they are collected from (for example, if collected according to risk-based sampling rather than random sampling). This has ripple effects on every other part of the system.
- A higher sample requirement would mean more time and money spent collecting those samples. It would also demand more from farmers, hunters and gamekeepers to provide carcasses for analysis. This could strengthen or damage relationships with these stakeholders, depending on their appetite for collaboration, and thereby increase or decrease their satisfaction with the system and their willingness to supply samples (45). Hunters, farmers and gamekeepers already deliver an excess of samples to APHA, and it was estimated they would be receptive to an increase in the number of carcasses asked of them if needed, though their specific upper-bound tolerance was unknown.
- More carcasses collected means more of all sample types are available for commercial collaborators.
- A higher sampling rate, or improvement in the geographical spread of collected samples will increase the overall confidence in the surveillance system. It will increase the probability that cases in wildlife will be detected before the disease becomes established in the wild population. This will reduce the number of human cases, and therefore provide a higher benefit to society at large.
- A change in the costs of maintaining the system, and the downstream effects on the benefit to stakeholders, will affect the benefit–cost ratio of the surveillance system. A higher benefit–cost ratio means the surveillance system generates greater value for money.

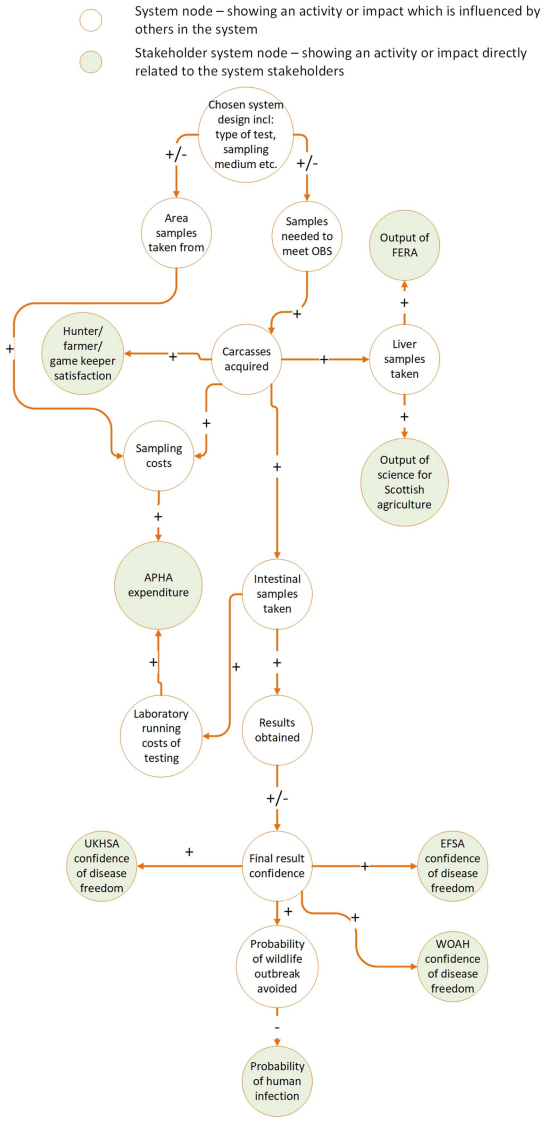

FIGURE 3
Example causal loop diagram illustrating the positive and negative interrelationships of different parts of the UK *E. multilocularis* surveillance system and the perceived stakeholder benefits and losses from changing aspects of the system.

3.3.3. Stakeholder analysis

This evaluation determines and depicts the level of interest and influence current stakeholders have in the system. Stakeholders have diverse views and roles. Thus, to understand them, it is a useful exercise to categorize them in order to identify the most influential stakeholders, or those who hold the largest stake in the system achieving its objectives. As a result, it is then possible to establish whether the position of individual stakeholders on the matrix is appropriate. A modified Mendelow matrix is an effective way to categorize stakeholders. This is a two-dimensional matrix plotting the interest and influence of stakeholders (20). It provides information about which stakeholders are the most engaged, and which are most influential.

Structured interviews should be used to determine the level of influence and interest in the system. Direct questions are a good starting point, for example 'what is your perceived level of influence on the system?'. It can be useful to follow up with more descriptive questioning. A question which asks the stakeholders how they might implement change to a system could return more tangible insights into

the barriers stakeholders face when trying to implement change. A stakeholder with high influence will likely have a strong idea of how to enact change to the system and may even have been directly involved in making prior changes to the system.

The level of interest in the system involves how stakeholders would be affected by changes to the system. When ascertaining the interest of stakeholders, questions that explore hypothetical scenarios may yield richer results, for example, asking how a stakeholder might be affected by increasing or decreasing the sample numbers taken, or by changing the objectives of the system. If their answers indicate they would need to take immediate action because of these changes, this illustrates a high level of interest in the system. For beneficiaries of output-based surveillance systems, such as the general public, who may not be aware of the implications of changes to it on their own health and wellbeing however, this can be a challenge. A judgment can be made in these cases based on the prior information compiled.

Another tool for collecting information from stakeholders could be survey-based questions rating interest and influence on a quantitative scale, for example from 1 to 10. With interviews and surveys, every effort should be made to contact as many stakeholders as possible from across the system. Where this is not possible, a proxy can be used to evaluate/assess the influence and interest these stakeholders have. This could be based on the perceptions of other stakeholders in the system, taking care to get input about missing stakeholders from as many other stakeholders as possible. Once the bulk of information has been compiled, they can be placed on the Mendelow's matrix. A completed matrix of all stakeholders should then be verified by the stakeholders.

Finally, you should evaluate whether the position of the stakeholders on the matrix is still appropriate, particularly regarding the influence they have on the system. This can be assessed by asking stakeholders whether they think they should have more or less influence on the system in the future. A desire to change their level of influence can be represented on the matrix with arrows. Arrows provide an indication of stakeholder satisfaction and suggest areas for improving stakeholder involvement.

Example: For the *E. multilocularis* surveillance system in GB, we reached out to stakeholders *via* email or through interviews, assembling information to plot these stakeholders on a Mendelow matrix. We interviewed the following stakeholders:

- APHA Parasitology discipline lead and laboratory coordinator for *E. multilocularis* surveillance in Great Britain.
- Carcass collection coordinator for *E. multilocularis* surveillance in GB.
- APHA discipline lead for wildlife epidemiology and modeling, leading *E. multilocularis* sample selection, and risk modeling.
- Science Advice for Scottish Agriculture research coordinator, rodenticide sampling in wildlife
- Fera Science research coordinator, rodenticide sampling in wildlife

Additionally, we contacted the UK Health Security Agency Emerging Infectious Zoonoses Team and DEFRA *via* email but were unable to reach WOAH. When interviewing, we discussed the following topics with each stakeholder:

- The role of the stakeholder within the system
- The perceived roles of other stakeholders in the system
- Their perceived understanding of how the surveillance system practically functioned to deliver outputs
- Their perceived influence on the system
- Their satisfaction with the system, particularly with regards to the level of influence they had on it.

For stakeholders that could not be contacted directly, attributes were estimated from the expert knowledge of the other stakeholders; from their past interactions with these stakeholders and their experience working within the system. With the information compiled in the interviews, it was possible to map each stakeholder on a Mendelow matrix (Figure 4).

In the future, DEFRA will receive the annual reports of the surveillance, therefore, they have both high interest and high influence on the matrix. APHA, and WOAH are also in this quarter of the matrix; APHA are responsible for carrying out the surveillance and WOAH are responsible for producing the annualized reports to prove disease freedom and publishing results shared by member states. With the current GB situation for *E. multilocularis*, the UKHSA is in the low interest, high influence quarter of the Matrix. However, this would likely change to high interest, high influence, if there were changes to the status of *E. multilocularis* in GB. When asked, satisfaction was very high: no stakeholder felt they needed more or less influence on the system.

3.3.4. Minimum sample size evaluation

This evaluation calculates the minimum sample size required to detect hazard at a set design prevalence and confidence level. This calculation is relevant for monitoring the hazard in the population. If the sample size is too big it will result in excess financial cost. If the sample size is too small, it can lead to the system not achieving its objectives. Scientific publications, international and governmental statistical data, hunting associations or other professional organizational data, expert opinions, and gray literature can all provide relevant population size data and information about test sensitivity. Furthermore, the sensitivity of the test can also be determined *via* validation studies and in the case of a commercial test, *via* the test manufacturer. This information can then be used to calculate the minimum sample size needed for surveillance using the online EpiTools calculator - "Sample size for demonstration of freedom (detection of disease) in a finite population" (29).

This tool can calculate the sample size needed to achieve the required probability of detecting disease or presence of a hazard (herd-sensitivity) at the defined design prevalence for a finite population, assuming a diagnostic assay with known sensitivity and 100% specificity. These calculations use an approximation of the hypergeometric distribution (29, 46). According to MacDiarmid (46) the probability (β) that there are no test-positive animals in the sample tested can be calculated as:

$$\beta = \left(1 - \frac{n\,SE}{N}\right)^{pN}$$

where:

- p = true prevalence of infection
- SE = sensitivity of the test
- N = herd size

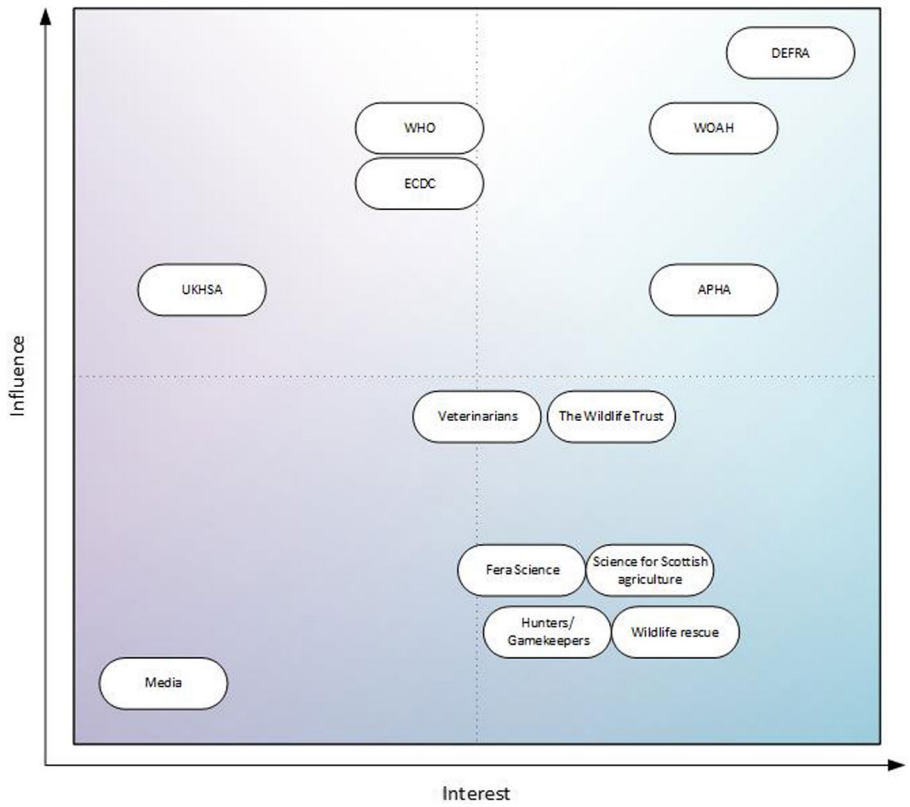

FIGURE 4
Stakeholders involved in GB *E. multilocularis* surveillance mapped to a Mendelow matrix, sorted by level of influence and interest in the surveillance system.

- n = sample size

The required parameters (inputs) for the calculator are:

- Population size
- Test sensitivity
- Desired herd-sensitivity
- Design (target) prevalence

The main output of this EpiTools analysis is the number of samples required to provide the desired herd sensitivity for a specified design prevalence. The results of this analysis are 383 sample required for both the SCT and IST, and 336 samples required for the PCR. The calculations concerned *E. multilocularis* in the red fox population in selected European countries. In these calculations, the EpiTools calculator inputs were set as follow:

- Red fox population size - defined according to the data from publications and reports (Table 2)
- Sensitivity of *E. multilocularis* detection test (sedimentation and counting technique (SCT) 0.78, intestinal scraping technique (IST) 0.78, or PCR method)- derived from publications and reports as reported in Table 3.
- Desired herd-sensitivity – was set at 0.95
- Design (target) prevalence – here was set in accordance with the calculated true prevalence

Furthermore, this EpiTools calculator can generate graphs of the sample sizes needed to achieve the desired herd sensitivity, for a defined test sensitivity and range of population size and design prevalence (Figure 5).

3.3.5. True prevalence evaluation

This section estimates the true prevalence to confirm or correct any previously calculated prevalence of disease (apparent prevalence). Most diagnostic tests have imperfect sensitivity and specificity. Calculation of true prevalence (the proportion of a population that is actually infected) considers the sensitivity and specificity of the applied test. Calculating the true prevalence can determine whether the choice of design prevalence for the system is still appropriate. This is more accurate than calculations of apparent prevalence (the proportion of the population that tests positive for the disease) which are reported in the majority of epidemiological studies/reports and do not include these parameters. Scientific publications, international and governmental reports, expert opinions, and gray literature can all be used to find these data.

A useful tool for calculating true prevalence is the EpiTools calculator – "Estimated true prevalence and predictive values from survey testing" (29). This tool calculates the true prevalence, as well as positive and negative predictive values, and likelihood ratios based on testing results using an assay of known sensitivity and specificity (29). For example, true prevalence of *E. multilocularis* in Poland was calculated by EpiTools calculator as 18.64% (95% CI, 16.64–20.82) while apparent prevalence was 16.5%. Based on this example one can

TABLE 2 Calculation of the number of samples required to detect *E. multilocularis* in the red fox population in selected European countries.

Country	References	Red fox population								Sample size for demonstration detection of disease					
		2009	2010	2011	2012	2013	2014	2022	2009	2010	2011	2012	2013	2014	2022
Poland	[1]				193,402	210,332	198,679					19	19	19	
Latvia	[2]	35,000	34,800						9	9					
Denmark	[3]						31,100							405	
Hungary	[4]							78,000							60
Romania	[5, 6]		53,292							63					
Finland	[7, 8]						150,000							384	
Ireland	[7, 9, 10]						150,000							339	
Great Britain	[7, 11]						240,000							353	
Norway	[7, 12]		70,000		70,000	70,000	151,000			475				476	

References: [1] – The Forest Data Bank (47); [2] – Kirjušina et al. (48); [3] – Danish Centre For Environment And Energy (49); [4] – European Health and Digital Executive Agency European Food Safety Authority (50) (HaDEA); [5] – Şuteu et al. (51); [6] – Romanian National Institute of Statistics (52); [7] – European Food Safety Authority (50); [8] – Kauhala (53); [9] – Hayden and Harrington (54); [10] – Marnell et al. (55); [11] – DEFRA Department for Environment and Agency (56); [12] – Sviland et al. (57).

see that number of tested samples, number of positive results, method sensitivity as well as method specificity effect on calculation result. For Poland and other selected countries of EU calculations of true and apparent prevalence are presented in Table 3. Furthermore, EpiTools calculator enables graphical visualization of output results.

Using *E. multilocularis* prevalence in Poland as an example, the inputs required to perform computations by the EpiTools calculator are as follows:

- Number of examined samples obtained from red foxes (intestines or faeces samples) and number positive samples - set according to data from publications and reports as indicated in Table 3.
- Sensitivity and specificity of the method (SCT, IST or PCR method)
- Confidence level – was set at 0.95
- Type of confidence interval for apparent prevalence – Wilson CI was used
- Type of confidence interval for true prevalence – Blaker was used

To determine the true prevalence (TP) from these data, EpiTools applies the Rogan-Gladen estimator, using the following formula:

$$TP = \frac{\left[AP + \left(SP - 1\right)\right]}{\left[SP + \left(SE - 1\right)\right]}$$

where:

- AP = apparent prevalence
- SP = specificity
- SE = sensitivity

3.3.6. Cost-effectiveness analysis

It is important that the testing process and the overall cost of the wider surveillance scheme is as cost effective as possible. This likely also affects stakeholder satisfaction and may affect the long-term sustainability of the system. To evaluate this, it is recommended to carry out a cost-effectiveness or cost–benefit analysis (or similar applicable economic analysis method). This example specifically looks at cost effectiveness analyses (CEA).

Cost effectiveness analyses measure the input cost required for the system to produce a given output. Unlike some other economic analysis approaches, the 'effectiveness' component of a CEA can be defined by the analyst. In output-based surveillance, the output is already defined at the operational level (to detect a stated design prevalence with a stated confidence). Cost effectiveness analysis can easily be applied in these cases, to measure the cost input required to meet these outputs. This can then be compared directly to alternative approaches. Gathering data on the cost inputs of a system first requires an inventory of all materials and reagents used, staff time required, and any transport and sample collection costs. Materials and reagents can be found using laboratory standard operating procedures (SOPs). The price of each cost component may be attainable through contact with stakeholders working within the system. Alternatively, these may be found on supplier websites. Staff time should ideally be derived through contact with the staff themselves, preferably staff who have a holistic view of the system from sample acquisition to result reporting.

TABLE 3 Calculation of the true prevalence of *E. multilocularis* in red foxes in selected European countries.

Country	Apparent prevalence calculation					True prevalence calculation				
	Survey references	No. of tested samples	Number of positive results	Method	Apparent prevalence (%)	Sensitivity and specificity references	Method sensitivity	Method specificity	True prevalence (%)	95% CI
Poland	[1]	1,546	255	SCT	16.5	[12]	0.885	1	18.64	16.64–20.82
Latvia	[2]	45	16	SCT	35.6	[12]	0.885	1	40.18	26.24–56.68
France	[3]	3,307	562	SCT	17	[12]	0.885	1	19.2	17.8–20.69
Germany (northern)	[4]	3,094	523	SCT	16.9	[12]	0.885	1	19.1	17.65–20.64
Denmark	[5]	546	4	SCT	0.73	[12]	0.885	1	0.83	0.32–2.11
Hungary	[6]	100	5	SCT	5	[12]	0.885	1	5.65	2.43–12.63
Romania	[7]	561	27	IST/SCT	4.8	[13]	0.78	1	6.17	4.27–8.86
Belgium	[8]	990	243	IST	24.55	[13]	0.78	1	31.47	28.16–35.03
Slovakia	[9]	660	49	IST/SCT	7.4	[13]	0.78	1	9.52	7.26–12.41
Estonia	[10]	17	5	SCT	29.4	[12]	0.885	1	33.23	15.01–60.04
Finland	[11]	265	0	PCR	0	[11]	0.78	1	0	0–1.83
Ireland	[11]	331	0	SCT	0	[12]	0.885	1	0	0–1.3
Great Britain	[11]	434	0	PCR	0	[11]	0.85	1	0	0–1.03
Norway	[11]	523	0	PCR	0	[11]	0.63	1	0	0–1.16

References: [1] – Karamon et al. (58); [2] – Bagrade et al. (59); [3] – Combes et al. (60); [4] – Berke et al. (61); [5] – Enemark et al. (62); [6] – Sréter et al. (63); [7] – Sikó et al. (64); [8] – Hanosset et al. (65); [9] – Bagrade et al. (59), 2001; [10] – Moks et al. (66); [11] – European Food Safety Authority (50); [12]–(67); [13] – Hofer et al. (68).

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Inputs	
Population size	198679
Test sensitivity	0.885
Confidence level	0.95
Desing prevalence	0.1864

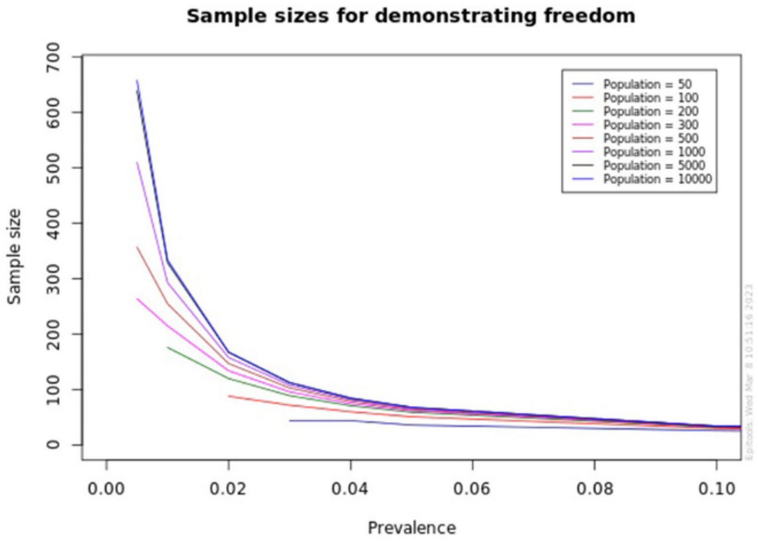

FIGURE 5
Plots generated by the EpiTools calculator showing predictions for different prevalence levels and population sizes for a specified test sensitivity.

When collecting data on alternative test types which are not yet in use, it may be useful to use proxies. Proxies can be similar tests already conducted for other pathogens, and hence already have internal costs listed in the organization. Data on alternative tests may also be found on supplier websites. Every test type will be different so it's important to avoid biases wherever possible. For example, if you are calculating costs over a year and a piece of key equipment needs maintenance every 4 years, then this cost needs to be considered fairly: it should not be ignored but should also not be considered in full for a single year of testing. A fair solution would be to divide this cost by the years between maintenance activities to make it a normalized annual cost output.

Data for each testing type must be calculated per test and multiplied by the required sample size based on the sensitivity of each test. This can be calculated using the EpiTools online resource. Doing so allows for direct comparison between the cost-effectiveness of each test type.

Example: In the design section, in test costing, we used hypothetical data as an example of the cost of the egg flotation test for *E. multilocularis* surveillance. An objective for this surveillance is to ensure that the system uses a method that is practically and financially feasible. This can be conducted by comparing the costs of the current testing method against the known surveillance budget. However, only a comparison of multiple surveillance design options can optimize value for money. For *E. multilocularis* we produced a CEA comparing the hypothetical costs of multiple testing methods; the egg flotation test, and two alternate methods identified in the sampling methods section. When working with estimated costs, the CEA can be used iteratively to generate a range of outputs or, if the upper and lower bounds of cost data are known, then this can provide a minimum and maximum cost for the surveillance.

Cataloging the other tests available was conducted through discussions with the stakeholders and through literature research. The annual EFSA report on *E. multilocularis* surveillance in Europe was an essential resource, summarizing how each country in Europe was conducting their tests, describing a range of alternative test-types (69).

We identified two alternative methods, the SCT and a real-time PCR method. APHA conducts the SCT as part of the external quality assurance and proficiency testing schemes provided by the European Union Reference Laboratory for Parasites (EURLP) for the detection of *Echinococcus* spp. worms in intestinal mucosa. The instructions and procedure provided by the EURLP for this testing was used to broadly determine the consumables, reagents and equipment required for this test (70). Prices per test were generated using hypothetical data. The staff time spent processing samples, 'lab time,' was calculated using an average sample throughput of 15 samples per day based on information from literature (71). The additional time costs including sample collection and post-mortems ('non-lab time') were assumed to be the same for all methods, and therefore are set at a blanket cost per sample (hypothetical data).

The real time PCR method used in this evaluation is the QIAamp Fast DNA Stool Mini Kit (QT) combined with a TaqMan PCR, the method for which has been previously described in literature (72, 73). A combination of this literature, and in-house SOPs were used to populate a list of consumables, reagents, and equipment (74) which were then assigned hypothetical costs.

The SOPs and information gathered for these tests were used to create the consumables, reagents and equipment lists. Each component was then assigned a hypothetical cost. Costs for two alternative methods of testing previously identified were also produced based on protocols found through literature searches, and the three methods were compared in a cost effectiveness analysis (Table 4). Hypothetical values were also generated for staff time, sample transport and post-mortems. All cost values were then added together to provide the annual costs of maintaining a surveillance system using each test type, including the costs for sample collection, post-mortem, testing, and epidemiological services linked to the system.

TABLE 4 Showing the cost-effectiveness of three different testing methodologies for *E. multilocularis* at detecting a 1% prevalence detection with 95% confidence (hypothetical data).

Parameter	Unit	Test		
		Egg flotation	SCT	qPCR
Species sampled	–	Fox	Fox	Fox
Throughput	–	Batch of 20 every 12 h	10–20 per day (Average 15)	12–30 min per sample (Average 21)
Test sensitivity	–	0.78	0.78	0.89
Test specificity	–	1	1	1
Consumables and reagents	Per test	€56.88	€3.74	€12.48
Staff time (testing)	Per test	€9.26	€17.57	€10.32
Operational costs (excluding testing)	Annual cost (800 tests)	€291,593.12	€291,593.12	€291,593.12
Equipment	Annual cost	€894.15	€625.05	€18,860.40
Tests required at 1% prevalence	No. of tests	383	383	336
Cost of testing at 1% prevalence	€	€165,823.53	€150,408.31	€148,989.54

The total annual cost for each testing methodology was converted into a mean cost per test. The number of samples to be taken was calculated using EpiTools, an online sample size calculator developed by AUSVET (6) with the test sensitivity, design prevalence, confidence level and host population size as inputs. Since positive results were assumed to be followed up and confirmed, the specificity of all tests was set to 1. The test sensitivity of 0.78 for the zinc egg flotation (EF) and SCT methods is the value recommended for use by EFSA for this type of testing, whereas test sensitivity for the qPCR method is the average of those sourced from literature. From these data the qPCR is the most sensitive of the testing methods with a sensitivity of 0.89.

The minimum number of tests required to detect a 1% prevalence with 95% confidence with the sensitivities specified by these tests was then multiplied by the cost per test to provide the overall cost of each testing methodology.

The costs of each methodology were compared. For annualized costs, such as sample collection and post-mortem, the per test cost was calculated based on the approximate number of samples collected in GB for the sampling year 2021–2022: 800 (75). This was multiplied by the number of tests required, determined using the EpiTools calculator.

For this hypothetical scenario, the SCT is the most economical when it comes to consumables and reagents, costing an estimated €3.74 per test compared to the €12.48 and €56.88 required for the PCR and EF, respectively. This is also true for the estimated annual cost of equipment and maintenance, with the SCT requiring an estimated €625.05 per year compared to €894.15 for the EF and €18.860.40 for the PCR equipment. This difference is mainly due to the comparatively large maintenance cost for real time PCR equipment. Where these outputs differ, however, is the cost of staff time associated with each test. We estimated the cost-per-test of both the EF and PCR at between €9–11 whereas due to the time intensive nature of the SCT, the per cost test was determined to be €17.57 based on staff processing an average of 15 samples per day (71).

Overall, with this model the qPCR is shown to be the most cost-effective testing method due to its lower number of tests required per year.

3.3.7. Propose improvements to the system (if applicable)

Each evaluation from the previous section will have developed an understanding of how well the surveillance system currently functions. This may have highlighted areas where the surveillance system needs improvement. Improvements do not necessarily mean increases in testing output, but rather changes to the system that make it more effective at achieving its objectives at the time of evaluation.

Examples of potential improvements include changes to test type to increase cost-effectiveness or accuracy of surveillance, changes to design prevalence to detect a higher or lower population prevalence with greater confidence or changes to sample number to better reflect the chosen design prevalence.

Any proposed improvements to the system constitute a change to the design proposal of the surveillance system. Hence, it may be necessary to go through the stages of design and implementation to ensure improvements are properly considered from all angles by the relevant stakeholders.

4. Discussion

Output-based standards can allow for variation in surveillance activities to achieve a universal objective and may be useful in the OH context where surveillance for animal pathogens can act as risk indicators for human health. In addition to the context of zoonotic pathogens, OBS may also be useful in other One Health Scenarios, for example in detecting a bacterial hazard at a particular design prevalence in a food product.

In the design section of this framework, we recommend a robust method of objective setting and highlight this as a reference point for all subsequent activities in the framework. We also emphasize the importance of identifying all the stakeholders acting within the OBS system and demonstrate how stakeholder engagement can guide the design of successful surveillance systems with their expertise and knowledge. We recommend the EpiTools calculator for determining sample size (29) in our worked examples. Later in the design section, we describe a method for estimating the costs of the available test

options, helping predict the feasibility of implementing the chosen test within the available surveillance budget.

In the implementation section, we show how systems mapping can be used to visualize the steps and stakeholders involved in surveillance, facilitating clear communication of the intended system design to all relevant stakeholders from an early stage. Later, we highlight the importance of left shift and operational risk analysis to effective project implementation.

The evaluation section described in this framework first establishes whether the stated objectives of the system are still relevant to the contemporary disease and legislative context. Then, the flexibility of the system to adaptation and change is analyzed to provide a holistic view of the relationships between system components and the system's capacity for change. By applying technical evaluation tools such as EpiTools, we can assess whether the chosen prevalence estimations and sample sizes remain accurate to the true disease situation. This provides an indication of whether individual surveillance streams should be upscaled or downscaled to meet the required output of the system. Along with a technical performance assessment, this guidance provides advice on how to evaluate the human factors within the system through stakeholder evaluation. Financial viewpoints are considered in the cost-effectiveness analysis section. This provides an example evaluation method for multiple testing options. In completing the full evaluation, the technical, human, economic, and practical elements of the system can be visualized in the wider context of the current disease situation.

However, there are limitations to some of the analyses described. For example, because of the variation across laboratories, countries, and sectors, the CEA did not consider the implementation costs of *changing* the testing type used. These are the additional costs required to move from one testing type to another, including the cost of retraining staff, and purchasing new equipment. Including implementation costs would provide a better understanding of the real costs of applying different test types. Any future expansions to this work could integrate the payback times for different tests following initial investment in them over a temporal dimension. This could say, for example, that moving to a PCR and fecal sample-based testing regime, while it would cost £3 M investment, would pay itself back in savings from reduced year-on-year sample collection and material costs in 10 years. Under this framework it was not possible to quantify the implementation costs of new training and equipment without knowing the existing laboratory capacity. Thus, to keep the analysis generic to a range of end-users, this aspect was not included.

Additionally, because this guidance is designed for OBS systems only, the recommendations it provides are more tailored than other surveillance evaluation tools such as SERVAL and RISKSUR EVA, which are generic to all forms of surveillance (4, 5). Its narrower scope provided an opportunity to ground this framework to worked examples that highlight immediate practical recommendations rather than top-level areas for improvement. However, we acknowledge that some elements of the framework may be prescriptive.

For instance, EpiTools is referenced throughout the guidance, without consideration of other epidemiological calculators. The calculator by Iowa state university, for instance, could equally be used for sample size and probability of detection calculation (76). We chose EpiTools for the examples because of its broad range of available analysis applications, including sample size estimations using both hypergeometric and binomial approaches and true prevalence estimations using Bayesian and pooled computational approaches. This range of analyses makes it applicable to OBS systems with large or small population sizes, and with a broad design prevalence range. In addition, the tool is free and has had usage across several published articles, making it readily accessible to analysts from a range of backgrounds (77–79).

Many of the ideas in the implementation section of this framework are tied to systems engineering practices. These have a good track record of use across a range of science and technology-focused projects (19, 80). However, several analyses in this framework could be conducted differently. For example, while causal loop diagrams have been used in a wide range of disciplines to represent dynamic systems (23, 44), analysts could equally use retrospective approaches for flexibility analysis as in the guidelines for evaluating public health surveillance systems produced by the United States Centers for Disease Control (81). We also acknowledge that not all sections of this framework will be relevant to all users and that, depending on the context of its users, there may be gaps that require additional research. This is expected given the broad scope of OBS in different situations, and as such this guidance should be considered alongside other training and literature from other sources. Nevertheless, we believe that the approaches described here encourage a holistic outlook on OBS systems throughout. Above all, they encourage extensive stakeholder engagement, not only with end users, but also with delivery and governance teams. We hope this framework will encourage cross-disciplinary implementations of OBS systems and thereby improve their performance and sustainability.

In summary, this framework provides a range of relevant activities and recommendations for the design, implementation, and evaluation of output-based surveillance systems. It is a holistic toolkit with applications from setting the objectives of a new system to analyzing the cost-performance of an established system. Not all sections will be applicable to all end users. However, its promotion of systems thinking, and stakeholder participation makes it a valuable tool in the cross-disciplinary implementation of OBS.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SR wrote the first draft of the manuscript. SR, MK, RD, AS, AZ-B, and VH wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work, carried out as part of the MATRIX project (Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards) has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830 and from the Department for Environment, Food and Rural Affairs (DEFRA) UK. Research at the National Veterinary Research Institute (PIWet),

Poland, was also partially supported by the Polish Ministry of Education and Science from the funds for science in the years 2018–2022 allocated for the implementation of co-financed international projects.

Acknowledgments

We acknowledge the important contributions made by Julia Coats and Izzy Rochester from the APHA wildlife team helping us establish key aspects of the sample collection pipeline for red foxes in Great Britain, and the insights provided by Richard Budgey from the APHA wildlife modeling team. We acknowledge Steve Campbell (SASA) and Libby Barnett (Fera Science) for providing insight into the external uses for red fox samples by scientific collaborators. We also acknowledge the valuable work of Mirek Rozycki (PIWET) in the initial direction-setting of this project.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Methods
PUBLISHED 11 May 2023
DOI 10.3389/fpubh.2023.1053986

OPEN ACCESS

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RECEIVED 26 September 2022
ACCEPTED 06 April 2023
PUBLISHED 11 May 2023

CITATION
Tegegne HA, Bogaardt C, Collineau L, Cazeau G, Lailler R, Reinhardt J, Freeth FTA, Taylor E, Prada JM and Hénaux V (2023) OH-EpiCap: a semi-quantitative tool for the evaluation of One Health epidemiological surveillance capacities and capabilities. *Front. Public Health* 11:1053986. doi: 10.3389/fpubh.2023.1053986

OH-EpiCap: a semi-quantitative tool for the evaluation of One Health epidemiological surveillance capacities and capabilities

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Although international health agencies encourage the development of One Health (OH) surveillance, many systems remain mostly compartmentalized, with limited collaborations among sectors and disciplines. In the framework of the OH European Joint Programme "MATRIX" project, a generic evaluation tool called OH-EpiCap has been developed to enable individual institutes/governments to characterize, assess and monitor their own OH epidemiological surveillance capacities and capabilities. The tool is organized around three dimensions: organization, operational activities, and impact of the OH surveillance system; each dimension is then divided into four targets, each including four indicators. A semi-quantitative questionnaire enables the scoring of each indicator, with four levels according to the degree of satisfaction in the studied OH surveillance system. The evaluation is conducted by a panel of surveillance representatives (during a half-day workshop or with a back-and-forth process to reach a consensus). An R Shiny-based web application facilitates implementation of the evaluation and visualization of the results, and includes a benchmarking option. The tool was piloted on several foodborne hazards (i.e., *Salmonella*, *Campylobacter*, *Listeria*), emerging threats (e.g., antimicrobial resistance) and other zoonotic hazards (psittacosis) in multiple European countries in 2022. These case studies showed that the OH-EpiCap tool supports the tracing of strengths and weaknesses in epidemiological capacities and the identification of concrete and direct actions to improve collaborative activities at all steps of surveillance. It appears complementary to the existing EU-LabCap tool, designed to assess the capacity and capability of European microbiology laboratories. In addition, it provides opportunity to reinforce trust between surveillance stakeholders from across the system and to build a good foundation for a professional network for further collaboration.

KEYWORDS

One Health (OH), evaluation, epidemiology, multi-sectoral collaboration, surveillance

1. Introduction

In recent years, the One Health (OH) concept has gained momentum, and international efforts have been made to strengthen the implementation of multi-sectoral surveillance to more effectively manage health hazards at the human, animal and environment interface (1). For decades, the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), and the World Organization for Animal Health (WOAH, formerly OIE), have been working together to address risks at the human–animal interface. In 2022, the United Nations Environment Programme (UNEP) joined the tripartite collaboration as an equal partner. The first joint plan signed by the quadripartite aims to create a framework to integrate systems and capacity to collectively better prevent, predict, detect, and respond to health threats of humans, animals, plants, and the environment with the objectives of strengthening OH surveillance, early warning and response systems (2).

OH surveillance is defined as a collaborative and systematic collection, validation, analysis, interpretation of data, and dissemination of information collected on humans, animals, and the environment to inform decisions for more effective evidence-based health interventions (3, 4). However, in spite of the efforts of the quadripartite alliance to promote collaboration in surveillance and laboratory networks and overpass professional silos, most surveillance systems remain compartmentalized, with limited interaction across actors in the system (5). For multiple reasons, implementing OH approaches in practice still proves challenging (6) and collaborations between health sectors occur mostly in crisis times (7).

There is a wide range of possible organizational models for collaboration, and its operationalization varies in terms of areas of implementation throughout the surveillance process (8–11). Collaboration is mainly driven by the epidemiological context and surveillance objective and is built according to actors' expectations (5). Regular evaluation of the organization and functionality of collaboration is crucial to assess the surveillance system's capacity and capability to produce relevant information, identify areas for improvement, and optimize added value gained by integrating efforts across sectors.

In recent years, several methods have been developed to assess whether collaborative efforts are appropriate and functional and whether it improves the impact of surveillance systems (12, 13). The Evaluation of Collaboration for Surveillance (ECoSur) tool targets the organization and functioning of multi-sectoral collaborations in a surveillance system (5). It relies on a semi-quantitative approach, with data collection based on interviews of the coordinators of the programs included in the surveillance system, requiring a 1–2-week evaluation period on average (5). The Network for Evaluation of One Health (NEOH) relies on the theory of change to identify the necessary preconditions and actions to be taken to reach long-term goals (14). The whole process is estimated to take 1–2 months and requires interviews of essential actors and stakeholders (13). The OH Assessment for Planning and Performance (OH-APP) focuses on multi-sectoral coordination mechanisms to inform planning and development assistance. The OH-APP complements the WHO Joint External Evaluation by providing specific indicators to measure the maturity of a multi-sectoral coordination mechanism and benchmark its progress toward a sustainable mechanism capable of coordinating multi-sectoral and multi-stakeholder collaboration for preparedness and response to public health threats (https://www.onehealthapp.org/about). Other tools were developed specifically for antimicrobial resistance (AMR) surveillance activities: the Progressive Management Pathway tool for AMR (PMP-AMR), the AMR integrated surveillance system evaluation project (ISSEP) tool, the Assessment Tool for Laboratories and AMR Surveillance Systems (ATLASS) (13) and the Integrated Surveillance System Evaluation (ISSE) framework (2). The different tools appear complementary in terms of evaluation objectives and provide generic science-based guidance for the evaluation of collaboration in surveillance systems. Yet, they also appear quite complex and require a lot of data, time, and human resources (13), limiting their (regular) implementation. There is therefore a need for a user-friendly tool to assess epidemiological surveillance interoperability and capacity across countries, with an aim to be repeatable.

The OH European Joint Programme MATRIX project aimed to produce guidelines and tools applicable at the national level to connect existing surveillance structures and resources, and strengthen integrated surveillance initiatives, ultimately adding value by building on existing resources, and creating synergies among sectors. In this context, we developed a generic evaluation and benchmarking tool (OH-EpiCap), implemented through an interactive online web application, for characterizing, monitoring, and evaluating epidemiological national surveillance capacities and capabilities for OH surveillance. This tool was designed to enable representatives of any surveillance system to conduct an evaluation of the multiple aspects of OH surveillance, in a short time and without requiring an external evaluation team. The evaluation addresses the multisectoral and multidisciplinary efforts to ensure communication, collaboration, and coordination among all relevant actors of the surveillance working locally, nationally, and globally to attain optimal health for people, animals, and our environment (https://extranet.who.int/sph/one-health-operations). Besides identifying areas that could lead to improvements in existing OH epidemiological surveillance capacities, the tool was designed to allow benchmarking (i.e., comparisons) with results from previous evaluations of that surveillance system, or other relevant systems, for example in other countries.

2. Methods

2.1. Identification, definition, and validation of indicators

Existing evaluation tools focusing on multi-sectoral and interdisciplinary collaboration aspects in epidemiological surveillance were used as a basis for the development of the OH-EpiCap tool. Besides, to structure our tool, we considered the format of the EU-LabCap tool, developed to assess bi-annually the capacity and capabilities of European microbiology laboratories (15).

Three dimensions of evaluation were considered in our tool: the organization of the collaborative system, the nature and functioning of collaborations for operational activities, and the impact of collaborations on surveillance (Figure 1). Each

dimension was then divided into four targets focusing on specific features of multi-sectoral collaborations, building from the existing evaluation frameworks. Finally, we established standardized indicators defining more accurately each target and we singled out the necessary criteria to support their evaluation. The definition of indicators in each target is available in Hénaux et al. (16).

The first dimension, about the organization of the OH surveillance system, includes the following targets and indicators: Target 1.1 *Formalization* focuses on the common aim of the system, support documentations, coordination roles, and leadership in the OH surveillance system; Target 1.2 *Coverage and transdisciplinary* addresses whether the surveillance covers all relevant sectors, disciplines, actors, geography, populations and hazards; Target 1.3 *Resources* addresses aspects related to financial and human resources, sharing of the available operational resources, and training; and Target 1.4 *Evaluation and resilience* focuses on internal and external evaluations, implementation of corrective measures, and the capacity of the OH surveillance system to adapt to changes.

The second dimension deals with OH aspects in operational activities: Target 2.1 D*ata collection and methods sharing* concerns the level of multi-sectoral collaboration in the design of surveillance protocols, data collection, harmonization of laboratory techniques and data warehousing; Target 2.2 *Data sharing* addresses data sharing agreements, evaluation of data quality, use of shared data, and the compliance of data with the FAIR principle; Target 2.3 *Data analysis and interpretation* addresses multi-sectoral integration for data analysis, sharing of statistical analysis techniques, sharing of scientific expertise, and harmonization of indicators; and Target 2.4 *Communication* focuses on both internal and external communication processes, dissemination to decision-makers, and information sharing in case of suspicion.

The third dimension deals with the impact of the OH surveillance system: Target 3.1 *Technical outputs* concerns the timely detection of emergence, knowledge improvement on hazard epidemiological situations, increased effectiveness of surveillance, and reduction of operational costs; Target 3.2 *Collaborative added value* addresses strengthening of the OH team and network, international collaboration and common strategy (road map) design; Target 3.3 *Immediate and intermediate outcomes* addresses advocacy, awareness, preparedness and interventions based on the information generated by the OH surveillance system; and Target 3.4 *Ultimate outcomes* focuses on research opportunities, policy changes, behavioral changes and better health outcomes that are attributed to the OH surveillance system.

The organization and definition of the targets and indicators were consolidated and validated through expert consultation. Experts were selected based on previous and ongoing involvement in research activities on OH aspects (e.g., One Health—European Joint Project (OH-EJP) program; Convergence in evaluation frameworks for integrated surveillance of AMR (CoEvalAMR) project) in national veterinary, public and/or environmental health institutes and from EFSA. The experts were asked to review and comment on all the proposed indicators and identify missing information. The list of indicators was refined based on experts' comments and validated with them through a back and forth process. Additional specific modifications were also carried out based on feedback from participants in case studies during the pilot phase (see below).

2.2. Questionnaire and semi-quantitative scoring options

A questionnaire was developed to facilitate the collection of information for the scoring of the indicators, with one question per indicator. A semi-quantitative scale was defined with four levels, describing the level of compliance of the system under examination compared to an ideal situation: higher values suggest better adherence to the OH principle targeted by the indicator (i.e., better integration of sectors) and lower values indicate improvements may be beneficial. In addition, the option of "Not applicable" (NA) was included to take into consideration the case where the indicator would not be relevant to the OH surveillance system under evaluation. The standardized scoring guide, detailing for each individual score, the situation in which that score should be awarded, is available in Hénaux et al. (16).

2.3. Data visualization and web application

A web application was developed (using R *shiny* and *shinydashboard* packages) (17, 18) with a user guide describing the different steps for completing the questionnaire and visualizing the results (16). The link to the application is: https://freddietafreeth.shinyapps.io/OH-EpiCap/. The interface enables users to complete the questionnaire interactively (and also to upload the answers from a questionnaire completed previously). Below each question, free text space is provided to add notes or justify the answer provided. These comments are saved and can be also visualized when reviewing the results of the evaluation. The application allows the user to save partially completed questionnaires in csv (human-readable) format, to revisit or complete the answers at a later time. To comply with the European General Data Protection Regulation, the OH-EpiCap team does not collect any data through the application, and the application does not ask any personal or identifying information regarding users or the surveillance system under evaluation. The application is hosted in the cloud with shinyapps.io (www.shinyapps.io), and questionnaire and benchmark data (files) are processed and temporarily stored on an external server for the duration of the user's session only. All data remain inaccessible to other users of the application. Users must save their work locally (i.e., in the machine they are using) before closing the application (to avoid any data loss).

The application facilitates the exploration of the completed (and/or uploaded) assessment and of the results of the evaluation by way of multiple visualizations. The answers to the OH-EpiCap questionnaire are analyzed at the target level for each dimension by averaging the scores across the indicators to get a final score (between 1 and 4), and at the dimension level by averaging target-level outputs (the mean scores over all questions are expressed as a percentage). Results are displayed in the form of interactive radar charts and lollipop plots to identify strengths and weaknesses at both dimension and target levels. Users may hover over data points to explore the breakdown of scores for each target and indicator. At the target level, this option displays for each data point the comments provided by the evaluators during the filling of the related question. Finally, users can download a two-page report

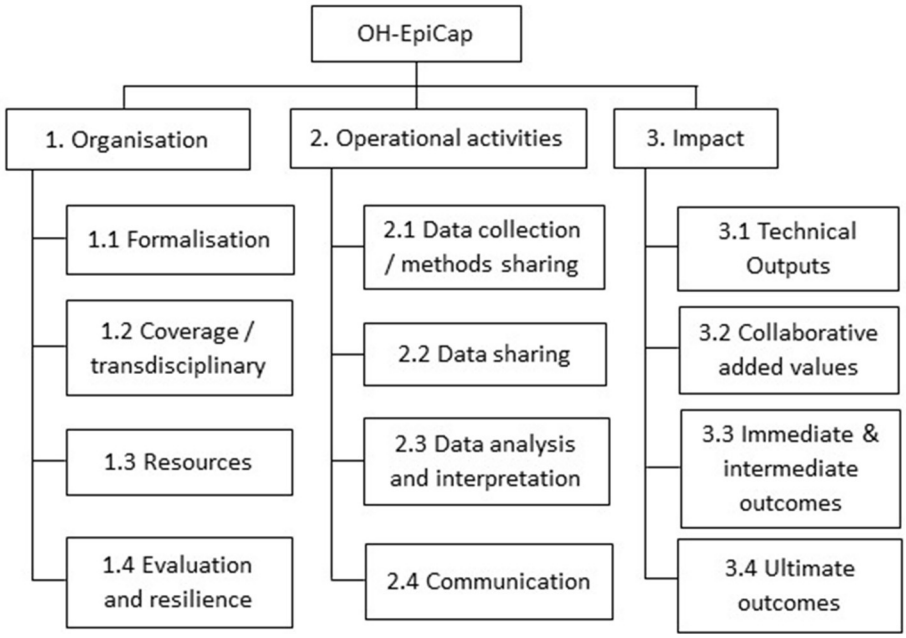

FIGURE 1
Structural overview of the OH-EpiCap targets, grouped by dimension.

(in html format) comprising the graphic outputs and comments highlighting the main strengths and weaknesses of the surveillance system examined. Moreover, the tool also includes a benchmarking functionality to compare results from the ongoing evaluation with a reference set based on results from previous OH-EpiCap evaluations. This reference dataset can be generated from other evaluations that the user has access to, using a specific tab of the web application. This function allows the integration of multiple evaluations (for example, from other countries for the same hazard), thus anonymizing the results for a given system/hazard.

2.4. Process to conduct a OH-EpiCap evaluation

The OH-EpiCap tool was designed to serve as a support for discussion and scoring of the OH aspects by a panel of representatives from the different sectors across the entire surveillance system of a specific hazard. We recommend to identify up to 8–10 participants who have a good knowledge of the system and encompass a range of disciplines and experiences regarding the functioning of collaborations among institutes and programs.

The selected surveillance representatives form an evaluation panel, which gathers during a 4-h workshop to complete the questionnaire, using the online application. For each question, the panel must provide one answer after reaching a consensus. In the case where it is not possible to organize a workshop to conduct the evaluation, the questionnaire may be filled sequentially by the surveillance representatives from each sector, with a back-and-forth process to reach a consensus. Once completed, the online application allows the panel to visualize the outcomes in real-time and to generate a OH-ness profile for the studied system.

2.5. Pilot phase

The OH-EpiCap tool was piloted through eight applications on surveillance systems of specific hazards targeted by the MATRIX consortium, including foodborne and other emerging zoonotic hazards. As a first step, for each surveillance system, a representative was identified directly within the MATRIX participants or their professional networks. Then, a 1-h meeting with the identified surveillance representatives was organized to present the tool and the evaluation process, and to answer questions. Participants were then asked to identify additional surveillance representatives to include in the evaluation panel. When available, a map of the targeted surveillance system (characterizing the institutes involved in the surveillance programs) was used to identify potential representatives. The choice of conducting a workshop or completing the questionnaire sequentially by representatives was left to the participants.

For three study cases, the evaluator panel chose to conduct the evaluation of their surveillance system through a workshop. These study cases focused on:

- Psittacosis surveillance system in Denmark: the workshop was held in person, and gathered seven surveillance representatives, from the public health sector with expertise in laboratory/bacteriology and epidemiology, and from the animal health sector from the official sampling, laboratory, and risk management unit. It lasted 3 h (including a round table of participants and a short introduction to the workshop, the filling of the three dimensions of the questionnaire, the results analysis, and debriefing).
- *Salmonella* surveillance system in Germany: the workshop was held online and gathered ten representatives, from the

public health (Robert Koch Institute), animal health (Friedrich Loeffler Institute), and food safety (German Federal Institute for Risk Assessment—BfR) sectors. It lasted 4 h; the two last targets of the third dimension were not completed during the workshop because of time constraints (and scoring for these indicators was provided at a later stage).

- *Campylobacter* surveillance system in Sweden: the workshop was held online and gathered five representatives from the public health (Folkhalsomyndigheten), animal health (National Veterinary Institute—SVA), and food safety (Swedish National Food Agency—SLV; Swedish Board of Agriculture) sectors. It lasted 3 h.

These OH-EpiCap evaluations were conducted in the language of the country to facilitate discussions. One or two persons from the MATRIX research team also participated as observers, to identify areas for improvement in the questionnaire and the online application, and to provide additional explanations if needed during the completion of the questionnaire by participants. At the end of the workshop, participants were asked to share their thoughts on the evaluation process, the relevance of the evaluation, and any feedback and comments to improve the tool. A checklist was provided for collecting this information regarding the questionnaire and its implementation (Supplementary material S1).

Other study cases were conducted through completion of the questionnaire (in a Word format), by one to four representatives of the surveillance systems, sequentially:

- AMR surveillance system in Portugal: the questionnaire was completed on the one hand by two representatives from the public health sector (Directorate General for Health—DGS and national health institute—INSA) and on the other hand by an expert from the animal health sector (National Institute for Agricultural and Veterinary Research—INIAV). Subsequently, a representative from the environmental health sector (Portuguese Environment Agency—APA), reviewed and commented the scores proposed by the other representatives.
- AMR surveillance system in France: the questionnaire was completed sequentially by one representative from the animal health and food safety sectors (National Agency for Food, Environmental and Occupational Health Safety—ANSES), and two representatives from the public health sector (Directorate General for Food—DGAL, and the national public health agency—SpF).
- *Salmonella* surveillance system in France: the questionnaire was completed by a representative from the public health and food safety sectors (ANSES), who is part of the coordination team at the national level.
- *Listeria* and *Salmonella* surveillance systems in the Netherlands: these two evaluations were conducted by two representatives from the National Institute for Public Health and the Environment (RIVM), who have a good knowledge of the surveillance across animal health, public health and food safety sectors and existing multi-sectoral collaborations.

Each evaluator spent between 2 and 3 h completing the questionnaire or reviewing and completing a pre-filled questionnaire. Then, the OH-EpiCap team filled the scores in the web application to generate the final report (displaying the results), that was sent back to the surveillance representatives.

2.6. Ethical approval

The MATRIX project obtained ethical approval from the ethical advisors of the One Health European Joint Programme. We informed verbally and through email the participants about the following points: (1) the use of the OH-EpiCap tool and application is voluntary; (2) the OH-EpiCap tool does not collect personal information, to comply with the European General Data Protection Regulation; (3) the web application does not keep the data regarding the OH surveillance system evaluated.

3. Results

3.1. OH-EpiCap report displaying results

Once the questionnaire is completed interactively (i.e., through the R-Shiny application), results from the evaluation are visually summarized. For confidential reasons, specific results and conclusions from the eight study cases are not presented. An example report, generated by the R-Shiny application using simulated data, is provided in Supplementary material S2.

Results are first presented through a radar chart showing average score across the indicators for each target, within the three dimensions (Figure 2), and a lollipop plot (Supplementary material S2) to identify strengths and weaknesses at both dimension and target levels. The graphs are accompanied by a short text, listing the targets demonstrating good adherence to One Health principles and the ones that would most benefit from improvement. Then, the OH-EpiCap report details the results per indicator within each target for each dimension: Organization (Figure 3), Operational activities (Figure 4), and Impact (Figure 5).

3.2. Questions and comments regarding the application of the OH-EpiCap tool

We detailed below the comments and questions raised by surveillance representatives during the meeting of preparation of the evaluation, and during and after the realization of the study cases. A first comment concerned whether the surveillance system targeted for the evaluation could be considered as a OH surveillance system in spite of a lack of formalization or of applicable legislation regarding the collaborations between sectors. We specified that the OH-EpiCap tool was developed for any surveillance system where some collaborations between sectors exist (at any step of the surveillance) even if those ones are not formalized or occur occasionally.

FIGURE 2
Example of OH-EpiCap results analyzed at the target level for each dimension (by averaging the scores across the indicators).

Another comment questioned whether integration could be considered from a system-wide perspective, including multi-sectoral collaborations but also inter-program collaborations even within the same sector (e.g., collaborations between a surveillance program targeting AMR and another one on antimicrobial use, in a specific sector). Although this vision appears different from the OH approach, the tool allows considering different levels of integration; however, such specificity should be clearly stated and understood by all surveillance representatives before the start of the evaluation.

During the filling of the questionnaire, for some indicators, the answers proposed for a specific question appeared to not fit with the OH surveillance system under evaluation or the epidemiological context. When such a comment occurred, we discussed it with the panel of evaluators to determine why the set of situations proposed for a specific indicator did not fit the system under evaluation. The feedback from the evaluators helped refine and complete the answers proposed for some indicators to consider specific OH surveillance contexts and situations not envisaged initially. In addition, the "NA" answer was added to all questions to be used if the question is not relevant for the OH surveillance system under evaluation. Overall, the NA option was selected few times by evaluation panels (between zero and four times among the eight study cases). We also suggested that if the answers proposed for a question did not fit the OH surveillance system under evaluation, the panel could define what would be the ideal situation and score the question accordingly by comparing the current situation to the ideal one. In this case, the panel can specify in the free comment space which alternative answers were considered (this would be useful for further result interpretation and dissemination).

Another question dealt with the amount of data saved in the web application and whether the data is accessible by stakeholders not involved in the evaluation, arguing that some information could be potentially confidential. We made it clear that the web application does not keep any data to comply with the European General Data Protection Regulation. Users must save their work locally (i.e., in the machine they are using) before closing the application (to avoid any data loss). They can also use the options offered by the web application to share the OH-EpiCap results with other stakeholders (as row data in csv format or through a final report in html format).

The last comment underlined the need for more time to further discuss and plan the actions to be taken to improve identified weaknesses. Participants are encouraged to further discuss and investigate underlying issues to improve collaboration in the system during another dedicated workshop.

4. Discussion

We present in this paper the design and the pilot study of the OH-EpiCap tool, which is a semi-quantitative evaluation tool developed for macro analysis of the OH capacities and capabilities of a system for surveillance of a specific hazard. This tool helps, without a priori consideration, characterize how multi-sectoral

FIGURE 3
Example of OH-EpiCap results at the indicator level for each target of dimension 1 (Organization).

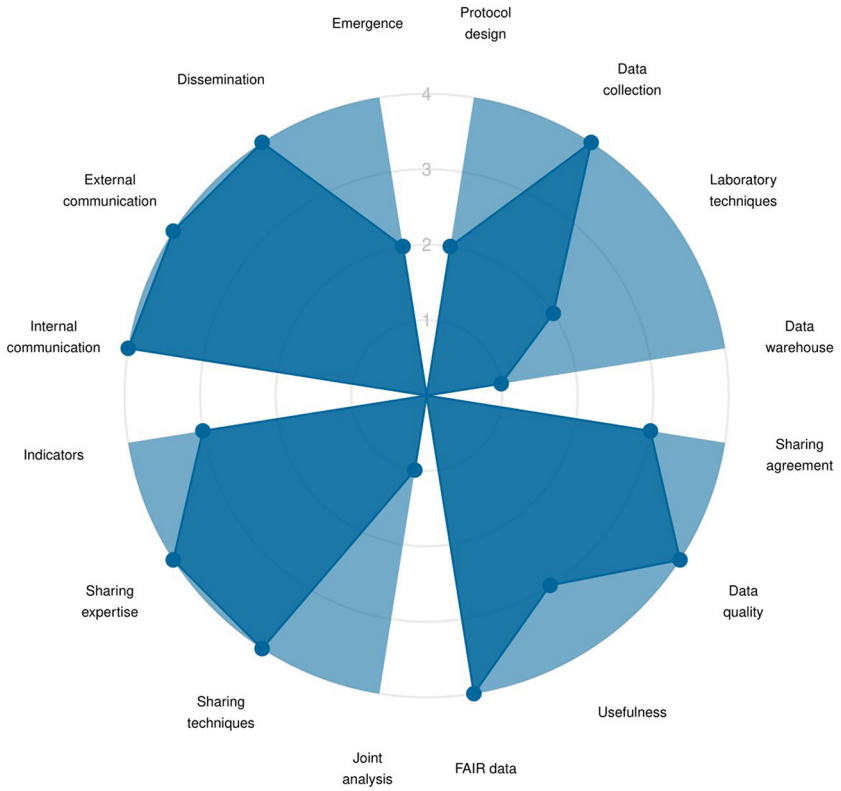

FIGURE 4
Example of OH-EpiCap results at the indicator level for each target of dimension 2 (Operations).

FIGURE 5
Example of OH-EpiCap results at the indicator level for each target of dimension 3 (Impact).

collaborations operate within surveillance systems. It facilitates the identification of strengths and weaknesses, focusing on the organization and functioning of existing collaborations, and of their impacts on the effectiveness of surveillance. The specific results of the evaluations regarding the strengths and weaknesses of the evaluated surveillance systems will be the topic of another paper.

The OH-EpiCap tool is generic and can be applied to the surveillance of any hazard. Accordingly, the tool was applied to a large range of hazards, including food-borne hazards (*Salmonella*, *Listeria*, and *Campylobacter*), other zoonotic hazards (psittacosis) and AMR. The questionnaire includes specific indicators oriented toward OH preparedness and response and is therefore of interest for surveillance systems targeting emerging or exotic zoonoses. The expert consultation and the pilot phase were beneficial to make the questionnaire more flexible to the diversity of contexts of surveillance, depending on hazards and countries, and to the level of integration of the system. Given that the tool is generic, the importance of clearly specifying the outline of the system under study and the levels of integration considered (e.g., inter-program collaborations), in addition to multi-sectoral integration, is a priority. We encourage the application of the OH-EpiCap tool to other hazards at the human-animal-plant-environment interface, in diverse contexts regarding technical infrastructure, surveillance capacity, and policy support.

Besides, the tool can address any surveillance system, whether it is well-formalized or at a low level of integration, as long as some multi-sectoral collaborations exist at any step of the surveillance even if they are not supported by official regulations, nor formalized through specific agreements and procedures. The formalization of the organization and functioning of the collaborations between sectors is considered an important aspect for OH surveillance (11), and therefore a lack of formalization will lead to low scores in some indicators of the OH-EpiCap tool (in particular in dimension 1). Depending on the aim of the OH surveillance system and if this lack of formalization is considered as an issue, surveillance representatives are encouraged to determine what elements would elevate the current multi-sectoral collaboration level to an official OH surveillance system.

The first step of a OH-EpiCap evaluation process is the identification of the panel of representatives of the surveillance system under study, i.e., who will conduct the evaluation. The composition of the evaluation team must be representative of the whole surveillance system (as much as possible). Thus, the panel should include experts from all sectors involved in the surveillance of the hazard under evaluation, and would encompass a large range of disciplines and experiences regarding the functioning of collaborations among institutes and programs. During the pilot phase, the experts who formed the panel for the OH-EpiCap evaluations encompassed several, if not all, sectors relevant for the surveillance systems, including the public health, animal health, food safety, and environmental health sectors, which aligns with the checklist for one health epidemiological reporting of evidence (COHERE) standards (19). We note that for most hazards evaluated in the pilot phase, the environmental sector is still poorly

or not included in the surveillance programs, which represents a challenge to identify an environmental health representative for the evaluation panels. Yet, when relevant for the hazard evaluated, the environment, non-domestic animal, plant, and ecosystem health should also be considered in the scoring of the indicators. A mapping of the surveillance system under study, characterizing the programs and institutes involved in the surveillance for each sector and collected data (20), would help identify surveillance representatives. This panel will then work closely together during the evaluation workshop, with ideally all representatives having the opportunity to express their views during the scoring of the indicators. Therefore, identifying respected and well-known members of the surveillance system under study is an asset to moderate respectful discussion and prevent any stronger opinions from monopolizing the exchanges over the quieter contributors.

The second step consists in the evaluation of the OH epidemiological capacities and capabilities following the three dimensions, through the web app. The evaluation is based on a semi-quantitative method; this is certainly marked by subjectivity, especially in the case of a limited panel of evaluators. Indeed, some indicators might be scored very differently across surveillance representatives with various backgrounds, perceptions, and expectations. Yet, we stress that only one answer can be provided to each question; therefore the surveillance representatives of the evaluation panel must reach a consensus to answer each question (based on their backgrounds, perceptions, and expectations). This constraint of having to reach a consensus for each question, within a standardized set of answers, limits the bias of subjectivity. Another limitation of this tool is that the current implementation assumes that all indicators are of equal importance (i.e., have the same weight). This is obviously a simplification and depending on the context of surveillance and the overall aim of the collaborations among sectors, some aspects of the evaluation may appear more important and should therefore get more focus during the result analysis and interpretation, as well as for prioritizing recommendations.

The organization of the evaluation in three distinct parts (one per dimension) helps the panel to articulate its reflection regarding the OH-ness of their surveillance system. It supports a collective and transparent evaluation approach, and facilitates identification of weaknesses and alternatives. Recommendations and concrete actions to improve the global systems can emerge from this process, facilitating in a second step prioritization among actions to improve OH-ness. The user-friendly web app provides a set of classical graphs (gauges, radar charts, lollipop plots) that enables users to easily visualize and analyze the strengths and weaknesses at the level of the indicators, and also of each target within the three dimensions. We underline the importance of taking careful notes during the workshop. Justifications provided by the panel in the comment spaces during questionnaire completion are displayed on the graph, facilitating the interpretation of the results at the end of the evaluation workshop, and also at a later stage as needed (thanks to the options to upload previously saved questionnaire answers in the web application). A careful documentation of how the questions were interpreted and answered is also recommended to follow changes in the monitoring system over time, through new evaluations by the same panel or by another panel of evaluators.

The pilot study showed that securing a half-day window for the workshop is needed to conduct the evaluation, generate a report, and analyze the results. However, we stress that further discussions regarding prioritization and planning of actions to improve identified weaknesses, should be scheduled at another time. Based on the evaluations conducted, we observed that the tool provides a manageable "first step for action" where there is an interest in upgrading or renewing existing collaborations across surveillance systems. The OH-EpiCap tool provides a macroscopic analysis of the overall organization, functioning and impact of multi-sectoral collaborations. In some cases, it may be relevant to complement the OH-EpiCap approach with a more thorough evaluation of the weaker OH aspects, using evaluation tools dedicated to the functioning and performance of surveillance (21) and/or OH aspects (13). Besides, the OH-EpiCap tool does not assess OH capacities related to laboratory activities; we recommend to consider applying the OH-LabCap tool (developed within the OH-HARMONY-CAP; https://onehealthejp.eu/jip-oh-harmony-cap/) for such aspects.

One important point highlighted by the evaluators is the simplicity of application of the tool, with limited time and human resources, without hindering the quality of the results. Indeed, the evaluation can be conducted through a half-day (3–4 h) workshop, and we suggest limiting the evaluator panel to a maximum of ten representatives. This aligns with recommendations in the literature regarding the sufficient number of representatives (or key informants) to obtain robust information about the evaluated system (22–25). When the evaluation cannot be conducted through a workshop, an evaluation by several experts sequentially or using a Delphi-like approach (i.e., each representative completes the questionnaire, then a facilitator collates and summarizes all responses, and provides the summary back to the participants for cross-checking/validation) can be alternative options to conduct the evaluation (26). These approaches do not enable surveillance representatives to share their views and experiences regarding OH surveillance, in contrast to a roundtable discussion. Therefore, such approaches should be preferred in situations where an evaluation would be requested by policymakers within a short delay, for example during surveys assessing the OH epidemiological capacities of EU countries for a specific hazard, or within a country for a large range of related hazards. As such, the tool will be very complementary to the existing EU-LabCap tool, designed to assess the capacity and capability of European microbiology laboratories (15). We emphasize that the benchmarking module of the OH-EpiCap web app enables each country to compare their results to a reference set that could be generated by the policymakers using a compilation of evaluation results for the same hazard from other countries, or for other hazards from the same country, depending on the context.

5. Conclusion

OH-EpiCap is a generic (i.e., applicable to multi-sectoral surveillance systems of any hazard), interactive (facilitating and supporting discussions among stakeholders from diverse sectors and disciplines), and standalone (thanks to the user-friendly web application) tool developed to conduct macro-level evaluation of epidemiological national capacities and capabilities for OH surveillance. It supports the diagnostic of strengths and weaknesses in multi-sectoral collaborations and helps to identify concrete and

direct actions to improve collaborative activities at all steps of surveillance. Besides, this evaluation framework strengthens trust between stakeholders across the systems, building a foundation for professional networks, acculturation to practices in other health sectors and disciplines, and long-term collaborations.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

VH and JP implemented the study. LC, HT, VH, RL, GC, and JP developed the tool. CB, FF, ET, and JP developed the web application. HT, JR, and VH conducted the pilot of the tool. All authors participated in drafting the manuscript and approved the final version.

Funding

This work was supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No. 773830: One Health European Joint Programme. Funding sources did not affect the design of this study, data collection, data analysis, decisions on publication, or preparation of the manuscript.

Acknowledgments

We thank the OH-EJP MATRIX consortium, experts from other OH-EJP projects, the CoEvalAMR project, and the European Food Safety Authority (EFSA) and the participants to the OH-EpiCap evaluations for their feedback and support to consolidate and validate the organization of the tool and the definition of the targets and indicators.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1053986/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Methods
PUBLISHED 30 May 2023
DOI 10.3389/fpubh.2023.1151568

OPEN ACCESS

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RECEIVED 26 January 2023
ACCEPTED 12 May 2023
PUBLISHED 30 May 2023

CITATION
Knijn A, Michelacci V, Gigliucci F, Tozzoli R, Chiani P, Minelli F, Scavia G, Ventola E and Morabito S (2023) IRIDA-ARIES Genomics, a key player in the One Health surveillance of diseases caused by infectious agents in Italy. *Front. Public Health* 11:1151568. doi: 10.3389/fpubh.2023.1151568

IRIDA-ARIES Genomics, a key player in the One Health surveillance of diseases caused by infectious agents in Italy

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Pathogen genomics is transforming surveillance of infectious diseases, deepening our understanding of evolution and diffusion of etiological agents, host-pathogen interactions and antimicrobial resistance. This discipline is playing an important role in the development of One Health Surveillance with public health experts of various disciplines integrating methods applied to pathogen research, monitoring, management and prevention of outbreaks. Especially with the notion that foodborne diseases may not be transmitted by food only, the ARIES Genomics project aimed to deliver an Information System for the collection of genomic and epidemiological data to enable genomics-based surveillance of infectious epidemics, foodborne outbreaks and diseases at the animal-human interface. Keeping in mind that the users of the system comprised persons with expertise in a wide variety of domains, the system was expected to be used with a low learning curve directly by the persons target of the analyses' results, keeping the information exchange chains as short as possible. As a result, the IRIDA-ARIES platform (https://irida.iss.it/) provides an intuitive web-based interface for multisectoral data collection and bioinformatic analyses. In practice, the user creates a sample and uploads the Next-generation sequencing reads, then an analysis pipeline is launched automatically performing a series of typing and clustering operations fueling the information flow. Instances of IRIDA-ARIES host the Italian national surveillance system for infections by *Listeria monocytogenes* (Lm) and the surveillance system for infections by Shigatoxin-producing *Escherichia coli* (STEC). As of today, the platform does not provide tools to manage epidemiological investigations but serves as an instrument of aggregation for risk monitoring, capable of triggering alarms on possible critical situations that might go unnoticed otherwise.

KEYWORDS

One Health surveillance, foodborne pathogens, genomic, multisectorial, molecular typing workflows, data integration

1. Introduction

The increasing application of Whole Genome Sequencing (WGS) in Public Health surveillance of infectious diseases, offers an excellent opportunity to employ the One Health approach (1) with the integration of both genomic and epidemiological data from different health domains (human, veterinary, food and environment). A One Health implementation allows for not only the precocious detection of outbreaks but also for a better understanding of the role of pathogen reservoirs, evolution and vehicles of transmission, enabling proactive prevention of public health threats.

The Italian National Institute of Health (Istituto Superiore di Sanità, ISS) deployed a genomic surveillance system for foodborne pathogens to shift from the existing typing system mainly based on the analysis of Pulsed Field Gel Electrophoresis (PFGE) profiles. This system is aimed at supporting the epidemiological surveillance of foodborne diseases in the population with specific short and medium/long term goals. The main short term goals were early detection of disperse outbreaks in the community, integration with genomic data from food/environment isolates to discriminate whether a certain food chain and vehicle is implicated or not in an outbreak. Likewise, integration of data and descriptive metadata from human and non-human isolates for source attribution and risk assessment studies were foreseen in the mid/long term to inform and evaluate the adoption of One Health control policies. This is particularly important for STEC control due to the large variety of hosts and sources that may play a role in the spread of infection to the most vulnerable population subgroups. For the purpose, the ARIES Genomics project planned to develop a platform as part of a One Health-Based Conceptual Framework (2, 3) starting with the existing collections of STEC and Lm. To guarantee adequate functionality for users with a wide variety of technical skills, the system had to have a low learning curve, a short chain of information exchange, and a simple but exhaustive user interface. This translated in a combination of essential comprehensive outcomes together with the detailed data available for users with more advanced bioinformatic knowledge. The system's stakeholders include public health professionals with different backgrounds. Laboratories and hospitals upload the data, but they also consume it because feedback of how their data relates to that of other Regions is returned as an incentive to participate to the system. In Italy public healthcare is federated at a regional level, so the platform has the important role to overcome data silos and provide horizontal (Hospitals/Laboratories between each other or Region-Region) as well as vertical (Hospitals/Laboratories-Regions-Central Health Authorities) spread of information. Here, the infrastructure, functionalities and usability of IRIDA-ARIES are described, an web-based platform for multisectoral data collection and bioinformatic analyses in support of a still to be formalized national One Health surveillance.

2. Methods

2.1. The IRIDA-ARIES platform

The IRIDA-ARIES genomic surveillance information system is built engaging two open-source platforms: A Galaxy instance (4) implemented as a cluster, ARIES (Advanced Research Infrastructure for Experimentation in genomicS) [preprint (5)] and an IRIDA (Integrated Rapid Infectious Disease Analysis) instance [(6), Figure 1].

The Galaxy Platform is a container for bioinformatic tools sharing a common workflow system, allowing each instance to focus on specific goals through the installation of appropriate tools. Each Galaxy instance is therefore different in function of the aims of the instances' managers. The code of the ARIES instance was installed as a role of the automation platform Ansible from the Galaxy Project (7) and was not changed. Customization of the platform consisted in the development and integration of specific tools and workflows for public health microbiology and molecular epidemiology.

The Galaxy software is not suitable for the collection of samples with genomic and epidemiological data, nor is it possible to implement automation to the analyses. To this means, the open source IRIDA platform fitted the purpose, providing an intuitive web-based interface for the collection of genomics data, utilizing ARIES as a workflow engine for the bioinformatic analyses. In this scenario, IRIDA communicates with ARIES through the latter's unified Applications Programming Interface (API), hiding the ARIES platform from the user, who only interacts with the IRIDA user interface which was translated in Italian.

2.2. Integration of heterogeneous genomic data

The IRIDA software package being open source under the Apache License 2.0 was essential for the project because it allowed to fork (copy) the code and adapt the properties of the overall system. The system as a national surveillance platform had to be open to data obtained with various sequencing platforms, not only the mostly used Illumina paired-end reads but also Ion Torrent single reads. Development of bioinformatic tools in pathogen genomics is heavily biased vs. paired-end data. In the Galaxy platform it is not possible to create collections of single reads together with paired-end reads. The IRIDA software was therefore adapted to mask single reads as paired-end and the bioinformatic tools in ARIES were modified correspondently to intercept and elaborate them appropriately. Since this required a matching intervention in ARIES as well as IRIDA breaking functioning of the code, this change could not be opened as a pull request for IRIDA in order to synchronize this feature to the original upstream repository.

Furthermore, since events of infectious diseases launched by European Union Member States on the European Centre for Disease Prevention and Control (ECDC) EpiPulse portal for the European surveillance of infectious diseases (8) frequently only share genomic assemblies (fasta files), the platform had to be able to accommodate and elaborate this type of sequences. Another adaption of both the interface of IRIDA as well as the workflows in ARIES was made. It is therefore possible to create a selection of heterogeneous samples (of both raw and assembled sequences) and launch a workflow using them.

10.3389/fpubh.2023.1151568

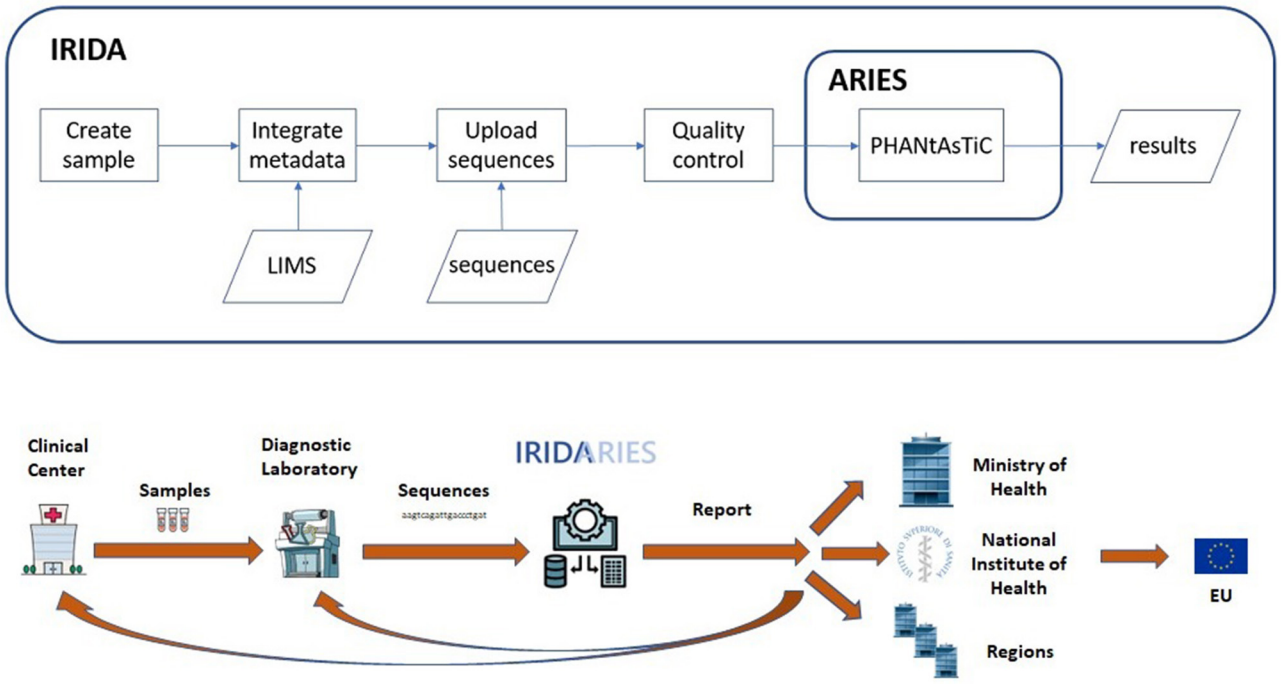

FIGURE 1
The IRIDA-ARIES platform: when a sample is created, metadata is recovered from both the local molecular and epidemiological information systems. Then, when genomic sequences are uploaded, quality control is performed and consequently the sequences are analyzed by ARIES using the pathogen-specific pipeline PHANtAsTiC. Results are returned to IRIDA and displayed in a specific analysis web page. Schematic design of the IRIDA-ARIES information flow. The flow is triggered at the local level by a clinical center which sends a sample of a case to a diagnostic laboratory (could be in-house) performing the whole genome sequencing. The sequences are uploaded to IRIDA-ARIES and the results are returned to the local level as well as forwarded to the regional and national level. If necessary, the data can be advanced to the European level.

2.3. The organization of IRIDA-ARIES

In Italy, healthcare is delegated at the regional level comprising nineteen Regions and two Autonomous Provinces, where each has local health authorities and manages its proper surveillance systems in an independent way, hindering the acquisition of a nation-wide overview (9). To reflect this reality, the organization of the surveillance platform was implemented in a federated way: Regional Projects were created for each pathogen, and the code of the interface was adapted to let these Projects partially share information with a National Project accessible for nation-wide analyses by all regional users (including Competent Authorities) and the Ministry of Health with read-only authorization. Sensitive data present in the Regional Projects is not shared in the National Project in compliance with the General Data Protection Regulation (GDPR, UE n. 2016/679). Upon request of the users, an additional system role was defined, authorized to view results but not to export data. In case of multi-regional clusters, users can see who the members of the other Regions that are involved in the cluster are and contact them directly. Although personalized for the Italian healthcare, this organization is general and may suit a wide variety of contexts.

2.4. Information flow

Several customizations have been introduced to automate as much as possible to lower the learning curve for unexperienced users while at the same time providing advanced tools for users with a genomics analysis background. The information flow is data-driven (Figure 1). To contribute to the platform, only two simple operations are required: creation of a sample by providing a unique sample name and upload of the sample's sequence(s). Upon creation of a sample, epidemiological metadata are added or retrieved for data integration from external sources, if available, using the sample name as a key value. Upon upload of the sequence(s), a pathogen-specific workflow is automatically launched performing assembly, typing and clustering elaborations.

After the automated workflow has concluded, an e-mail is sent to Project members containing concise information in function of the pathogen: the end-of-analysis message contains either core genome Multi Locus Sequence Typing (cgMLST) clustering results (whether the sample is part of a cluster, i.e., its genetic profile is similar to those of other samples within a certain cut-off) or variant typing results. In case of a cluster involving more than one Region, the mail is also sent to any other Region involved, to the Ministry of Health and to the ISS to support coordination and outbreak management. A JSON file (Table 1) containing the analytical results is sent attached to the e-mail to allow for automated acquisition of the data by the receiver. Further automation is possible for the user, since the IRIDA batch uploader (10) published by the IRIDA developer team, was adapted to the specific metadata introduced in the IRIDA-ARIES instance and integrated into the system as an FTP service. If necessary, data can be forwarded to the European level.

2.5. Molecular typing workflows

All analysis workflows have been designed specifically for the IRIDA-ARIES platform combining both existing as well as in-house developed tools. The workflows used for the automatic elaboration of samples are the most complex. The PHANtAsTiC (Public Health Analysis of Nucleotides through Assembly, Typing and Clustering) workflow [preprint (4)] has been developed to perform a series of pathogen-specific typing tools. All bioinformatic tools that have been integrated into the workflow are listed in Table 2. The assembly phase only applies when raw sequences are uploaded. The sequences are assembled with specific parameters for Ion Torrent or Illumina data and a quality report is generated. In case pre-assembled sequences are provided for samples, this step is skipped. During the typing phase, generic as well as pathogen-specific tools are applied to obtain as much information on the sample as possible. These include serotyping, Multilocus sequence typing (MLST), virulotyping, antimicrobial resistance (AMR) prediction. Aside the molecular typing, a cluster analysis is performed on the distance matrix of the core genome cgMLST profile of each sample with respect to those of all samples present in the platform resulting in a phylogenetic tree. A warning is triggered in case samples are found within a given allele distance threshold which is set at 4 for Lm and 10 for STEC. These values have empirically shown to reflect actual clusters when compared with phylogenetic analyses.

The workflow is in its second version since the code has recently been adapted to match the cgMLST typing method performed at a European level by the European Food Safety Authority (EFSA) One Health WGS System (31). In fact, Mentalist (32) has been replaced by chewBBACA (28) as the allele typing method for Lm, while the latter was already used for analyzing STEC samples.

TABLE 1 Example JSON file containing the analytical results produced by the automatic pipeline PHANtAsTiC for a sample of *Listeria monocytogenes*.

{"coverage": "153.63", "read_mean_length": "139", "q30_rate": "0.831981", "total_bases": "499125548", "information_name": "H_706", "qc_status": "Passed", "qc_messages": "Passed.", "serotype_serogroup": "1/2a,3a", "serotype_amplicons": "lmo0737,Prs", "mlst_ST": "ST155", "mlst_CC": "CC155", "mlst_lineage": "II", "region": "Lombardia", "year": "2022", "core_genome_schema_size": 1743, "sample_genes_mapped": 1729, "Cluster_Id": "-"}

TABLE 2 The bioinformatic tools used in the PHANtAsTiC v2.1 pipeline.

Phase	Step	Software/database	Version	Refences
Assembly	Trimming	fastp	v0.23.2	(11)
	Assembly Ion Torrent	SPAdes	v3.15	(12)
	Assembly Illumina	INNUca	v4.2.2	(13)
	Assembly quality assessment	QUAST	v.5.0.2	(14)
Typing	Serotyping STEC	BLASTn	v2.11.0	(15)
		Statens Serum Institute database	2022-05-16	(16)
	Serotyping Lm	LisSero	v0.1	(17)
	Multilocus sequence typing (MLST)	mlst	v2.16.1	(18)
		PubMLST typing schemes	7 loci	(19)
	Virulotyping	patho_typing	v0.1	(20)
		Statens Serum Institute database	2022-12-02	(16)
	Shiga toxin subtyping	duk	v0.1	(21)
		Trimmomatic	v0.39	(22)
		SKESA	v2.4	(23)
		SPAdes	v3.15	(12)
		fastq_pair	v1.0	(24)
		MUSCLE	v3.8	(25)
		BLASTn	v2.11.0	(15)
		Statens Serum Institute database	2022-10-18	(16)
	Antimicrobial resistance prediction	ABRIcate	v1.0.1	(26)
		ResFinder	2023	(27)
Clustering	Core genome MLST	chewBBACA	v3.1.2	(28)
		INNUENDO *Escherichia coli* schema	2023	(29)
		Pasteur *Listeria monocytogenes* schema	2023	(30)

To give users the possibility to further investigate selected samples within the system, several workflows have been added to the platform. These workflows comprise: cgMLST cluster analysis of the previously calculated allele profiles, reference-free Single-Nucleotide Polymorphism analysis using the PopPUNK software v1.1.2 (33), Minimum Spanning Tree analysis of the previously calculated allele profiles with the GrapeTree software v2.2 (34), creation of an HTML summary of the samples with some simple pivot charts, multi virulotyping (calculation of a matrix of samples-virulence genes for the selected samples) for easier comparison between samples, a tool for the creation of an official analysis report in PDF. Expert users can use a workflow to directly export sequences to the ARIES Galaxy instance (5), where a wide variety of genomic and molecular epidemiology bioinformatic tools can be readily used. A copy of the manual of the platform is available as Supplementary material.

2.6. Data sharing

Sequences as well as metadata can be easily shared with other systems for further analyses. The IRIDA platform by default features a tool to assist in uploading sequence files to NCBI's Sequence Read Archive. A tool for the export of samples' metadata has been added to the platform. Currently, a collaboration agreement framework is in the process of being finalized, regulating the exchange of human and animal/food/feed Listeriosis data between the National Listeriosis Surveillance Working Group at ISS and the National Reference Laboratory (NRL) for *Listeria monocytogenes* based at the Abruzzo and Molise Veterinary Public Health Institute (IZSAM). Moreover, a tool is under development for the programmatic submission of cgMLST allele profile data to the EFSA One Health WGS system database. Locally, the associated analytical results of STEC data are visible in the web application of the NRL developed for the STEC collection.

2.7. Limitations

The customizations to the platform have broken the encapsulation of the two underlying software packages. In fact, masking the heterogeneous data that is shared between them has limited the generality of both systems. Also, ARIES analysis workflows consume data directly from the IRIDA database.

The IRIDA database is implemented on a single server but could be scaled up as a cluster. ARIES is relying on a SLURM (35) cluster for computational capacity and cluster nodes can be easily added if needed using the Ansible automation software. At the moment, ARIES is configured to run all jobs locally, using the file system that is shared between cluster nodes and IRIDA. The installation of a Pulsar server (36) is planned to allow for the execution of jobs on remote High-Performance Computing clusters (HPCs) overcoming the need for a shared file system.

The IRIDA platform is scaled up to four servers for high load deployment, dividing different tasks between them. With this configuration, batch uploads of several thousands of samples have been managed by the system. Currently, no further scaling of the system is possible.

IRIDA-ARIES has to be considered as a component of the applications and protocols to be used in the ecosystem of surveillance, prevention and risk management. Its modular structure and the implemented APIs do allow for the flexible development of personalized interfaces vs. heterogeneous outputs.

3. Results

Although the platform is not designed to manage the whole process of surveillance and outbreak management, it comprises features for risk monitoring and is capable of automatically detecting clusters and triggering alarms on possible critical situations. Users are immediately aware of which Regions are involved in the warning and can readily establish connections while keeping information chains short. Regional data is shared to allow for a constantly updated national overview of pathogen diffusion. Feedback is returned to the regional users engaging them to participate actively with their data, creating a virtuous circle avoiding the danger of data silos at the regional level.

Sharing of genomic data facilitates timely detection of clusters and, in general, situations of concern. Furthermore, the exchange with the veterinary public health Institutes (Istituti Zooprofilattici Sperimentali, IZSs) in a One Health view to receive human, animal, food and environmental samples, allows for direct comparison of genomic profiles in order to rapidly exclude possible contamination sources avoiding unnecessary high economic impact and to provide objective arguments to risk management for the timely activation of prevention measures. The exchange of sequence data without its metadata in case of suspect samples would avoid issues with data sharing. Should a situation of suspected outbreak occur, then an integrated data exchange protocol could be activated.

The IRIDA-ARIES platform is currently hosting the Italian national surveillance system for infections by *Listeria monocytogenes* and the local surveillance system for infections by Shiga toxin-producing *Escherichia coli* and counts 71 users, including personnel of the regional Public Health Services. For Listeriosis as of 14/12/2022, a total of 1,453 samples have been uploaded to the platform spanning the period 2002–2022, comprising 1,295 human samples, 61 animal/food/feed historical samples and 97 samples from outbreak events shared through European channels. The platform identified 108 clusters comprising 695 samples (73% of the clusters were composed of 5 or less samples). For STEC as of 14/12/2022, a total of 1,540 samples have been uploaded to the platform spanning the period 1989–2022, comprising 683 human samples, 798 animal/food/feed samples and 59 samples from outbreak events shared through European channels. In this case, 192 clusters have been identified by the platform including 664 samples (90% of the clusters consisted of 5 or less samples). Since PFGE typing was performed only in the presence of an epidemiologically identified suspect cluster and there was no collection of PFGE profiles from the territory, a comparison of cluster detection before and after the switchover is impossible.

The platform has been used to analyze the sequences of 42 STEC and 97 Lm isolates (accessed on 11/11/2022) appended to the

information on the events of infectious disease, mainly outbreaks of infections, launched through the ECDC EpiPulse portal or to Urgent Inquiries launched on the former platform Epidemic Intelligence Information System for food- and waterborne diseases (ECDC-FWD-EPIS). The sequences were processed automatically by the platform upon upload and compared with the sequences of all the samples of the same species (for Lm) or serogroup (for STEC) isolated from human cases of disease in Italy already present in the database. This system was used to investigate 30 different events involving STEC strains and 71 involving Lm isolates, allowing to quickly reply on the ECDC FWD system about possible correlations among Italian isolates and those part of ongoing international events.

The platform has proven particularly useful in the investigation of two large outbreaks of Listeriosis that have occurred in Italy in 2022. The presence of two growing clusters, of sequence type 8 and 155 respectively, was noted as evidenced by the platform. Consequently, in particular for the ST155 outbreak, on August 1st 2022 a Working Group was formed by the Ministry of Health, comprising the ISS, the IZSs, the NRL for *Listeria monocytogenes* and the Regions/Autonomous Provinces. The work of this Group supported the epidemiological investigation on the correlation between the clinical cases and the consumption of certain meat products. During the investigation, analysis of the cgMLST profiles allowed for the rapid identification of samples belonging or not to the specific cluster, narrowing the analytical process. The phylogenetic pipelines integrated into the platform have been used by the Working Group for the redaction of the periodic reports as well as autonomously by the regional users themselves. As stated by the Italian undersecretary of the ministry of Health in a parliamentary interrogation: "*The current situation linked to Listeriosis has emerged thanks to the work of the Ministry of Health, through ordinary surveillance and through the IRIDA database of the Istituto Superiore di Sanità, which has made it possible to verify the increase in human cases throughout the national territory.*" (37).

4. Discussion

The introduction of the IRIDA-ARIES platform has made the transition from PFGE-based to WGS-based surveillance of listeriosis and STEC infections in Italy smooth, allowing concomitantly to obtain a better overview of the existence of clusters with respect to geographical location as well as to temporal occurrence. In fact, it facilitated the move to a solution joining sample management and user collaboration to combine regional efforts and create a nation-wide view of pathogens' monitoring. Routine sequencing, together with collection of typing data on the territory, has made cluster identification proactive because often the identification of a cluster occurs before the epidemiological suspicion or in the absence of a specific unexpected increase of cases in a given time frame and area. Moreover, the analytical results are shared in real-time to stakeholders in various information systems without being copied by hand, speeding up the process and eliminating repeated tasks and possible errors during transcription. By applying genomics-based surveillance to infectious diseases, One Health practitioners can identify the specific genetic makeup of a pathogen, providing information on the hazard characterization and use this information to predict its potential for spread and to develop targeted interventions. The possibility to upload pre-assembled sequences from European outbreaks originating from both human (ECDC) and animal/food/feed (EFSA) concerning food- and waterborne diseases and zoonoses for a direct comparison with national samples, allows to integrate the Italian surveillance of foodborne diseases within an international One Health perspective. The objective is to align the typing workflows for each pathogen in collaboration with these European Agencies to obtain compatible results that can be readily exchanged.

In 2019, a face-to-face course was organized for the future regional users of the system. The feedback has been very positive, before the end of the course many participants had become confident with the system and acquired the ability to use most applications of the platform. Also, several requests from the participants could be readily implemented. A helpdesk has been set up to assist users running into problems. Now that the restrictions due to the COVID-19 pandemic have been largely lifted, an annual in-person meeting of the *Listeria* network has been foreseen, so the regional users get to know their counterparts from the other Regions, facilitating contacts in case of inter-regional clusters.

The system has been well-accepted by all different types of users because it has proven intuitive enough for those without specific computer skills, while yet powerful for the needs of the users with advanced bioinformatic experience. Although submission of data is on a voluntary basis in Italy, the system is now used by the majority of the Regions. Several clusters persistent in time and/or location have been highlighted by the system, indicating the platform as a powerful tool in support of future preparedness of early detection of food safety risks.

Integration of human genomic data with samples originating from other One Health domains allows the platform to act as a key player in the surveillance of diseases caused by infectious agents in Italy. Not only in Italy though, the platform has been designed as multi-language and can readily be used in any (inter-)national context upon addition of a language dictionary. Issues with data sharing include data ownership, privacy regulations and legal considerations and have been tackled on several levels. The collection of the data has been approved by the Data Protection Officer of the ISS. The Regions remain owners of the genomic data they provide, their sequences cannot be accessed by others but only used in aggregated analyses.

The platform has been used for STEC and Lm because the ISS already collected data for these pathogens and therefore the expertise for analyzing these genomes had been previously acquired. The surveillance of other pathogens could be implemented without much effort since the bioinformatic tools of the platform can be flexibly adapted. An IRIDA-ARIES instance named ICoGen (Italian COVID Genomics), is actually in use at the ISS for the national surveillance of genomic variants of the SARS-CoV-2 virus.

In the mid/long term, the IRIDA-ARIES infrastructure is meant to become the national platform for the genomic surveillance of infectious diseases. In this respect, the established networks providing data on Lm and STEC isolates from the different Italian regions, will be the starting point for expanding and consolidating the data providers' network for other foodborne infections. The

analytical metadata of the sequenced strains will represent the central elements for the prompt identification of outbreak events as well as for source attribution and exposure risk assessment. Further development will focus on the integration of the platform as a component of an overall infrastructure for the surveillance and management of infectious diseases. The hope is that IRIDA-ARIES through the establishment of an inclusive cross-sector network will serve as a basis and stimulus for the creation of a national systemic approach enabling source attribution studies such as those carried out in the DiSCoVeR project (38) and possibly adapting solutions already implemented and new tools for surveillance and risk assessment still under development in projects such as COHESIVE (39) which is part of the One Health European Joint Program (40). Furthermore, the next step will include a FAIRification process of the produced datasets to enhance machine findability, accessibility, interoperability and reusability (41). The latter will be crucial for the integration of the heterogenous data collected during the various levels of a One Health surveillance and risk assessment infrastructure. FAIR principles for data and software are generally applicable, but need to be extended in order to address the processual nature of workflows, which will pave the way for standardized trustable data with the added value of being ready for secondary data reuse and exploitation by third parties (42).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories can be found below: IRIDA-ARIES: https://github.com/aknijn/irida PHANtAsTiC: https://github.com/aknijn/phantastic-galaxy.

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The members of the European Union Reference Laboratory for *Escherichia coli* collected STEC samples and sequenced the DNA for molecular typing. The members of the National Listeriosis Surveillance Working Group collected Listeriosis samples and sequenced the DNA for molecular typing. The members of the IRIDA-ARIES user group STEC collected STEC samples and sequenced the DNA for molecular typing. The members of the IRIDA-ARIES user group Listeriosis collected the samples and sequenced the DNA for molecular typing. The members of the Italian Registry of Hemolytic Uremic Syndrome collected STEC samples.

Author contributions

AK and SM were responsible for the concept and design of the study, interpretation of results, writing, and critical review of the manuscript. AK was responsible for the design and development of the IRIDA-ARIES platform. AK, VM, FG, and SM were responsible for the design of the bioinformatic workflows. RT, PC, and FM were responsible for the collection and curation of data from the

STEC registry. GS and EV were responsible for the collection and curation of the epidemiologic data of the Italian Registry of Hemolytic Uremic Syndrome. All authors contributed to the article and approved the submitted version.

Funding

Funding was provided by intramural funds of ISS.

Acknowledgments

This publication made use of the PubMLST website (https://pubmlst.org/) developed by Keith Jolley (Jolley & Maiden 2010, BMC Bioinformatics, 11:595) and sited at the University of Oxford. The development of that website was funded by the Wellcome Trust. We wish to thank Manuela Marra and Maria Carollo from the Core Facilities Technical-Scientific Service of the Istituto Superiore di Sanità for the whole genome sequencing of part of the isolates whose genomes populate the IRIDA ARIES database.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1151568/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 13 June 2023
DOI 10.3389/fpubh.2023.1121522

OPEN ACCESS

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RECEIVED 11 December 2022
ACCEPTED 11 May 2023
PUBLISHED 13 June 2023

CITATION
Alves F, Artursson K, Bloch J, Brisabois A, Imberechts H, Jokelainen P, La Ragione R, Lindblad M, Forss RL, Marston DA, Parvizi O, Tuominen L and Omazic A (2023) A multi-country One Health foodborne outbreak simulation exercise: cross-sectoral cooperation, data sharing and communication. *Front. Public Health* 11:1121522. doi: 10.3389/fpubh.2023.1121522

A multi-country One Health foodborne outbreak simulation exercise: cross-sectoral cooperation, data sharing and communication

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Introduction : The awareness of scientists and policy makers regarding the requirement for an integrated One Health (OH) approach in responding to zoonoses has increased in recent years. However, there remains an overall inertia in relation to the implementation of practical cross-sector collaborations. Foodborne outbreaks of zoonotic diseases continue to affect the European population despite stringent regulations, evidencing the requirement for better 'prevent, detect and response' strategies. Response exercises play an essential role in the improvement of crisis management plans, providing the opportunity to test practical intervention methodologies in a controlled environment.

Methods: The One Health European Joint Programme simulation exercise (OHEJP SimEx) aimed at practicing the OH capacity and interoperability across public health, animal health and food safety sectors in a challenging outbreak scenario. The OHEJP SimEx was delivered through a sequence of scripts covering the different stages of a *Salmonella* outbreak investigation at a national level, involving both the human food chain and the raw pet feed industry.

Results: A total of 255 participants from 11 European countries (Belgium, Denmark, Estonia, Finland, France, Italy, Norway, Poland, Portugal, Sweden, the Netherlands) took part in national level two-day exercises during 2022. National evaluations identified common recommendations to countries aiming to improve their OH structure to establish formal communication channels between sectors, implement a common data sharing platform, harmonize laboratory procedures, and reinforce inter-laboratory networks within countries. The large proportion of participants (94%) indicated significant interest in pursuing a OH approach and desire to work more closely with other sectors.

Discussion: The OHEJP SimEx outcomes will assist policy makers in implementing a harmonized approach to cross-sector health-related topics, by highlighting

the benefits of cooperation, identifying gaps in the current strategies and suggesting actions required to better address foodborne outbreaks. Furthermore, we summarize recommendations for future OH simulation exercises, which are essential to continually test, challenge and improve national OH strategies.

KEYWORDS

Salmonella, simulation exercise, zoonosis, One Health, foodborne outbreak, public health, food safety, animal health

1. Introduction

Detecting and responding to current and emerging zoonotic threats increasingly requires involvement from more than just one sector. Therefore, fostering cross-sector collaboration and disease response preparedness under the framework of One Health (OH) has become a priority for many countries (1). The awareness of the scientific community and policy makers to the emerging risk that infectious pathogens pose to health has increased due to the efforts made in the OH field, with multiple international projects showing the way to further developing this area (2). OH is defined as "an integrative and systemic approach to health, grounded on the understanding that human health is closely linked to the healthiness of food, animals and the environment, and the healthy balance of their impact on the ecosystems they share, everywhere in the world" (3). Several international reports reveal a general agreement among stakeholders regarding the benefits that a One Health approach brings to society, contributing to tackle food and water insecurity and shortage, supporting a sustainable development, and helping in the management of financial and natural resources toward future risk prevention (4–7).

While the theoretical aspects of OH have been well established and embraced within the scientific community, practical implementation has been hindered due to the complex requirement of political, ethical, economical, and societal engagement (8), rendering a truly unified and efficient One Health based system far from being delivered. Initiatives which aim to achieve a tangible transformation should primarily focus on improving communication, coordination, collaboration, and capacity building across all sectors of society and to align with the fundamental principles of inclusivity, parity and stewardship (9). The One Health European Joint Programme (One Health EJP)[1] was conceived to move toward a holistic global approach to health threats, with the primary goal of promoting international and interinstitutional collaboration to improve preparedness. The One Health EJP consortium promotes transdisciplinary collaboration across sectors by supporting collective research activities and developing tools and guidelines in the fields of foodborne zoonoses, antimicrobial resistance, and emerging threats. In addition, by providing education and training initiatives, the consortium facilitates the harmonization of the approaches taken by different institutes. Congregating 44 partners across Europe, building upon the collaborations from the Med-Vet-Net-Association[2], the One Health EJP is based on the concept that no transmissible disease can be addressed as a problem constricted to any individual country or sector (10). The consortium strives to employ the outputs delivered and promote them across the scientific community, thereby implementing tangible changes that can be sustained beyond the duration of the programme.

Food safety and security are considered an overarching subject in the OH international agenda for a roadmap toward sustainable development (2, 11, 12). Despite the rigorous regulation enforced within the European Union (EU), foodborne outbreaks continue to significantly affect the population with a sustained number of reported outbreaks each year (13). This impact showcases the importance of equipping response systems with improved tools to address and mitigate foodborne infections. For example, despite the strictly regulated control programmes implemented in poultry production units and the regulation on food safety and process hygiene criteria for *Salmonella enterica* serotype Typhimurium in several food categories, it remains as an important gastrointestinal pathogen in EU, accountable for 22% of all foodborne outbreaks in 2020 (13). Despite egg and egg products being the most common sources of *Salmonella,* other foodstuffs such as meat products also contribute to human infections (14), highlighting the need to identify additional infection routes (15). Only by linking together the sector specific activities, thereby embracing the ethos of the OH approach, will we improve our response to less predictable outbreaks of disease.

While disease incursions remain a constant and significant threat, our ability to adequately respond to them defines their scale and impact on the community. An essential tool within emergency preparedness plans is the conduction of simulation exercises, exposing existing gaps in a controlled environment and assessing the current crisis management strategies without the negative consequences of a real-life emergency. Improvement plans drawn up after such an exercise provides detailed and tangible documentation for each sector and motivation to deliver the improvements required. The nature and scale of the exercise may vary depending on the aims and objectives, budget, and resource availability, ranging from discussion-based exercises (orientation exercise; table-top exercise) to more complex operation-based exercises (drill; functional or command post exercise; full-scale exercise). Table-top exercises are a common format for simulation exercises, offering the opportunity to be completed in an informal and stress-free environment where the participants are guided by a facilitator and encouraged to engage in a roundtable discussion based on a simulated scenario. A series of scripted injects are given to the participants, presenting the problems that need to be tackled. This type of exercise stimulates the participants' problem-solving capacities and develops the communication strategies required

1 https://onehealthejp.eu/, 2018–2023.

2 http://www.medvetnet.org/

to respond effectively in the event of an actual disease incursion. Although table-top exercises lack the full realism of functional or full-scale exercises, they provide an effective and efficient way to become familiar with procedures and policy. Moreover, this format is not necessarily timebound, therefore allowing the participants to allocate time to focus on the critical elements of the scenario (16–18).

Within the remit of the One Health EJP, the multi-country OHEJP SimEx was designed with an overall aim to practice the OH capability, capacity, and interoperability at a national level, across public health (PH), animal health (AH) and food safety (FS) sectors. To succeed in this aim, a challenging outbreak scenario with a zoonotic disease that typifies the OH concept and that was relevant across Europe was required. Therefore, a *Salmonella* outbreak scenario was developed, which included both human food and pet feed supplies specifically to provide the opportunity to share experiences and perspectives across sectors, evidencing the added value of applying a OH approach, while also providing an opportunity to test a food tracing software tool: The FoodChain-Lab (FCL) web application. By assisting countries to identify current gaps in their OH approach to a foodborne outbreak and defining strategies to tackle them, the OHEJP SimEx outcomes have resulted in recommendations suitable for all countries to assist in defining a national roadmap for future outbreak preparedness plans.

2. Methods

2.1. Organization and planning

Within the One Health EJP, a Joint Integrative Project (JIP) priority topic was identified: "Sharing best intervention practice – twinning and simulation exercises." To address this topic, the OHEJP SimEx project was designed. A OHEJP SimEx Steering Board was formed with representatives from the One Health EJP Project Management Team. The Steering Board provided the Project Directive. Relevant stakeholders, European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA), Food and Agriculture Organization of the United Nations (FAO), World Organisation for Animal Health (WOAH) and World Health Organization (WHO) were represented in an Advisory Board. The timeline for the project was constrained within the overall OHEJP project timeframe. Preparation for the project began in January 2021 with the appointment of a project leader and included recruitment of an international project team of nine specialists with complementary expertise in the areas of PH, AH and FS and emergency response exercises. The team was responsible for planning, supporting the national conductions, evaluating the outcome of OHEJP SimEx and dissemination of the outcomes which began in September 2021 and completed in December 2022 (Figure 1). Meetings between the project team and the Steering Board were held on a regular basis throughout this period to ensure the scenario and outputs remained relevant and applicable to the overarching One Health EJP aims. Dissemination of the project outcomes continued after the project completed through the continuing communication channels within One Health EJP.

The OHEJP SimEx project team developed a realistic scenario which could be executed in multiple European countries including the following criteria:

1. The pathogen must be relevant across Europe
2. The pathogen must satisfy the One-Health focus spanning AH, PH and FS
3. Country level focus
4. Cross sectoral collaboration focus
5. Data sharing focus

This exercise was included as an implementation activity in One Health EJP. All One Health EJP partner institutes were invited to participate in the OHEJP SimEx. In addition, the institutes were encouraged to invite other institutes from outside the One Health EJP consortium, e.g., to cover up for missing sectors or to better represent the national outbreak management. Participation of institutes was on a voluntary basis. The original request and subsequent reminders to participate in the exercise were sent out by email to the following groups within the One Health EJP: Scientific Steering Board members, representatives from the Programme Managers' Committee and Project Leaders. All partners that decided to participate selected a contact person who become the link between the institute and the One Health SimEx Project Team. The contact person from each institute was then fully involved in the decision-making process about participation. In November 2021, 15 countries had expressed their willingness to participate in the exercise. By the time of the preparatory workshop (see Section 2.3) for the National Exercise Leaders (NELs) and Local Exercise Leaders (LELs) in March 2022, two countries had decided to withdraw due to their national outbreak teams being heavily involved in the COVID-19 pandemic and avian influenza outbreak responses. A further two countries withdrew due to difficulties in involving all sectors and changes in leadership, respectively. Thus, conduction of the scenario involved eleven countries (Belgium, Denmark, Estonia, Finland, France, Italy, the Netherlands, Norway, Poland, Portugal, and Sweden).

Then, in order to conduct the exercise at national level, it was necessary for each participating country to assemble a national team, including representatives from PH, AH and FS. Each national team was composed of a NEL, LELs, Local Evaluators (LEs), and a Training Audience (TA) (Figure 2). Each participating institute appointed a LEL, whose role was to organize and facilitate the institute's participation in the initiative by establishing a connection between the OHEJP SimEx project team and the institute, identifying and convening a TA, and guiding the country conduction. The NEL had overall responsibility for the coordination of the team at country-level and for most countries the NEL was also one of the LELs. The NELs and LELs had the option to adapt the scenario to the relevant local setting and to add further institutional and national objectives to the OHEJP SimEx. The NEL and LELs assembled the TA ensuring inclusion from each sector and varying levels of experience and seniority. Typically, the TA included epidemiologists, medical experts, veterinarians, laboratory personnel, communication experts and other representatives from the relevant sectors. Each institute appointed a LE, responsible for the evaluation of the exercise both during and after conduction. The LEs were critical in the success of the project, providing key observations that identified the existing gaps hindering a true One Health cooperation.

The OHEJP SimEx was designed as a table-top exercise in which the participants were encouraged to meet in person for the conduction. Final decisions regarding organization of the conduction were made by the national teams. The OHEJP SimEx ran for 2 days and was

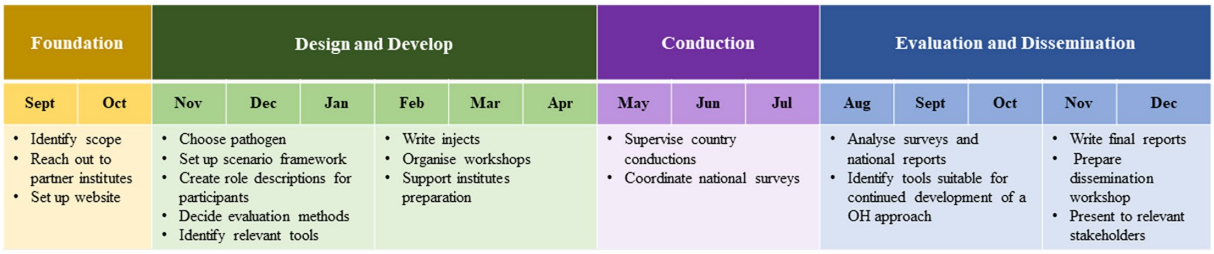

FIGURE 1
Timeline of the OHEJP SimEx project planning, conducting, evaluation and dissemination activities. The project began in September 2021 and ended in December 2022.

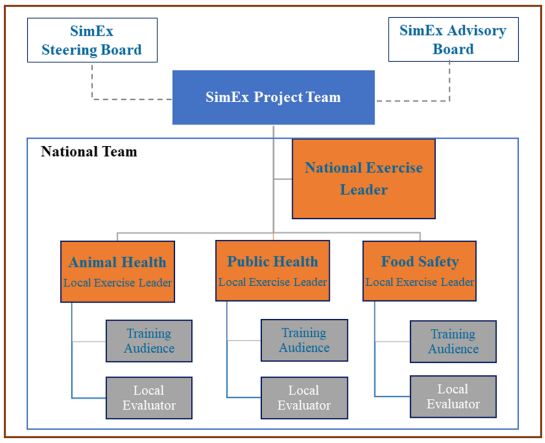

FIGURE 2
Organogram of the OHEJP SimEx project. The SimEx team was supported by both the Advisory board (including experts in outbreak exercises) and the Steering board. Each national team (denoted by the blue box) is led by the National Exercise Lead (NEL), coordinating the sector Local Exercise Leads (LEL) who in most cases represent a specific Institute. The Local Evaluators (LEs) were chosen based on their specialist knowledge in each sector, and the Training Audience (TA) chosen by the LELs.

conducted in the participating countries during the period of May to September 2022.

2.2. Scenario, objectives and conduction

The OHEJP SimEx was built following the ECDC guidelines on simulation exercises (18). The exercise was designed to replicate a *Salmonella enterica* serotype Typhimurium outbreak at a national level involving both the human food chain and the raw pet feed industry. To ensure that a cohesive language was used between sectors, the OH glossary produced within the One Health EJP was used (19, 20). The criteria listed in 2.1 were defined at the beginning of the project to guide the OHEJP SimEx project team in the scenario design with the purpose to help participating countries to identify gaps in their national outbreak contingency plans. The scenario covered all stages of a foodborne outbreak investigation and considered different possible routes of transmission between humans and animals. As the scenario unfolded, the TA was challenged with a sequence of injects covering relevant outbreak related information (i.e., number of cases, epidemiological data, laboratory results). Each inject was designed to trigger discussion and encourage the sectors to work together, showcasing the added value of employing a OH approach in a zoonotic outbreak situation.

The finalized exercise scenario was delivered through a sequence of 15 scripted injects divided into three parts. The first part of the exercise focused on increasing knowledge with objectives that highlighted the role and functionality of each sector and the availability of guidelines and systems in the event of a zoonotic outbreak. The second part of the exercise was designed to emphasize the importance of data sharing in an outbreak situation and help national teams identify possible gaps in the cohesiveness of current data collection practices. The final part of the exercise was designed to promote intersectoral cooperation and communication in an outbreak situation, helping the TA improve their understanding on how to create common main messages and identify relevant target audiences.

Each inject consisted of two parts, one to be delivered exclusively to the LELs covering the purpose of the inject, the expected outcomes, critical conditions for TA to achieve in order to proceed, and some follow up questions. The other part was for the TA with the event to be worked on. While most injects targeted the whole TA, some were directed toward a specific sector, to mimic a real-life situation and assess the flow of information between the sectors. The exercise scenario is available from the corresponding author upon request.

Prior to conduction, all NEL and LELs attended a workshop held by the OHEJP SimEx project team, during which the scenario was presented NELs and LELs were encouraged to review and adapt the scenario to reflect their national setting, if necessary. Providing the flexibility to tailor the scenario allowed the NEL and LELs to ensure maximum relevance for the training audience.

FoodChain-Lab web application is a food tracing software jointly developed by the German Federal Institute for Risk Assessment (BfR), EFSA, One Health EJP COHESIVE and other European projects, which allows to model, visualize, and analyze complex food supply chain networks (21, 22). This tool was included in the OHEJP SimEx as a practical tracing exercise, for the TA to establish possible contamination sources and transmission chains. Inclusion of FCL which could be accessed by all sectors highlighted the advantages of having an intersectoral tool when deciding on the implementation of control measures like product sampling and batch recall.

A final meeting at the end of the exercise allowed the TA to review and discuss the challenges encountered during the simulated outbreak investigation and management.

2.3. Evaluation of outcomes

The OHJEP SimEx evaluation was designed to assess the success of the objectives to identify any limitations on data sharing, develop intersectoral communication strategies and increase the mutual understanding between sectors. By identifying cooperation gaps, the evaluation also provided evidence to support the improvement of future foodborne outbreak management strategies with a OH approach.

The LEs attended a training session, delivered by the OHEJP SimEx project team prior to conduction to prepare and support them. This included how to conduct After-Action Review (AAR; Hot debrief). The guided AAR discussions covered the chronological narrative of the conduction, focusing on the most relevant decisions to highlight the strengths and weaknesses identified. Hot debriefs provided participants with the vital opportunity to share their thoughts while the experience is still fresh, avoiding missing relevant details. Post-conduction, a link to a survey was sent to all participants (i.e TA, NELs, LELs and LEs), to provide the project team with invaluable feedback on their experiences. To guarantee representative value, a minimum response rate of 80% was aimed for. The majority of questions were posed according to the Likert scale with four different options: strongly disagree, disagree, agree, strongly agree. To facilitate the interpretation of the feedback, these options were reduced into two categories: disagree and agree. Answers were processed in Microsoft Excel (version 2210 Build 16. 0. 15726. 2018816.43; Microsoft, Washington, USA) and presented as descriptive statistics.

The LEL of each institute was responsible for analyzing its own outcomes which were combined by the NEL to deliver a national report covering the experiences of the conduction, main lessons learned and recommendations for future improvement. The OHEJP SimEx project team provided a template for the national report to ensure a level of consistency in the information provided. The OHEJP SimEx project team analyzed the national reports to identify common problems, major gaps, and current best practices. However, because the report template did not explicitly request answers to a series of questions, the data presented below was compiled from the information provided and may not represent a complete picture.

By compiling and analyzing data from all the evaluation outcomes, we have provided a comprehensive analysis and summarized a list of recommendations for the improvement of OH approach to foodborne outbreaks as well as suggestions for future OH simulation exercises. In addition, the national experiences were shared at an internal One Health EJP Scientific Steering Board meeting (28th of September 2022) and at a dedicated Joint SimEx/Dissemination Workshop 'A One Health simulation exercise as a roadmap for future foodborne outbreak preparedness' (6th December 2022) that was targeted to stakeholders.

2.4. Ethical statement

This research was conducted in accordance with the principles embodied in the Declaration of Helsinki and in accordance with the One Health EJP Consortium agreement, project number 773830, Version 4, 2017-12-13 (signed version) with Amendment #1–2020. This consortium agreement is based upon regulation (EU) No 1290/2013 of the European Parliament and of the Council of 11 December 2013 laying down the rules for the participation and dissemination in "Horizon 2020 – the Framework Programme for Research and Innovation (2014–2020)." The data in the post-exercise survey, completed by the participants, were collected *via* an electronic questionnaire in EUSurvey (23) set in anonymous mode, and no personal data were collected. Participant were informed at the start of the survey that the results would be collated and published publicly. Individual written informed consent was not required from the participants.

3. Results

In total, 255 participants from 42 institutions from 11 countries (Belgium, Denmark, Estonia, Finland, France, Italy, Norway, Poland, Portugal, Sweden, the Netherlands) completed the OHEJP SimEx (Table 1; Figure 3), from which 205 answered the post-conduction survey. Four countries achieved the desired 80% response rate, and all countries had response rates above 60%. The overall response rate was 80% ($n = 205$), confirming that the results can be considered representative.

Based on the post-conduction survey results, there was a balanced representation across the three sectors, with 23% ($n = 47$) of participants belonging to the AH sector, 35% ($n = 71$) to the FS sector and 37% ($n = 75$) to the PH sector. Twelve participants (6%) did not identify with a sector. The overall opinion on the exercise was positive, with 94% ($n = 192$) of participants reporting feeling encouraged to pursue a OH approach by working more closely with other sectors in future outbreak situations.

3.1. Exercise planning and conduction

The majority of the participating countries decided that the scenario was suitable to utilize as provided. However, minor

TABLE 1 Number of participants and post-conduction survey response rate of national teams.

Country	Number of participants	Post-conduction survey respondents	Response rate (%)
Belgium	37	29	78.4
Denmark	23	19	82.6
Estonia	20	15	75.0
Finland	19	13	68.4
France	25	16	64.0
Italy	52	45	86.5
Norway	23	18	78.3
Poland	13	10	76.9
Portugal	11	8	72.7
Sweden	21	21	100
The Netherlands	11	11	100
Total	255	205	80.4

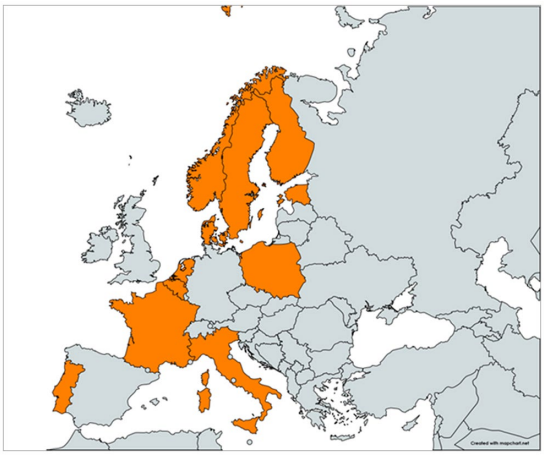

FIGURE 3
Map of the participant countries. Participating countries are highlighted in orange.

adaptations were made by four out of the 11 countries. Modifications included adding information to explore topics of national relevance or providing supporting documents to the TA. In addition, one country made more significant changes to the scenario to replicate the structure of their national system. Four countries chose to translate the documents into a local language prior to conduction. Over 95% of participants reported finding the scenario ($n = 195$) and chosen pathogen ($n = 201$) relevant, 89% ($n = 183$) expressed that the scenario equally covered all the sectors, and 95% ($n = 194$) considered the scenario was in line with the exercise objectives. Sixteen percent ($n = 32$) of participants reported a lack of reality in the way the outbreak unfolded and 26% ($n = 54$) of participants did not think the injects fully mimicked a real-life outbreak situation.

At a country level, the TA size ranged from 5 to 40 individuals, with a median of 11. One challenge reported during the planning phase was the assembling of a TA with sufficient expertise to conduct a fruitful discussion while balancing the inclusion of less experienced staff that could benefit from this training opportunity. Two countries were not able to assemble representatives from all the relevant sectors which likely reflected some of the TA responses regarding their satisfaction of the exercise.

Organization of the facilities during conduction varied amongst the participating countries. Using one large room and seating the TA according to sector was reported as beneficial by resembling the reality of interinstitutional collaboration. The majority of countries either used a single large table or separated the TA around smaller tables by sector whilst ensuring intersectoral communication was still possible. The LEs mostly assumed a position separated from the TA. Five countries opted to conduct the OHEJP SimEx online. The importance of having a cohesive TA from the beginning to the end was evidenced by the problems reported by countries ($n = 2$) that experienced changes in the TA members during the exercise conduction, hindering continuity from 1 day to the next.

The post-conduction survey results noted positive feedback on the exercise organization, with over 94% of all participants either agreeing or strongly agreeing with aspects related with the time ($n = 197$) and venue ($n = 191$) logistics and 98% ($n = 201$) expressing their satisfaction with the performance of the NELs and LELs. The time frame for the sequence of events and the discussion time allocated to each inject worked well for the majority of countries, of those that did not agree included that the time frame did not resemble the country's reality and that not enough time was allowed for discussion.

Holding preparation and planning meetings prior to the conduction was considered a benefit by the LEL and NELs for a successful conduction and was translated to a higher understanding amongst the TA of their role in the exercise and on the expected outcomes. Dividing the responsibilities of conduction between the LELs, depending on their expertise, was considered advantageous, as it reinforced the sense of a shared responsibility among different sectors.

Inclusion of FCL in the exercise was considered an opportunity for participants from AH and PH to better understand FS tracing procedures. The overall opinion on FCL varied, with most, 93% ($n = 191$), considering it useful for the exercise and some even requesting a more extensive practical exercise. Participants not directly involved in outbreak investigations and tracing, e.g., the communication experts, were less integrated in this part of the exercise.

Inevitably the multi-country approach revealed differences in perception of the scenario between national TAs. While one country reported the scenario as not challenging enough, another country deemed it unrealistic.

3.2. Scenario part 1: roles and functionality

The overall opinion among the participants, 88% ($n = 180$), stated the exercise was successful in highlighting the role of each sector in an event of a foodborne outbreak, and in showcasing the functionality of the systems in place (85% ($n = 174$) agreement) (Figure 4). Five countries also identified OHEJP SimEx as an opportunity for young professionals to familiarize themselves with the standard operating procedures and institutional routines to be followed during an outbreak. The exercise acted as a knowledge transfer platform between the less experienced and more experienced participants. OHEJP SimEx also provided institutes with the opportunity to revise their internal coordination practices including collaboration between different structural units of the same institute.

Three countries noted that an outbreak management team is only assembled once an outbreak has been declared, resulting in a fragmented decision-making process in the absence of a cohesive multidisciplinary team. One country reported that they have a long-standing collaboration for outbreak investigations and management.

Several countries highlighted the role of OHEJP SimEx in bringing people together and helping to strengthen interpersonal relations between professionals across sectors. In particular in the countries where the sector organization was more dispersed, OHEJP SimEx provided a unique opportunity for people to meet and clarify their roles. Moving toward or strengthening a single cooperating food safety governance structure, including both the human food chain and animal feed seemed to be the preferred system.

The need for further training initiatives covering institutes at different hierarchical levels to promote a common understanding between all parties involved and a quicker implementation of the necessary legal actions (e.g., product recall and inspections) was

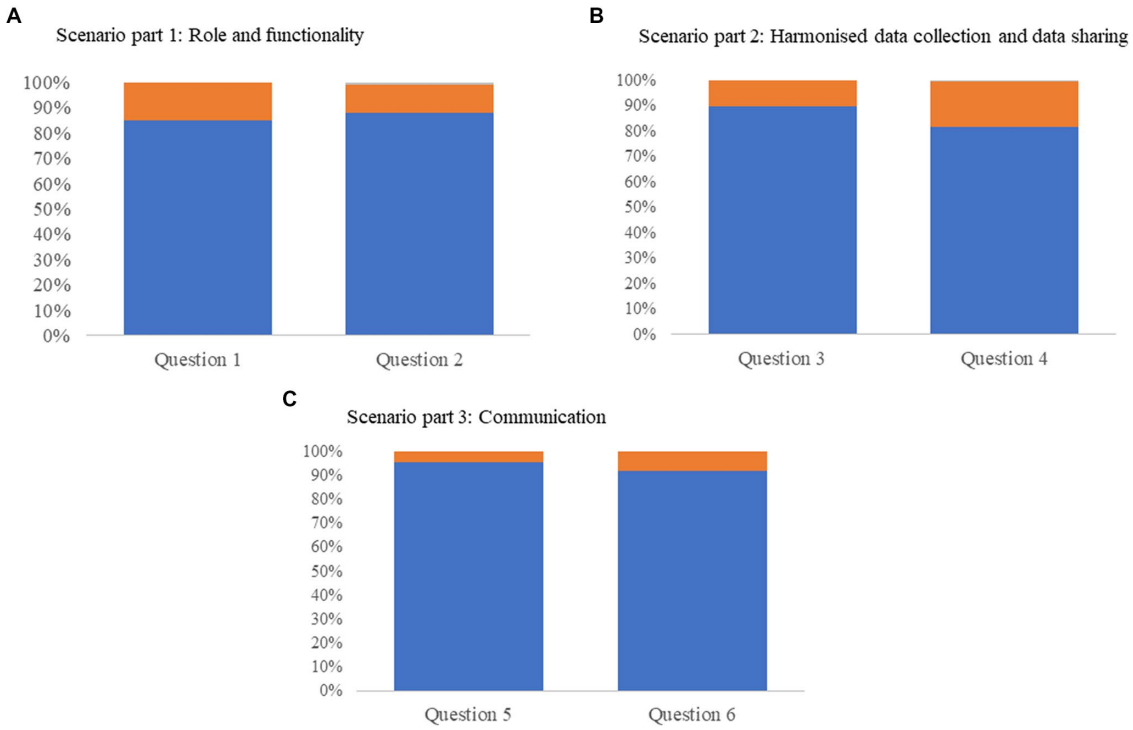

FIGURE 4
Post-conduction survey results graphically represented as percentage of respondents where blue indicates the respondent agrees, orange indicates the respondent disagrees, and gray indicates data missing. **(A)** Data from questions relating to scenario part 1: Role and functionality. Question 1: 'This exercise has helped you to be more aware of the currently available warning systems and emergency action plans in place (both at national level and in the European Union) and when they should be activated,' question 2: 'Your understanding of what other sectors expect from your sector has increased.' **(B)** Scenario part 2: Harmonized data collection and data sharing. Question 3: 'You have gained an increased understanding of the need to have a harmonized approach for data collation when dealing with a foodborne zoonosis outbreak', question 4: 'Solving problems associated with data sharing is something your institute prioritizes.' **(C)** Scenario part 3: Communication. Question 5: 'The exercise clarified the importance of having a coordinated action plan,' and question 6: 'You gained a better understanding of the different communicational needs and different target audiences.'

highlighted in several country reports. Four countries mentioned the key role of National Reference Laboratories in bridging the gaps between the different sectors and authorities, revealing the advantages of including them in the training initiatives.

One gap highlighted by four countries was that most interinstitutional communication was based on personal contacts and established through informal communication routes. The advantages of contacting professionals from other sectors using a personal contact network are largely recognized as a quick and efficient communication method, but a dependence on private networks is vulnerable particularly when there is a change in personnel.

Although not specifically focused on as it is outside the remit of a national response, it was evident that most countries had excellent knowledge and functionality of international early warning systems, e.g., the EpiPulse ECDC tool (24), which appeared well implemented in most countries. However, lack of full understanding of the available tools at national level and on how and when to activate them was evident.

3.3. Scenario part 2: harmonized data collection and data sharing

The second part of the scenario focused on harmonized approaches to data collection. The results showed that the OHEJP SimEx allowed different sectors to explore their data sharing procedures and to identify possible gaps that may hinder a coordinated and common data management plan. While 90% ($n = 184$) of the participants reported to have improved their understanding of the importance of data harmonization practices after conduction, 18% ($n = 36$) indicated that their institute did not prioritize the implementation of such practices (Figure 4). Furthermore, it was interesting to note that the degree of implementation of data sharing routines prior to the exercise conduction varied greatly amongst the participating countries. The majority of the participating countries (73%, $n = 8$) reported a requirement for an interinstitutional data sharing and data collection platform accessible to all sectors. Fragmented data collection structures, designed and implemented at an institute level, were considered to result in incompatible outputs and/or restrictive sharing policies, and proved inadequate for the OH scenario explored in this exercise.

Further investigation revealed that with the systems available in most countries, a sector is only contacted by other sectors at the point in which it becomes directly involved in an outbreak. This 'need to know' approach results in early-stage information being excluded from certain sectors and promotes inconsistency in accessible data to the investigation teams, reinforcing their dependence on informal data sharing routes to gain a OH perspective. In particular, data system gaps were evidenced in countries where the official control plans for raw pet feed are

currently under development as pet raw food feeding is not widely practiced. Extrapolating these results to an emerging zoonotic disease scenario or an outbreak with an obscure source of infection, the benefits of a cross-sectoral surveillance network will become markedly evident, allowing for a more efficient, rapid, and adaptable response system.

One of the major challenges identified was how to comply with the General Data Protection Regulation (GDPR) that applies to different data. The participants raised legal questions on data accessibility, under which circumstances they can be accessed, and for what they can be used. Most participants also noted a need for clearer guidelines on GDPR in relation to pathogen isolates and microbial genomic information. Other challenges identified by the participants included the lack of political will, the absence of harmonized data collection methodologies and the need for further training in data analysis, particularly in the area of whole genome sequencing (WGS).

3.4. Scenario part 3: communication

Three countries expressed the importance of an early communication strategy during an outbreak investigation, and one country also stated that it is important to ensure a unified message across all sectors. To achieve this, it is necessary to involve communication experts from an early stage of the outbreak investigation and to clarify the role of each authority. Indeed, three countries mentioned that good communication between authorities has been previously established by holding regular joint meetings. Among participants, 92% ($n = 189$) expressed having increased their understanding of communicational needs and target audience identification (Figure 4).

Communication at an early stage of an outbreak can be challenging particularly when there is limited information available. One country discussed the effect of circulating misinformation to their public messaging strategy.

Through the scenario, the countries recognised successful communication occurs when the message clearly indicates the known, acknowledges what is still unknown, and indicates what is being carried out to acquire further information. This format reassures the public that the authorities are working in accordance with their duties and helps to reduce public concerns. In addition to this simple communication formula, uncertainties should also be communicated appropriately. Clear and transparent communication is expected to support and maintain trust in the authorities.

Variation in the perception of 'severity' between the different sectors during the early stages of outbreak was highlighted by one country. Concerns about the possibility of conflicting opinions arising between sectors and also between the outbreak investigation team and the communication experts were discussed. Indeed, a different country reported friction regarding whether to hold a press conference or not. Another country's communication team also expressed concern that it may be unclear to the public which authority has primary responsibility for the outbreak investigation. The major gaps relating to risk communication were associated with a lack of structure for supporting communication strategies. Improving communication was highlighted by one country as the main action needed to further improve cross-sector cooperation.

3.5. Recommendations for One Health improvement

Regardless of the level of OH experience and maturity level of OH structures in the participating countries, there was an overall agreement on the major gaps and needs for improvement amongst the participants and countries (Table 2). These conclusions can be used by decision makers when reviewing the outbreak investigation and management plans in place at national or regional level and to define strategies to improve them. Furthermore, those planning future simulation exercises, wishing to integrate One Health coordination when responding to a health crisis, can benefit from the leant experiences from this exercise by considering the recommendations identified (Table 3).

4. Discussion

Well-functioning preparedness plans for responding to unexpected events are a high priority for many countries due to increased health threats posed by climate change and globalization. As part of a broader contingency, conducting exercises should be considered a fundamental element, together with allocating resources, investing in equipment, and drawing action plans. Training initiatives such as OHEJP SimEx play an essential role in the national contingency, bringing relevant professionals from different sectors with appropriate expertise albeit with different level of experience together to promote a cohesive approach to future health emergency situations (16–18).

Regardless of the topic and scope, a successful outbreak exercise requires detailed planning and organization. This begins with recruiting a team and setting up detailed aims and objectives. Thereafter, creating a realistic scenario and planning the conduction

TABLE 2 Recommendations for the improvement of the One Health approach to foodborne outbreaks.

Focus	Recommendation
Role and functionality	Create One Health strategies, guidelines and procedures at institutional level
	Hold regular meetings and training with authorities from all sectors
	Improve coordination between regional and central authorities
	Implement official communication channels between institutes
Harmonized data collection and data sharing	Harmonize typing methods and reinforce inter-laboratory networks
	Strengthen the links between human and veterinary primary health care practitioners and the official laboratories
	Implement common data collection and data sharing platforms that can be used across sectors
	Provide training in genomic data analysis and interpretation
Communication	Create a communication plan for outbreak situations

TABLE 3 Recommendations for future One Health simulation exercises.

Focus	Recommendations
Exercise Logistics	Hold preparation and planning meetings prior to conduction
	Divide the responsibilities of conduction between the facilitators from different sectors
	Incorporate practical tasks in the exercise
One Health	For a more detailed multi-country exercise, identify countries with similar systems for the scenario chosen for analysis
	Consider including more sectors (e.g., environmental sector)
	Have stronger focus on communication strategies
	Consider evaluating One Health-ness before and after the exercise

are important steps. The length and complexity of these stages depends on the nature and scale of the exercise. Considering the OHEJP SimEx was primarily a discussion-based exercise, the major challenge was to meet the expected needs of eleven countries that had different prerequisites. The scenario had to be generic enough for the exercise to be relevant to each country's response framework and organizational structure, yet detailed enough to be realistic and capable of resulting in relevant discussions.

To align with the local conditions, the participating countries were given the option to adapt the scenario. Despite this, there were some conflicting opinions from the TA on the exercise content and delivery. Indeed, the variations in *Salmonella* status and relevant regulations and structures amongst the participating countries meant the scenario was more compatible with some countries than others. Further, the main reasons for the lack of reality stated by some participants were the source attribution of a *Salmonella* outbreak to a cattle production unit (usually not regarded as a primary *Salmonella* source), and the inclusion of raw pet food in the exercise. The latter was not yet a relevant market in some of the participating countries, therefore the structures and regulations relating to raw pet food were not well defined. Rather than view this as a disadvantage, the experience for the TA in these countries is uniquely placed to assess the issues and successes each country encountered and provide recommendations for future One Health initiatives. If designing a more detailed exercise, it might be useful to identify countries with a similar prevalence for the selected pathogen and similar relevant systems (e.g., utilizing WGS for surveillance or not), allowing more detailed analysis into specific areas. If countries wish to participate without this alignment, then excluding them from the analysis, or separating countries according to how well they align to the pre-requisites will enable them to benefit from the exercise and provide some important data without affecting the main aims of the exercise. It is important to have a strong representation of all the different OH sectors in the exercise planning team to avoid a biased representation of a specific sector over the others, thus guaranteeing that the exercise can explore relevant topics for each sector.

By including the environmental health sector, future exercises could explore additional aspects of this from a OH perspective often-overlooked sector. When building the scenario, environmental pathogen dissemination was not included as a key event to explore in this exercise, but we noticed that the topic arose during some team discussions and that the absence of environmental health professionals in the TA hindered the development of such discussions. Therefore, the environmental health sector should be considered as an essential part of the holistic OH approach for pathogens that are known to transmit *via* the environment or for novel pathogens where limited knowledge exists and be encompassed in future simulation exercises. Furthermore, in one of the countries unable to secure representation from all three sectors, the TA noted more dissatisfaction regarding the relevance of the scenario, as they could not fully engage in all the injects, an observation that stresses the importance of including all sectors represented in the scenario. Although communication was central to this scenario, an even stronger focus on communication is recommended for future simulation exercises. Indeed, participants identified in some countries the communication staff only had a secondary role during the majority of the OHEJP SimEx. Including from the onset communication experts within the exercise planning team could address this concern. Incorporating more practical tasks (e.g., data sharing exercises) should also be considered to increase the overall participation and engagement.

Based on the weaknesses and strengths identified amongst the different countries it is possible to note common topics requiring improvement to implement the OH strategy to outbreak investigation and management. Countries should strive to set up a OH coordination strategy before a specific need for it is identified, ensuring a well-established organization able to support a prompt and efficient response (1). Interinstitutional guidelines covering relevant authorities and their responsibilities is useful when assembling an interdisciplinary outbreak investigation team, with the relevant authorities working together throughout the different stages of a foodborne outbreak and constructing a suitable joint action plan. During an outbreak, it is important to ensure continuity from beginning to end and maintain collaboration beyond the outbreak investigation, so that the team reviews their strategy and improves it accordingly. Additionally, institutes should implement updated and standardized procedures that can support the outbreak management team, including clarifying the role and responsibilities of each party. All participating countries are in a strong position to understand how far through this process they are and the required steps to achieve the ambitious aim of One-Healthiness. The One Health EJP Joint Integrative Project MATRIX has developed an online tool OH-EpiCap[3] (25) to facilitate characterizing and improving national One Healthiness through the evaluation of the surveillance system's capacities and capabilities. Indeed, we would recommend any future OH exercises to encourage the participants to use the OH-EpiCap tool before and after the conduction as one option to quantify the benefits of the exercise.

Establishing a routine of meetings with representatives from the different sectors should be considered a priority for countries aiming to improve their OH strategy. Meeting regularly builds trust and promotes transparency between and within sectors, which is fundamental for a successful cross-sectoral cooperation. To develop an efficient health emergency response system which is capable of quickly adapting to different scenarios, all relevant sectors must

3 https://freddietafreeth.shinyapps.io/OH-EpiCap/

be identified and equitably included. Ideally, to enable a swifter decision-making process, at least one member of each sector should have a direct link to governmental bodies to facilitate the communication with policy makers when needed (26, 27). The inclusion of networking and training activities should be considered when planning regular activities to make sure that any updates to the national contingency plans are covered and that new colleagues are included. The action plan should be tested in simulation exercises and reviewed periodically.

The successful implementation of OH structures requires a good understanding of the national and regional context and priorities (27). Implementing actions at local level was overall considered helpful by allowing to better contain the outbreak spread and adapt any needed actions to the regional reality. Nevertheless, OH structures require coordination with central authorities to avoid duplication of resources and efforts.

It was noteworthy that almost all participating institutes declared a dependance on personal and informal communication routes, which compromises the sustainability of interinstitutional communication networks. An effort should be made to implement efficient and official structural communicational channels that can be sustained regardless of personal contacts. These should include contact points at several key organizations, be regularly updated and be easily accessible to all relevant parties while still avoiding complex instructions that diminish compliance.

By centralizing the typing data of pathogen strains from different sources, the data can be made available to the investigation team without delays, helping to move forward with the outbreak investigation. Fragmentation of laboratory services can be time and resource consuming, hinder the harmonization of the results and increase the risk of information delays in the communication between laboratories. When centralization is not feasible or preferred, an effort should be made toward the harmonization of the characterization methods used in the different laboratories so that the results can be transferable and comparable (28). To support the work of reference laboratories, it is important to raise awareness at primary care level to the need of sending isolated strains and epidemiological information to the central laboratories, as well as reinforcing the hospital to laboratory network. In addition, the AH sector should attempt to improve the contact with primary care veterinary services and to establish a stronger network with veterinary practitioners so that isolates from companion animals can be included in national surveillance programmes. Countries needing technical support can reach out to international laboratory networks.

The need for a common data sharing platform that can be used across sectors was a common outcome across the participating countries and its implementation is pivotal in achieving a OH approach. Efficient tools are needed for earlier identification of outbreaks and quicker access to data for analytical studies and source attribution. Ideally, new data sharing platforms should build from and integrate already existing databases, be able to support large amounts of data and allow for multiple users to access simultaneously. To assist in the transition to an integrated strategy, institutes could develop guidelines on interinstitutional data sharing practices. Harmonization efforts could start at an institutional level by promoting a standardized use of internal data management tools by the professionals to avoid the vulnerability of a system dependent on a limited number of people. Ideally, national surveillance systems would be standardized internationally, thereby facilitating a coordinated approach to cross-border foodborne outbreaks.

It was noteworthy that GDPR was highlighted as a major barrier to the implementation of shared data collection across sectors. All personnel with access to the data related to an outbreak will need to be aware of, and comply with, the GDPR that applies to the different data categories and be authorized to work with it. Based on the comments from the participating countries, restricting access to common data sharing platforms to the central authority of each sector was considered preferential. Nevertheless, an efficient communication route needs to be established with the authorities at a regional level to guarantee the quick and efficient implementation of any actions that may be required.

Outbreak investigations comprise of both epidemiological and pathogen-related data. The majority of recommendations and gaps identified were concerning epidemiological data. However, it is important to raise awareness regarding inclusion of pathogen data, in particular genomic data, in national data sharing plans, which are used for cluster identification and source attribution. Ideally, national databases that connect AH, PH and FS laboratories should be created in countries that lack these databases and extended to include more pathogens in countries that already have them in place. To ensure genomic data is used optimally, the protocols used to generate the data and output formats need to be standardized across the different laboratories prior to implementation. As the demand for better and quicker typing techniques increases, there is a need to invest in WGS technologies and building the capacity of skilled teams that can generate and analyze large amounts of genomic data in real time.

Data visualization tools like the FCL, can prove helpful during an outbreak situation by allowing to visualize and analyze complex food networks, help in data collection, tracing back analysis and source attribution. Nevertheless, some points were raised regarding the complicated and time-consuming process of entering data into the FCL platform, and that it may be hard to adapt the tool to a more complex OH incident where the data is too heterogeneous. For the optimal implementation of the tool, it is necessary to improve its interoperability with other information systems and databases (possibly including sequencing data) and offer training on how to use the platform. Countries that showed interest in implementing FCL in their national action plan for foodborne outbreak investigations were given the opportunity to attend a workshop with the tool designers to help with the process. Future multi-country simulation exercises should take the opportunity to include different practical tools such as FCL as it provides a unique platform to fully test the complexities of country specific requirements. Providing a more robust range of suggested improvements benefitting future users.

As noted by many of the countries, the inclusion of communication experts from the different sectors in the outbreak management team is essential to ensure the public perception on the cohesiveness of the team and to promote internal mutual understanding. To assure consistency, their inclusion should precede the assembly of the emergency team and considered in the early construction of OH mechanisms (27). It is important to note that a good communication plan requires flexibility to adapt to rapidly changing situations and should be a dynamic process that involves feedback from both the stakeholders and the communities (27, 29, 30).

The One Health EJP is composed of public institutes in the AH, PH and FS sectors and therefore has close collaboration with national

and international stakeholders, including those represented in the OHEJP SimEx Advisory Board. This collaboration has enabled sharing the experiences from OHEJP SimEx to policy makers, whose support is essential for establishing and strengthening the structures needed to implement a OH approach to investigation and management of outbreaks. For the successful implementation of the actions identified here, they need to be assessed taking into consideration each national reality and adapted accordingly. There are several tools and resources available to support decision makers in making the transition to better OH structures and support them in drawing national action plans that can address the major gaps (6).

5. Conclusion

The OHEJP SimEx was a successful multi-country national simulation exercise. The results revealed the need for initiatives that can support countries in the practical implementation of OH. With the persistent risk of zoonotic foodborne outbreaks there is a continuous need to invest in prevention and contingency, as well as building capacity to respond to a health emergency, using an OH approach. Future OH simulation exercises can build on the OHEJP SimEx structure and experiences and should try to address the limitations identified. All participants acknowledged the essential tasks to engage with stakeholders and policy makers in order to ensure the framework of practical implementation of a OH approach is supported.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AO and KA conceptualized the project. AB, AO, DM, FA, JB, LT, ML, OP, and RF contributed to scenario design and development, evaluation, and reporting and dissemination of results. AB, AO, DM, FA, JB, LT, ML, OP, PJ, and RF contributed to data collection. OP analyzed the survey data. AO and FA drafted the manuscript. DM contributed to communication, information sharing, and language editing. HI, KA, PJ, and RR contributed to the project design and editing and writing the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 773830.

Acknowledgments

We sincerely thank all national teams who participated in the OHEJP SimEx, in particular NELs, LELs and LEs for their dedication throughout the different phases of this project. We would also like to extend our gratitude to the members of the OHEJP SimEx Advisory Board for all the advice and guidance provided. Finally, we thank the One Health EJP Project Management Team and Communications Team for their support and help.

Conflict of interest

The authors declare that the OHEJP SimEx project was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1121522/full#supplementary-material

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Glossary

AAR	After-Action Review
AH	Animal health
BfR	German Federal Institute for Risk Assessment
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCL	FoodChain-Lab
FS	Food safety
GDPR	General Data Protection Regulation
LEs	Local Evaluators
LELs	Local Exercise Leaders
NEL	National Exercise Leader
OH	One Health
OHEJP SimEx	One Health European Joint Programme simulation exercise
One Health EJP	One Health European Joint Programme
PH	Public health
TA	Training Audience
WGS	Whole Genome Sequencing
WHO	World Health Organization
WOAH	World Organisation for Animal Health

frontiers | Frontiers in Public Health

TYPE Brief Research Report
PUBLISHED 19 June 2023
DOI 10.3389/fpubh.2023.1138645

OPEN ACCESS

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RECEIVED 05 January 2023
ACCEPTED 26 May 2023
PUBLISHED 19 June 2023

CITATION
Moura P, Collineau L, Sandberg M, Tomassone L, De Meneghi D, Norström M, Bennani H, Häsler B, Colomb-Cotinat M, Bourély C, Filippitzi M-E, Mediouni S, Boriani E, Asaduzzaman M, Caniça M, Aenishaenslin C and Alban L (2023) Users' perception of the OH-EpiCap evaluation tool based on its application to nine national antimicrobial resistance surveillance systems. *Front. Public Health* 11:1138645. doi: 10.3389/fpubh.2023.1138645

Users' perception of the OH-EpiCap evaluation tool based on its application to nine national antimicrobial resistance surveillance systems

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Introduction: Antimicrobial resistance (AMR) is a One Health (OH) challenge. To achieve or maintain an effective and efficient AMR surveillance system, it is crucial to evaluate its performance in meeting the proposed objectives, while complying with resource restrictions. The OH-EpiCap tool was created to evaluate the degree of compliance of hazard surveillance activities with essential OH concepts across the following dimensions: organization, operational activities, and impact of the surveillance system. We present feedback on the application of the OH-EpiCap tool from a user's perspective, based on the use of the tool to evaluate nine national AMR surveillance systems, each with different contexts and objectives.

Methods: The OH-EpiCap was assessed using the updated CoEvalAMR methodology. This methodology allows the evaluation of the content themes and functional aspects of the tool and captures the user's subjective experiences via a strengths, weaknesses, opportunities, and threats (SWOT) approach.

Results and Discussion: The results of the evaluation of the OH-EpiCap are presented and discussed. The OH-EpiCap is an easy-to-use tool, which can facilitate a fast macro-overview of the application of the OH concept to AMR surveillance. When used by specialists in the matter, an evaluation using OH-EpiCap can serve as a basis for the discussion of possible adaptations of AMR surveillance activities or targeting areas that may be further investigated using other evaluation tools.

KEYWORDS

One Health, antimicrobial resistance, antimicrobial consumption monitoring, system evaluation, surveillance system

1. Introduction

International organizations are calling for a One Health (OH) approach to tackle antimicrobial resistance (AMR). The One Health High Level Expert Panel (OHHLEP) defines OH as "an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems" recognizing that the health of these populations is closely linked and interdependent (1).

AMR genes and microbes know no border, and certain antimicrobial agents are cross used in humans, animals, and plants. Hence, AMR is one of the quintessential examples of a OH challenge (2). Therefore, an integrated, multisectoral approach is necessary to address the issue (3, 4). Integrated surveillance, according to Aenishaenslin et al., is the "systematic collection, analysis, interpretation of data, and dissemination of information collected from different components of a system to provide a global, multidisciplinary, multi-perspective understanding of a health problem and to inform system-based decisions" (5). These actions should be coordinated between the human, animal and environmental sectors (6).

The application of this concept to national surveillance systems is essential to better understand AMR emergence and dissemination and to sustain risk mitigation decisions (7). The OHHLEP has recently released a theory of change for OH that can help to support intersectoral collaboration in national strategies for OH challenges, including those aiming to keep antimicrobials (AM) effective for future generations of people and animals. This theory of change includes the goals, objectives, desired impact at country level, intermediate outcomes, and related functions (1).

Conducting regular evaluations of a surveillance system's processes and performance is crucial to assess if the established objectives are being met in the most cost-effective way (8). OH initiatives should preferably be evaluated using a methodology that targets all disciplines encompassed and estimate the potential added value of the current approach over a less integrated one (9). The objectives of the evaluation should be made clear from the start, and an overview of the systems' surveillance components should be produced to guide it, and to balance the objectives of the evaluation with the available resources to perform it (8).

The international network CoEvalAMR was established in 2019 with the goal of providing guidance to help users in choosing an assessment tool from a catalog of tools available to evaluate antimicrobial use (AMU) and AMR surveillance systems (10). Moreover, the network aimed to guide future applications and improvement of the tools assessed and the development of new tools. To meet these aims, a methodology focusing on the users' perception of the tool was developed during Phase 1 of the CoEvalAMR network (11). The original methodology was used by Sandberg et al. (11) to provide feedback on six different evaluation tools based on their application in eight countries. Based on the experience gained, the methodology has recently been updated and further refined, as part of the work undertaken in Phase 2 of the CoEvalAMR network (12). The methodology encompasses the evaluation of descriptive and functional aspects, together with an assessment of content themes and questions on strengths, weaknesses, opportunities, and threats (SWOT) (12).

OH-EpiCap is among the catalog of tools being assessed in Phase 2 of the CoEvalAMR network. This tool has recently been developed by the MATRIX consortium, funded by the One Health European Joint Program to systematize the characterization of epidemiological surveillance activities in a national surveillance system (13). OH-EpiCap is presented as an easy-to-apply tool, covering previously overlooked aspects such as the impact of integrated surveillance. More specifically, the purpose of OH-EpiCap is to facilitate the evaluation and reinforcement of national capacities and capabilities for OH integrated surveillance of zoonotic hazards (13).

In this study, we applied and evaluated OH-EpiCap using the updated CoEvalAMR user's perception methodology and presented feedback on the application of the OH-EpiCap tool to nine national AMR surveillance systems, with different monitoring contexts and objectives.

2. Materials and methods

2.1. Description of OH-EpiCap

The OH-EpiCap tool is composed of three thematic domains (called "dimensions"), each with four different targets that are again segmented into four indicator questions, leading to a total of 48 indicators, briefly presented in Table 1. Each indicator is scored from 1 (no compliance) to 4 (full compliance), with the possibility to select "non-applicable" in case the indicator is not relevant to the system under evaluation. All indicators have the same weight, and for each target, the average value of the indicators' scores is converted into a target score (13).

Different respondents can have diverging opinions on the scoring of the indicators that compose OH-EpiCap, according to their backgrounds, perceptions, and expectations. To reduce the possible bias that the subjectivity of the scoring method may create, a consensus among respondents within one working group is required to select a final score among those described in the scoring guide (13).

The tool also includes a graphical interface developed in RShiny, where the results of the evaluation are presented in a dashboard that can be exported as a report. The OH-EpiCap tool is available on the following website: https://freddietafreeth.shinyapps.io/OH-EpiCap/.

2.2. Data collection

The nine surveillance systems evaluated were selected by members of the CoEvalAMR network. The selection was made by convenience

TABLE 1 Dimensions, targets and indicators evaluated by the OH-EpiCap tool—modified after (14).

Dimension 1: Organization			
Target 1.1 Formalization: common aim, support documentations, shared leadership, and definition of roles/composition of coordination committees	**Target 1.2 Coverage:** inclusion of all relevant actors, disciplines, sectors, geography, populations, and related hazards	**Target 1.3 Resources:** budget and human resources, program training, and sharing of resources	**Target 1.4 Evaluation and resilience:** internal and external evaluations, development/ implementation of corrective measures, and adaptability to change
Dimension 2: Operational activities			
Target 2.1 Data collection and methods sharing: multisectoral collaboration in the design of surveillance protocols and data collection, harmonization of laboratory techniques and data warehousing	**Target 2.2 Data sharing:** data sharing agreements, assessment of data quality, usefulness of shared data, and the compliance of data with the FAIR (findability, accessibility, Interoperability and Reusability) principle	**Target 2.3 Data analysis and interpretation:** multisectoral integration for data analysis, sharing of analysis techniques, sharing of scientific expertise, and harmonization of indicators	**Target 2.4 Communication:** internal and external communication, dissemination to decision-makers, and information sharing in case of suspicion/particular events
Dimension 3: Impact			
Target 3.1 Technical outputs: timely detection of emergence, epidemiological knowledge improvement, increased effectiveness of surveillance, and reduction of operational costs	**Target 3.2 Collaborative added value:** strengthening of the OH team and network, international collaboration, and common strategy (road map) design	**Target 3.3 Immediate and intermediate outcomes:** advocacy, awareness, preparedness, and interventions based on the information generated	**Target 3.4 Ultimate outcomes:** research opportunities, policy changes and behavioral changes and better health outcomes

of the members, due to direct acquaintance with the systems evaluated or close personal contacts. The evaluations were conducted from August to November 2022.

The number of respondents involved in the evaluation of each case study varied from one to five; these respondents are referred to as "assessors" throughout the text. The assessors filled in the OH-EpiCap evaluation questions during either a single or repeated workshop session that lasted a total of 2–8 h. All assessors involved had expertise in AMR surveillance in the country they represented for this study, scoring the indicator questions according to their own experience or knowledge from previous activities. This methodology makes the evaluation outputs somewhat subjective. In the country case studies that were conducted by more than one assessor, the subjectivity was reduced because of the requirement to reach consensus within the group of assessors who formed part of the country case. Whenever needed, additional experts and information sources were consulted.

The OH-EpiCap tool was used to evaluate national AMR surveillance systems in Bangladesh, Belgium, Canada, Denmark, France, Italy, Norway, Portugal and the United Kingdom (Table 2). The number of assessors and their affiliation, the type of workshop conducted, and the total duration of the evaluation are described for each country in Supplementary Table S1. The surveillance system evaluated in each country including its main aims can be found in Table 2.

2.3. Data analysis

The updated CoEvalAMR users' perception methodology was used to evaluate the OH-EpiCap tool (13). The methodology consists of a series of questions related to: (1) a general description of the case study and the tool, (2) two standardized scoring schemes, one regarding functional aspects, and another for content themes, as well as (3) a SWOT analysis (12). The functional aspects encompassed in the methodology are grouped into: Ease of use, Scope, Prerequisites before use, Time and resources, and Outputs. The content themes related to the tool's scope are: AMU and AMR, Collaboration, Resources, Output and use of information, Integration, Adaptivity, Technical operations, Impact and Governance. The definitions of the content themes and functional aspects can be consulted in Alban et al. (12).

Both functional aspects and content themes of OH-EpiCap tool were scored semi-quantitatively using a scale from 1 to 4 or "non-applicable." Groups composed of several functional aspects or content themes were averaged. Next, the median, maximum and minimum of the scores given by the assessors for functional aspects and content themes were displayed in a radar diagram in Figures 1A,B, respectively. Due to the skewness of the distribution of the answers' scores, which were not normally distributed, the decision was made to show the median of the scores. Microsoft Excel® was used for data analysis and visualization of the outputs.

The SWOT analysis was undertaken to capture the assessors' subjective experiences when applying OH-EpiCap. More specifically, the following wording accompanied each component: Strengths: "The strengths of this tool are," Weaknesses: "The weaknesses of this tool are," Opportunities: "The added value(s) of using this tool is" and Threats: "This tool might be criticized because of." A qualitative analysis of the feedback provided by the assessors was performed following the same principles as described by Sandberg et al. (11), which were based on grounded theory (15): all individual sentences were collected, then, sentences with similar content were simplified and condensed into one sentence. The synthesis was performed by three of the assessors and later verified by the remaining assessors.

10.3389/fpubh.2023.1138645

TABLE 2 National AMR surveillance systems evaluated using the OH-EpiCap tool.

Country	Name of the system	Main aims of the system
Bangladesh	One Health Event Based Surveillance (EBS)	• Develop a 'One Health surveillance system platform' to enable early detection of disease outbreaks. Coordinated joint response to disease outbreaks
Belgium	AMR-AMU surveillance program in the context of developing the OH AMU-AMR national report (OH belmap)	• Summarize results and trends of existing monitoring programs: related to the consumption of antibiotic agents for food animals and humans and to the monitoring occurrence of antimicrobial resistance in bacteria isolated from food animals, humans and food of animal origin • Identify blind spots in monitoring programs and make recommendations to improve future monitoring
Canada	Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS)	• Provide an integrated approach to monitor trends in antimicrobial resistance and antimicrobial use in humans & animals and help identify appropriate measures to contain the emergence and spread of resistant bacteria between animals, food, and people in Canada • Facilitate assessment of the public health impact of antimicrobials used in humans & agriculture to support the creation of evidence-based policies to control AMU in hospital, community, and agricultural settings • Provide timely analysis and dissemination of surveillance data to stakeholders, and facilitate knowledge translation via targeted communications products • Allow accurate comparisons with other countries that use similar surveillance systems (NARMS, DANMAP) • Provision of data for Health Canada—Veterinary Drugs Directorate for new antimicrobial drug approval processes and post-approval monitoring
Denmark	Danish Program for surveillance of antimicrobial consumption and resistance in bacteria from food animals, food and humans (DANMAP)	• Monitor the consumption of antimicrobial agents for food animals and humans and the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin and humans • Study associations between antimicrobial consumption and antimicrobial resistance • Identify routes of transmission and areas for further research studies
France	Surveillance system for AMR, AMU and antimicrobial residues	• Monitor trends of AMU and AMR in humans and animals, incl. in diseased animals • Assess what is common to several sectors and what is not • Inform policy recommendations and assess the impact of interventions
Italy	ClassyFarm	• Risk categorization of farms according to an integrated approach containing biosecurity, welfare, AMU/AMR, animal health and lesions at slaughterhouse
Norway	The surveillance program for antimicrobial resistance in human pathogens (NORM) and the monitoring program for antimicrobial resistance in bacteria from feed, food and animals (NORM-VET)	NORM: • Collect and process data about antibiotic resistance of microbe isolates to determine the incidence and prevalence of antibiotic resistance and monitor changes over time • Drive, promote and provide a basis for research to understand why microbes develop antibiotic resistance, with a view to promoting and developing preventive measures in the treatment of infectious diseases • Provide a basis to give health advice and information on measures that could prevent development antimicrobial drug resistance to the public and local, regional and central health authorities • Give the Norwegian health authorities a foundation to contribute to international statistics within specific areas NORM-VET: • Provide and present data on the occurrence and distribution of antimicrobial resistance over time. • Describe the relationship between the use of antimicrobials and occurrence of resistance in the veterinary and food production sectors. • The information generated is used for research, setting policies, assessing risks, and evaluating interventions
Portugal	Infection Prevention and Control and Antimicrobial Resistance Program (PPCIRA)	• Monitor the occurrence of antimicrobial resistance in bacteria isolated from humans • Identify routes of transmission • Detect and monitor outbreaks caused by bacteria with antimicrobial resistant genes • Prevent the emergence and transmission of bacteria with antimicrobial resistant genes
United Kingdom	Surveillance system for AMU and AMR in the UK	• Monitor AMU in humans and animals • Monitor trends of AMR in bacteria isolated from humans, food producing animals, and food of animal origin • Detect new and emerging AMR threats • Inform policy recommendations and assess the impact of interventions

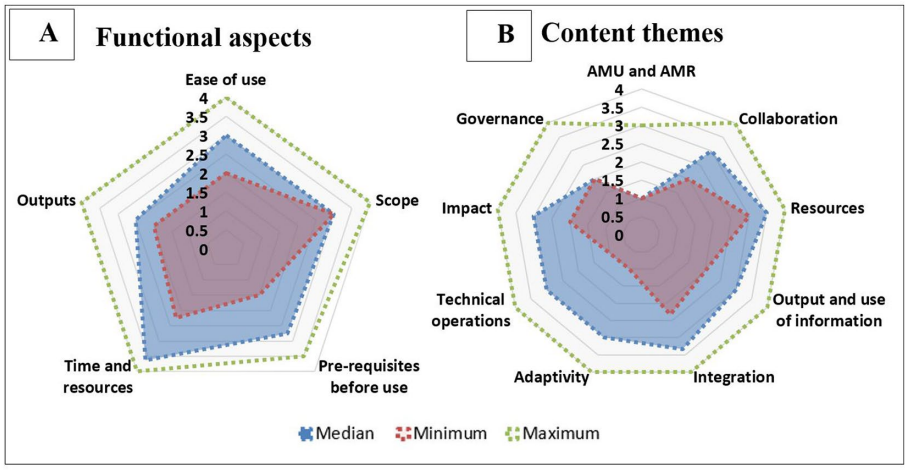

FIGURE 1
Evaluation of the functional aspects **(A)** and content themes **(B)** of the OH-EpiCap tool according to the CoEvalAMR user's perception methodology based upon nine country case studies.

3. Results

3.1. Functional aspects

Regarding ease-of-use, OH-EpiCap scored highly due to its user-friendly interface with checkboxes to answer the questions that formed these indicators. The scope of the tool is defined as the ability to address the stated evaluation objectives and is further divided into the content themes evaluated (12). OH-EpiCap was not created with the objective of covering all the national capacities and capabilities for OH integrated surveillance. OH-EpiCap does not cover certain content themes in the level of detail perceived as relevant by the assessors, even for a macro evaluation, as addressed in section 3.2.

The OH-EpiCap tool is free of use. As for prerequisites to use it, no previous data collection is required, and the answers can be given based on the evaluators' experience connected with the surveillance system. Most indicators require that the evaluation is conducted with specialists in the surveillance system, or that they are consulted in the process, given that an in-depth perspective of the whole surveillance system is needed. No training is necessary to get acquainted with the tool.

OH-EpiCap can—in most cases—be successfully applied by a small group composed of for example three or four persons, providing that the group can form a clear cross-sectoral picture of the surveillance system. Based on our experience, and depending on the expertise of the stakeholders gathered, the evaluation can be conducted in half a day or slightly longer. If additional stakeholders need to be consulted after the initial workshop, the evaluation process will be prolonged. In the case that supplementary information that may impact a given answer is gathered via extra communications, outside of the stakeholder workshop, it should be further discussed with all the assessors.

The graphical outputs generated by the tool were found to provide an easily accessible overview of the responses given. However, given the superficiality of the evaluation content (Table 1), the output of the evaluation need to be further discussed and investigated with relevant actors before it can be translated into specific changes in the surveillance system. Please see the section 3.2 for an elaboration of this issue.

3.2. Content themes

The tool does not encompass indicators specifically addressing AMU and AMR surveillance. Even though not covered to a complete extent, OH-EpiCap still provides an overview of the thematic areas connected with the human and budget resources needed to maintain the surveillance activity, as well as the collaboration in the governance structures of the system and in the technical surveillance activities. It also encompasses indicators related to the possible adaptation of the surveillance activities to new challenges and in an efficient manner. The overall impact of the surveillance system is also covered, but without details on how the information generated by the surveillance system could lead to changes in the health outputs. Moreover, the tool does not go into details in the governance domain, specifically the accountability of stakeholders, the coordination of activities and the transparency of processes are only superficially covered.

3.3. SWOT analysis

The subjective experience of the application of OH-EpiCap by the assessors captured via SWOT analysis is presented in Table 3 in a summarized format.

4. Discussion

4.1. Overall perception of the tool

During the development of OH-EpiCap, several pilot applications on various surveillance activities were conducted. Due to the generic design of OH-EpiCap, it has been successfully applied to surveillance activities connected with food-borne hazards, such

TABLE 3 Outcome of SWOT analysis of the OH-EpiCap tool, based on an application of the tool to nine country cases.

Topic and meaning	Synthesis of the comments provided
Strengths: The strengths of this tool are	A feasible compromise between comprehensiveness in quantity of information captured and human/time resources required to carry out evaluation. Simple and well-organized design, following a user-friendly step-by-step approach with boxes to check. No previous extensive training is needed to use it. The provided glossary encompassing explanations of what is meant by an expression is very helpful and increases the ease and swiftness of use. Produces visually attractive figures, encompassed in a report, which provide a good overview of the answers given and make it easy to share and communicate the results. An example of which can be seen in **Supplementary Figure S1**. In the report, general suggestions for further improvements and indicators of good adherence to OH principles are provided. It is available for free, useful for single or multidisciplinary settings and suitable for any country. It could produce a lot of food for thought, if people with a deep understanding of the surveillance system and all the main processes are consulted.
Weaknesses: The weaknesses of this tool are	Some of the indicator questions could be further simplified to facilitate their interpretation. Although comprehensive, the evaluation products are superficial, and they cannot be directly translated into action, requiring further investigation. If surveillance initiatives are based on one dominating OH pillar, it is not easy to answer some indicator questions, which are structured to catch multi-sectoral/disciplinary collaborations. Some indicators are difficult to score without dedicated *ad-hoc* studies. Sometimes difficult to delineate which impacts comes from OH surveillance versus sectoral surveillance (Dimension 3). Some indicators aiming at evaluating effectiveness refer more to technical performance of surveillance (sensitivity, timeliness) than its capacity to inform decision-making. The tool is sometimes hard to apply to a system which integrates data from multiple domains such as AMR and AMU in animals and humans, but is managed by only one institution, as several items refer to inter-institutions collaboration and governance.
Opportunities: The added value(s) of using this tool is	Helpful to identify new areas that should be further investigated and to initiate discussion around the possibility of adapting the existing systems. Provides a good overview of a surveillance system targeting one hazard, or a component of a complex system. Evaluation can be performed in a short time, so it may be done frequently, and after relevant updates. Provides an evaluation at a macroscopic scale of the overall "OH-ness" of the system and facilitates an overall description of the system. Can be used pragmatically for preliminary assessment. Useful to identify key areas for improvement that can be evaluated into more details with a different tool.
Threats: This tool might be criticized because of	The tool is not well adapted to evaluation of complex surveillance systems that encompass multiple hazards and components, such as AMU and AMR, given that the surveillance of different AMR bacteria may differ in the same surveillance system. If results of evaluation or its application are not discussed with key people, its simplicity may lead to a superficial evaluation of certain aspects. Some indicators are not applicable to country or program context, e.g., added value of OH integration in the case a system was integrated from its beginning. Because data collection is expected to be short (e.g., no interviews), it is critical to have the right experts around the table to provide the required knowledge. Not suitable for end-users of the system. To ensure full comprehension of some indicators, previous clarification of their aim may be required, giving special attention to the terminology used, before conducting a meeting with relevant stakeholders. While the tool provides output figures describing the level of OH-ness, it does not allow to visualize the actual system (distribution of surveillance programs by sector and domain) or collaboration between actors/programs (e.g., via social network analysis). Adding this feature would be an asset.

as *Salmonella*, *Campylobacter, Listeria*, and other zoonotic hazards such as *Chlamydia psittaci* (15). With this study, we illustrate its application to the evaluation of integrated surveillance systems for AMR.

According to the information collected in the nine case studies, OH-EpiCap can provide an overview of several crucial topics connected with AMR integrated surveillance, even though the tool was not specifically designed to evaluate these activities. The OH-EpiCap tool provides a summary assessment of the three dimensions targeted, which cover most of the elements that are important for assessing integrated surveillance systems, as described in the Integrated surveillance systems evaluation (ISSE) framework (5). The ISSE framework identified five levels of assessment for such surveillance systems, which include the integration of a OH approach, the production of integrated information and expertise, the generation of actionable knowledge, the influence on decision-making and the contribution to desirable outcomes. Evaluating these five levels in a comprehensive manner requires considerable time and resources, and

OH-EpiCap constitutes a good first step toward evaluation of all of them.

Simplistic design and user friendliness, without requiring training of evaluators, are highly appreciated, not just by our assessors but also among users in general as shown in a survey recently undertaken among surveillance program practitioners and evaluators (16). To make the workshop more time efficient, it is recommended that at least one of the evaluators gets acquainted with the indicator questions and clarifies any possible doubts before organizing a session with the specialists involved in the evaluation and other relevant actors.

The outputs generated by OH-EpiCap may not lead directly to actions, however these can provide the basis for discussing further improvements with relevant stakeholders, as presented in a case study by (17). The MATRIX project also encompassed other activities that are complementary to the development of OH-EpiCap, such as a "Roadmap to develop national One Health Surveillance" which aims to function as a guideline for the development of OH Surveillance activities according to needs and resources in different countries (18).

An evaluation using OH-EpiCap can be conducted in a short period of time and with a small group of stakeholders, making it feasible to conduct an evaluation in situations with low resources. Moreover, evaluations can be done recurrently, when changes are implemented, benchmarking the system with itself over time. This can be made easily as OH-EpiCap contains benchmarking functionalities. These functionalities were not investigated in the present study, because of the different aims and purposes of the systems evaluated as noted in Table 2. For example, the Danish DANMAP program serves the purpose of integrated monitoring of AMU and AMR for both the animal and human sectors. In contrast, the Italian ClassyFarm encompasses mainly farm-level risk categorization components (e.g., biosecurity and animal welfare, besides AMR and AMU) whereas AMR surveillance in the human sector is conducted by different Italian institutions (19). Given the above-mentioned differences in the aims of the surveillance activities which we evaluated, indicator questions connected to real-time response capacity were considered not relevant in some surveillance activities.

AMR surveillance systems are complex and encompass multiple hazards, e.g., surveillance of clinical isolates in human health, bacterial isolates from animals at slaughter lines or in slurry, or AMR genes in sewage systems, each with their own particularities and logistics (5). So, when answering some of the questions representing an individual indicator in OH-EpiCap, interpretations need to be considered. This approach can justify the application of OH-EpiCap to several surveillance components, while focusing on one hazard at a time.

We applied the OH-EpiCap tool in nine different countries, by different native language users, providing important feedback to the developers regarding the phrasing of the indicator questions. We found that most of the indicator questions were considered simple and straight-forward. However, considering the expected worldwide application of the tool by users, who may have different use of the English language and, hence, familiarity with the terminology used, materials should be developed to unequivocally clarify the meaning of all indicators. With the publication of case studies evaluations and the scientific paper accompanying the tool (13), this should be accounted for. At the time of writing, the OH-EpiCap tool was still in a Beta Version, so the phrasing of indicators was not final.

4.2. Contribution of OH-EpiCap to the evaluation of integrated AMR surveillance systems

Except for Bangladesh, all country cases presented here were conducted in high-income countries. Hence, we have only limited experience regarding the applicability of the tool to low- and middle-income countries (LMICs). In LMICs, AMR surveillance is often hindered by deficient health system governance and restrictive financing of health data producing systems and laboratory capacities (20, 21). In addition, more efforts are needed to improve the capacity, quality standards, and integration of AMR surveillance in LMICs, which often have focus on human health. Due to its generic design, OH-EpiCap does not require that an integrated surveillance system is already established. However, at least primary surveillance activities need to be established and run. If this is not the case in a country, engagement in other tools such as the FAO Progressive Management Pathway for Antimicrobial Resistance (FAO-PMP-AMR), which aims to guide countries in the implementation of national action plans against AMR and early surveillance efforts (22), may be considered.

By highlighting components which may be improved in a hazard integrated surveillance system, OH-EpiCap can be considered as a valuable new addition to the current catalog of tools to evaluate integrated AMR surveillance systems (11). Moreover, OH-EpiCap can act as a simple gateway to conduct a more in-depth evaluation of certain surveillance system components as considered relevant. This may be done by using other pre-established tools designed to evaluate OH integration, such as the Evaluation of Collaboration for Surveillance (ECoSur) or The Network for Evaluation of One Health (NEOH).

The ECoSur tool has been developed to facilitate an in-depth analysis of the organization and functioning of collaboration taking place in a multisectoral surveillance system, aiming to evaluate the overall quality and relevance of such collaboration in meeting the objectives envisioned by stakeholders to produce the expected outputs of the program (23). From a user's perspective, this tool gives a detailed evaluation of multisectoral collaboration in OH surveillance activities, however it requires a high level of abstraction to understand the indicator questions listed in the tool. Still, conducting a full ECoSur evaluation is rewarding regarding quality of output, but remains time and resource demanding (11).

The NEOH tool allows the evaluation of the coherence between operational and organizational aspects of OH activities, with the aim of identifying the added value of the integration across disciplines and sectors (24). From a user's perspective, this tool is a comprehensive, multi-faceted fit for a transversal and detailed analysis of OH initiatives. However, conducting an evaluation using NEOH may be difficult and time consuming given that users should have specific training in systems thinking to make the most of it (11).

One of the ongoing activities in the CoEvalAMR network aims to simplify the application of the NEOH and ECoSur tools, using a modular approach. Given the complexity of evaluating integrated AMR surveillance systems, this could be of great value, targeting the evaluation to certain components which need to be prioritized.

Within the CoEvalAMR network, case studies have already been conducted from a user's perspective on the application of

the ECoSur (25) and NEOH tools (26–29). Other tools and frameworks that have been specifically designed to evaluate integrated AMR surveillance have also been evaluated: the FAO-PMP-AMR tool (30–33) as mentioned above (34); the FAO Assessment Tool for Laboratories and AMR Surveillance Systems (FAO-ATLASS) (35) developed to facilitate the assessment and definition of targets to improve national AMR surveillance systems in the food and agriculture sectors (36) and the ISSE framework (37, 38) developed to structure an assessment of the added value of integration in AMR surveillance systems (39). The interactive selection tool developed by the CoEvalAMR network can help users to select an appropriate tool for their needs (40).

5. Conclusion

The OH-EpiCap tool is a new addition to the portfolio of existing tools to evaluate integrated AMR surveillance systems. It provides a brief macro-overview of relevant OH topics, such as the perceived added value of establishing a OH team as a governance structure. This can serve as a basis to discuss possible adaptations of AMR surveillance activities, or targeting areas that may be further investigated using other established tools. It is free and easy to use, does not require training, and can be performed in less than a day provided that the group performing the evaluation has detailed knowledge on the surveillance system to be evaluated.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

PM drafted the first version of the manuscript together with LA and LC. All other authors commented on the first version. PM took lead in revising the submitted manuscript together with LA and LC. All authors read and approved the final version of the revised manuscript and contributed with data from their national case study.

Funding

This study was funded by the Canadian Institutes for Health Research through the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR). The Portuguese case study was supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme (MATRIX project).

Acknowledgments

The authors acknowledge Henok Ayalew Tegegne, Carlijn Bogaardt, Géraldine Cazeau, Renaud Lailler, Johana Reinhardt, Emma Taylor, Joaquin M. Prada, and Viviane Hénaux for the development of the OH-EpiCap tool and for making it available to conduct this work, as well as the stakeholders who provided information to conduct the evaluations in the different country cases.

Conflict of interest

LC was involved in the development of the OH-EpiCap tool. LA works for an organization that gives advice to farmers and meat-producing companies.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1138645/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 11 July 2023
DOI 10.3389/fpubh.2023.1123189

OPEN ACCESS

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RECEIVED 13 December 2022
ACCEPTED 05 June 2023
PUBLISHED 11 July 2023

CITATION
Bourély C, Rousset L, Colomb-Cotinat M and Collineau L (2023) How to move towards One Health surveillance? A qualitative study exploring the factors influencing collaborations between antimicrobial resistance surveillance programmes in France.
Front. Public Health 11:1123189.
doi: 10.3389/fpubh.2023.1123189

How to move towards One Health surveillance? A qualitative study exploring the factors influencing collaborations between antimicrobial resistance surveillance programmes in France

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Introduction: Antimicrobial resistance (AMR) is a major public health issue, against which international organisations and governmental bodies call for integration between surveillance programmes involved in human, animal, and environmental sectors. Collaborations are the primary feature of integration and deserve to be supported. However, little is known about the factors that can foster collaborations between surveillance programmes. This study aimed to provide a better understanding of the factors for setting-up collaborations between AMR surveillance programmes in France.

Methods: We performed a qualitative study based on 36 semi-structured interviews with programmes' coordinators and 15 with key-informant experts involved in AMR surveillance.

Results: The implementation of collaboration between sectors was multifactorial: we identified 42 factors grouped into six categories (i.e., characteristics of the overall AMR surveillance system, features of the collaborating programme, profile of the actors involved, characteristics of the collaboration itself, broader context, and AMR research activities). Collaborations were mainly fostered by good interpersonal relationship between actors, their interest in transdisciplinary approaches and the benefits of collaboration on the programmes involved. Limited resources and the complexity of the AMR surveillance system hindered collaboration. Paradoxically, coordinators generally did not perceive collaborations as a resource-pooling tool since they generally set them up only after consolidating their own programme.

Discussion: Since most factors identified were not specific to AMR, these results can be useful for other collaborative surveillance system. Ultimately, they provide a better understanding of stakeholders' motivations and influences driving collaboration, and can help researchers and risk managers promoting a One Health approach against public health threats.

KEYWORDS

One Health surveillance, collaboration, integration, antimicrobial resistance, antibiotics, qualitative research

1. Introduction

The increasing occurrence of zoonoses, and recently the COVID-19 crisis highlighted the importance of having close links established between surveillance programmes in humans and animals to guide operational decision-making and serve appropriate risk management. Through the collection and analysis of temporal and spatial data on health events, surveillance is a cornerstone for guiding mitigation measures and for early detection of worrying trends, hence ensuring optimal management. This last decade, international organisations have advocated for an integrated approach of surveillance, so called One Health approach, for dealing with public health threats at the nexus of the human, animal, food and environmental sectors; this especially applies to antimicrobial resistance (AMR) (1–3).

In 2015, the World Health Organization (WHO), through its Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) published a guideline with basic information required to establish an integrated surveillance of AMR, including antibiotic use in humans, food-producing animals and retail food (4). More recently in October 2022, the publication of the "One Health joint plan of action" by the Quadripartite Organizations (FAO, UNEP, WHO, and WOAH) strengthened the One Health approach with the full integration of environmental challenges, and provided a formal and legal framework to tackle complex health challenges such as AMR at the human, animal, and ecosystem interface (3).

Collaborations are the primary feature of integration. They are considered as an interprofessional process by which surveillance programmes actors address together an issue with members of the team respectfully sharing knowledge and/or resources (5). Collaboration can occur at any step of the surveillance process, from the governance to the implementation of operational surveillance activities (e.g., sample collection, data analysis) (5). However, little is known about the factors that can foster collaborations, especially between the various surveillance programmes composing a surveillance system (6–8). A recent study pointed out that the French antimicrobial resistance surveillance system was resourceful and varied yet complex and fragmented, involving 48 surveillance programmes [targeted the human ($n = 35$), animal ($n = 12$), food ($n = 3$) and/or the environment ($n = 1$) sectors] from different domains [AMR, antibiotic use (AMU) and antibiotic residue] (9). Furthermore, collaborations among several programmes were observed, including cross-sectoral collaborations [among human (hospital and community), animal, food or environment sectors] and cross-domain collaborations (AMR and AMU). This first descriptive study indicated that the French surveillance system could be appropriate to explore reasons for collaborations.

Hence, the aim of this study was to investigate factors influencing collaborations between surveillance programmes for antimicrobial resistance in France. Ultimately, this work aimed to provide a better understanding of actors' motivations and influences driving integration between surveillance programmes, to help researchers and risk managers promoting a One Health approach.

2. Materials and methods

2.1. Study design

We carried out a qualitative study, based on semi-structured interviews with coordinators of surveillance programmes (actors in charge of the programme with a representative role) and key-informants (experts in French AMR surveillance), to investigate the factors for the set-up of collaborations between surveillance programmes within the AMR surveillance system in France. Based on the previous identification of all AMR surveillance programmes and the description of the collaborations in place by Collineau et al. (9), coordinators of all domains (AMR, AMU, antibiotic residues) and sectors [human (hospital and community), animal, food and environment] were interviewed. Coordinators were interviewed on every single collaboration in which they were involved. Coordinators, whose surveillance programme(s) was not involved in a collaboration, were also interviewed. Moreover, in order to ensure broad investigation of factors and to cross-validate opinions, key-informants were interviewed. The eligibility criteria of key-informants were based on their expertise in AMR surveillance, their awareness of collaborations in place and their implication in the structuration of the French AMR surveillance system. The key-informants were selected through snowball sampling (both programmes coordinators and selected experts provided referrals for this recruitment).

2.2. Data collection

The selected participants were contacted individually by email to provide information on the study (purpose, nature, background) and were informed that their opinions and speech would remain anonymous, and that any material potentially leading to individual identification would be removed. Written consent to be part of the study was obtained ahead of the interviews.

In order to maximise both the quantity and quality of data collected, an interview guide, specific for each type of participants (coordinators versus key-informants), was drafted following the framework of the Theory of Planned Behaviour (10). The guide was pre-tested through an exploratory interview with a first coordinator. Addressed topics are presented in Table 1. The questions of the interviewer changed to delve into participants' individual responses and to adapt to the type of surveillance programme.

Given the travel restrictions related to the COVID-19 pandemic, all interviews were conducted remotely, by videoconference, using Microsoft Teams® software. In order to ensure the comparability of the information collected, one of the interviewer (L. R.) was systematically present at all the interviews and was assisted by one or two other interviewers (other co-authors) depending on the number of people interviewed. Interviews with key-informants were systematically individual ones, whereas the number of respondents for the interviews with coordinators varied from one to four, depending on the main coordinator's willingness to be accompanied by other co-coordinators (from the same programme).

At the beginning of the interview, the aim and background of the study were explained, as well as the interview's confidentiality rules, and the roles of the respondents were collected. Although the interviewers used interview guides, respondents were free to introduce any other information they felt was relevant. Interviews were recorded to facilitate the dialogue and subsequent analysis.

TABLE 1 Topics and underlying topics of discussion during the interviews.

Topic	Underlying topics
Opening questions	Description of the surveillance programme and its role in the surveillance system (for coordinator) Description of their role in the AMR surveillance system and its integration (for key-informant)
Decision to take part in a collaboration	Factors involved in the implementation of collaboration Presentation of the decision-making process Role-players involved/people influencing the decision Evolutions regarding the decision to collaborate
Perception and opinion on the collaboration	Purpose of the collaboration Opinion and view on the organisation and the management of the collaboration Impact of collaborative activities on the surveillance programme Relationships with other actors Outputs and outcomes of the collaboration Expectations regarding the collaboration
Motivation and interest behind participation in a collaboration	Factors that influenced participation Personal interests Third-party opinions or arguments that influenced the decision
Drawback and obstacle for participating	Factors that influenced refusal to collaborate Reasons for dissatisfaction Changes in viewpoint
Impact of the collaboration	Benefits for surveillance programmes involved Added value for other actors
Closing questions	General feeling on the French surveillance system of antimicrobial resistance and its *One Health-ness* Any further elements

The interviewers' notes were shared among the co-authors after each interview. Data continued to be collected until saturation occurred (i.e., a point where collecting more data would not lead to new information related to the research questions) (11).

2.3. Data analysis

The interview recordings were manually transcribed and compiled with the notes. At first, data analysis involved reading through all of the transcripts to get a sense of the dataset as a whole (12). Then, the transcripts were subjected to thematic analysis, as described by Beaud and Weber (13). Specifically, thematic analysis is a method of examination of the content of discourses to identify, analyse and interpret meanings gathered in themes. The analysis was conducted inductively in a circular process and used a constant comparative method (14): repetitions of forward and backward movements from transcripts, gathering of text fragments, attribution of codes and introduction of inferences (11). Before making any inference, evidence to the contrary was sought. The data were examined in regard to the research questions, significant text fragments were identified, coded and grouped into categories, i.e., groups of content that share common feature. Similarly, categories were organised around themes. When a collaborative factor was identified to be linked with another one (i.e., mentioned together), this link was search for in other interviews. Factors were considered as mutually dependent once cross-validation was achieved (links in Figure 1). The triangulation principle (i.e., cross-checking information to validate each inference) and iteration principle (i.e., looking for repeats and synergy in transcripts) were strictly applied (15). To protect respondents' confidentiality, all results in this paper were anonymized. Note that all verbatim quotes cited in this paper have been translated from French (Supplementary Table).

2.4. Ethical statement

Since this qualitative study was not a clinical trial, it did not require the formal consent and approval by the 'Comité de protection des personnes' in France (French ethics committee). Nevertheless, this research followed ethical rules in compliance with the Statement of Ethical Practise of the British Sociological Association (16), and was validated by the legal affairs department of the French Agency for Food, Environmental and Occupational Health and Safety (ANSES).

3. Results

In total, 51 semi-structured interviews were conducted for 68 participants, including 53 coordinators and 15 key-informant experts (Table 2). Some of the interviews with coordinators were multi-participants (14 interviews out of 36), whereas all interviews with key-informants were solo. Interviews were performed from March to June 2021 and lasted between 27 and 119 min (median: 57 min). Four of the participants were

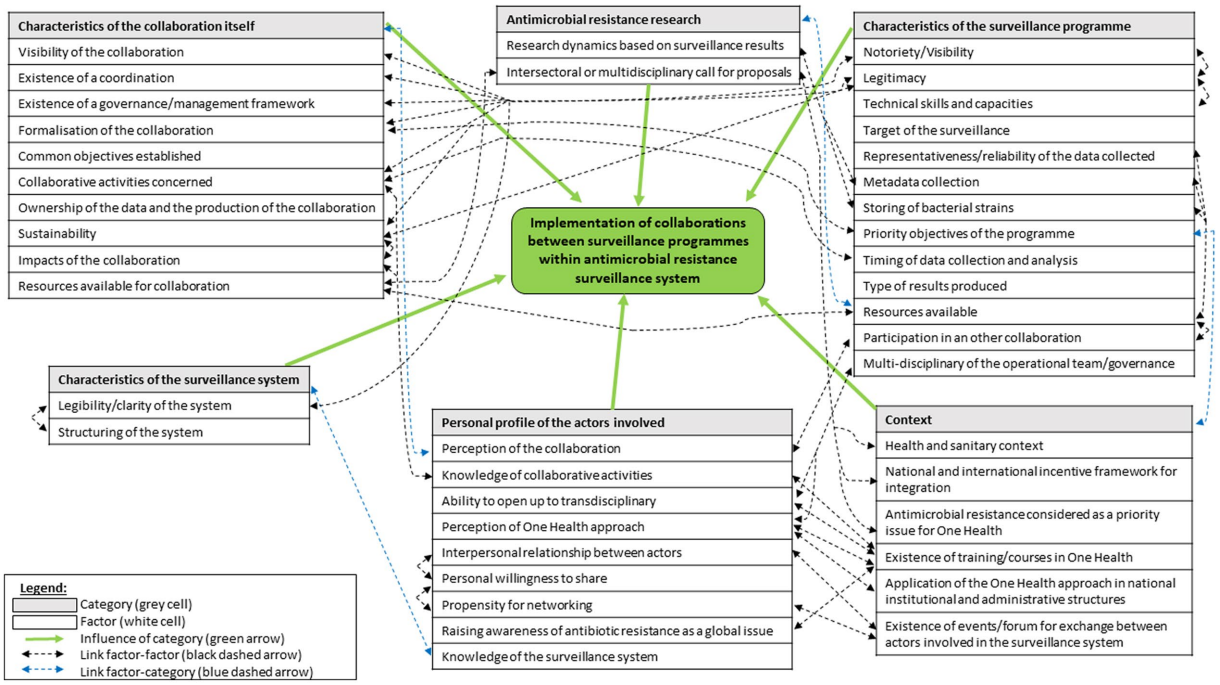

FIGURE 1
Map demonstrating the relationships between factors and categories of drivers mentioned by respondents as influencing collaborations between AMR surveillance programmes.

TABLE 2 Characteristics of the 51 interviews.

Type of actors	Number of interviews	Number of actors interviewed*	Length of interview in minutes: median [min; max]
Coordinator of surveillance programme	36	53	59 [27; 90]
Key-informant expert	15	15	55 [41; 119]

*From 1 to 4 coordinators interviewed per session.

coordinating two surveillance programmes and, therefore, were interviewed about both programme simultaneously. In total, we collected points of views of 53 (co-)coordinators representing 40 surveillance programmes, among 48 operational surveillance programmes of AMR in France in 2021. We interviewed all coordinators, whose surveillance programme(s) was involved in a cross-sectoral or cross-domain collaboration in 2021 (Supplementary Figures 1, 2).

Thematic analysis revealed three major themes corresponding to the two research aims (Table 3). Results are presented according to this frame. Note that there were no differences between the views of the respondents from the different sectors or of coordinators involved in collaborations or not.

3.1. Key to setting up collaboration and actors influences

Among the reasons for and limits to setting up collaborations, our study identified 42 factors grouped into six categories (Figure 1). The Figure 1 illustrates that most factors were linked together, according to the respondents.

3.1.1. Personal profile of actors involved and importance of interpersonal relationships

Thematic analyses revealed that collaborations depended on the profile of actors involved, especially their willingness to share their data or expertise, to open up to transdisciplinary approaches, their perception of the One Health approach, as well as their awareness of antimicrobial resistance as a global issue. Moreover, all respondents reported that a history of good quality relationship between actors fostered the implementation of collaborations between surveillance programmes. These interpersonal relationships were formed because people had common educational backgrounds, used the same disciplinary language or worked in the same sector or on the same pathogens. This appeared to lead to a trustful relationship between them, which facilitated exchanges, mutual understanding, and thus collaboration. Therefore, collaborations were first based on people relationships and second on shared sectors or disciplines.

> "*They are people we have known for years and with whom we get on well. The collaboration between us is natural*" No. 1.
>
> *"I would say that it is more collaborations based on individuals, than collaborations let's say of organisations"* No. 44.

TABLE 3 Overview of the research aims linked to the themes and categories that emerged during data analysis.

Research aim	Theme	Categories
To improve understanding of the factors that influence the implementation of collaboration between surveillance programmes	Key to setting up collaboration and actors influences	Personal profile of actors involved and importance of interpersonal relationships
		A fragmented surveillance system with a lack of legibility
		Characteristics of the surveillance programmes influencing collaborations
		Collaborations' characteristics influencing collaborations
		Antimicrobial resistance research as a springboard for collaborations
		The influence of the broader context
	Impacts of collaboration	Benefits of collaboration at different levels
		A necessary balance between collaboration and stand-alone existence
To explore challenges of the One Health approach for the surveillance system	Perception of the One Health approach	Plural visions of a theoretical approach that is difficult to grasp
		A need for engagement of diverse stakeholders
		A need for common indicators

Respondents reported that implementation of collaboration between different surveillance sectors, or between coordinators of different disciplines was difficult because of the lack of knowledge of the people involved, who felt that they did not share the same issues.

> *"The coordinators of programmes A and B are people […] who are in the field of public health epidemiology. In programme C, they are pure microbiologists. […] And so on the one hand it's the long view on a few pathogens and on the other hand a much more transversal vision. That's perhaps why it's difficult to get them to communicate!"* No. 17.

Respondents mentioned that knowledge and interpersonal relationships were mainly achieved through networking at scientific events. Thus the propensity of coordinators to network, as well as the existence of events facilitating exchanges between actors, influenced the implementation of collaborations.

> *"Through this symposium, which takes place every two years, we share our work and it is an opportunity to forge links and collaborations"* No. 7.

Among the potential collaborative activities for surveillance, respondents were more frequently engaged in joint dissemination of the results, than in governance or other operational activities (e.g., design of the surveillance protocol, data collection, or data analysis). Furthermore, several respondents (about one-third of the coordinators) only envisaged joint communication of the results between surveillance programmes, due to a lack of awareness of other possible collaborative activities, which limited the scope of collaborations.

> *"We don't work on the same bacteria at all. […] They work on bacteria* A *and they work on bacteria* B*, so there you go. And so we can't collaborate with them"* No. 40.

3.1.2. A fragmented surveillance system with a lack of legibility

The respondents did not have a good knowledge of the antimicrobial resistance surveillance system in France, of other surveillance programmes existing in other sectors or domains, and, subsequently, of what the latter could bring to them in the framework of collaboration. According to them, this lack of knowledge was linked to the complexity of the French surveillance system, which was fragmented and lacked legibility (numerous programmes, and all the surveillance programmes were not known by stakeholders) (9). In addition, the surveillance system was perceived as being very sector-based, impacting working habits and collaborations, that were primarily set up within the same sector (e.g., human health) or within the same surveillance domain (e.g., antibiotic consumption).

> *"It's really hard to get people to work together because of the number of programmes and also because of corporatism. I find that the world of human health is really many, many silos, with people who don't talk to each other, with different labels"* No. 15.

3.1.3. Characteristics of the surveillance programmes influencing collaborations

Thematic analysis revealed that 13 factors related to the characteristics of the surveillance programmes influenced collaboration (Figure 1).

3.1.3.1. Structural aspects

Regarding the mechanism of implementation of collaboration, we observed that they were organised according to sectors and domains (surveillance targets) of the programmes. Indeed, either the surveillance programmes were in the same sector (e.g., human health) and collaborated because their surveillance domains were different (resistance in different settings, or antibiotic use versus resistance), or the collaborating programmes focused on the same domain but in different sectors (e.g., enterobacterales in human and animal health).

Additionally, it was interesting to note that the existing collaborations supported the development of new collaborations. In particular, the participation of programmes to national or supranational networks (usually well recognised), placed them in a dynamic that made the coordinators more inclined to collaborate.

> *"In the scientific committee of subsystem X, we will actually exchange in terms of methodology, or participate to studies between surveillance programmes […] We have sometimes collaborated within the subsystem itself, on particular themes that are of interest to us"* No. 34.

3.1.3.2. Operational aspects

First, programmes with notoriety and legitimacy (via mandatory surveillance, national recognition, or long history of

existence) were those mainly involved in collaborations. Moreover, surveillance programmes known to collect good quality data (good representativeness, large coverage), or metadata (such as geographical indication, socio-demographic or clinical information on patients), or to store bacterial strains were more engaged into collaborations. Indeed, these characteristics allowed them to compare their data more easily or relevantly between each other, or to do more in-depth analyses with other programmes with specific resources (e.g., WGS). Indeed, our study showed that for establishing joint analysis and valorization of surveillance data, it was necessary for the programmes involved to have comparable methods, compatible data collection and analysis timelines, and similar geographical coverage.

> *"With programmes A and B you have an idea of the prevalence of resistance, but you don't know which strains are circulating. So necessarily there are these collaborations, there have to be shipments of strains to programme C, because it has the expertise in characterising the strains so that we can tell which clones are circulating"* No. 9.

3.1.4. Collaborations' characteristics influencing collaborations

The impact of collaboration, particularly in terms of benefits for actors or surveillance programmes, emerged as a key element in the implementation and sustainability of collaborations. While collaborations were first established based on interpersonal links or informal relationships, the coordinators were more inclined to collaborate if the collaboration was then structured and formalised (with an agreement or a charter for example). This formalism helped to reassure the actors involved. Moreover, respondents reported that the existence of coordination, of a governance or management framework for collaboration, and the formal definition of common objectives between surveillance programmes also influenced the establishment of collaborations.

> *"Everyone found their interest in it. We drew up a charter, of course! We were very careful as we wanted it to be very respectful; there is a charter of commitment, and of rights and duties of each participating surveillance programme"* No. 36.

The thematic analysis also revealed that the type of collaborative activities implemented also influenced the setup of collaborations. Collaborative activities with high visibility (e.g., external joint communication) increased both visibility and legitimacy of surveillance programmes and fostered collaborations. Finally, coordinators reported that the visibility of the collaboration itself influenced the implementation of collaboration. In fact, coordinators had interest in implementing it, since its visibility contributed to both the reputation and legitimacy of the surveillance programmes involved, and it also created the desire for other programmes to collaborate.

3.1.5. Antimicrobial resistance research as a springboard for collaborations

Respondents indicated that cross-sectoral or multidisciplinary calls for research projects encouraged collaboration between surveillance programmes. It was also a way of obtaining the resources necessary for initiating a collaboration. Respondents indicated research projects could thus be the first step to kick start more permanent collaborations between surveillance programmes.

> *"We have already done several research projects with programme A […] We have to continue to move in that direction. It's not yet an organised routine activity, if you like. It's taking shape more around research projects, which are in essence on-offs, than by something in continuous flow. I think we need to move towards this now. This is an essential aspect of this One Health approach"* No. 2.

More broadly, research was seen as a means of enhancing the value of the collaborations and programmes involved (e.g., publications reinforced the reputation of programmes and coordinators). Research also supported collaborations as it helped to improve surveillance and thus strengthen surveillance programmes that were then better able to collaborate. Ultimately, the thematic analysis showed that the dynamics of research that built on the surveillance data produced by the surveillance programmes was a factor for collaboration.

3.1.6. The influence of the broader context

According to the respondents, the broader health context (including the COVID-19 pandemic) played a role in the establishment of collaborations, influencing both the surveillance priorities and the resources allocated for these collaborations. Moreover, collaborations between surveillance programmes were strengthened when antimicrobial resistance was considered as a One Health priority by coordinators and funders. More generally, collaborations were enhanced by various elements that fostered integration, such as a national or international framework to support integrated surveillance systems, the application of the One Heath concept in institutional or administrative bodies, and the existence of specific One Health trainings in the university or academic curricula of coordinators.

> *"The Covid crisis has helped quite a bit in terms of awareness of the interconnection between human and animal health and the health of ecosystems […]. The topic of antimicrobial resistance should benefit from this general "One Health" impetus, even if at first glance it may seem to suffer a little from it. Because emerging infectious diseases have come to the forefront, and antimicrobial resistance, which was considered the number one threat in the "One Health" field, has taken a back seat"* No. 30.

3.2. Impacts of collaboration

3.2.1. Benefits of collaboration at different levels

According to the respondents, the benefits of implementing collaborations were diverse and occurred at various levels (Table 4). For example, for the surveillance programmes involved, the collaboration led to an increased efficiency in surveillance through the

TABLE 4 Benefits identified from collaboration within the surveillance system and associated level.

Level	Benefit
Surveillance programme	Pooling of material, human and financial resources for surveillance activities and deliverables (reduced surveillance costs) Broadened range of surveillance activities Improved surveillance (reactivity, accuracy, etc.) Increased visibility Strengthened legitimacy Improved sustainability of surveillance programme
Surveillance system	Greater coherence Efficiency gain – optimisation of surveillance (improved and harmonized methods, timeliness, reactivity etc.)
Actors involved in surveillance	Strengthened professional network Strengthened interpersonal relationships Increased awareness regarding antimicrobial resistance Gain in skills or expertise Better understanding and knowledge of other disciplines and sectors Gain in reputation
Public interest	Expanded scientific knowledge Improved prevention and control strategies

pooling of resources, such as the sharing of experience or expertise between coordinators. While collaborations were primarily based on quality and trusting relationships between actors, it was interesting to note that collaborations also helped to strengthen the link between them.

> *"That's it, ideally collaborating would be to build stronger and longer lasting connections with people from the programme A"* No. 7.

3.2.2. A necessary balance between collaboration and stand-alone existence of surveillance programmes

The availability of resources was one of the major factors for the implementation of collaborations and was mentioned by almost all respondents. For a collaboration to work and be sustainable, specific resources (time and funding) had to be found or allocated from the budgets of the programmes involved. However, it emerged from the interviews that the majority of surveillance programmes were operating on a just-in-time basis with limited or even insufficient resources to maintain high quality of their own data collection or analysis. Consequently, the set-up of collaborations appeared to be of secondary importance, compared to maintaining the operations of their own surveillance programme.

> *"It's very difficult to set up collaborations with the budget we are currently being allocated"* No. 2.

> *"People are quite willing to collaborate in both directions, so that's really good. The big difficulty is the priorities of each programme, and therefore the time allocated to this collaboration"* No. 18.

Paradoxically, even if the pooling of resources enabled by collaboration was perceived as a potential benefit, it did not counteract the view of the respondents that collaborative activities came only after the surveillance programme' own activities. However, an exception occurred when collaboration was included in the priority activities of the programmes (three programmes). By formalising them in this way, collaborations were more legitimate and their funding was simpler.

> *"It is certain that the fact that it is in our mandate encourages us to set up [collaborations] and encourages us, probably in a subjective way, to carry out collaborations"* No. 44

For the respondents, in essence, the collaboration should bring an added value for each of the parties, comparable to a win-win approach between programmes.

> *"Putting together these data, juxtaposing them, making them talk, while respecting the surveillance programmes, was an extremely strong motivation! Because for each member it was a demonstration that they existed, it valued them"* No. 36.

While collaboration was a way to gain visibility and legitimacy, it was also seen by some respondents as a threat to the visibility or sustainability of the programmes involved, if they were to give way to the collaboration itself or to one of the parties.

> *"It was a bit complicated. They had constraints, which we can understand. Because of the fear that the surveillance programme A would completely absorb the programme B. There was already a need to really clarify the collaboration"* No. 44.

Finally, it was interesting to observe that some programmes, far from positioning as collaborators, saw themselves more as competitors in the search for funding or in responding to project calls (the latter remaining mostly sectoral or monodisciplinary).

> *"I guess that it's not very easy to get programmes to work together, except for those that already work very well, because for personal or friendly reasons they work together. But there is quite a lot of competition eh, for surveillance programmes!"* No. 17.

3.3. Perception of the One Health approach

3.3.1. Plural visions of a theoretical approach that is difficult to grasp

It emerged from the interviews that the One Health approach appeared difficult to grasp in a concrete way and remained a relatively abstract notion. Firstly, it appeared that the One Health approach was difficult to translate and explain. Secondly, the respondents had different visions of it, ranging from complete integration between all sectors up to minimum integration to improve human health only. As a consequence, there were differences in orientation of coordinators towards what One Health means for their surveillance programme.

> *"The One Health for me is a concept, alright, that I would say a little bit of a facade. What may be behind it seems much less clear to me"* No. 17.

> *"There are two ways of conceiving One Health. There are those who say: 'One Health is human health to which other healths must contribute', which is the very medical approach of One Health, very anthropocentric. And then there are those who say: 'No, One Health is putting the three sectors at the same level of importance, because the poor health of one will influence the health of the other two in any direction'"* No. 12.

3.3.2. Need for engagement of diverse stakeholders

According to the respondents, for the One Health concept to become a reality in surveillance of antimicrobial resistance, it should not only be implemented by all actors at different levels of organisation (ministry, administration, university, research centre, laboratories, etc.), but also supported by all actors in the system (risk managers, researchers, teachers, etc.). All the respondents testified that there is room for improvement in the application of this concept for antimicrobial resistance surveillance.

> *"We do not have the impression that there's anything there. There's not something integrated between the animal and the human sectors. At a time when we are very One Health, I think we could do better. Already we depend on two different ministries, that does not help I think"* No. 9.

It was interesting to note that, according to the respondents, the impetus for the One Health approach in antimicrobial resistance surveillance currently comes mainly from surveillance programmes (where operational or governance teams are multidisciplinary, for example) or from the academic and scientific world (via the organisation of interdisciplinary or cross-sectoral events, which make it possible to create interpersonal relationships). They mentioned that this impetus should also come from transdisciplinary education or training of actors so that they better understand each other.

> *"It's complicated to structure a One Health team locally, you have to get it accepted! In other words, they tell you that you are scattered. And just in a single team, try to integrate a sociologist, you'll see!"* No. 13.

> *"That's the limit of One Health, in fact, you want to integrate everything and at the same time you're not ready to understand everything […] They [the disciplines] don't have the same language: when a sociologist talks to me I don't understand anything, and I think he's going to smoke me out"* No. 25.

Besides, the respondents highlighted the importance, in the short term, to implement a national coordination of integrated surveillance, via, for example, the creation of a cross-sectoral operational team with dedicated resources. They also emphasised the importance of transdisciplinary and cross-sectoral training and education. The latter could contribute both to acculturate the actors to this approach and to foster links between coordinators from different sectors.

> *"One way of improving this is to take the problem at its roots and create a common core of training […] Eventually, if the vets, pharmacists, doctors, in short, if all these people meet in a form of common training, friendships will be created and people will follow each other, and perhaps, in addition, there is a common understanding"* No. 42.

> *"Beyond surveillance, it is a more general issue, and we have asked for this several times without success, that there really is an interministerial delegate for antimicrobial resistance who has authority over the ministries to obtain results […] In terms of showing the importance of the topic it would be a positive signal"* No. 30.

Finally, several respondents (a third of all respondents) regretted that the One Health global approach was insufficiently considered as a prerequisite and was still too often taken into account only at the very end of the process.

> *"In fact, during specialty training, we already try to teach them their own specialty and we consider that this One Health topic is a luxury"* No. 18.

3.3.3. A need for common indicators

According to the respondents, a concrete way to implement the One Health approach would be to have a few common indicators across sectors, which all surveillance programmes could calculate, in order to make all collected data interoperable. These indicators should be simple, operational and relevant to be used widely.

> *"That's typical for the prescription data, it would be good to have an indicator in common between the small animals or the big animals and the humans at the same time. Because here we don't really understand the parallel, apart from the direction of the trends, well, between the ALEA [Animal Level of Exposure to Antimicrobials] and the DDD [Defined Daily Dose]"* No. 22.

However, the respondents stressed that the implementation of such indicators could jeopardise the operations of surveillance programmes should they require a profound change in the methodology used. These changes would also have to be made in agreement with programme funders (government, agency, private sector, associations, professional organisations), who do not

necessarily consider the One Heath approach. In the end, in a context where major changes would be necessary, the respondents were divided between the need for either a change of practise driven by the coordinators in a collegial manner, or by a regulatory, national or supranational demand.

4. Discussion

To our knowledge, this study is the first to investigate levers and impediments to collaboration between antimicrobial resistance surveillance programmes. This study identified a large number of factors (that could act as incentives or barriers), gathered into six categories (Figure 1).

According to our results, actor-related factors played a decisive role in the willingness to collaborate, which can be perceived as a change in the way they implement surveillance. The process of change itself relies on the consent and commitment of coordinators, who need to acknowledge a certain legitimacy of the collaboration to accept and implement it (17). In addition, current collaborations were based more on the network of actors and good interpersonal relationships between them, than on lead institutions or a national or supranational demand for collaboration. As collaborations were built primarily on interpersonal relationships rather than on structures, coordinators who were open to cross-sectoral approaches appeared as powerful drivers for collaborations. However, fragility resulted from this interpersonal mode of operation: collaboration could fade if coordinators who were the leaders of collaboration were to leave their function. Besides, all the contextual elements (i.e., conferences, workshops, education, training) that encouraged actors from different sectors or disciplines to better know each other, understand each other, exchange and learn from other disciplines ultimately facilitated collaborations. Therefore, in order to move towards the concrete application of a One Heath approach, all initiatives aiming at bringing coordinators together should be sustainably supported to ensure that coordinators know each other and can converse regularly. In addition, the increasing development of One Health courses related to AMR should be encouraged. The study also highlighted that coordinators lacked knowledge of the surveillance system, the existing programmes, and the role of each actor. To help in this direction, we previously published a comprehensive mapping and characterisation of all the programmes that constitute the French surveillance system (9); this was lacking so far. We are confident this work will contribute to improve the understanding of the surveillance system, hence facilitating the potential kick-off of new collaborations.

Over the last decade in France, cross-sectoral or One Heath scientific events on antimicrobial resistance that brought together programme coordinators from different sectors or disciplines have been limited. Each year during the World Antimicrobial Awareness Week, a cross-sectoral conference gathers the different ministries and public health agencies involved in surveillance, but programmes' coordinators are no necessarily invited. In November 2021, two large French meta-networks, PROMISE and ABRomics funded through the French Priority Research Programme on antimicrobial resistance (18), were launched. They constitute an excellent opportunity to foster knowledge exchange, and improve synergies between programmes. The meta-network PROMISE aims to build a One-Health community of actors on antimicrobial resistance; it includes a data warehouse to share surveillance data between programmes, the identification of common indicators as well as pilot studies with joint data analysis. To overcome possible limitations to data sharing (e.g., data ownership regulations, internal programme rules), data can be anonymised or even aggregated for analysis at different scales. In addition, the meta-network ABRomics aims to build a One Health cross-sectoral online platform to facilitate sharing of bacterial (meta)genomics data among researchers from different sectors and disciplines, hence fostering collaboration between surveillance communities. Furthermore, at the European level, the European research agenda is moving towards a One health approach - in the steps of the One Health European Joint Programme (OHEJP, 2018–2022) – encouraging transdisciplinary research, innovation, surveillance, both at national and European levels (19). While the context is increasingly favourable to holistic approaches, Benedetti et al. (20) stressed the importance of largely promoting and communicating joint actions and transdisciplinary activities, to ensure that they are known and useful to both the scientific community and policymakers.

In terms of barriers to collaboration between programmes, the lack of human, financial and/or technical resources dedicated to collaboration, the siloed surveillance system, and the sectoral priority of programmes, appeared as challenges difficult to overcome. Moreover, the poor legibility of the surveillance system led to a lack of knowledge of existing programmes by the coordinators. In this context, the work of characterising and mapping surveillance systems appears particularly useful to support synergies between programmes (9). Although calls for proposals are a way for programmes to join forces to obtain resources, they can also encourage competition between them, and subsequently deteriorate the relationships among coordinators. This highlighted the need for intersectoral calls for proposals, with the selection of projects based on collaboration between research teams from different domains and/or disciplines to ensure a comprehensive approach of a scientific issue, and thus increase collaboration between surveillance programmes. Even if collaborations could be seen as a pooling of resources, they were still largely considered as a costly additional activity, since collaborative activities were not necessarily in line with the sectoral objectives of the programmes. Thus, the inclusion of intersectoral collaborative activities within the objectives of the surveillance programmes could improve alignment between resources and objectives, and support the development and sustainability of collaboration. All these elements underlined the need for an impetus for One Health to be given at different levels to become a reality, and not just by coordinators, but more broadly by programme funders and risk managers. According to our results, a context favourable to intersectoral/interdomain collaboration is crucial to encourage One Health collaborations. It implies that collaboration should be an intrinsic objective of surveillance, to overcome structural and operational barriers that cause difficulties to collaborate. The activities of surveillance programmes (and therefore collaborations) mainly depend on the orientations given by their funding bodies. To support the development and sustainability of collaboration, it is therefore essential that the collaborative activities are in line with the programmes' own objectives.

In addition, administrative structural barriers between ministries of Health, Agriculture and the Environment are obstacles to progress to One Health (6, 19). More exchanges and coordination between ministries (through meetings, joint working programmes, or even the setup of an interministerial coordination body) could

foster integration between siloed directorates of ministries. At the European level, a recent study highlighted that the research needs should be defined from a One health perspective, requiring the involvement of European Union agencies and including both policy cooperation and transdisciplinary coordination, similarly to the OHEJP approach (19). The authors also underpinned that fragmented research activities and in-silo regulations limit transdisciplinary and interagency cooperation, requiring a more horizontal approach to regulatory frameworks to fully integrate the One Health principle.

This study also enabled us to identify numerous benefits to the setup of collaborations (Table 4). Several of them were consistent with previous results dealing with the impact of integrated surveillance (6–8) and of One Health networks (21). These authors also identified the improvement of scientific knowledge, in particular a better understanding of transmission routes across sectors, the identification of the relative importance of the different reservoirs in the emergence and maintenance of resistance in humans, the identification of correlations between antibiotic use and resistance within and between sectors, and the assessment of intervention impacts within and between sectors. We believe that the use of qualitative approach applied to a dense system was particularly relevant to progress in the identification of benefits resulting from collaborative surveillance. Since impacts are seen as drivers for implementing collaborations, it would be interesting to further investigate and characterise the impacts and benefits of collaborations within the surveillance system, not only for programme coordinators, but also for all stakeholders involved.

Our study pursued a qualitative sociological approach, which is a valuable way of understanding the diversity and extent of opinions (22, 23). Although this approach does not lend itself to the quantification of each opinion in the broad population, nor to statistical inference, it helped to answer and understand why collaborations occur. The qualitative approach was therefore well suited for gaining insight into coordinators' decisions making. Although ideally interviews should have been conducted individually without witnesses to facilitate expression of personal opinions, several interviews with coordinators were not individual, upon the coordinators request to supplement their responses with those from co-coordinators. While this allowed us to collect and reinforce diverse views on the factors of collaborations, it could hinder the spontaneity and exhaustivity of information provided by the respondents due to hierarchical link between them. Besides, all interviews were conducted remotely due to the travel restrictions, making it more difficult to analyse the gestures and reactions of respondents. Despite those limits, we believe this qualitative approach was a valuable way to capture novel information regarding reasons for collaboration that cannot be obtained using a quantitative questionnaire methodology. It was a necessary first step before possibly considering further quantitative research to weigh each factor.

The French antimicrobial resistance surveillance system appeared to be a particularly relevant case study to explore the reasons for collaborations. It was varied and fragmented, with numerous surveillance programmes involved or not in collaborations (9). Moreover, since AMR is a major public health concern involving four sectors (human, animal, food, and environment), three domains (antibiotic resistance, antibiotic use or consumption, and residue of antibiotic), several disciplines (among others epidemiology, microbiology, infectiology, hygiene, pharmacology, ecology, sociology) this system enabled us to investigate collaborations from various sights (collaborations involving two to 14 surveillance programmes). Finally, since this system was dense and complex, with heterogeneous programmes, we believed it was a better case study to identify multiple factors for collaboration than a surveillance system focusing on one particular disease.

By following analysis and sampling rules (triangulation, iteration and saturation) and thank to the confidentiality of interviews ensuring the trustworthiness of respondents answers (11, 23), we were able to identify relevant factors for collaboration between AMR surveillance programmes at the French level. Since most factors were neither specific to the French context nor the antimicrobial resistance threat, we believe these results could be useful to other collaborative surveillance systems dealing with other diseases, in other countries. However, since we were only able to identify factors of collaboration within the French AMR surveillance system, it would be interesting to perform similar studies for other surveillance systems and in different contexts (for example in a non-European country) to identify other relevant factors impacting the implementation of collaboration between programmes. Considering that we focused on a particularly complex surveillance system, it would be relevant to explore and compare which collaboration factors occur in a less fragmented surveillance system. Finally, by providing incentives to foster integration and clues to understand coordinators' positions, our findings can be of interest to any surveillance system in other countries, for researchers, programmes' coordinators, and risk managers to move globally towards a One Health approach of surveillance.

Data availability statement

The datasets presented in this article are not readily available because the data used for this study were obtained through semi-structured interviews of programmes' coordinators and key informant experts. Conditions of approval (respecting the anonymity of respondents) do not allow us to distribute or make available data directly to other parties. Data analysed are included in this published article and its supplementary information files. Requests to access the datasets should be directed to lucie.collineau@anses.fr.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to be part of the study was obtained from the participants before interviews.

Author contributions

CB and LR analysed the data. CB wrote the original draft. CB, LR, MC-C, and LC conceptualised, designed, performed the study,

interpreted the data, read, and approved the final manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the French Ministry of Agriculture (http://agriculture.gouv.fr) as part of the Surv1Health Project (Plan EcoAntibio2: research grant 2019-124). No additional external funding was received for this study. The funders had no role in study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

The importance of the participants in this project cannot be overemphasised. The authors would like to thank all of the respondents for their time and insight, which were essential to this project. The authors also thank all the experts contacted during this study for their suggestions, advice and review of the manuscript, especially Cécile Aenishaenslin and Nikky Millar.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1123189/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Brief Research Report
PUBLISHED 20 November 2023
DOI 10.3389/fpubh.2023.1127701

OPEN ACCESS

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RECEIVED 19 December 2022
ACCEPTED 31 October 2023
PUBLISHED 20 November 2023

CITATION
Moura P, Borck Høg B, Alban L, Sönksen UW, Ribeiro Duarte AS and Sandberg M (2023) Evaluating the OH-EpiCap tool using the Danish integrated surveillance program for AMU and AMR as a case study.
Front. Public Health 11:1127701.
doi: 10.3389/fpubh.2023.1127701

Evaluating the OH-EpiCap tool using the Danish integrated surveillance program for AMU and AMR as a case study

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Antimicrobial resistance (AMR) is considered a One Health (OH) challenge, ideally demanding concerted efforts from the animal, human and environmental side. DANMAP, the Danish Integrated Antimicrobial Resistance Monitoring and Research Program, is monitoring AMR and antimicrobial use in animals and humans. OH-EpiCap is an evaluation tool, developed to address essential elements in OH surveillance systems, such as the dimensions of the organization, operational activities and the impact of the surveillance activities. We aimed to evaluate DANMAP using OH-EpiCap and hereby assessed the suitability of OH-EpiCap to evaluate integrated AMR surveillance systems. During the evaluation, the strengths and weaknesses of DANMAP concerning the "OH-ness" of the program were discussed. Furthermore, possible adaptations of the standard operating procedures and governance structure were addressed. Attention was paid to the ability and easiness of DANMAP to cope with current and future challenges connected to integrated AMR surveillance. It was concluded that DANMAP has a strong OH approach covering relevant aspects for humans and animals, whereas environmental aspects are missing. OH-EpiCap proved to be straightforward to use and provided valuable insights. The authors recommend OH-EpiCap to be used by health authorities and stakeholders. It is not suitable for the technical evaluation of a surveillance program.

KEYWORDS

One Health, surveillance, antimicrobial, resistance, consumption, stewardship

Introduction

Antimicrobial resistance (AMR) has been defined a cross-sectoral problem due to it affecting both animals and humans, carrying an inbound risk of circulation within and between both domains. In addition, the environment can serve as a "melting pot" for both antimicrobial resistant bacteria and genes (1). The exchange among populations often happens sporadically, but AMR may accumulate over time, and at the time of detection, the root causes are not always easy to establish (2–4). Nonetheless, the antimicrobial use (AMU) in one sector may contribute to the development of resistance in another sector, as it has been demonstrated by both scientific publications (5–7) and

the joint report on antimicrobial usage and antimicrobial resistance in humans and food producing animals in the European Union (8).

Integrated surveillance programs are based on multi-sectoral collaborative activities such as the collection, analysis and dissemination of the results to relevant stakeholders across sectors for action and policymaking (9). In AMR surveillance programs, multi-level integration is considered crucial to untangle the consequences related to AMR. Connecting the political decision level to the technical surveillance activity level is essential to sustain risk mitigation decisions against the defined health hazards (10).

In concordance with what is described above, the One Health High-Level Expert Panel (OHHLEP) of the World Health Organization (WHO) defines One Health (OH) as "an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems," recognizing that they are closely linked and interdependent (11). The OHHLEP supports the described horizontal, e.g., cross-sectoral, and vertical, multi-level, approach to integrated surveillance systems and encourages the upscaling of intersectoral collaboration in national strategies against AMR by use of the strategic framework developed by the Panel (12).

The Danish program for surveillance of AMU and AMR in bacteria from food animals, food, and humans (DANMAP) was established in 1995. Collaboration and cross-sectoral decision-making have always served as a fundament of the program (13). The objectives of DANMAP are: (i) to monitor AMU in food animals and humans, as well as the occurrence of AMR in bacteria isolated from food animals, food of animal origin and humans; (ii) to study associations between AMU and AMR; (iii) to identify routes of transmission and areas for further research.

The ultimate objective of the program is to produce information that sustains risk mitigation actions connected to AMR hazards that might affect humans and/or animals. Several bacterial hazards with different types of AMR are under surveillance, i.e., bacteria isolated from patients, zoonotic bacterial pathogens (*Salmonella* spp. and *Campylobacter* spp.), and indicator and pathogenic bacteria from food producing animals (pigs, cattle and broilers) and from food products thereof (13).

According to Aenishaenslin et al. (9), the added value of integration in AMR surveillance can be projected or recognized in the system's performance in different outcomes: (1) immediate outcomes as increased epidemiological knowledge from the combination of collected data; (2) intermediate outcomes, by causing behavior and policy changes that can be reflected in AMR trends; (3) ultimate outcomes such as reductions in overall AMU and AMR, leading to measurable improvements of health in the affected domains (9). In line with Aenishaenslin et al. (9), the epidemiological knowledge generated by the DANMAP program itself and associated research have contributed significantly to actions in the Danish livestock farming industry, such as the voluntary ban on the use of third- and fourth-generation cephalosporins in the Danish pig industry (14), which ultimately led to these substances not being used at all in Danish pig production (13).

In 2017, a new Danish National OH Strategy Against Antibiotic Resistance was issued by the Ministry of Food, Agriculture and Environment together with the Ministry of Health, reiterating existing initiatives on AMR prevention, mitigation and control (15). In Denmark, due to the large size of the pig sector compared to the size of the country, the AMU in pigs has been in focus for decades. This has resulted in a relatively low AMU per pig as shown by Moura et al. (16). The many actions implemented in Denmark to guide AM stewardship can be consulted in the latest DANMAP report (13).

Scientific and technological advancements together with emerging or potentially threatening health challenges can justify changes in a surveillance program. Therefore, it is important to evaluate a program's performance in meeting the defined objectives, while operating under a set budget (17).

Given the sheer complexity of designing and operating a multi-sectorial national-scale program, the aim of evaluating the One Health-ness" (OH-ness) of DANMAP has been recognized as relevant by the program's steering committee. As mentioned above, integration and collaboration are essential parts of OH. However, it is possible that increasing the level of integration and collaboration in all components of AMR surveillance would neither add value to the information generated nor improve decision-making. Therefore, the cost-effectiveness of changes in the integration and collaboration should be understood and evaluated critically (18).

The MATRIX consortium, funded by the One Health European Joint Program, developed the OH-EpiCap tool to systematically characterize epidemiological surveillance activities in a national surveillance system. The main aim of OH-EpiCap is to facilitate the assessment and improvement of national capacities and capabilities for integrated surveillance of zoonotic hazards (19). So far, due its generic design, the OH-EpiCap tool has been successfully applied to surveillance activities connected with food-borne hazards, such as *Salmonella*, *Campylobacter, Listeria* and other zoonotic hazards such as *Chlamydia psittaci*, however, it has so far not been used to evaluate AMR surveillance activities.

To provide guidance in choosing among evaluation tools for AMU and AMR surveillance systems, an international network called CoEvalAMR was established in 2019 (20). The CoEvalAMR network has recently evaluated nine AMR surveillance systems using OH-EpiCap (21). This case study is part of the aforementioned work. With the main objective of determining whether OH-EpiCap is suitable to evaluate a complex integrated AMR program, we evaluated DANMAP using OH-EpiCap. The outcomes of this evaluation serve as the basis for the secondary aim of this work, which was to present and briefly discuss the strengths and weaknesses of DANMAP in what concerns the OH-ness of the program.

Abbreviations: AM, Antimicrobial; AMR, Antimicrobial resistance; AMU, Antimicrobial use; DANMAP, Danish Integrated Antimicrobial Resistance Monitoring and Research Program; DTU, Technical University of Denmark; FAO, Food and Agriculture Organization of the United Nations; MiBa, Danish Microbiology Database; OH, One Health; SSI, Statens Serum Institut; UNEP, United Nations Environment Program; WHO, World Health Organization; WOAH, World Organization for Animal Health.

Materials and methods

DANMAP

As proposed by the OH-EpiCap tool, a surveillance system can be decomposed into the following activities: (1) planning and

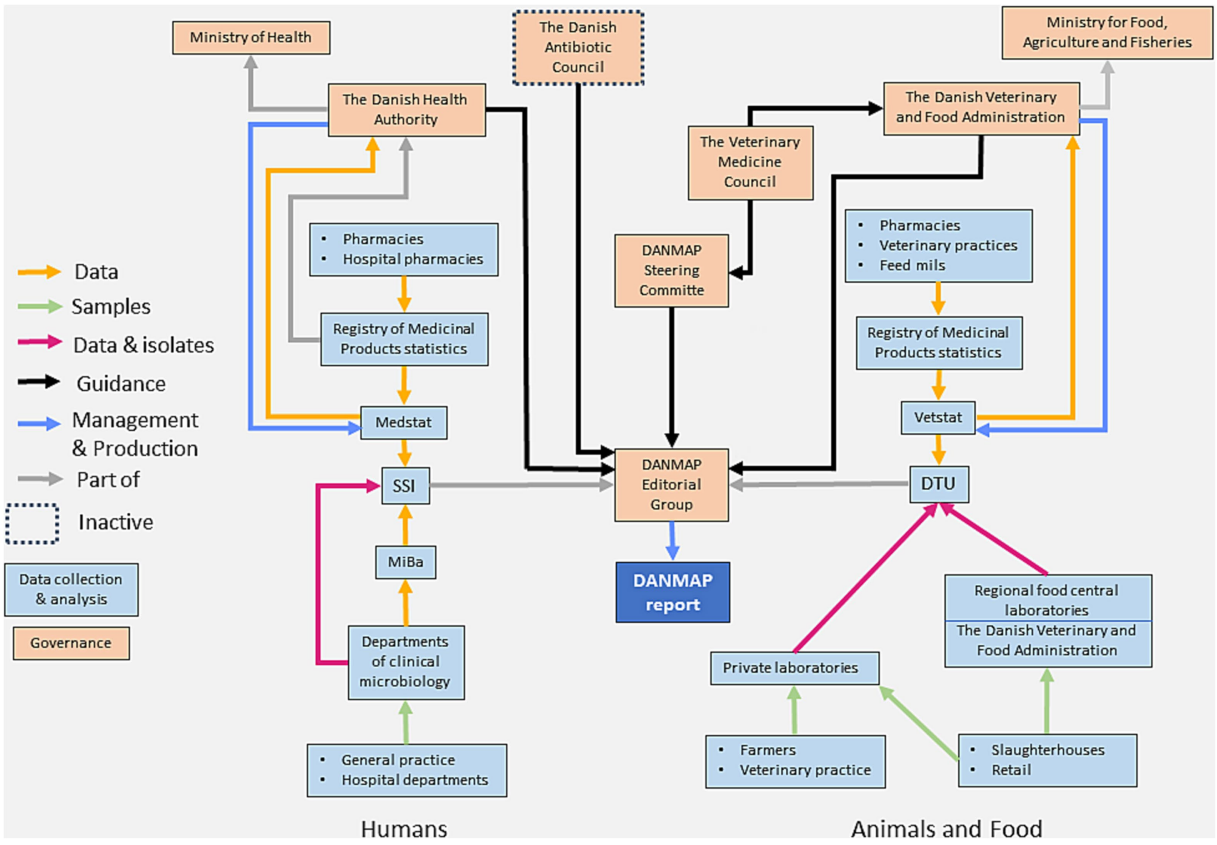

FIGURE 1
Organigram showing the governance structure and activity flow in DANMAP, as it was in 2022.

management, (2) data collection, analysis and interpretation, and (3) distribution of the information generated (22).

The governance structure and main activities that compose DANMAP are presented in Figure 1. DANMAP is managed by a collaborating team from the National Food Institute at the Danish Technical University (DTU) (EU Reference Laboratory for antimicrobial resistance) and the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI). The program and its overarching goals are set by order of the Ministry of the Interior and Health of Denmark and the Ministry of Food, Agriculture and Fisheries of Denmark and financed via state funds. The Danish Antibiotic Council, currently not in function, was instructed to oversee the program. This task is now the responsibility of the two ministries and the Danish parliament. The governance structure of the program aims to ensure that all relevant parties can express their science-based advises and/or political views on the program's design and on the implementation of activities. Currently, the Danish Veterinary Medicine Council (23), which is composed of experts from the animal and human side, provides advise to the Danish authorities and to DANMAP. In addition, DANMAP receives input and recommendations to guide the program's priorities by multiple stakeholders with expertise in animal and public health. The stakeholders are the Danish Veterinary Association and the Danish Medical Association, the livestock producers, as well as other farmers' organizations, animal health organizations, food and drug regulators and researchers. These stakeholders are invited to the annual stakeholder meeting, at which the results of the surveillance activity to be released in the yearly DANMAP report are presented.

DANMAP has no formalized evaluation methodology apart from regular technical reviews of data inclusion, quality and flow. After receiving scientific guidance from several parties, the steering committee, composed by representatives from DTU Food and SSI, coordinates, describes and prioritizes the program's activities. The Danish Veterinary and Food Administration and the Danish Health Authority are the risk managers of the Danish AMR activities. Based on results from DANMAP, these agencies ultimately define and decide the priorities for the different initiatives and actions, such as, e.g., adjusting the permit limits that form part of the yellow card system for reduction of AMU in the pig sector and updating the guidelines for the management and control of highly resistant pathogens in the Danish healthcare system (24, 25).

In our evaluation, we assessed the AMU and AMR surveillance components of DANMAP, as a whole, whereas the management/execution part was only evaluated for the animal sector (Target 1.1 Formalization, as seen in Table 1).

The OH-EpiCap tool

The OH-EpiCap tool is composed of three thematic domains/dimensions, each with four targets. These are further segmented into four questions, leading to a total of 48 standardized questions,

which are used as indicators (Table 1). The 48 questions are answered using a semi-quantitative scale, ranging from 1 to 4, with 4 representing the best scenario regarding integrated OH surveillance. Respondents need to be familiar with the surveillance program of interest to answer the questions. The OH-EpiCap tool also comprises a dashboard, in which components are reported and where the average scores per target are presented in a radar diagram like the one presented in Figure 2.

TABLE 1 Dimensions and targets, each composed of four questions, evaluated by the OH-EpiCap—adapted after Tegegne et al. (19).

Dimension 1: organization			
Target 1.1 Formalization	Target 1.2 Coverage	Target 1.3 Resources	Target 1.4 Evaluation and resilience
Dimension 2: operational activities			
Target 2.1 Data collection and methods sharing	Target 2.2 Data sharing	Target 2.3 Data analysis and interpretation	Target 2.4 Communication
Dimension 3: impact			
Target 3.1 Technical outputs	Target 3.2 Collaborative added value	Target 3.3 Immediate and intermediate outcomes	Target 3.4 Ultimate outcomes

Application of the OH-EpiCap tool

The OH-EpiCap tool was last used by the authors on 18th of August 2022. Hence, the discussion reflects the questions as they were formulated and included in the OH-EpiCap version applied and the standard operating procedures of DANMAP at that time. The evaluation was conducted in two rounds, where the OH-EpiCap evaluation scheme was applied and discussed between representatives from the DANMAP management ($n = 2$), academia ($n = 2$), and the Danish livestock industry ($n = 1$). The assessors who formed part of the group provided answers based upon their work experience and views on the components of the system, Subsequently, the answers were discussed in the group and the final scores were obtained by consensus. Overall, when answering the individual questions that form part of OH-EpiCap, a conservative approach was chosen. Hence, when in doubt between two options, the lowest score was chosen to raise awareness and promote discussion around the identified target areas. The most relevant points identified in the evaluation are presented in the Result and Discussion Section.

Results and discussion

Evaluation of DANMAP using the OH-EpiCap tool

Given that integrated surveillance has been a pillar of DANMAP for more than 25 years, the program scored highly in all three

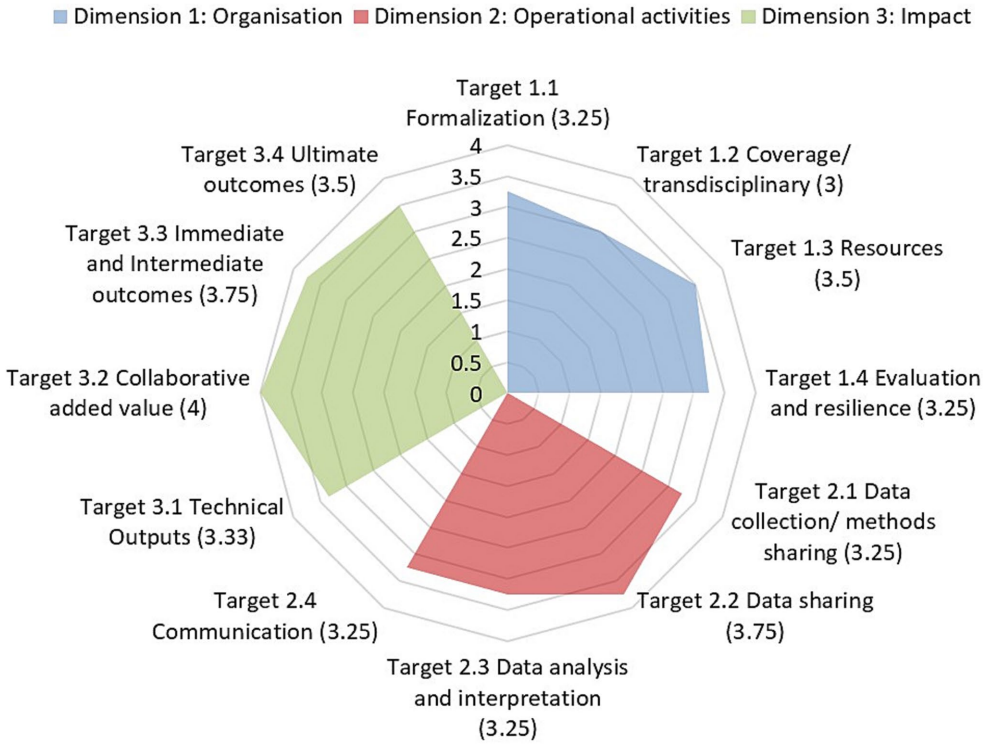

FIGURE 2
Average scores of DANMAP for the target areas covered by the OH-EpiCap tool, segmented into three dimensions. The average scores for each of the target areas are indicated in brackets. The figure was adapted from the report generated by the OH-EpiCap.

dimensions identified by OH-EpiCap, with averaging scores above 3 in all target areas. The average evaluation score of DANMAP among all questions that composed each of the target areas covered by the OH-EpiCap can be seen graphically in Figure 2.

Since a perfect level of "OH-ness" in a surveillance activity does not exist (26), changes in the DANMAP program should be carefully evaluated in relation to their added value to the program's objectives and cost effectiveness (10). Moreover, changes must be at the level at which their impacts can be understood or estimated (9) using some of the recognized metrics to evaluate the benefits of OH (27).

Dimension 1: organization

Regarding the dimension organization, DANMAP scored ≥3 in each of the following areas: formalization, coverage/transdisciplinary, resource, evaluation and resilience (Figure 2).

DANMAP operates with a clear OH integrated cross-sectoral aim, based on the views of all stakeholders. Still, the program's leadership and the steering committee do not include all sectors and stakeholders who could potentially be relevant to OH surveillance of AMR, as the environment is not represented. Therefore, other representatives in these governance structures, from the environmental sector and perhaps the livestock industry, could reinforce the OH approach. To safeguard the impartiality of the decisions and discussions, and to maintain an organization that facilitates the needed action(s), the role and operational methods of new additions to the governance structures of the program should be carefully considered before implemented.

DANMAP fully covers Denmark's territory. Regarding the populations under surveillance, AMU in the human sector is entirely covered and so are the clinical bacterial isolates tested for AMR. On the animal side, AMU is systematically monitored in all food producing and pet animal species. Moreover, AMR is monitored in food producing animals via pathogenic bacterial isolates collected from diseased animals and from caecum samples of healthy broilers, fattening pigs and bovines (calves), randomly selected at the slaughter lines. On the food side, AMR is monitored in bacterial isolates from retail meat consisting of broilers, pork and beef, of nationally and imported origin. AMR in bacteria from food-producing animals and their meat is monitored according to sampling schemes following the European Union legislation for the harmonized monitoring of AMR in zoonotic bacteria (28). Collection of more consistent information about AMU and AMR in companion animals is under development (29).

Previous national research performed around the turn of the century led to the non-inclusion of environmental data into the surveillance program, because no evidence was found to highlight the importance or necessity of such data and adjoined actions. Since then, Danish livestock production has intensified, and human hospital activity has increased. Hereby, the role of wastewater or manure for the spreading of AMR could potentially have increased. The importance of such transmission sources could be further investigated, and if judged as part of relevant exposure pathways, they could be included in the AMR surveillance in a systematic way. Moreover, the consequences of interaction between resistant bacteria originating from the human and animal components with the ubiquitous bacteria in soil could also be considered and investigated, where and if judged relevant. The necessary data, methodologies, and analyses to address this issue are currently being considered. Co-operation with the existing surveillance of diseases/infections in wild game and bi-valved mussels using the samples for analysing bacteria and their resistances might also be a cost-effective way of monitoring presence and potential for spread of AMR in the environment. This is addressed in ongoing DANMAP projects.

Over time, DANMAP has evolved, adapting to new challenges and optimizing content and processes, most of it following internal evaluations or recommendations based on expert opinions. Nonetheless, more regular evaluations could have been performed using a standardized methodology, which could probably have eased a timelier implementation of various proposed corrective measures.

Resources are shared in DANMAP, whenever relevant, but given the multi-institutional nature of the program, this is not always efficient when it comes to sharing equipment or highly trained staff. Appropriate training to manage the tasks at hand is given to the staff involved in the data management and analytical activities. Still, investment in future development and improvement of existing methods and analytical skills should be considered to maintain the high dataflow and adapt to changing methods and datatypes generated.

For the current aims of the program, economic and human resources are considered as sufficient and sustainable, as DANMAP encompasses most of the disciplines that are currently considered relevant to the OH surveillance. The program has successfully and efficiently adapted to previous critical situations, bringing in more expertise whenever needed. To consistently investigate in emerging issues and include them in the surveillance program objectives, an extension of the budget would be required, which would allow more staffing resources for investigation of relevant areas to include. This would also be the case for addition of already considered new components to the surveillance program, e.g., environmental monitoring, the continued expansion of molecular-based surveillance and its integration in the different monitored sectors and the transition to more real-time surveillance than seen at current.

Relevant supporting documentation related to DANMAP, including standard operating procedures, data collection and analytical procedures should preferably be compiled and shared at one common digital point, increasing the public accessibility to the generated results. This should also include a description of governance procedures and stakeholders' roles and responsibilities. In addition, it should be evaluated whether the program needs more visibility to increase its impact and usefulness for, e.g., antibiotic stewardship programs. This would contribute to the overall transparency of the program including improved applicability of the generated results into mitigating actions.

Dimension 2: operational activities

Regarding the dimension operational activities, DANMAP scored >3 for each of the areas that formed part of this dimension: data collection/methods, data sharing, data analysis and interpretation, and communication (Figure 2).

The design of surveillance protocols on the animal side is mainly established by the European Union requirements; when new additions to this are being considered, there is an effective collaboration between

the sectors involved. As an example, DANMAP 2022 includes for the first time, the results of Extended Spectrum Beta-Lactamase, Ampicillin Class C beta-lactamases -, and Carbapenemase-producing *E. coli* monitoring in turkey meat, as defined in the recently implemented Decision 2020/1729/EU (13). Moreover, the lines of intra- and cross-sectoral communication were improved during the COVID-19 pandemic, which demanded close collaboration, not only within the human health sector, but also with inclusion of the animal production and environmental sector. Regarding data collection protocols, only intra-sectoral collaboration is considered relevant to conduct the current activities of the program.

Laboratory techniques and procedures are coordinated between the responsible actors. Harmonization of indicators for data analysis across sectors and methodology for sampling the animal population for AMR surveillance could possibly be improved. The selection of indicators monitored in animals and humans could, perhaps, be harmonized in a more meaningful way, e.g., *Enterococcus faecalis* is currently monitored in both animals and humans, but only *Enterococcus faecium* is being whole genome sequenced and typed in samples from humans. While both species are jointly responsible for most human infections by enterococci, the proportion of vancomycin resistant invasive isolates has remained stable among *E. faecalis* but increased markedly among *E. faecium* from approximately 2% in 2012 to 14% in 2022. Whole genome sequencing also of *E. faecalis* and *E. faecium* isolates from animal populations would ensure the detection of cross-sectoral transmission. Also, in a truly all-encompassing program, healthy humans and wildlife could be regularly monitored, but this would inevitably come with multiple challenges related to budget and logistics.

When relevant, sectors share data warehouses and digital analytical tools. Joint multi-sectorial analysis could potentially be improved in the future, given that cross-sectoral data sharing agreements would be developed. Frequent and systematic evaluations of data quality are taking place and handled according to the FAIR principles, implying findability, accessibility, interoperability, and reusability.

Scientific expertise is always shared across sectors and upon request, which contributes to the overall transparency and internal/cross-sectoral communication. Data and findings are shared among sectors whenever this is found relevant by the actors. Still, despite the publication of data in the annual report and upon request, communication of findings to the political level could be evaluated and improved for sustained political attention and support. Making data more accessible to the stakeholders by developing dissemination platforms that are fast and easy to consult and understand is on the agenda. Even though not in real-time, the steering committee is informed in a timely manner about the emergence of possible hazards, but translation into action takes time.

Dimension 3: impact

Regarding the dimension impact, DANMAP scored >3 for each of the areas that form part of this dimension: technical outputs, collaborative added value immediate and intermediate outcomes, and ultimate outcomes (Figure 2).

The program follows a clear national (15) and European OH strategy (30). The steering committee and coordinating actors from the livestock and human sectors are actively involved in the public communication of results, with the annual release of the national report on AMU and AMR and many scientific publications, which frequently involve multi-sectoral national and internationally established collaborations (3, 4). Given the dimension of the entire DANMAP program and the multiple actors it involves, there are no clear figures regarding the full operational costs of the program.

Integrated surveillance has been the foundation of DANMAP from the very beginning. Therefore, questions in OH-EpiCap regarding the added value of adapting to a OH response were not considered relevant in our evaluation. Given the more than 25 years of the program, its impact on epidemiological knowledge of AMR is clear (16). DANMAP has guided sector-specific interventions and policy changes, which have highly contributed to achieve the goals established in the national action plan. One example is the current use of AM in the Danish pig sector, which shows almost no use of 3rd and 4th generation cephalosporines and fluoroquinolones (16). In Denmark, there is a strong will for working collaboratively, which may be one additional explanation for why the OH networks function well in Denmark, and the level of awareness among the stakeholders is very high, even if translation into action could be further improved.

Using OH-EpiCap tool to evaluate integrated AMR surveillance systems

The OH-EpiCap tool facilitates a quick assessment of certain essential components in OH collaboration that could lead to possible reforms, as has been experienced in Denmark. The tool allowed the actors involved in DANMAP to pinpoint certain components of the program where there is room for improvement to increase the OH-ness of the system. Nevertheless, a more detailed and precise analysis should be conducted to complement the evaluation provided by OH-EpiCap. Some of the identified components have already been previously recognized by program's management. Still, discussing these issues again, considering recent technological and scientific advancements was considered a positive and valuable experience.

Stakeholders from distinct backgrounds, with diverging perceptions and expectations can be involved in the evaluation processes of a surveillance system using the OH-EpiCap tool. To reduce the possible bias and the overall subjectivity of the evaluation, a consensus among respondents is required to select a final answer (19). The simple and efficient design of the OH-EpiCap makes it a user-friendly addition to the field of existing tools and frameworks to evaluate AMR surveillance (31). However, when assessing complex systems such as DANMAP, in relation to a topic as broad as OH, one should be mindful about the aspects and activities thereof that are not being evaluated (26).

As stated in the Introduction, a full review of OH-EpiCap is presented in a separate paper reporting from nine case studies, all using the CoEvalAMR user's perspective methodology (22, 32) focusing on the functional aspects and content themes as well as a SWOT-like analysis to provide feedback on the application of the OH-EpiCap (21).

Conclusion

The process related to evaluating DANMAP using the OH-EpiCap tool provided a good overview of the program's degree

of OH-ness. Moreover, it facilitated the identification of components that could potentially be further improved or included in future reforms, to possibly increase the integrated nature of the program, such as environmental AMR surveillance components Therefore, the authors recognize the value of using OH-EpiCap to initiate an evaluation of the OH-ness of national AMR surveillance programs. Still, as OH-EpiCap is mainly providing an overview, feasibility, requirements and relevance of any additional activities in a program should be considered carefully before implementation. The OH-EpiCap tool not suitable for the technical evaluation of a surveillance program, i.e., sample sizes, matrices etc.

It was concluded that DANMAP demonstrates a high level of adherence to the OH concept, covering relevant aspects for humans and animals, whereas environmental aspects are missing. If it is judged that incorporation of environmental sampling would be valuable, budgetary implications should be foreseen and handled.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

PM drafted the paper with inputs from all authors. LA, MS, and US conducted the evaluation. All authors contributed to the article and approved the submitted version.

Funding

This study was partly funded by the Danish Food Safety Authorities and the CoEvalAMR (https://coevalamr.fp7-risksur.eu/).

Acknowledgments

The authors acknowledge Henok Ayalew Tegegne, Carlijn Bogaardt, Lucie Collineau, Géraldine Cazeau, Renaud Lailler, Johana Reinhardt, Emma Taylor, Joaquin M. Prada, Viviane Hénaux for the development of the OH-EpiCap and for making it available to conduct this work.

Conflict of interest

LA works for an organization that gives advice to farmers and meat-producing companies.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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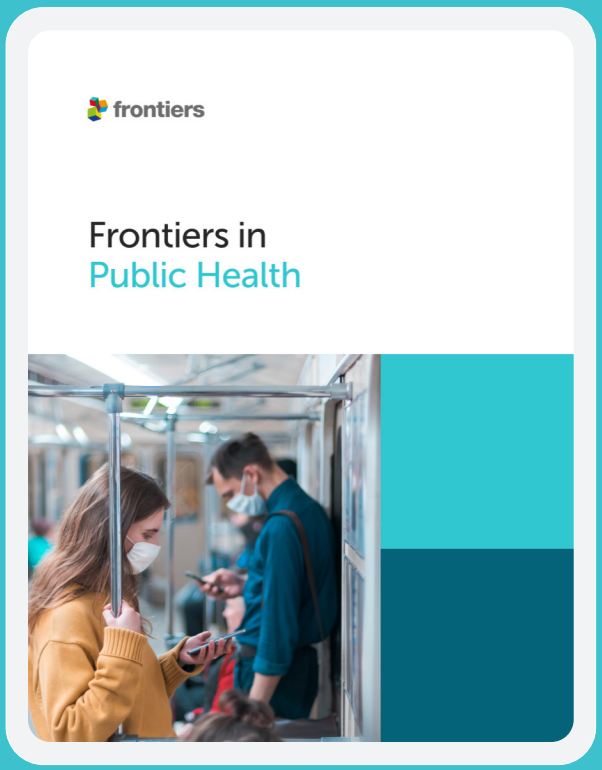

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