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Bridging science and policy for animal health surveillance: ICAHS4

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Bridging science and policy for animal health surveillance: ICAHS4 2022

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Table of contents

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Editorial: Bridging science and policy for animal health surveillance: ICAHS4 2022

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surveillance, methodology, animal health, food safety, decision-making, policy

Editorial on the Research Topic
Bridging science and policy for animal health surveillance: ICAHS4 2022

The ICAHS4 conference took place in Copenhagen, Denmark, in May 2022, 2 years later than planned due to the COVID-19 pandemic. The conference provided an opportunity for meetings, learning and sharing between all stakeholders involved in surveillance and control of animal health and food safety issues, across sectors such as government, academia and livestock industries.

The COVID-19 pandemic has taught the society the importance of surveillance and early detection of infectious diseases. We have learnt that it is insufficient simply to act on disease emergence and spread. Instead, focus is needed on prevention, surveillance, and early detection of the precursors of emerging infectious disease. Since budgets are limited and the challenges plenty, it is a necessity to collaborate across sectors—academia, governments, industry and the public—in a transdisciplinary way.

Globalization has created a situation where animals are transported across long distances to ensure economic productivity, and foods are traded internationally to keep prices low for consumers. The downside is that with movements of people and goods, hazards may also travel unnoticed, leading to unwanted events. The ongoing spread of African swine fever (ASF) shows the challenges of risk mitigation not only in domestic animals, but also in wildlife. The culling of all mink in Denmark in November 2020 due to fear of spreading of COVID-19 virus resulted in thousands of livestock producers suddenly faced with their life's work disappearing. In addition, the development of antimicrobial resistance (AMR) and the spread of zoonotic pathogens from one part of the world to another demonstrate that the challenges in veterinary public health are global.

To combat these threats, veterinary authorities are under increasing pressure to effectively allocate resources for animal health surveillance and associated risk mitigation; therefore, it is critical to understand why, where and which actions are needed to prevent new threats to animal and public health. Additionally, socio-economic factors influence how actions taken by authorities or livestock industries are perceived by the public. Lack of public involvement may lead to poor understanding and lack of support; for example, vandalism of fences erected in forests to stop ASF from spreading in wild boar. The way forward demands dynamic solutions. Prioritization and feasibility will differ between countries, dependent on local context as well as economic and social values. Therefore, we require global, transdisciplinary collaboration to mitigate global threats, and it is critical to learn from each other to achieve successful prevention, control and mitigation.

This Research Topic contains a selection of the work presented at the ICAHS4 conference, covering the latest experiences in novel research within surveillance for animal health and food safety and security. The intention was to inspire the development of new ways of collaboration; for example, through Public-Private-Partnerships, and interdisciplinary or transdisciplinary approaches. Many novel collaboration models were demonstrated at the conference which allowed participants to learn from each other regarding implementation in practice. Such alternative governance models may lead to cost-effective and successful collaborations.

The areas covered include:

- Surveillance for epidemics and emerging diseases.
- Cross-sector and One Health surveillance.
- Translating surveillance outcomes into policy, decisions and actions.
- Surveillance data.
- Integrating novel methods in surveillance.

The Research Topic consists of 16 original contributions: nine original research articles (including one methods article), four brief research reports, two perspective contributions and one mini-review. The contributions report work undertaken in Denmark, Italy, Lithuania, the Netherlands, Scotland, Spain, Sweden, Thailand, The United States of America, or by international institutions like FAO and WOAH as well as international networks.

Four papers investigate surveillance for epidemics and emerging diseases. Gao et al. focused on the role of empty livestock vehicles returning to Denmark after exports of pigs for the introduction of ASF. Analyses of strengths, weaknesses, opportunities, and threats (SWOT) were conducted related to export of livestock and in particular, of pigs. It was concluded that washing and disinfection, as required and undertaken at the designated stations, are the most important among all risk-reducing measures identified. Hinjoy et al. studied risk perceptions regarding avian influenza among poultry farmers and traders in three border provinces of Thailand adjacent to Laos. According to the 346 respondents' answers, experience in poultry farming was associated with greater risk perception. Regular training could be a way to improve risk perception, and experienced poultry farmers and traders could be part of a community mentorship program to share their experiences and knowledge on avian influenza. Žigaitė et al. evaluated the passive surveillance of SARS-CoV-2 in mink farms in Lithuania. The results showed a prevalence of 23% viral RNA-positive mink farms, and that 84% of the mink farms had been exposed to the virus. The widespread exposure of mink farms to SARS-CoV-2 suggests that passive surveillance is ineffective for early detection of SARS-CoV-2 in mink. Arede et al. described surveillance activities for anthrax, brucellosis, Crimean Congo hemorrhagic fever, foot-and-mouth disease, lumpy skin disease, and peste des petits ruminants that are present or threaten to emerge in the region Black Sea Basin, which consists of Armenia, Azerbaijan, Belarus, Bulgaria, Georgia, Moldova, Romania, Türkiye, and Ukraine. It was concluded that there is a need for stronger international partnerships and resources to strengthen veterinary health capacity, protect animal health and improve ruminant production.

Two papers research cross-sector and One Health surveillance. Moya et al. explored government veterinarians' perception of routine biosecurity in livestock production systems in Spain. The respondents stressed the limited availability of staff and time. The veterinarians interviewed considered that farmers only implement biosecurity measures to avoid being sanctioned, and not because they are aware of the importance of biosecurity. Alban et al. reported from an international network called CoEvalAMR, which is developing guidelines for selection of tools for evaluation of integrated AMU and AMR. Moreover, evaluation tools are systematically assessed using a methodology with a focus on user's experience. Hereby, tool users can share their experience, assisting other users in identifying the most suited tool for their evaluation purpose.

Two papers explore ways of translating surveillance outcomes into policy, decisions, and actions. de Vos et al. described a rapid incursion risk assessment tool for multiple livestock diseases, including the main sources for incursion, and the changes in risk over time. The tool calculates a semi-quantitative risk score for the incursion risk of each disease, and the results enable prioritization. Scollo et al. reported a semi-quantitative risk assessment methodology, developed to classify Italian pig farms in terms of the probability of introduction of ASF, based on farm data collection. The estimation of frequency and levels of non-compliance with biosecurity measures was used to identify weak points in risk prevention at farm level.

Four papers investigate analysis of surveillance data. Schrag et al. explored a method of benchmarking AMU use in the context of farm-level therapeutic incidence (a proxy for disease incidence), and the outcome of that therapy. Reporting AMU in this format addresses multiple primary questions on recording of disease and AMU, necessary for evaluating on farm antimicrobial stewardship in sufficient details. Keck et al. presented the "Assessment Tool for Laboratories and AMR Surveillance Systems" (FAO-ATLASS), which consists of a surveillance and a laboratory assessment module. FAO-ATLASS allows national authorities to systematically assess their AMR surveillance system in food and agriculture and implement a strategic stepwise approach to improve their systems. Marrana et al. reviewed the Laboratory Twinning Programme created in 2006 by the World Organization for Animal Health (WOAH), to balance the global distribution of veterinary laboratory expertise. The review shows that there has been broad uptake and diversity in the focus of the twinning projects implemented in WOAH Member Countries. The programme would benefit from an evaluation that looks at its outcomes and quantifiable impact in beneficiary countries. Comin et al. raised the question of whether meat inspection data can be used for animal health and welfare surveillance. The results covering

Swedish pigs and beef cattle showed that some findings are consistently detected and other less. Moreover, calibration and training activities are necessary to enable correct conclusions and for producers to experience an equivalent likelihood of deduction in payment.

Four papers integrate novel methods in surveillance. Moura et al. described the Vet-AMNet system, which was recently developed to collect and analyze national AMU data in Portuguese dairy farms. Outputs were generated by the Portuguese system using Dutch AMU data. The Vet-AMNet system was validated by comparing these outputs with the Dutch result. Duncan et al. evaluated the functionality of the Scottish Animal Disease Surveillance Center. In this recent evaluation, they developed a new denominator using a combination of agricultural census and movement data, to identify relevant holdings more accurately. This provides information that could help policy makers and surveillance providers make decisions about service provision, as well as evaluate the impact of future changes. Dórea et al. discussed how to design analytical workflows focused on decision support. They conclude that the value of data-driven surveillance depends on a "needs-driven" design approach to data digitalization and information delivery. Finally, Gustafsson et al. described the Swedish National Veterinary Institute's workflows and visualization for epidemiological analysis and dynamic report generation to improve disease surveillance. The workflows are designed to be flexible and adaptable to changing data sources and stakeholder demands, with the goal to create a robust infrastructure for the delivery of actionable epidemiological information.

Author contributions

LA: Conceptualization, Writing—original draft, Writing—review and editing. VB: Writing—review and editing. FD: Writing—review and editing. CS-L: Writing—review and editing. SD: Writing—review and editing.

Conflict of interest

LA works for an organization that gives advice to the Danish farmers and meat industry.

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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Rapid risk assessment tool (RRAT) to prioritize emerging and re-emerging livestock diseases for risk management

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Increasing globalization and international trade contribute to rapid expansion of animal and human diseases. Hence, preparedness is warranted to prevent outbreaks of emerging and re-emerging diseases or detect outbreaks in an early stage. We developed a rapid risk assessment tool (RRAT) to inform risk managers on the incursion risk of multiple livestock diseases, about the main sources for incursion and the change of risk over time. RRAT was built as a relational database to link data on disease outbreaks worldwide, on introduction routes and on disease-specific parameters. The tool was parameterized to assess the incursion risk of 10 livestock diseases for the Netherlands by three introduction routes: legal trade in live animals, legal trade of animal products, and animal products illegally carried by air travelers. RRAT calculates a semi-quantitative risk score for the incursion risk of each disease, the results of which allow for prioritization. Results based on the years 2016-2018 indicated that the legal introduction routes had the highest incursion risk for bovine tuberculosis, whereas the illegal route posed the highest risk for classical swine fever. The overall incursion risk *via* the illegal route was lower than *via* the legal routes. The incursion risk of African swine fever increased over the period considered, whereas the risk of equine infectious anemia decreased. The variation in the incursion risk over time illustrates the need to update the risk estimates on a regular basis. RRAT has been designed such that the risk assessment can be automatically updated when new data becomes available. For diseases with high-risk scores, model results can be analyzed in more detail to see which countries and trade flows contribute most to the risk, the results of which can be used to design risk-based surveillance. RRAT thus provides a multitude of information to evaluate the incursion risk of livestock diseases at different levels of detail. To give risk managers access to all results of RRAT, an online visualization tool was built.

KEYWORDS

incursion risk, animal trade, animal products, travelers, livestock diseases, risk ranking, Netherlands

Introduction

Increasing globalization and international trade contribute to rapid expansion of animal and human diseases. Introduction of animal diseases into naive livestock populations can result in large-scale epidemics with serious economic and socio-ethical impact. Illustrative examples include the introduction of foot and mouth disease (FMD) in the United Kingdom in 2001 (1) and subsequent spread to the Netherlands (2), the introduction of bluetongue (BT) in the Netherlands in 2006 with subsequent spread to neighboring countries (3, 4), and the introduction of African swine fever (ASF) into Georgia in 2007 (5). ASF subsequently spread into Europe and Asia (6), and in 2021 the disease was also introduced into the Americas (7). Recent incursions of diseases that had not been reported before in Europe, such as lumpy skin disease (LSD) in South-Eastern Europe in 2014-2017 and peste des petits ruminants (PPR) in Bulgaria in 2018 highlight the continuous threat of emerging and re-emerging disease outbreaks (7–9). Preparedness is thus warranted to prevent outbreaks of livestock diseases in new territories or to detect outbreaks in an early stage. Risk assessment is a useful tool to inform risk managers on the incursion risk of livestock diseases that can not only provide information on the magnitude of the risk, but also on the main sources of risk and the change of risk over time.

Most introduction risk assessments performed over the last decades focused on a single disease and a single introduction route [e.g., (10–15)] and were initiated to address specific risk questions. Those risk questions often arise in response to new disease events to evaluate the increased incursion risk from such an event. In recent years, several generic risk assessment tools were developed that accommodate multiple diseases and/or introduction routes (16). An important asset of these generic risk assessment tools is the ability to prioritize diseases or risk regions for their incursion risk, enabling the targeted use of limited resources for prevention and surveillance. The repeated use of these tools to inform risk managers is, however, limited, either because the tools do not have an underlying database and need to be populated with data before each use, or because expert opinion is needed to update results. One of the earliest prioritization tools was developed by Roberts et al. (17) for the United Kingdom. This tool integrates expert opinion with data on disease outbreaks and trade into a semi-quantitative risk score for each disease. Updates are performed manually, and expert opinion is key to keep the tool up and running. An automatic update of data and calculations when new data becomes available would facilitate repeated use of generic risk assessment tools.

The incursion risk of a livestock disease is largely determined by the distribution of the pathogen in the world and the connections of a disease-free territory with these regions. These connections are the so-called introduction routes and can either be trade in livestock or their products, trade in exotic animals, migrating wildlife, movements of people if the disease is zoonotic, or introduction of vectors if the disease is vector-borne. Data on the worldwide distribution of animal diseases and on the volume of introduction routes is largely available from global databases such as WAHIS (World Animal Health Information System) (7), EMPRES-i (Global Animal Disease Information System) (18), Comtrade (19) and Comext (20). Integration of this data is mostly done by disease experts leaving it a labor-intensive and subjective exercise to evaluate the incursion risk. The increased accessibility and interoperability of most of these global databases has opened the door to a more automated risk assessment approach. To fully exploit the available data, we developed a rapid risk assessment tool (RRAT) that combines the data from global databases into an automated estimate of the incursion risk for multiple livestock diseases. The main objective of this tool is to support risk managers in prioritizing diseases for risk management. Furthermore, RRAT can indicate high risk trade flows and source countries, the results of which can be used for risk-based surveillance. In this paper, RRAT is described and results for the incursion risk for the Netherlands are presented and discussed.

Materials and methods

RRAT has been built as a relational database in R (21) and SQLite (22) with the main tables in the tool describing: the worldwide occurrence of animal diseases; the volumes of the introduction routes; and disease-specific parameters to assess the risk of each introduction route. RRAT is a semi-quantitative risk assessment tool that provides the user with a risk score for the probability that a specific disease enters a new region or country ("target area") and will result in a first infection of local livestock animals. Introduction routes considered in RRAT comprise the legal trade in live animals ("animal route"), the legal trade of animal products ("product route"), and animal products illegally carried by air travelers ("traveler route"). The introduction routes are all subdivided into multiple pathways to account for diversity in animal species and animal products. Up till now, RRAT has been parameterized for 10 diseases that are considered a potential threat to the Netherlands, viz. African horse sickness (AHS), ASF, Aujeszky's disease (Auj), BT, bovine tuberculosis (bTB), classical swine fever (CSF), equine infectious anemia (EIA), foot and mouth disease (FMD), LSD, and PPR (Table 1). Calculations have been performed for the years 2016, 2017 and 2018 with the Netherlands as target area.

Calculations in RRAT are based on the Binomial process considering (1) the number of animals or products entering the target area, (2) the probability that an individual animal or product is infected, and (3) the probability that entry of an infected animal or product results in a first infection of local

TABLE 1 Overview of causing pathogens, reservoir livestock hosts and main transmission routes of ten diseases in RRAT.

Disease	Pathogen[a]	Reservoir livestock host	Main transmission route
African horse sickness	AHS virus (*Orbivirus*, *Reoviridae*)	Horses	Biological vector (*Culicoides* spp.)
African swine fever	ASF virus (*Asfivirus*, *Asfarviridae*)	Pigs	Direct and indirect contact, swill feeding, biological vector (*Ornithodorus* spp.)
Aujeszky's disease	Suid herpesvirus 1 (*Varicellovirus*, *Herpesviridae*)	Pigs	Direct and indirect contact, venereal transmission, swill feeding
Bluetongue	BT virus (*Orbivirus*, *Reoviridae*)	Bovines, sheep, goats	Biological vector (*Culicoides* spp.)
Bovine tuberculosis	*Mycobacterium bovis*[b]	Bovines, pigs, goats	Direct contact, respiratory transmission, ingestion of raw meat and milk
Classical swine fever	CSF virus (*Pestivirus*, *Flaviviridae*)	Pigs	Direct and indirect contact, venereal and congenital transmission, swill feeding
Equine infectious anemia	EIA virus (*Lentivirus*, *Retroviridae*)	Horses	Mechanical vectors (*Tabanidae* family, *Stomoxys calcitrans*)
Foot and mouth disease	FMD virus (*Aphthovirus*, *Picornaviridae*)	Bovines, pigs, sheep, goats	Direct and indirect contact, airborne transmission, swill feeding
Lumpy skin disease	LSD virus (*Capripoxvirus*, *Poxviridae*)	Bovines	Mechanical vectors (mosquitoes, biting flies, *Culicoides* spp., hard ticks), venereal and congenital transmission
Peste des petits ruminants	PPR virus (*Morbillivirus*, *Paramyxoviridae*)	Sheep, goats	Direct and indirect contact

[a]Genus and family of pathogen given between brackets.
[b]Some outbreaks of bovine tuberculosis are caused by *M. caprae*.

animals. An overview of the model parameters in RRAT is given in Table 2.

The overall risk score R_P for a target area by a single introduction route *i* is calculated as:

$$R_{P\,i} = 1 - \prod_{C=1}^{c} \prod_{P=1}^{p} \prod_{D=1}^{d} \left(1 - P_{entry_{CPD}} \times P_{est_{PD}}\right)^{N_{CP}} \quad (1)$$

where N_{CP} is the number of pathway units (animals for livestock, pets and exotic mammals; batches for poultry, exotic birds and germplasm; kg for animal products) of pathway *P* that enters the target area from source country *C* in the time period considered, $P_{entry_{CPD}}$ is the probability of entry of disease *D* from source country *C* by pathway *P*, and $P_{est_{PD}}$ is the probability that entry of disease *D* by pathway *P* results in a first local infection (establishment) in the target area. Although the overall risk score of RRAT is calculated as the probability of a successful introduction of any disease in the tool, it cannot be interpreted as such, because input into the tool is partly based on proxy values that were assigned to risk classes, rather than strictly quantitative data derived from e.g., scientific literature or animal experiments. Proxy values were defined as approximate values that represent the—sometimes unknown, and mostly uncertain – actual values of input parameters. The risk score is thus a semi-quantitative score, that can be calculated at different levels, e.g., per disease, source country or pathway, and can as such be used to rank diseases, source countries and pathways for their incursion risk.

Due to its asymptotic nature, the overall risk score R_P is not discriminating if its value approaches one for multiple diseases, source countries or pathways. Therefore, a second risk score indicating the number of successful introductions, R_N, is calculated as:

$$R_{N\,i} = \sum_{C=1}^{c} \sum_{P=1}^{p} \sum_{D=1}^{d} N_{CP} \times P_{entry_{CPD}} \times P_{est_{PD}} \quad (2)$$

Again, although being calculated as the number of successful introductions of any disease in the tool, this risk score cannot be interpreted as such given its semi-quantitative nature.

Trade of live animals

Data on the numbers and batches of animals traded to the Netherlands from each source country was derived from TRACES (23) (Supplementary Table S1.1). RRAT not only considers the trade in livestock, but also trade in equines, dogs, cats, and exotic mammals and birds. Animals were grouped based on species and destination (for life or for slaughter). This resulted in a total of 38 animal species groups (pathways) considered for this introduction route.

TABLE 2 Overview of model parameters in RRAT.

Parameter	Description	Introduction route	Reference
N_{CP}	Number of pathway units of pathway *P* from source country *C*	Animal, Product, Traveler	Supplementary Tables S1.1; S1.2; S1.3
$P_{entry_{CPD}}$	Probability of entry of disease *D* from source country *C* by pathway *P*	Animal, Product, Traveler	Eq. 3; Eq. 6
$P_{est_{PD}}$	Probability that entry of disease *D* by pathway *P* results in a first local infection (establishment)	Animal, Product, Traveler	Eq. 5; Eq. 8
Inc_{CD}	Incidence of disease *D* in source country *C*	Animal	(58)
Inc_{abs_D}	Proxy value to estimate incidence of disease *D* for countries where disease is absent from domestic livestock (risk class 1, 2 or 3, Figure 3)	Animal	Supplementary Table S3.2
Inc_{unk_D}	Proxy value to estimate incidence of disease *D* for countries where presence of disease is unknown (risk class 5 or 6, Figure 3)	Animal	Supplementary Table S3.2
P_{infsus_P}	Susceptibility-class dependent probability of infection of animal species *P*	Animal	Supplementary Table S3.1
T_{inf_D}	Average infectious period of disease *D* in reservoir hosts	Animal	Supplementary Table S3.2
$P_{inf_{CPD}}$	Probability of animal species *P* from country *C* being infected with disease *D*	Animal, Product	Eq. 4
$P_{det_{CPD}}$	Probability of animal species *P* infected with disease *D* being detected before transport in country *C*	Animal	(31–38)
$P_{contact_P}$	Probability that an imported infected animal of animal species *P* comes into contact with susceptible livestock	Animal	Supplementary Table S3.3
$P_{trans_{PD}}$	Probability that an infected animal of animal species *P* will transmit disease *D* if in contact with susceptible livestock	Animal	Supplementary Table S3.1
$P_{infan_{CPD}}$	Probability that product *P* from country *C* is derived from an animal infected with disease *D*	Product, Traveler	Eq. 7; Eq. 10
$P_{cont_{PD}}$	Probability that product *P* is contaminated with disease D	Product	Supplementary Table S3.5
P_{detsl_D}	Probability of detection of infection with disease *D* at slaughter	Product	Supplementary Table S3.4
P_{exp_P}	Probability that a local animal is exposed to product *P*	Product	Supplementary Table S3.6
$P_{contex_{PD}}$	Probability that product *P* is contaminated with disease *D* at exposure to a local animal	Product	Supplementary Tables S3.7; S3.8; S3.10
$P_{infex_{PD}}$	Probability of infection of product *P* with disease *D* upon exposure to a local animal	Product	Supplementary Table S3.8
Nt_C	Number of travelers arriving in the Netherlands from source country *C*	Traveler	(40)
Pt_C	Fraction of travelers carrying products of animal origin when arriving from source country *C*	Traveler	(41–50)
RP_{CP}	Probability that an animal product carried by a traveler arriving from source country *C* is of product type *P*	Traveler	(41–50, 52); Supplementary Table S2.3
W_{CP}	Average weight (kg) of product type *P* carried per traveler arriving from source country *C*	Traveler	(52); Supplementary Table S2.3;
P_{hm_P}	Proportion of "homemade" product *P*	Traveler	(53)

For each disease in RRAT, the animal species groups were assigned a susceptibility class based on information derived from factsheets and scientific literature (24–30). Five susceptibility classes were used: (1) reservoir host, (2) spill-over host possibly contributing to transmission (3) host in which only experimental infections have been described, (4) dead-end host, and (5) not susceptible (Supplementary Tables S2.1, S3.1).

To estimate the probability of entry ($P_{entry_{CPD}}$), two main parameters were used: (1) the probability of an individual animal being infected ($P_{inf_{CPD}}$), and (2) the probability of an infected animal not being detected before transport ($1-P_{det_{CPD}}$) (Figure 1). $P_{entry_{CPD}}$ was calculated as:

$$P_{entry_{CPD}} = P_{inf_{CPD}} \times \left(1 - P_{det_{CPD}}\right) \quad (3)$$

$P_{inf_{CPD}}$ was estimated using data on disease incidence in the source countries and disease-specific parameters. $P_{inf_{CPD}}$ was calculated as:

$$P_{inf_{CPD}} = Inc_{CD} \times T_{inf_D} \times P_{infsus_P} \quad (4)$$

where Inc_{CD} is the incidence of disease *D* in source country *C*, P_{infsus_P} is a proxy value to account for the probability of infection of animal species *P* with disease *D* dependent on its susceptibility class, and T_{inf_D} is a proxy value to account for the average infectious period of disease *D* in reservoir hosts. The calculation of Inc_{CD} was based on all cases reported to the OIE in a one-year period (see Section "Disease incidence" for more details). However, for most diseases, animals are only

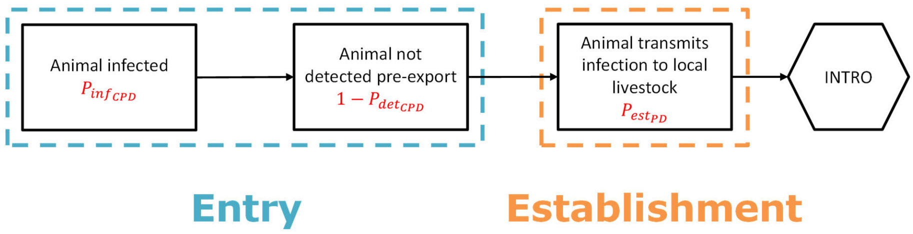

FIGURE 1
Scenario tree outlining the steps to assess the probability of entry and first infection for the legal trade in live animals ("animal route").

infectious for a relatively short period. Therefore, T_{inf_D} was used to correct for the average infectious period of infected animals, using four classes and accompanying proxy values ($T_{inf_D} = 0.05$ if infectious period $<$ 2 weeks; $T_{inf_D} = 0.1$ if infectious period $>$ 2 weeks and $<$ 1 month; $T_{inf_D} = 0.25$ if infectious period $>$ 1 month and $<$ 1 year; $T_{inf_D} = 1$ if infectious period $>$ 1 year) (Supplementary Table S3.2). As Inc_{CD} was based on reported cases in reservoir livestock hosts only, P_{infsus_P} was used to correct for the expected incidence of disease in non-reservoir hosts with the value of P_{infsus_P} dependent on the animal's susceptibility class (Supplementary Table S3.1).

$P_{det_{CPD}}$ was estimated using data on European legislation regarding both intracommunity trade (between European Union (EU) member states) and importation of animals from non-EU countries (31–38). Legal requirements such as clinical inspection, quarantine and testing, or importations from disease-free regions only were listed per disease, pathway (animal species) and source country. Individual measures were rated with a score between 0 and 1 for their effectiveness using information on e.g., length of the incubation period, severity of clinical signs, and test sensitivity. If $>$ 1 measure was in force, $P_{det_{CPD}}$ was set equal to the effectiveness of the most effective measure.

The probability of a first infection ($P_{est_{PD}}$) was estimated considering the infectiousness and the destination of the imported animals. $P_{est_{PD}}$ was calculated as:

$$P_{est_{PD}} = P_{contact_P} \times P_{trans_{PD}} \tag{5}$$

where $P_{contact_P}$ is a proxy value to account for the probability that the imported infected animal comes into contact with susceptible livestock in the target area, and $P_{trans_{PD}}$ is a proxy value to account for the probability that the infected animal will transmit the disease if in contact with susceptible livestock. The value of $P_{contact_P}$ depends on the destination of the imported animal (Supplementary Table S3.3). The value of $P_{trans_{PD}}$ depends on the susceptibility class of the imported animal (Supplementry Table S3.1). Although the infection can be carried by dead-end hosts, they do not contribute to transmission of the disease and will as such not result in a successful introduction.

Trade of animal products

Data on the import of live animal products (germplasm and hatching eggs) and manure was derived from TRACES (23), because TRACES provided most detail on the animal species from which these products were derived. Imports of germplasm (semen, embryos and ova) were, however, only available at batch level. Data on the import of other animal products was derived from Comext (20) (Supplementary Table S1.2). Animal products were assigned to animal product groups considering product type (meat, casings, milk and dairy products, eggs and egg products, hides, feathers and down, etc.), treatment (fresh, frozen, dried, salted, heated, etc.) and the animal species from which the product was derived (bovines, pigs, sheep, goats, equines, poultry, etc.). This resulted in a total of 139 pathways considered for this introduction route. For presentation purposes, results were aggregated in 16 summarizing product groups (Supplementary Table S2.2).

To estimate the probability of entry ($P_{entry_{CPD}}$), two main parameters were used: (1) the probability that the product is derived from an infected animal ($P_{infan_{CPD}}$), and (2) the probability that the product itself is contaminated ($P_{cont_{PD}}$) (Figure 2). $P_{entry_{CPD}}$ was calculated as:

$$P_{entry_{CPD}} = P_{infan_{CPD}} \times P_{cont_{PD}} \tag{6}$$

$P_{infan_{CPD}}$ was estimated taking into account the infection probability of the animal from which the product was derived in the source country ($P_{inf_{CPD}}$) and the probability of detection of the infection in the animal at ante-mortem or post-mortem inspection in the slaughterhouse (P_{detsl_D}, Supplementary Table S3.4). The latter only applied to products derived from slaughtered animals such as meat and hides, but not to products derived from live animals such as milk, eggs, germplasm and manure (Supplementray Table S2.2). To retrieve the value of $P_{infan_{CPD}}$, the animal species from which the products were derived were linked to the 38 animal species groups used for the introduction route of live animal imports. A worst-case approach was applied here, i.e., if a product could have been derived from $>$ 1 animal species, it was linked to all relevant animal species groups and given the value of $P_{inf_{CPD}}$ of

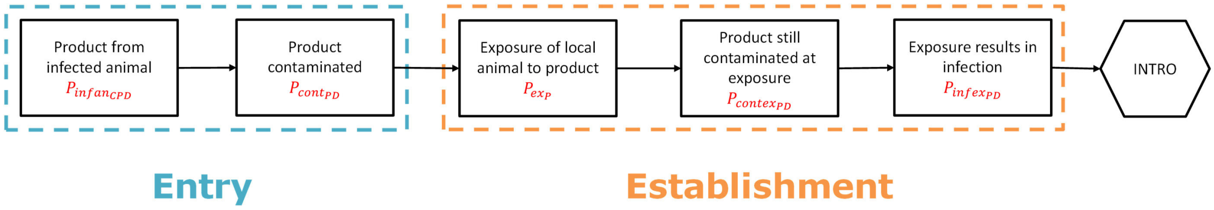

FIGURE 2
Scenario tree outlining the steps to assess the probability of entry and first infection for the legal trade of animal products including germplasm ("product route").

the animal species group that was most susceptible to the disease. $P_{infan_{CPD}}$ was calculated as:

$$P_{infan_{CPD}} = P_{inf_{CPD}} \times \left(1 - P_{detsl_D}\right) \tag{7}$$

$P_{cont_{PD}}$ was based on data derived from factsheets and scientific literature and entered into RRAT as an absence/presence score (Supplementary Figure S3.1, Supplementary Table S3.5).

To estimate the probability of first infection ($P_{est_{PD}}$), three parameters were used: (1) the probability that a local animal is exposed to the product in the target area (P_{exp}), the probability that the product still contains viable pathogen when local animals are exposed to it ($P_{contexp_{PD}}$), and (3) the probability that such exposure results in infection ($P_{infexp_{PD}}$) (Figure 2). $P_{est_{PD}}$ was calculated as:

$$P_{est_{PD}} = P_{exp} \times P_{contexp_{PD}} \times P_{infexp_{PD}} \tag{8}$$

P_{exp} is given by a proxy value accounting for the probability that the imported product will end up with local livestock animals and was made dependent on the intended use of the product (Supplementary Table S3.6). $P_{contexp_{PD}}$ depends on the survival time of the pathogen in the product and the average time it will take for the product to reach local animals, which is dependent on e.g., shipping time and shelf life. The latter is difficult to estimate and will probably be quite long for most products. $P_{contexp_{PD}}$ was therefore based on risk classes accounting for survival time and products were assigned to a risk class based on reported survival time in factsheets and literature (Supplementary Table S3.7, Supplementary Figure S3.1). The risk class was reduced by one level if import of the product from infected territories was subjected to import restrictions according to OIE (25) or EU legislation (39). Each risk class was given a probability score on a $\log_{10}$ scale to obtain proxy values for $P_{contexp_{PD}}$ (Supplementary Table S3.8). $P_{infexp_{PD}}$ was also given a proxy value based on risk classes, using the same $\log_{10}$ scale as for $P_{contexp_{PD}}$. The risk classes for this parameter were assigned considering the most likely exposure route to the pathogen dependent on its intended use (Supplementary Table S3.6). The probability that exposure results in infection depends on the exposure route and is disease specific, with some diseases more readily transmitted by e.g., aerosols whereas others are more readily transmitted by oral ingestion. The risk class assigned to each exposure route was therefore made disease-dependent (Supplementary Table S3.9).

Animal products carried by air travelers

No database was available to directly input the amount of animal products carried by air travelers into RRAT. To estimate the volume of this introduction route, data on air passenger transport between the main airports of the Netherlands and their main partner airports (40) was combined with data from Great Britain on seizures of animal products (both meat and dairy products) (41–50) and input from scientific literature. Carrying animal products for own use into the Netherlands is illegal only if imported from non-EU countries. Therefore, customs do not search luggage of people traveling within the EU and hence no data was available to estimate the flow of products coming from EU member states. The incursion risk by this introduction route was thus evaluated for non-EU countries only.

Seizures of animal products were classified according to the type of product (meat; dairy; eggs), the animal species of which the product was derived (bovines; pigs; sheep; goats; poultry; buffalo; bushmeat), and the treatment of meat (fresh and frozen; dried and salted; heated). This resulted in a total of 21 pathways considered for this introduction route (Supplementary Table S2.3). No equine products were considered for the traveler route, and hence the incursion risk of the equine diseases AHS and EIA was not estimated for this introduction route. Data on seizures of animal products was not available at country level, but for 14 geographical regions comprising multiple countries (41) (Supplementary Figure S3.2). In RRAT, each source country was assigned to one of those 14 regions to extract the corresponding values from the database and calculations were performed at country level. Results of this introduction route are, however, presented at regional level, matching the lowest spatial resolution in the data.

The amount of animal products carried by people traveling to the Netherlands (N_{CP}) was calculated as:

$$N_{CP} = Nt_C \times Pt_C \times RP_{CP} \times W_{CP} \quad (9)$$

where Nt_C is the number of air travelers arriving in the Netherlands from source country C during a one-year period, Pt_C is the fraction of travelers carrying products of animal origin when arriving from source country C, RP_{CP} is the fraction of products carried by travelers from source country C that is of product type P, and W_{CP} is the average weight (kg) of product type P carried per traveler arriving from source country C. Note that values for Pt_C, RP_{CP} and W_{CP} were only available at regional level (Supplementary Table S2.3). The calculated amounts are given in Supplementary Table S1.3.

The number of air travelers (Nt_C) was derived from the Eurostat database table *avia_par_nl*, where the transport measurement (tra_meas) was *passengers carried – arrivals* (PAS_CRD_ARR) (40). This table reports on all passengers on a specific flight (with a single flight number) that terminate their journey at the reporting airport. Therefore, it was assumed that all passengers would have the Netherlands as destination (no transit passengers included). For journeys including multiple flights, the airport of embarkation was not known, resulting in an underestimate of the number of travelers arriving from non-EU countries.

Very little information was available to estimate the fraction of air travelers carrying products of animal origin (Pt_C) and most estimates from literature were biased, i.e., passenger checks were risk-based, likely resulting in an overestimate of the probability that travelers will carry animal products. In RRAT, Pt_C was set to 15.5% based on estimates from Great Britain that 63.8% of the travelers that carry products of animal origin bring meat (43–50), and that 9.9% of all travelers bring meat (42). The value of 15.5% was used for all source regions. The RRAT is, however, flexible to include source region-specific values for this parameter. The probability that an animal product carried by a traveler arriving from source country C is of product type P (RP_{CP}) was based on the proportion of seizures per product type from travelers arriving from the 14 different regions. Estimates for the proportions of meat from bovines, pigs, small ruminants, buffaloes and bushmeat were derived from VLA (42). These were complemented with data from Defra (43–50) to estimate the ratio of meat to dairy products. This ratio varied widely between regions with dairy constituting only 5% of seizures from Southern Africa and as much as 60% of seizures from Southern Asia (Supplementary Table S2.3). Estimates for the proportion of poultry in total meat were derived from scientific literature, with several publications reporting proportions of approximately 40% (51, 52). Eggs and egg products were estimated to be only 1% of animal products carried by travelers (51–55). Reported average weights per seizure (W_{CP}) are mostly between 2 and 4 kg (42, 51, 53, 54, 56, 57), although seizures of bushmeat tend to have a higher weight than seizures of livestock meat (52, 57). In RRAT, region-based weights to estimate W_{CP} were derived from a study from Switzerland (52). No detail was available to estimate the weights for the different product types. The RRAT is, however, flexible to include product type-specific values for this parameter if new data would become available.

The calculations to estimate the probability of entry ($P_{entry_{CPD}}$) and establishment ($P_{est_{PD}}$) of pathogens via animal products carried by travelers were analogous to the calculations for the product route. Input parameters were derived by connecting each of the 21 pathways of the traveler route to one of the 139 pathways of the product route that had similar characteristics with respect to product type, treatment and animal species. Because it was assumed that part of the products carried by travelers were derived from animals slaughtered at home, an additional parameter (P_{hmp}) was introduced to account for the fact that detection of infected animals at the slaughterhouse was less likely for products carried by travelers than for legally imported products. P_{hmp} is the proportion of products carried by travelers that is "homemade" and its value was set to 0.29 for all product types derived from dead livestock animals (53), and to 1 for bushmeat. The RRAT is, however, flexible to include product type-specific values for this parameter. The probability that the product carried by a traveler was derived from an infected animal ($P_{infan_{CPD}}$) was therefore calculated as:

$$P_{infan_{CPD}} = P_{inf_{CPD}} \times \left(1 - P_{detsl_D} \times \left(1 - P_{hmp}\right)\right) \quad (10)$$

Products carried by travelers were assumed to have the same intended use as legally imported products, i.e., the probability of exposure to local animals is equal for both introduction routes. However, products carried by travelers escape import controls and therefore cannot be assessed for compliance with OIE standards or EU legislation. Therefore, the input values for the probability that the product still contains viable pathogen when local animals are exposed to it ($P_{contex_{PD}}$) were separately estimated for this introduction route (Supplementary Table S3.10, Supplementary Figure S3.1).

Disease incidence

Data on disease presence in the world was based on annual reports of individual countries in WAHIS (58). These reports were obtained using web scraping, because the data was not downloadable at the time RRAT was built. A decision tree was used to assign each country to a risk class considering the information the country had provided in the annual report to the OIE (Figure 3), and the reported disease incidence by countries in the same UN subregion (59). The decision tree distinguishes three main groups of countries: those that reported presence of disease in either wildlife or domestic animals (upper branch),

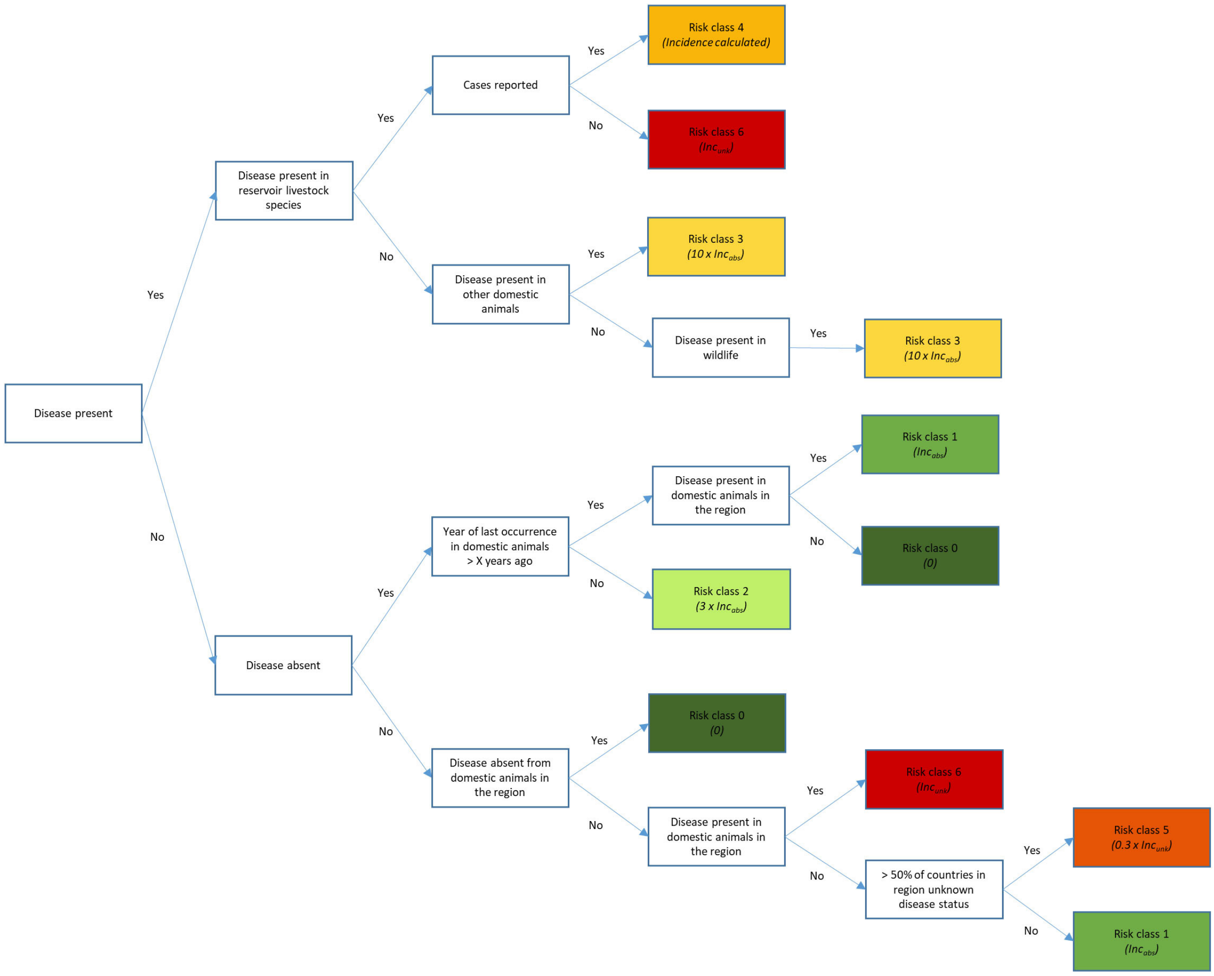

FIGURE 3
Decision tree to assign countries to one out of seven risk classes regarding disease incidence, considering the information available in the OIE annual reports (7).

those that reported absence of disease (middle branch), and those that had not provided any information on the disease for the year considered (lower branch). Only for countries that had reported cases to the OIE (risk class 4), the incidence of disease *D* in source country *C* (Inc_{CD}) could be calculated by dividing the number of cases of disease *D* in livestock reservoir hosts in source country *C* (58) by the population of affected livestock reservoir hosts for disease *D* present in source country *C* (58, 60). For EU member states, the OIE data was complemented with data from the Animal Disease Information System (ADIS) (61) and reports from the European Commission (EC) on diseases in bovines and swine (62–64). These sources only provided the number of outbreaks, not the number of cases. The number of outbreaks was therefore multiplied with the median number of cases per outbreak as reported by WAHIS (58) (Supplementary Table S3.2) to arrive at an estimate of the number of cases in order to calculate Inc_{CD}. If no data was available on the number of cases at all, a proxy value was used for Inc_{CD} based on the assigned risk class for disease presence. If a country had reported absence of disease, the year of last occurrence was considered for the risk classification, where we assumed that real absence was more likely if the disease had not been reported for a longer period (X years, where X was disease-dependent, Supplementary Table S3.2) and if the disease was not present in the UN subregion either. If no information was available on the disease status of a country, information on disease occurrence in the UN subregion was used to assign this country to a risk class. Only for countries assigned to risk class 0, Inc_{CD} was set to 0 as we deemed presence of the disease in those countries extremely unlikely. For the countries assigned to other risk classes, proxy values were used for Inc_{CD} that were either based on Inc_{abs_D} or Inc_{unk_D}. Inc_{abs_D} equaled an incidence 100 times lower than the minimum incidence for disease *D* calculated for countries in risk class 4 (disease present and cases reported), whereas Inc_{unk_D} equaled the maximum incidence calculated for disease D for countries in risk class 4 (Supplementary Table S3.2).

Baseline scenario

In the baseline scenario, model input as described above was used to estimate the incursion risk of 10 livestock diseases (AHS, ASF, Auj, BT, bTB, CSF, EIA, FMD, LSD, PPR) for the Netherlands for the years 2016, 2017, and 2018. Model calculations in RRAT are deterministic resulting in a point estimate for each output parameter. Main output parameters considered were the risk scores for individual diseases ($R_{Pi,D}$) for each introduction route per year, and the contribution of source countries and pathways to these estimated risk scores.

Sensitivity analysis

Sensitivity analysis was performed to explore the impact of assumptions and input parameters on the results of RRAT. Three main areas of input uncertainty were investigated: (1) the incidence of disease in source countries, (2) the use of proxy values to estimate probabilities, and (3) the databases used to derive the volume of animals and animal products for the animal and traveler pathway, respectively (Table 3). A total of 12 alternative scenarios was run and results were compared to the baseline scenario for the overall risk score R_{Ni} (Eq. 2) per introduction route. Considering that the main objective of RRAT is to prioritize diseases for risk management, changes in the overall risk score might be of less concern than changes in ranking of diseases, source countries, or pathways with respect to their incursion risk. To analyze the impact of uncertainty on ranking, the risk scores for individual diseases ($R_{Ni,D}$) and individual source countries ($R_{Ni,C}$) were ranked for both the baseline scenario and the alternative scenarios and compared using Spearman's rank correlation coefficient. The number-based risk score R_N was used for the sensitivity analysis rather than the probability-based risk score R_P, because differences between scenarios cannot be observed for high probability-based scores due to the asymptotic nature of R_P.

Results

Baseline scenario

Model calculations returned a risk score ($R_{Pi,D}$) for each disease and each introduction route in RRAT for the Netherlands for the years 2016-2018 (Figure 4). The overall risk was highest for bTB with risk scores approaching 1 for both the animal route and the product route. The incursion risk of AHS, LSD and PPR, on the other hand, was very low for all introduction routes. Trade in live animals also posed a risk for EIA incursion, although the risk decreased over the years considered, and – to a much lesser extent – for BT incursion (Figure 4A). Despite the threatening ASF situation in Europe in the period 2016-2018, the probability of ASF incursion by the animal route was very low, because no live pigs were imported from infected countries. The relatively low incursion risk of most diseases for the animal route is explained by the fact that livestock animals were almost exclusively imported from European countries in which most of the diseases considered were reported absent. Trade of animal products entailed an incursion risk for a larger number of diseases than trade in live animals, since products were imported from a much wider geographical range including sometimes infected areas. The highest incursion risks for the product route were observed for bTB, Auj, BT and FMD. The incursion risk for ASF had increased tremendously in 2018 if compared to previous years which is explained by the expansion of ASF-infected territories in 2018, both in Europe and South-East Asia (6). Calculated risk scores for the traveler route were much lower than for the product route. Diseases most likely introduced *via* the traveler route were CSF, ASF, FMD and bTB. Although travelers are not allowed to carry animal products from outside the EU, products were carried from all over the world including regions from which legal import of products is restricted. This resulted in a different ranking of diseases for the traveler route than the product route.

As the RRAT calculates individual risk scores for each disease, pathway and source country, results can be explored in more detail to elucidate the countries and/or pathways contributing most to the incursion risk for a specific disease. Figure 5A shows the incursion risk of bovine tuberculosis per source country for the animal route and indicates that the incursion risk mainly originated from Ireland, Poland, Belgium, the United Kingdom and Spain. This was either related to a high incidence of bTB in those countries, high numbers of bovines imported from those countries (mainly veal calves), or both. Remarkably, there was also a risk of introducing bTB by trade of animals originating from Chile. This was related to the importation of camelids (lamas and vicunas). Horses entering the Netherlands more frequently originated from countries outside Europe than livestock animals. This is reflected by the countries contributing most to the incursion risk of EIA, not only being Bulgaria and Italy, but also the United States of America (Figure 5B).

The incursion risk due to legal trade of animal products was explored in more detail for bTB, Auj, BT, FMD, and ASF. Whereas the incursion risk of bTB *via* the product route was high for multiple countries, with Ireland, United Kingdom, Spain, Belgium, Italy and China contributing most to the risk, the incursion risk of the other diseases was largely due to importations from a few countries only (Figure 6). The countries

TABLE 3 Alternative scenarios explored in the sensitivity analysis to evaluate the impact of uncertain input parameters on the results of RRAT.

No.	Scenario	Parameter	Baseline value	New value	References
	Incidence of disease				
1A	Regions	Regions used to assign countries to risk classes for disease incidence	UN subregions	Adjusted UN subregions	(59, 76); Supplementary Figure S4.1
1B	Minimum incidence	Proxy value to estimate disease incidence for risk classes 1, 2 and 3 (Inc_{abs_D})	Value 100 times less than minimum incidence calculated for countries in risk class 4	Value equal to minimum incidence calculated for countries in risk class 4	Supplementary Table S3.2; S4.1
1C	Maximum incidence	Proxy value to estimate disease incidence for risk classes 5 and 6 (Inc_{unk_D})	Value equal to maximum incidence calculated for countries in risk class 4	Value of 0.1 or 0.3 dependent on disease characteristics such as incubation period, transmission rate, and clinical signs	Supplementary Table S3.2; Supplementary Table S4.1
1D	Scaling factor for risk classes	Multiplication factor to calculate disease incidence for risk classes 2, 3 and 5	risk class 2 = 3 × Inc_{abs_D}; risk class 3 = 10 × Inc_{abs_D}; risk class 5 = 0.3 × Inc_{unk_D}	risk class 2 = 10 × Inc_{abs_D}; risk class 3 = 100 × Inc_{abs_D}; risk class 5 = 0.1 × Inc_{unk_D}	Figure 3
1E	Underreporting	Underreporting factor	No underreporting assumed	Inclusion of an underreporting factor of 2.5 or 4 to calculate disease prevalence for countries in risk class 4; value dependent on disease characteristics such as incubation period, transmission rate, and clinical signs	(42, 76); Supplementary Table S4.1
	Proxy values				
2A	Probability infection	Probability of infection (P_{infsus_P}) for non-reservoir hosts	10^{-2} for spill over hosts; 10^{-3} for experimental hosts; 10^{-2} for dead end hosts	10^{-3} for spill over hosts; 10^{-4} for experimental hosts; 10^{-3} for dead end hosts	Supplementary Table S3.1
2B	Probability transmission	Probability of transmission ($P_{trans_{PD}}$) for non-reservoir hosts	0.3 for spill over hosts; 0.1 for experimental hosts	0.1 for spill over hosts; 0.03 for experimental hosts	Supplementary Table S3.1
2C	Probability contact with susceptible livestock	Probability of contact ($P_{contact_P}$) for all destinations but reservoir hosts going to livestock farms	10^{-1} for household, trade, approved body or livestock farm if non-reservoir host; 10^{-2} for slaughterhouse	10^{-2} for household, trade, approved body or livestock farm if non-reservoir host; 10^{-3} for slaughterhouse	Supplementary Table S3.3
2D	Probability product contaminated at exposure	Proxy value for the risk classes for the probability of contamination at exposure ($P_{contex_{PD}}$)	high = 1; moderate = 0.1; low = 0.01; very low = 0.001	high = 1; moderate = 0.3; low = 0.1; very low = 0.03	Supplementary Table S3.8
2E	Probability infection upon exposure	Proxy value for the risk classes for the probability of infection upon exposure ($P_{infex_{PD}}$)	high = 1; moderate = 0.1; low = 0.01; very low = 0.001	high = 1; moderate = 0.3; low = 0.1; very low = 0.03	Supplementary Table S3.8
	Databases				
3A	Eurostat (animals)	Number of imported live animals (N_{CP})	Data from TRACES	Data from Comext	(20, 23)
3B	PAS_BRD_ARR (travelers)	Number of travelers (N_{CP})	Data filtered for PAS_CRD_ARR (passengers carried – arrivals) in Eurostat database *avia_par_nl*	Data filtered for PAS_BRD_ARR (passengers on board – arrivals) in Eurostat database *avia_par_nl*	(40)

contributing most to the incursion risk for Auj were Italy, Bulgaria and Romania; for BT it was the USA; for FMD it were Pakistan, Thailand and South Korea; and for ASF it was Romania. Importations of meat products (fresh and frozen) contributed most to the incursion risk for Auj and ASF, whereas for bTB and FMD importations of milk and dairy products constituted a high incursion risk. The incursion risk of BT was mainly related to the import of products

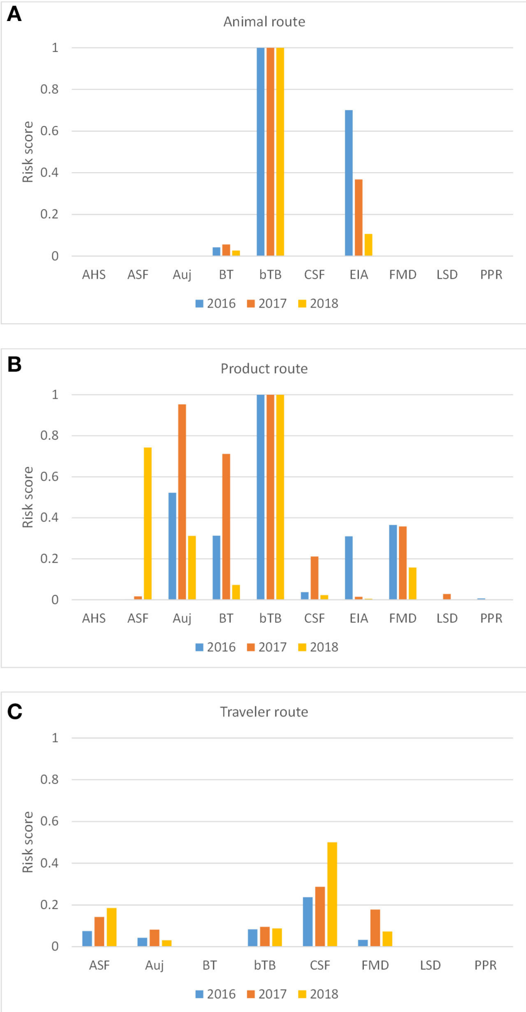

FIGURE 4
Probability-based risk score for the incursion risk of 10 diseases for the Netherlands in 2016, 2017, and 2018 for the animal route **(A)**, the product route **(B)**, and the traveler route **(C)**. The incursion risk of AHS and EIA were not considered for the traveler route. Diseases: AHS, African horse sickness; ASF, African swine fever; Auj, Aujeszky's disease; BT, bluetongue; bTB, bovine tuberculosis; CSF, classical swine fever; EIA, equine infectious anemia; FMD, foot-and-mouth disease; LSD, lumpy skin disease; PPR, peste des petits ruminants.

for pharmaceutical use (containing blood-derived products) (Figure 7A). It is noteworthy that import of litter and manure contributed considerably to the incursion risk of bTB and that import of hides contributed considerably to the incursion risk of FMD. Litter and manure were imported mainly from Belgium and Germany in large quantities ($>2 \times 10^3$ tons annually). This combined with bTB reported in Belgium resulted in a non-negligible incursion risk, albeit the probability of bTB infection of local animals upon entry of manure ($P_{est_{PD}}$) was low. Hides were imported from all over the world with FMD-infected countries such as Thailand, India, Pakistan and South Korea

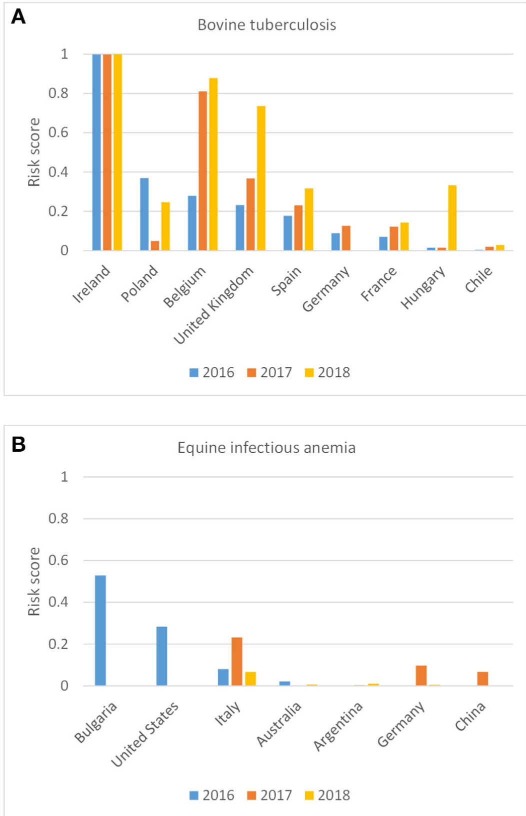

FIGURE 5
Probability-based risk score for the incursion risk of bovine tuberculosis **(A)** and equine infectious anemia **(B)** for the Netherlands in 2016, 2017, and 2018 for the animal route per source country (only source countries included with a risk score > 0.01 in any year).

being main suppliers from outside the EU. Although this contributed to the incursion risk of FMD, the overall incursion risk by hides was scored as low.

Figure 8 shows the incursion risk due to travelers per source region for CSF, ASF, FMD, and bTB. CSF was most likely introduced from the Caribbean and Eastern Asia, although a steep increase in the incursion risk from Eastern Europe was observed in 2018. A similar risk profile was observed for ASF, although Western Africa was also a risk region for incursion of ASF *via* the traveler route. Overall risk scores for ASF were, however, lower than for CSF. For FMD, Eastern Asia and the Near and Middle East were the most likely source regions. bTB was most likely to be introduced from Northern Africa and the Near and Middle East. The incursion risk of CSF and ASF was completely related to carriage of pig meat, with fresh and frozen meat contributing approximately 90% to the risk and dried and salted meat approximately 10%. The incursion risk of FMD and bTB was related to both meat and dairy products, with dairy contributing approximately 50–60% of the risk (Figure 7B).

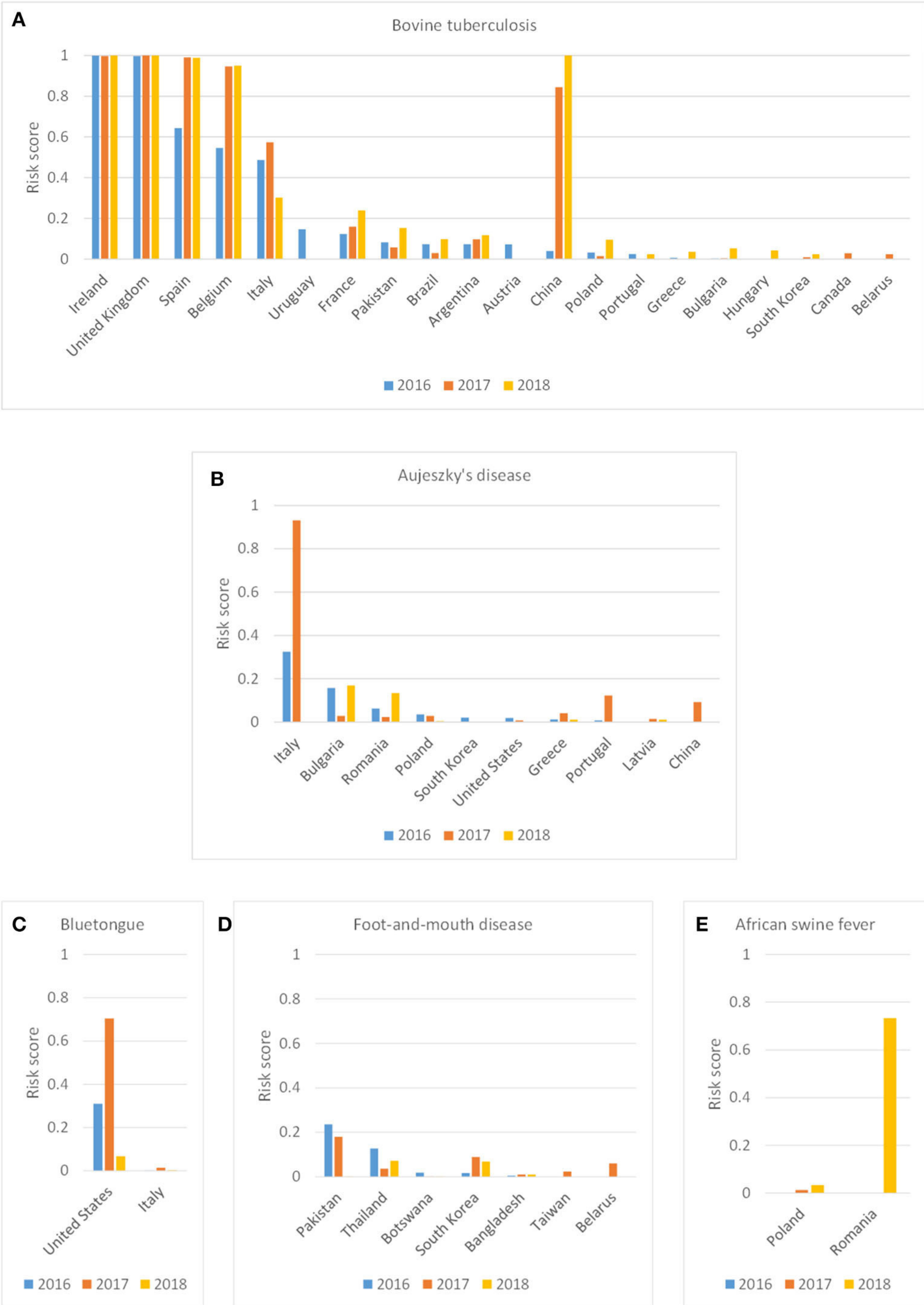

FIGURE 6
Probability-based risk score for the incursion risk of bovine tuberculosis **(A)**, Aujeszky's disease **(B)**, bluetongue **(C)**, foot-and-mouth disease **(D)** and African swine fever **(E)** for the Netherlands in 2016, 2017, and 2018 for the product route per source country (only source countries included with a risk score > 0.01 in any year).

Sensitivity analysis

Results of the alternative scenarios (Table 3) were compared to the baseline scenario for the overall risk score R_{Ni}, which indicates the incursion risk of any of the diseases in RRAT to the Netherlands for each of the introduction routes i for the years 2016, 2017 and 2018. The number-based risk score was used rather than the probability-based risk score,

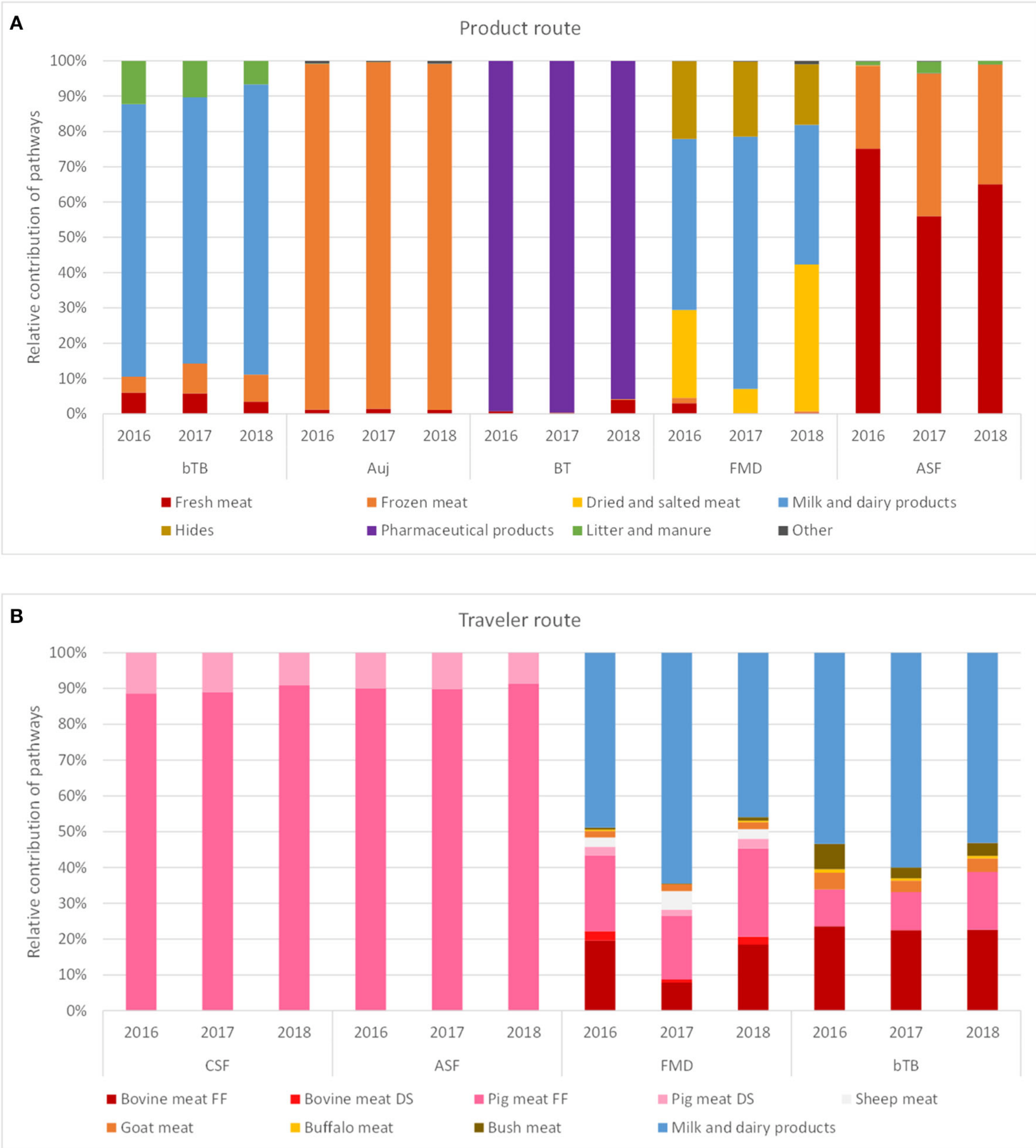

FIGURE 7
Contribution of different pathways to the incursion risk of selected diseases for the Netherlands in 2016, 2017, and 2018 for the product route **(A)** and the traveler route **(B)**. Diseases: bTB, bovine tuberculosis; Auj, Aujeszky's disease; BT, bluetongue; FMD, foot-and-mouth disease; ASF, African swine fever; CSF, classical swine fever. Products: FF, fresh and frozen meat; DS, dried and salted meat.

as the probability-based risk has an asymptote at 1, making a comparison of results useless. The results of the animal route were most sensitive to the database used for the trade figures (Scenario 3A) with the use of Comext data (20) resulting in a 10-fold higher overall risk score (Figure 9). The other introduction routes were not affected by this scenario, since only the database for trade in live animals was changed. Scenario 1C affected the overall risk score most (Figure 9). In this scenario the value for Inc_{unk_D} was increased 10- to 100-fold, resulting in a similar increase for the overall risk score of the product and traveler routes. The impact on the animal route was less pronounced, because imports of live animals mostly originated from source countries for which disease was absent or the incidence was known (i.e., Inc_{unk_D} was not needed to estimate disease incidence for these countries). Scenario 1E also affected the overall risk score of all three introduction routes, although

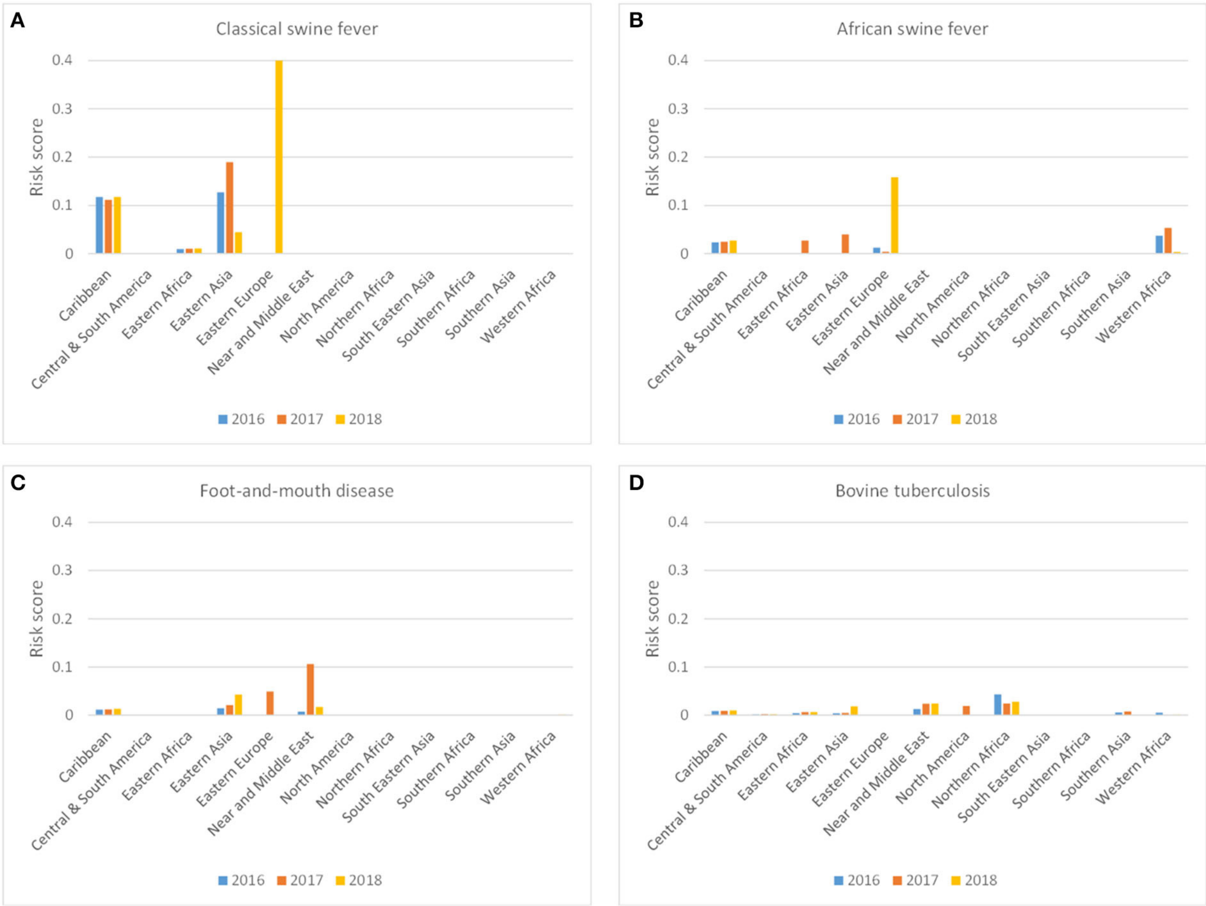

FIGURE 8
Probability-based risk score for the incursion risk of classical swine fever **(A)**, African swine fever **(B)**, foot-and-mouth disease **(C)** and bovine tuberculosis **(D)** for the Netherlands in 2016, 2017, and 2018 for the traveler route per source region.

to a lesser extent. In this scenario, an underreporting factor was included to estimate disease incidence for countries that had reported cases to the OIE, resulting in higher incidence estimates for these countries. Scenarios 2D (proxy values for probability of contamination of a product at exposure) and 2E (proxy values for probability of infection upon exposure to a contaminated product) resulted in an increased overall risk score for the product and traveler routes. This was not unexpected as higher proxy values were used in the alternative scenarios. These scenarios did not affect the animal route. All other scenarios had limited effect on the calculated overall risk scores.

Changes in ranking of diseases and source countries (source regions for the traveler route) when running the alternative scenarios were evaluated using Spearman's rank correlation coefficient. Figure 10 shows the correlation coefficients between the baseline and the alternative scenarios for the source countries/regions (x-axis) and the diseases (y-axis). Correlation coefficients for the product route were all > 0.9, indicating that changes in ranking were limited. For the animal route, only scenario 3A (trade figures based on Comext database) resulted in considerable changes of the ranking of both source countries and diseases for all 3 years evaluated. For the traveler route, results were slightly less stable than for the other two routes, but only scenario 1C (higher value for Inc_{unk_D}) resulted in considerable changes of the ranking of both source countries and diseases for all 3 years evaluated. The relative sensitivity of this route to the value of Inc_{unk_D} is explained from the fact that travelers could come from any country in the world, including those countries with an unknown disease status, whereas imports of live animals and animal products were mostly limited to countries with a known disease status, although not exclusively.

Discussion

Interpretation of results

RRAT is a useful tool to assess the incursion risk of multiple diseases and results can be used to prioritize diseases for risk management and early warning. RRAT provides a multitude of

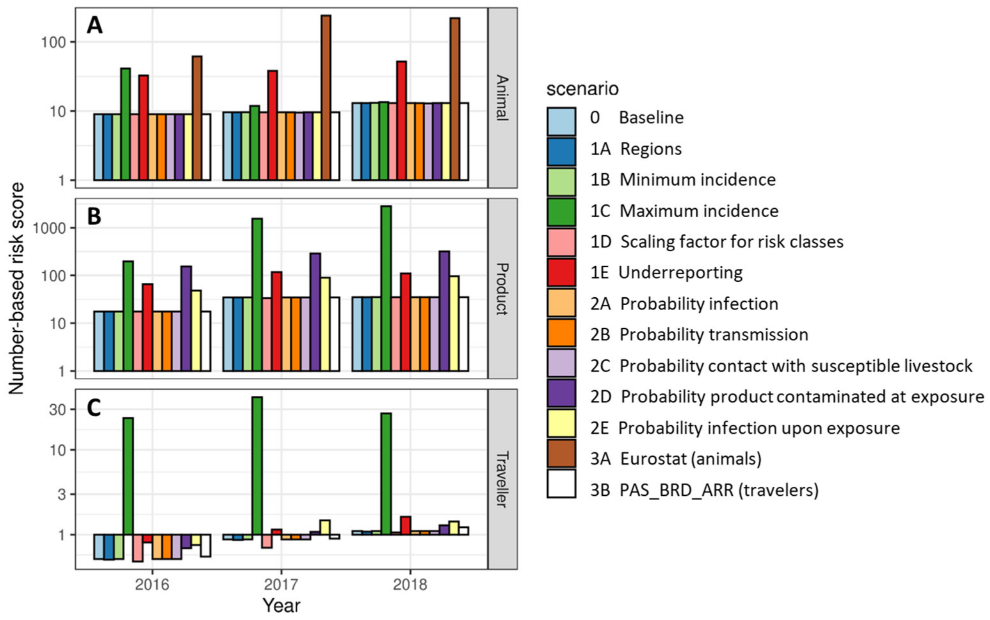

FIGURE 9
Number-based risk scores for the incursion risk of any disease for the Netherlands in 2016, 2017, and 2018 for the animal route **(A)**, the product route **(B)**, and the traveler route **(C)** for the baseline scenario and each alternative scenario. Risk scores are given on a $\log_{10}$ scale.

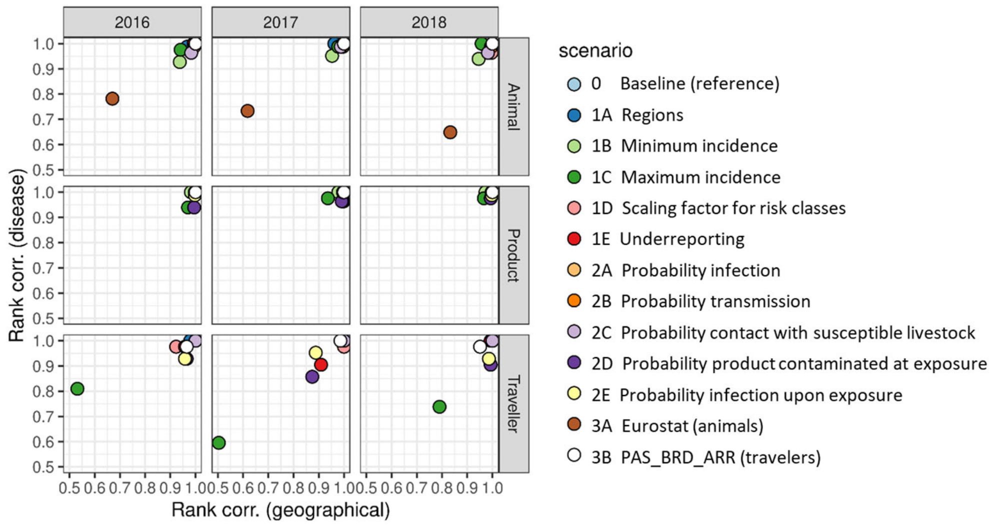

FIGURE 10
Spearman's rank correlation coefficients indicating the agreement in ranking of risk scores for individual source countries[a] (x-axis, "geographical") and individual diseases (y-axis, "disease") between the baseline scenario and each alternative scenario. [a] Ranking for the traveler route was based on source regions.

information to evaluate the incursion risk of livestock diseases at different levels of detail. Results of the tool can be queried to indicate the pathways (animal species or product types) and source countries/regions contributing most to the risk (as shown in Figures 5–8). This information is useful input for the design of risk-based surveillance. To give risk managers access to all results of RRAT, an online visualization tool was built (https://shiny.wur.nl/content/941b9565-64d1-490c-b11b-d5f2cc45c44e/).

RRAT was built such that it can be automatically updated when new data becomes available. Input data from WAHIS (58), TRACES (23) and Comext (20) are automatically processed into risk scores for the diseases included in the tool. Adding a new disease to the tool is relatively easy as it only requires an update of disease-specific parameters. Adapting the tool to assess the risk for other target areas (countries) is also relatively easy, since this only requires an update of the volumes of trade and travel. The tool does, however, not provide the full remit of the incursion risk as introduction routes related to e.g., vector and wildlife ecology are not considered. This might explain the relatively low risk calculated for ASF despite the presence of ASF virus in Europe. Inclusion of additional introduction routes will increase the accuracy of the estimated incursion risk. Counotte et al. (65) designed a complementary module for RRAT using a generic approach to assess the animal disease incursion risk *via* wildlife migration. They showed that also the incursion risk of ASF *via* migration of wild boar is very low for the Netherlands given the relatively large geographical distance between reported outbreaks in wild boar and the Dutch border for the years analyzed. Results of the wildlife module can, however, not be directly compared to the results obtained for the introduction routes in RRAT, because the wildlife module only estimates the probability of entry of infected wild boar into the Netherlands and not the subsequent exposure of local livestock. It must be noted that also the results of the introduction routes in RRAT itself cannot be compared directly, as the risk estimates for the animal route are based on individual animals, whereas the risk estimates for the product and traveler route are based on kilograms of product. This might have resulted in a slight overestimate of the incursion risk by the product and traveler route, since from a single slaughtered animal more than one kilogram of product is harvested. There is no easy way to account for this in the risk assessment tool, as it is not known whether imported animal products are mostly derived from the same animals or from different animals, i.e., 10 kg of pork could have been derived from the same pig or from 10 pigs or even more. The ratio between slaughtered animals and the weight of imported products will probably also differ for different animal product types and source regions. To guide policy makers in interpreting the results of RRAT, we translated the semi-quantitative risk scores into qualitative risk levels. When doing so, we accounted for the fact that animal products will mostly present a lower incursion risk than live animals (Supplementary Table S5.1,S5.2). The qualitative risk levels were used to present results in the online visualization tool.

In contrast to the animal route, results of the product and traveler route can to some extent be compared, as both are based on kilograms of products. From Figure 4 it is clear that calculated risk scores for the traveler route are much lower than for the product route. This is mainly explained from the volumes for both introduction routes, with the quantities of products carried by travelers being approximately 10^3 times less than the quantities imported legally (Supplementary Table S1.2,S1.3). On the other hand, products carried by travelers have a more diverse geographic origin and are not subjected to import controls, resulting in a potentially higher incursion risk per kilogram of product. The incursion risk *via* the product route might have been slightly overestimated by RRAT, as we had quite some uncertainty on the animal origin of products not intended for human consumption (e.g., casings, hides, products for pharmaceutical use). Most CN codes (combined nomenclature) (66) for these products represent composite groups and a worst-case approach was used considering all products a risk when these were derived from at least one susceptible domestic livestock species. Although products not intended for human consumption only made up about 10% of the total legal import flows, they had a very high contribution to the incursion risk of BT (Figure 7). In contrast to the product route, the incursion risk of the traveler route was based only on animal products for human consumption. The incursion risk *via* this route has definitely been underestimated by RRAT. We only included products carried by air passengers from outside the EU, since no data was available on products carried by travelers within the EU, because bringing products of animal origin from other EU member states is not illegal and thus not checked at customs. In addition, the incursion risk *via* animal products carried by travelers over land (train, bus, car) is not considered in RRAT.

Validation of results

Validation of the results of RRAT is difficult as the tool estimates the incursion risk of diseases that are not introduced into the Netherlands regularly. The only exception is bTB for which 23 introductions occurred in the period 1999-2013 by trade in live animals (67). RRAT indeed indicated that trade in live animals entails a high risk of bTB introduction, indicating to a large extent the same source countries as high risk as the study of De Vos et al. (67). The estimated EIA incursion risk by legal trade in live animals was very high for 2016 and decreased in the years after. In 2017, the first (and until now only) case of EIA in the Netherlands was detected by serology, the moment of introduction of the infection being unknown (68). The estimated ASF incursion risk was relatively low, although a steep increase of the incursion risk by the product route was seen for 2018. Despite the increasing threat of ASF in Europe in recent years, at the time of writing (June 2022), ASF was absent from the Netherlands. The most likely introduction route for ASF, based on results of RRAT, is *via* legal trade of animal products. Although it cannot be excluded that contaminated pork products have been imported in recent years, this has not resulted in disease outbreaks. The probability that contaminated pork products end up with pigs is expected to be very low, as swill feeding is not allowed in the EU (69). The results of RRAT can also be partly validated by comparing the results of RRAT to those of bespoke RA models, although one should keep in mind that the risk estimates given by RRAT are semi-quantitative risk scores rather than absolute numbers. The incursion risk of AHS was, e.g., estimated to be very low by RRAT (Figure 4), which is in agreement with a quantitative risk assessment for movements of live equines by De Vos et al. (11).

RRAT was cross-validated against other generic risk assessment tools that recently were developed in Europe by applying all tools to the same case study on ASF (16). Results indicated that the generic tools largely agreed on the relative risks across countries and scenarios, resulting in the same ranking. RRAT was primarily designed for prioritization purposes, the ranking of diseases, source countries and pathways being thus the most important output of the tool. Therefore, the cross-validation contributed to the credibility of results obtained with RRAT. In addition, results for the years 2016-2018 were face validated by risk assessors and risk managers and any unexpected results were queried by investigating the underlying data in the tool. For instance, contrary to our expectations, China did not contribute much to the ASF incursion risk in 2018, despite presence of ASF in China since August 2018 (6, 70) and large volumes of pork products being imported from China (20). The huge pig population (4.3×10^8 heads) (60) in China resulted, however, in a low estimate for the incidence of ASF and consequently also for the incursion risk posed by pig products imported from China.

Robustness of results

RRAT can be classified as a semi-quantitative risk tool. The output of RRAT is presented as risk scores between 0 and 1. Although the risk score is calculated as if it were the probability of at least one introduction per year, the absolute value of the risk score cannot be interpreted as such, because input values for probabilities in RRAT are to a large extent based on risk classes rather than quantitative data derived from literature or experiments. These risk classes have been translated into proxy values to allow for the calculation of risk scores. Results of RRAT thus give an indication of relative risks rather than absolute risks and are therefore most useful for prioritization.

The impact of the proxy values was evaluated in the sensitivity analysis and appeared to be limited. In most scenarios, the change in proxy values did not affect the estimated risk scores. However, higher values for the probability of contamination of products at exposure, $P_{contex_{PD}}$, and the probability of infection upon exposure, $P_{infex_{PD}}$ (Figure 9; scenarios 2D and 2E), resulted in higher risk scores for the product and traveler route. The ranking of diseases, pathways and source countries/regions was, however, only slightly affected in these scenarios (Figure 10 and Supplementary Figure S4.2). Changing of the proxy values used to estimate the incidence of disease if countries had an unknown disease status (Inc_{unk_D}) had a large impact on the estimated risk scores (Figure 9; scenario 1C). For the traveler route, the change of Inc_{unk_D} also had an impact on the ranking of diseases, pathways and source countries/regions (Figure 10 and Supplementary Figure S4.2).

Even though data from global databases is inputted into RRAT as purely quantitative data, these also contain uncertainty. Numbers of livestock imported, e.g., differ considerably between TRACES and Comext. The effect of using data from Comext (20) rather than TRACES was explored in scenario 3A. Results indicated that risk estimates based on Comext were much higher than based on TRACES (Figure 9). Ranking of diseases, pathways and source countries was also highly affected by the global database used (Figure 10 and Supplementary Figure S4.2). Similarly, data from WAHIS on disease occurrence worldwide is biased due to underreporting or non-reporting. Scenario 1E of the sensitivity analysis indicated that risk estimates were higher, especially for the animal route, when correcting for underreporting (Figure 9). Ranking of diseases, pathways and source countries/regions was, however, not affected (Figure 10 and Supplementary Figure S4.2). In this scenario we assumed equal underreporting for all geographic regions, whereas in reality there might be differences depending on, e.g., surveillance in place. Disease incidence could only be calculated for a subset of countries in which disease was present. Therefore, a decision tree was used in RRAT to classify countries for their disease risk based on quantitative and qualitative data available from WAHIS (Figure 3). If countries did not report at all (neither absence nor presence), they were classified as high risk, unless we had evidence that disease was likely to be absent based on information from other countries in the same region. For the EU, data on disease outbreaks from WAHIS was complemented with data from the Animal Disease Information System (ADIS) (61) and EC reports (62–64) if available. For countries in other regions in the world, the data in RRAT was solely derived from WAHIS. To account for the fact that disease might be present unnoticed, we also considered the disease status of neighboring countries (based on UN subregions) (59) to assign a disease status to countries that reported absence of disease. This sometimes resulted in a likely overestimate of the incursion risk, e.g., when considering the ASF incursion risk from Denmark, that is clustered with the Baltic states in which ASF has been present since 2014 (71).

Based on the results of the what-if analysis, we conclude that risk estimates given by RRAT are more sensitive to uncertainties in data reported by global databases than uncertainty introduced by expert opinion when using proxy values to assign quantitative probabilities to risk classes. Uncertainties in global databases can directly be traced to reporting issues, both when considering disease outbreaks and trade of animals and animal products. Where TRACES was built to track and trace animal movements within the EU from the perspective of animal and public health, the data in Comext is primarily obtained from import and export flows as declared by customs from an economic perspective.

We also calculated Spearman's rank correlation coefficients to compare the ranking of diseases, pathways and countries/regions among different years (Supplementary Figure S4.3). Strikingly, the differences between years were in general bigger than the differences observed between scenarios in the sensitivity analysis. This emphasizes that historical data cannot directly be used to predict future incursion risks. When we conceptualized RRAT, we aimed at regular updates of the risk assessments in an automated fashion to ensure that the estimated incursion risks reflect the current conditions. Therefore, RRAT has been designed such that updates of the assessment can be easily made when new data becomes available. RRAT is, however, dependent on data from global databases on disease outbreaks, and trade and travel, making the tool vulnerable to changes in these databases. In 2021, the OIE has launched a new WAHIS interface (7), making the R scripts that we prepared to scrape the annual reports off their website useless. This, and the delay in the launch and realization of the new WAHIS interface, has hampered timely updates of RRAT with 2019 and 2020 data. The next step in the development of RRAT is to adapt the R scripts such that we can easily import data on disease outbreaks from the new WAHIS website. The availability of application programming interfaces (api) to import data facilitates the use of global databases in estimating disease incursion risks. The development of generic risk assessment tools such as RRAT also illustrates the importance of building and maintaining global databases using the FAIR principles (findable, accessible, interoperable and reusable). Disease-specific parameters in RRAT have been entered once and are considered not to be subjected to change at short notice. The only exception is the legislation for import of live animals. EU requirements for importations of live animals have been regularly updated in recent years, especially for equines. Most changes had, however, little effect on the estimated incursion risks as they concerned source countries and animal species with low-volume trade flows. However, with the implementation of the Animal Health Law (72) in 2021, an update of the legislation tables in RRAT is needed. Unfortunately, we have not been able to design an automated procedure for this task.

Comparison with other generic risk assessment tools

Several other generic risk assessment tools have been developed in recent years [e.g., (16, 17, 73–79)]. Each of these tools were developed with different objectives, and different approaches were used (16). Some of these tools can be used for rapid risk assessment in response to disease events and have expert opinion as input [e.g., (74, 75, 80)]. However, only few of these tools have, like RRAT, the data needed for the risk estimates available in the tool [e.g., (17, 76)], allowing for a rapid response without the need to bring disease experts together. The main asset of these tools is that risk assessments can be updated relatively easy, making the tools suitable for horizon scanning. Another difference is that some of the tools only address the probability of entry into a new area [e.g., (76)], whereas others also include epidemiological [e.g., (77)] or economic consequences [e.g., (75, 78, 79)]. RRAT has an in-between position by including the exposure assessment and the probability of a new infection, but not estimating subsequent spread of disease, or impact on animal health and economics. We deemed the inclusion of a first infection in local livestock a minimal requirement to make results of the tool meaningful, as import of contaminated products does not by definition result in disease outbreaks, nor does import of animals for slaughter or import of exotic animals in case of subclinical infections and no contacts with livestock farms. A shared challenge for these generic risk assessment tools is to keep them up and running and to have added value to policy makers in setting priorities for preventive measures and surveillance. Bianchini et al. (81) did a survey on the use of animal health information systems and risk analysis tools among professionals in animal and public health around the world. They concluded that the main areas of interest from these systems and tools are information on where diseases are present, pathways of introduction, and spread assessment. RRAT provides insight into the first two areas of interest. Results of RRAT are easily accessible *via* the online visualization tool, allowing for independent use by policy makers.

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

CV and MS initiated and conceptualized the research and drafted the equations and algorithms for RRAT. CV and RP designed the outline of RRAT. CV and RP collected data from global databases. RP cleaned and analyzed all data inputted into RRAT. CV, MS, and EK collected data on disease-specific parameters, legislation for animal trade and travelers. RP built the online visualization tool. CV analyzed the results of RRAT and drafted the manuscript. All authors contributed to the article and approved the submitted version.

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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/fvets.2022.963758/full#supplementary-material

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Improving farm-level antimicrobial stewardship benchmarks by reporting antimicrobial use within the context of both the magnitude of disease pressure and the outcome of therapy

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This manuscript explores a method of benchmarking antimicrobial use within the context of farm level therapeutic incidence (a proxy for disease incidence), and the outcome of that therapy. This is reported both within the same farm over time (2016–2019), as well as evaluated across participating farms. Reporting antimicrobial use in this format addresses multiple primary questions necessary for evaluating on farm antimicrobial stewardship: How much disease is recorded? How much antimicrobial use is recorded? How often are antimicrobials included in therapy for each disease? What is the outcome of therapy? The three primary metrics reported are: therapeutic events per 100 cow years (TE/100CY), antimicrobial regimens per 100 cow years (REG/100CY), and the percent therapeutic success (% Success). Success was defined as: the cow remained in the herd and had no further TE recorded within 30 days of the end of the TE being evaluated. These measures identify opportunities for change on an individual farm, such as improvement in disease prevention, or a change in choices about when to include an antimicrobial in the treatment protocol. Therapeutic outcomes provide additional context, in some instances demonstrating differences in recording practices and case definitions, while in other cases serving to safeguard animal welfare as efforts are made to decrease antimicrobial use in the future. Although developed for farm level reporting, the metrics may also be more broadly summarized to meet future reporting requirements for marketing chain or national level antimicrobial use reports. The process outlined here serves as a prototype to be considered when developing antimicrobial use reporting systems where farm level antimicrobial stewardship is the primary objective.

KEYWORDS

antimicrobial use, livestock, benchmarking, monitoring, antimicrobial stewardship, dairy, pharmacoepidemiology

Introduction

Benchmarking farm antimicrobial use has been promoted as a mechanism for improving antimicrobial stewardship (1). Although numerous methods for quantifying antimicrobial use have been described and implemented, metrics vary greatly in their level of granularity and the amount of farm level context available to improve interpretation (2–5). If benchmarking at the farm level is to be used as a tool for improved stewardship, metrics should be sufficiently detailed at the level of the farm where use occurred (5). Creating actionable change is a challenge, but it has been suggested that improvement in both veterinarian and farmer confidence in making treatment decisions will likely improve antimicrobial use on farms (6). This manuscript explores a method of benchmarking antimicrobial use within the context of farm level therapeutic incidence (a proxy for disease incidence), and the outcome of that therapy. This is reported both within the same farm over time (2016–2019), as well as across participating farms.

Numerous parameters related to cow health in dairy systems have been benchmarked, including cow longevity, mastitis, lameness, milk production, milk quality, reproductive efficiency, and metabolic disease (7–11). It is common to benchmark dairies across multiple measures to provide as accurate and complete picture of these complex processes as possible. It has been stated that "it is advisable to use different benchmarks in combination for monitoring health, as well as for deciding on strategies to improve overall herd health management" (9); these authors, in a paper utilizing the Austrian dairy data collection system, concluded that "single parameters are not sufficient to evaluate complex parameters, such as fertility, udder health, or metabolic health." Due to the multitude of factors that can contribute to antimicrobial effectiveness and resistance development, we argue that single parameters are not sufficient for evaluating antimicrobial use.

Our main objective was to develop antimicrobial use benchmark reports using metrics that provide veterinarians and animal caretakers with indicators of antimicrobial use within the context of recorded disease therapies as well as the outcome of these therapies within and across farms. This allows for a more nuanced interpretation of measures rather than indicating a simple increase or decrease of a single value, providing a more accurate and actionable tool to be used at the farm level to drive antimicrobial stewardship decisions.

Materials and methods

Data collection and analysis

Data for the calendar years 2016–2019 were collected from a convenience sample of 27 dairy farms in the United States. Herd size based on inventory of adult cows (>0 lactations) ranged from 211 to 6,676 cows, with a farm mean of 1,195 and a median of 952 (lactating and dry cows combined). All herds were Grade A farms (farms meet quality standards to market fluid milk in the United States), and none marketed organic product. Breed was predominantly Holstein, but two farms had 100% Jersey, and 5 others had <100% Holstein with considerable variation in non-Holstein percentage. All dairies used parlors for milk harvesting. Dairies were recruited through their veterinarians with whom the investigators had a previously established relationship. Efforts were made to include dairies in multiple regions of the United States (West, Midwest, Northeast); however, there were no restrictions for participation other than provision of data, a willingness to work with the investigators, and allowing publication of summarized data, while providing for confidentiality of individual farm-level data. Collection of data was accomplished at yearly farm visits by the investigators where a farm software data backup and collection of any non-electronic treatment records were conducted. These data were then submitted to standardization and quality assurance protocols as previously described (12), by standardizing record format (condensed vs. long), disease, treatment, dose.

In this report 4 farms were selected as examples which are representative of variation encountered in both cow health management and record keeping practices. Referred to as "Red Dairy," "Cyan Dairy," "Blue Farm," and "Yellow Dairy," the identity of these real farms has been masked. The farms selected as examples demonstrate a variety of different antimicrobial use patterns and context surrounding their antimicrobial use measures.

Fundamental constructs: Therapeutic events and standardized treatment regimens

There are 2 constructs fundamental to the values reported: therapeutic events (TE) and standardized treatment regimens (REG). They are hierarchical, with REG nested within TE.

As previously described, REGs were defined by grouping treatment records by individual cow, disease syndrome, and active drug substance, where the time between product administrations was not >7 days (12).

Therapeutic events were identified by grouping regimens only by individual cow and date with the same 7-day maximum between one regimen and another; neither disease nor active substance was utilized a basis for grouping. Therefore, a single therapeutic event may contain multiple REG, both antimicrobial-containing regimens and regimens without antimicrobials, and be associated with a single disease or multiple diseases. When there are multiple standardized treatment regimens within one therapeutic event, the time frame of each can overlap in any manner, or they can be consecutive provided that there is no gap in therapy (no regimen being administered) where final administration of one regimen is separated from the first administration of another by more than 7 days. In order to accurately report documented disease, non-antimicrobial regimens were included as part of therapeutic events. Examples of non-antimicrobial regimens include documentation of "no treat," documentation of disease without documentation of treatment administered ("unknown"), and documentation of non-antimicrobial treatments ("non-antimicrobial") such as flunixin meglumine and calcium. In order to efficiently identify unique sequences in the original data that belonged to the same REG or TE, a function was written in R to assign unique identifiers to the original data rows within the same REG or TE. Details of this function are available in Supplementary material 3.1.

This approach facilitated analysis in two ways. First, it allowed counting the number of unique REG and TE while grouping the data by desired variables such as dairy, disease syndrome, antimicrobial class, or calendar year. These counts could then be put over any desired denominator, such as counts of cow years. Secondly, a description (rather than a total count) of standardized treatment regimens [as published in Schrag et al. (12)] or therapeutic events can be produced. These summaries describe the distribution of different characteristics of each construct. Descriptions of REG may be of importance to those interested in defining doses to apply to antimicrobial sales data, or who have research questions about dose or duration. Descriptions of TE may be of particular interest at the farm level to identify differences in recording methods, protocol adherence, or further details about which drugs are included in therapies. This is particularly important when attributing antimicrobial use to common diseases. These descriptions are provided in the Supplementary material 1.

One additional metric, antimicrobial regimen to therapy ratio (RT-ratio), was calculated as an indicator of the frequency with which antimicrobial regimens were included in therapeutic events. Calculation is performed by dividing the number of antimicrobial regimens by the number of therapeutic events: (REG/TE) = RT-ratio.

Therapeutic outcomes

Therapeutic outcomes were calculated for each therapeutic event. The outcome was evaluated at a maximum of 30 days after the final administration date in the sequence. For example, if the final administration date of ceftiofur HCl was January 1, the outcome was evaluated on January 31. There were 4 possible outcomes: Relapse, Died, Sold, and Success. An outcome of "Relapse" was assigned if the cow received another TE prior to day 30. "Success" was defined as the cow remaining in the herd at the time of outcome evaluation (day 30) without relapse. An outcome of "Sold" or "Died" was assigned if a "Sold" or "Died" event was detected for that cow prior to day 30. Reasons for the cow dying or being sold were not consistently recorded. Individual therapeutic events could only have a single outcome. Assignment of outcomes was accomplished by using a similar R function (details in Supplementary material 3.1) to the one which created unique groups for each therapeutic event, but where the time gap was defined as 30 days rather than 7.

Therapeutic event sequences and disease syndromes

By definition, a TE can include multiple regimens and therefore, may have multiple disease syndromes tied to it in the original treatment record. When only one disease syndrome was associated with a therapeutic event, that disease syndrome was assigned. If multiple diseases were identified as belonging to a single TE, then the disease syndrome associated with that sequence was defined as "complex disease." An exception to this was made if there were two disease syndromes and one was "unknown." In this case the known disease syndrome was assigned as the identified disease syndrome. More details about which disease combinations were common in complex disease can be found in the Supplementary material 1.3.

Denominator of cow years

The denominator of cow years (CY) was calculated from the average inventory of adult cows present on each dairy during a calendar year. In the dairy record systems, CY is an average count of cows who have a lactation number greater than zero measured at multiple time points throughout the year. The method of obtaining this count varied slightly based on the type of production records available. For farms that utilized Dairy Comp 305 systems (26/29 herds; Valley Agricultural Software, Tulare, CA), CY was calculated as a weighted weekly average of the farm inventory of adult cows (LACT>0) for the given year. For all other farms, it was calculated as a weighted average of the farm inventory on Dairy Herd Improvement Association (DHIA) test days which varied from 6 to 12 times per year.

Reporting format—Main questions addressed

All outputs from these analyses were formatted with the goal of providing information important for the evaluation and improvement of farm level antimicrobial stewardship. All visualizations were created using the ggplot2 package in R (13).

Seven specific questions that could be addressed with these data include:

A) How much disease is recorded? (TE/100CY)
B) How much antimicrobial use is recorded? (REG/100CY)
C) What are the outcomes of therapy? (%Success, %Relapse, %Sold, %Died)
D) How often are antimicrobials included as part of therapy? (RT-ratio)
E) How might recording practices be influencing results?
F) What variation exists across farms, or within a farm over time?

In addition to these farm level questions, broader reporting at the national or commodity level was briefly explored:

G) What variation exists across years when data are summarized?

Scatter plots

Scatter plots of the rates of Antimicrobial Regimens (REG/100CY) by Therapeutic Events (TE/100CY) were created using the data from all farms. The axis scales were log transformed to facilitate visualization of points. Each small black dot in Figure 1 represents one calendar year from 2016 to 2019 for each dairy in the study. Each colored dot represents one calendar year from each example farm. The solid black lines creating crosshairs near the center of the graph, represent the median value (middle dairy) on each axis for all years and all dairies combined. This creates 4 quadrants each representing a different combination of values on each axis, with "high" and "low" not representing a judgment on appropriateness of use, but rather being above or below the median:

- Upper Left
  Low Disease (Therapeutic Event Rate),
  High Antimicrobial Use (Antimicrobial Regimen Rate)
- Upper Right
  High Disease (Therapeutic Event Rate)
  High Antimicrobial Use (Antimicrobial Regimen Rate)
- Lower Left
  Low Disease (Therapeutic Event Rate)
  Low Antimicrobial Use (Antimicrobial Regimen Rate)
- Lower Right
  High Disease (Therapeutic Event Rate)
  Low Antimicrobial Use (Antimicrobial Regimen Rate)

The dark gray shaded region in the background represents the area of the middle 50% (25–75th percentile) of the values on each axis. The light gray shaded region represents the area of the middle 80% on each axis. A dashed line was added for visual reference only and represents a 1:1 ratio between the x and y axis (RT-ratio = 1).

When individual farm reports were created, the reported farm's points were enlarged, shape was mapped to year, and color was mapped to the percent success for that year. Examples of individual farm benchmarks are presented in Supplementary material 5. This manuscript focuses on reporting formats generated for a veterinarian, or veterinary clinic, where multiple farms are presented in the same report. In this case rather than mapping color to percent success, color is mapped to a unique farm identifier (Red Dairy, Blue Dairy, Cyan Dairy, Yellow Dairy as examples of 4 farms for a report to a veterinary practice), and percent success is only reported in the tabular format rather than the scatter plot. In addition to a summary plot where all diseases are combined (Figure 1), scatter plots are also created for each disease individually (Figure 2). A more graphical explanation of scatter plots can be found in Supplementary material 4.1.

When used for multi-farm reporting, scatter plots inform main questions A, B, and F. When used for individual farm reporting, they additionally inform main question C. Scatter plots can also be used to summarize data across broader categories such as for use across all dairies by year (Figure 3). When utilized in this manner they inform main question G, and the shaded areas are generated by summarizing at the year level and mapping the color of the shaded region and median cross hairs to year.

Tabular format and recording details

All 11 metrics calculated are presented in 3 sets of tables reporting how much disease and antimicrobial use is reported, the outcome of therapy, and what data are recorded. Each table is subdivided by disease and calendar year. The numerical values within the tables are the value for the individual farm reported. The background color of

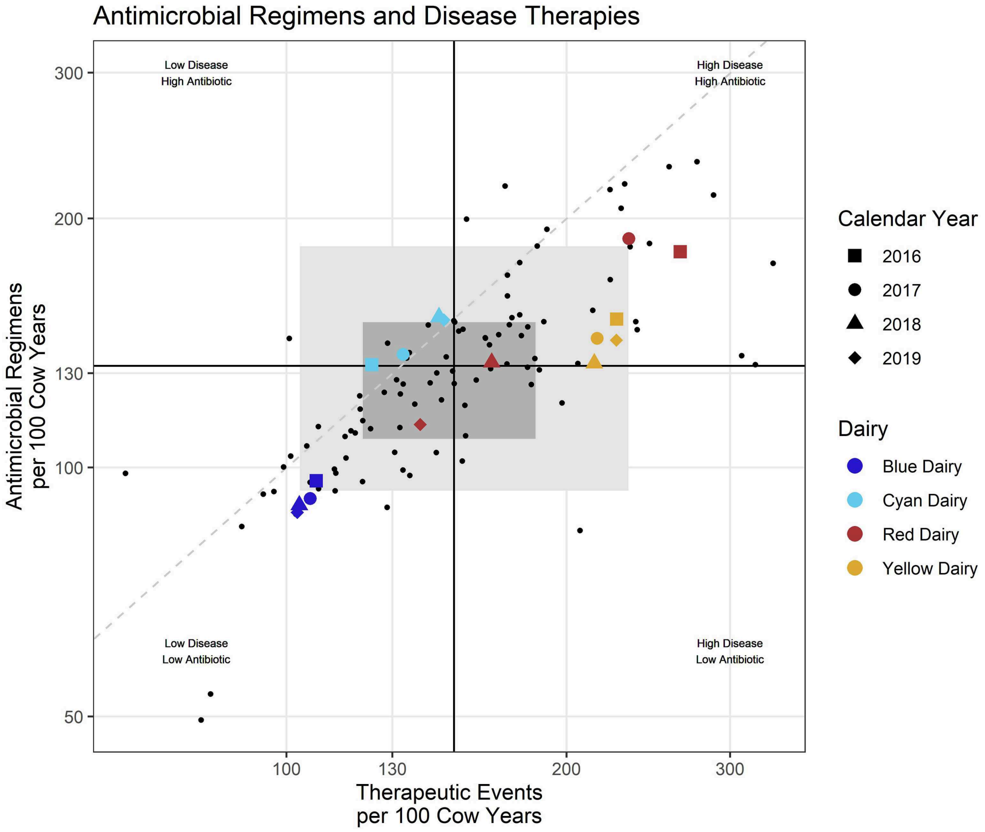

FIGURE 1
Each small dot represents an individual dairy during one calendar year/Colored shapes represent selected example daries with shape indicating calendar year. Black lines represent the intersect of the medians (middle value) for the entire dataset. Dashed diagonal line provides visual reference of a 1:1 relationship between the x and y axis. Shaded rectangles represent the middle 50% (dark) and 80% (lighter) of participating dairies all years combined.

each square is shaded according to where that value ranks across the rest of the farms in the data set for each metric. This 'rank' is referred to as "Percentile Rank" throughout the reports. It is calculated by grouping values by metric, and then utilizing the percent_rank()[1] function in base R to assign a percentile rank to each value in the data set. Classifications were then defined as "very low" (0-≤20th percentile), "low" (>20-40th percentile), "average" (>40-60th percentile), "high" (>60-80th percentile), or "very high" (>80th percentile). Each time these percentile rank classifications are utilized in the benchmarks they are each tied to a corresponding background color in the tabular format, or point color in the individual farm scatterplots.

Some farms had extremely low incidence (<30 total TE) within a disease/year category. In these cases, calculating a percent success and a percentile rank was not interpretable due to the low counts. This is indicated by a gray color in the background (listed as "not calculated" in the legend) of the tabular output (Figures 4–6) and warns that the count was low and the value should be interpreted carefully.

1 Hadley Wickham, Romain François, Lionel Henry and Kirill Müller (2021). dplyr: A Grammar of Data Manipulation. R package version 1.0.6. https://CRAN.R-project.org/package=dplyr.

Results

The figures in this manuscript represent examples of the final report format generated iteratively over the 4 years of the study. This final format was arrived at after revising the benchmark reports each year based on discussions with the participating farm's veterinarians and animal health teams. The report format presented in the main manuscript is aimed at veterinarians or animal health teams with the need to efficiently evaluate multiple farms. The example farms presented are: Red Dairy, Blue Dairy, Cyan Dairy, and Yellow Dairy. These example farms are each an actual farm in the study, renamed to protect anonymity. Section Discussion of the supplemental materials contains additional

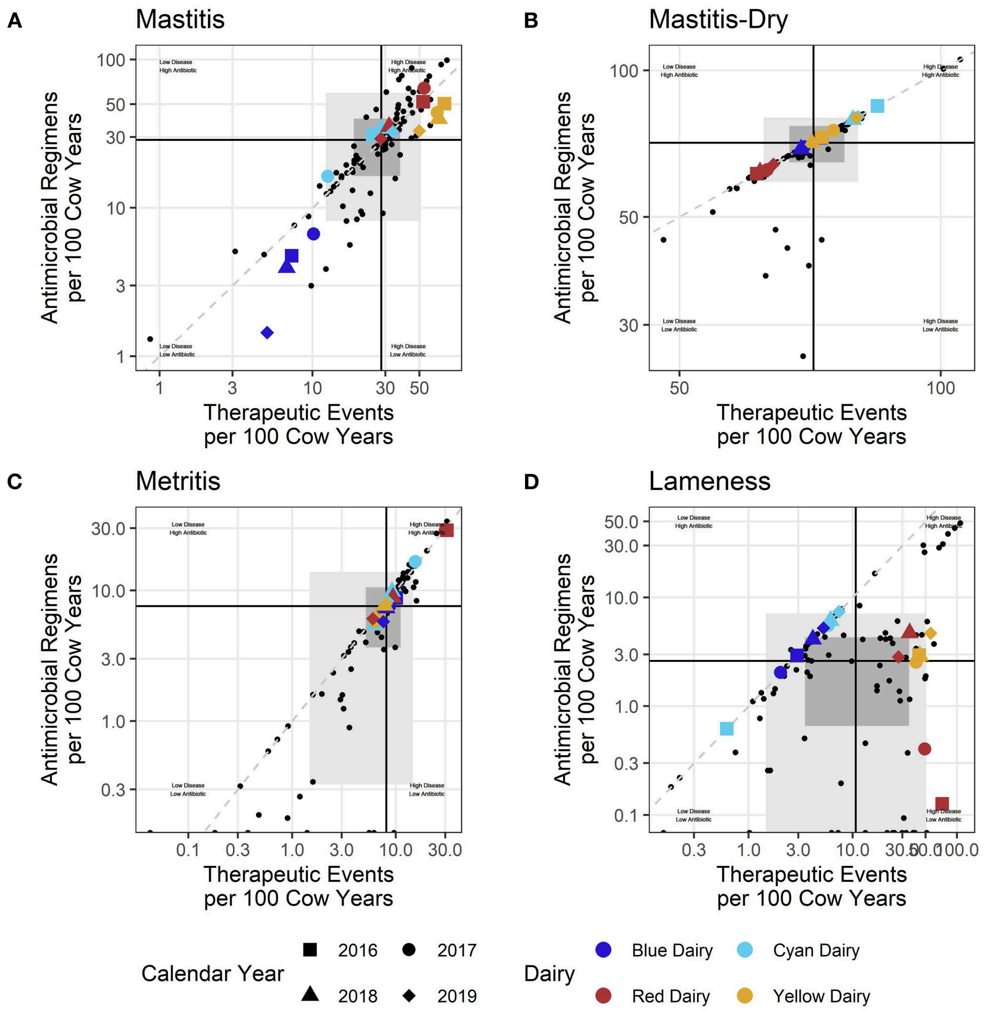

FIGURE 2
(A–D) Each small dot represents an individual dairy during one calander year/Shaded rectangles represent the middle 50% (dark) and 80% (lighter) of participating dairies. Black lines represent the intersect of the medians (middle value) for the entire dataset. Colored shapes indicate the value of example dairies and respective calendar years.

example reports for these farms where the individual farm is the primary audience.

Scatter plots

Scatter plots allow efficient evaluation of REG/100CY within the context of TE/100CY. The visual aids in the graph can be used to rapidly categorize and compare farms according to both their magnitude of recorded disease pressure and antimicrobial regimens used. For example, in Figure 1, both Red Dairy and Cyan Dairy fall within the middle 50% of dairies (dark gray shaded region) for some or all study years. Yellow Dairy falls within the middle 80% (light gray) region for all years, as does Blue Dairy for 2016. For years 2017–2019 Blue Dairy falls outside (below and left of) all gray shading indicating that they are below the 10th percentile for values on both axes. Likewise Red Dairy falls outside (above and right of) the gray shading indicating that compared to other study dairies for 2016 and 2017 their values were above the 90th percentile.

Dairies and their veterinarians can also observe the magnitude of variation in their metrics across years. For example, both Blue Dairy and Yellow Dairy show little variation between years, while Cyan Dairy shows somewhat more variation across calendar years. Red Dairy shows the most variation with 2016 and 2017 far above the gray middle 80% region, 2018 is still in this quadrant but well within the shaded middle 50% region, and 2019 enters the low disease—-low antimicrobial use quadrant but remains within the middle 50%. However, this is very difficult to interpret with all diseases combined.

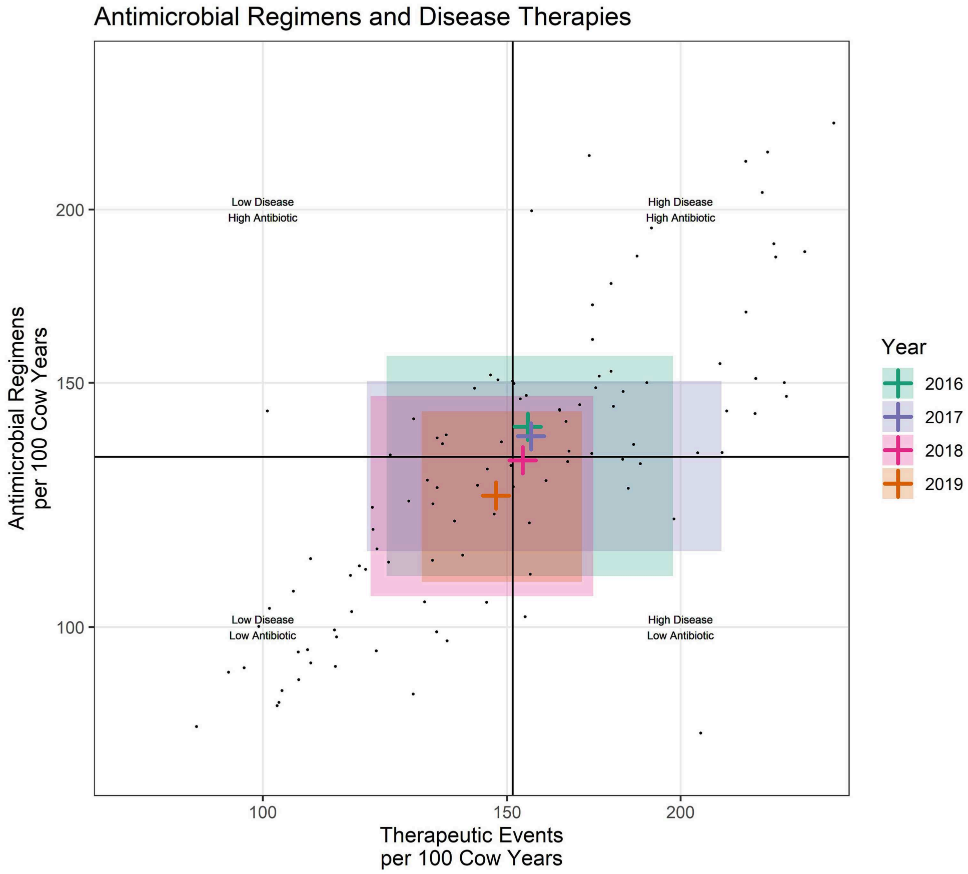

FIGURE 3
Each small dot represents an individual dairy during one calendar year. Shaded rectangles represent the middle 50% of participating dairies for each calendar year. Black lines represent the intersect of the medians (middle value) for the entire dataset. Colored crosshairs represent the intersect of the median (middle value) for each year.

Similar observations can be made for each disease individually (Figure 2). For each disease, questions can be asked about why there are changes within a dairy over time, why a dairy falls within a particular quadrant, or why its values are on the edges of the distribution of farms (outside the gray shading). To simplify discussion, only 4 diseases were selected for inclusion in this manuscript: mastitis, dry cow treatment (mastitis-dry), lameness, and uterine disease (metritis). However, 9 disease syndromes were reported in the individual dairy benchmarks which can be found in the Supplementary material 5.

Broad data summaries

While this study primarily focused on farm level reporting, the same measures useful for farm level reporting might also be utilized for fulfilling reporting requirements at the market chain or national level. For example, scatter plots can be created by summarizing values from all dairies within year (Figure 3). When data are summarized by year, there are now 4 cross hairs created by the median values on each axis calculated for each calendar year. These cross hairs indicate the midpoint of the distribution of values within each calendar year. The shaded area represents the middle 50% of values for each year, creating a reporting format where antimicrobial use can be broadly reported across years within the context of all diseases. This informs a fundamental goal of stewardship, reduction in the need for antimicrobials (as indicated by TE/100CY), to be presented along with the antimicrobial use (REG/100CY). Figure 3 is included in this manuscript only to demonstrate possible methods for broad level reporting. No attempt should be made to interpret trends over time in U.S. dairies from this small convenience sample.

Frequency of antimicrobial inclusion

There are two ways to evaluate how often antimicrobials are included in a therapeutic event. The first is by observation

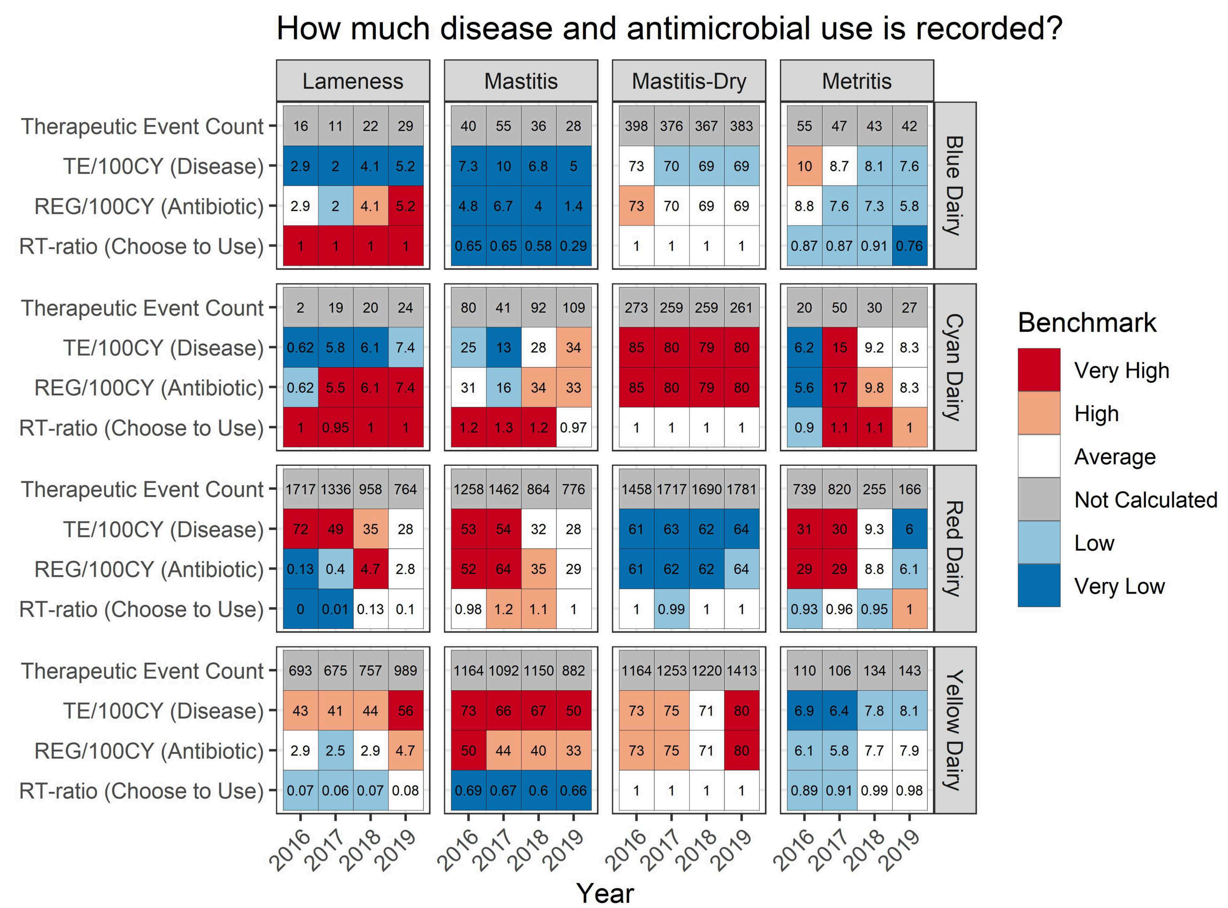

FIGURE 4
For each measure listed on left the value is reported numerically within the cell. The background color indicates how that value ranks compared to other participating dairies.

of where a point falls in relation to the dashed line in the scatter plots. For example, in the mastitis plot (Figure 2A) Blue Dairy and Yellow Dairy fall below the dashed line, indicating that on average not all mastitis TE include an antimicrobial. Red Dairy and Cyan Dairy fall above this line, indicating that for those farms, on average, every mastitis therapeutic event includes at least one antimicrobial, and some include more than one. Farms falling far below the dashed line (a 1:1 RT-ratio) compared to farms falling very near to it are sometimes making different decisions about when or how they include antimicrobials in their treatment protocols. For example, some farms are using pathogen-based treatment protocols (Blue Dairy and Yellow Dairy) where only certain mastitis cases receive antimicrobial therapy and the farms fall well below the dashed line in Figure 2A. For each disease in Figure 2, the location of the cross hairs in relation to the dashed line indicates the median RT-ratio for each disease. For mastitis, mastitis-dry, and metritis the cross hairs fall very near 1. For lameness the crosshairs fall well below the dashed line indicating that not all therapies for lameness include an antimicrobial. Here Red Dairy and Yellow Dairy record all lameness events, while Blue Dairy and Cyan Dairy record only those animals treated with an antimicrobial. When interpreting the scatter plots, care must be taken to recognize the influence that styles of record keeping might have on observed differences across farms.

RT-ratio

A second way to evaluate how often antimicrobials are included is indicated by the RT-ratio in the tabular format (Figure 4, row 4 for each dairy). It indicates the frequency with which antimicrobials were chosen for inclusion in a therapeutic sequence for each disease syndrome. For mastitis both Blue Dairy and Yellow Dairy have very low RT-ratios ranging from 0.29 to 0.69, as indicated by the blue shading in the background of their tabular format. Both of these dairies were confirmed to be using pathogen-based treatment strategies. For mastitis,

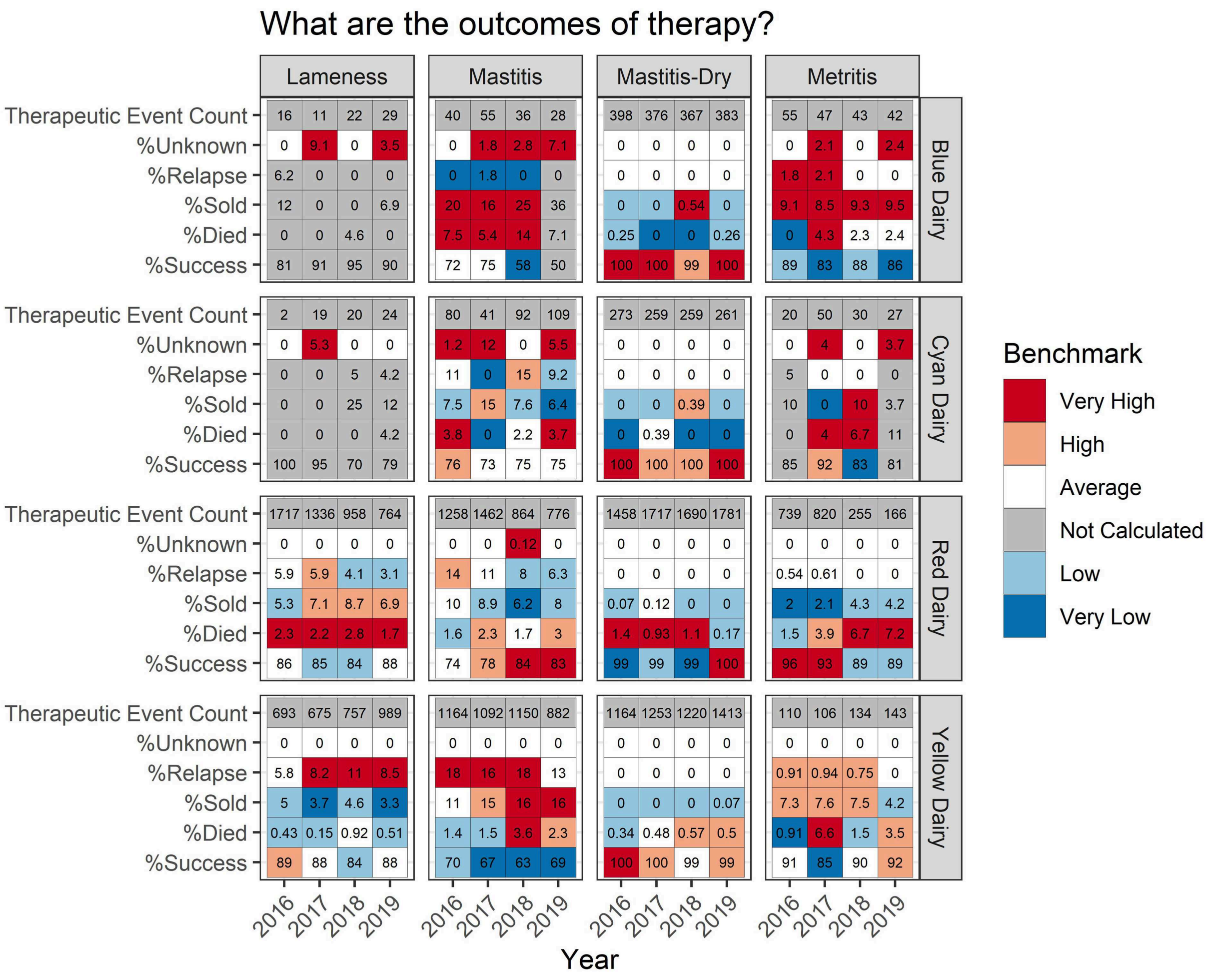

FIGURE 5
For each measure listed on left the value is reported numerically within the cell. The background color indicates how that value ranks compared to other participating dairies. Occasionally outcomes could not be accurately determined due to/data quality issues associated with cow identity. This occurred more frequently for dairies with hand written treatment records i.e., Blue Dairy and Cyan Dairy.

Red Dairy and Cyan Dairy had RT-ratios ranging from 0.98 to 1.32. Neither of these farms were utilizing pathogen-based treatment protocols.

Therapeutic outcomes

Figure 5 shows the percent therapeutic success, and reasons a therapeutic event failed, the cow died (% Died), the cow relapsed (% Relapse), or the cow was culled (% Sold). Focusing on the % success for mastitis, Red Dairy has a success of 74, 78, 84, and 83% for years 2016–19, respectively. The background color indicates how Red Dairy ranks compared to other study farms. Background values range from white indicating average rank in 2016, to light red indicating high rank in 2017, to dark red indicating a very high rank in 2018 and 2019. The background color can be used to rapidly and comparatively access the performance of the other example farms for mastitis % success (the bottom row for each dairy under the mastitis heading). Yellow Dairy has the lowest mastitis % success across all years: light blue 70% in 2016, and dark blue 67%, 63%, 69% in 2017–19, respectively. When examining the details of therapy failures on Yellow Dairy, it can be observed that the low % success can be attributed to both relapses (% Relapse) and culling decisions (% Sold). This is in contrast to Blue Dairy where relapses are very uncommon (dark blue background for % Relapse), but the % Died ranks very high compared to other dairies for 2016–2018.

An example of a pattern which may be worth investigating further can be observed in the metritis % Success for Red Dairy. In 2016 and 2017 it was very high, 96% and 93%, respectively. However, in 2017 and 2018 it was lower, at

FIGURE 6
For each measure listed on left the value is reported numerically within the cell. The background color indicates how that value ranks compared to other participating dairies. The measures listed in this table are not mutually exclusive and therefore do not sum to 100% within dairy/year.

89%. While these seem like small changes numerically, they are relatively large shifts (top quintile vs. second to bottom quintile) in where this dairy lies within the distribution of success for metritis across all farms and all years. There are many potential drivers of this, both within this farm and related to changes that may have happened on other farms contributing to the distribution. Figure 4 adds more context for interpretation, indicating that the disease incidence was very high in 2016-17 (31 and 30 TE/100CY, red background), average in 2018 (9.3 TE/100CY, white background), and very low in 2019 (6 TE/100CY, dark blue background). However, interpretation requires extensive farm level knowledge.

Occasionally an outcome could not be calculated for a therapeutic event. These are reported as "Unknown" outcomes. This occurred more frequently for the dairies with handwritten treatment records (Blue Dairy and Yellow Dairy) where data quality did not always allow for reliable matching of a therapeutic event to an outcome.

Recording practices

Presenting data in tabular format provides several other details (Figure 6) which aid in interpretation of measures or trends. Continuing with the example of Red Dairy's metritis therapy, Figure 6 indicates that in 2016–2018, 8–10% of metritis therapies were documented as unknown, but in 2019, 0% of therapies were documented as unknown. If this shift occurred because record keeping practices became more complete, and those therapies which previously failed to document the specific therapy provided now specify it, then this change would not influence the overall TE/100CY, but it might influence the REG/100CY. However, if instead this farm had a serious labor shortage and recording practices were revised to reduce the amount of employee time devoted to recording causing these once documented but incomplete records to simply be never documented, this could decrease the TE/100CY in the records artificially. Alternatively, if a farm previously never documented retained placenta events that received no therapy, and then revised their protocol to begin recording them, this might increase their apparent TE/100CY even though the farm level incidence remained stable.

Lameness recording practices are variable across dairies (Figure 6). For Blue Dairy and Cyan Dairy, zero percent of recorded lameness therapies included a non-antimicrobial. However, on Red Dairy and Yellow Dairy >88% of lameness therapies indicated that a non-antimicrobial was used. This information should be related back to the disease specific scatter plots (Figure 2D) where there are 2 distinct populations of dairies, those who fall very near to the dashed line (like Blue Dairy and Cyan Dairy) and those who fall below and to the right of it (Red Dairy and Yellow Dairy). Proper interpretation of changes within a farm over time, or comparisons across farms must acknowledge the context of recording practices and its influence on a farm's values.

Discussion

Several studies have compared antimicrobial use metrics specifically within dairy systems. While there are some nuances across metrics which might relate to different stewardship program priorities, the differences are slight with the exception of measuring mass-based grams compared to a dose-based measure of treatment incidence (14–16). In a study looking at benchmarking both large and small animal clinicians in the United States it was found that "Prescribing frequency, durations of therapy, and ranking of antimicrobial classes appear to be the metrics most well-received by veterinary clinicians, while dose-based metrics associated with the ADD [animal daily dose] are less intuitive." (17); even in a hospital setting with clinicians interpreting the benchmarks, dose-based metrics were perceived as "less intuitive."

The selection of antimicrobial use metrics in this manuscript was driven by consideration of 5 basic steps in antimicrobial stewardship: (1) characterization of disease pressure through appropriate case definitions and diagnostics, (2) consideration of alternatives to antimicrobials for prevention, control, and treatment of disease, (3) when necessary, selection of the appropriate antimicrobial regimen for each disease, (4) continuous monitoring of antimicrobial use and therapeutic outcomes, and (5) continuous re-evaluation of the need for the instituted antimicrobial regimens. The reporting format was guided by a quest to find efficient methods for generating more specific and actionable questions about antimicrobial stewardship on an individual farm.

The consideration of antimicrobial stewardship step 1 drove selection of REG/100CY and TE/100CY based on their direct and intuitive relationship to the component of detected disease (12). In this context, detected disease may lead to either therapy of acute disease or the use of an antimicrobial for prevention or control. The link between recorded disease pressure and recorded antimicrobial use also allowed the reporting of the RT-ratio, which provides information about the decision that an antimicrobial intervention is necessary, the third step in antimicrobial stewardship.

In a recent and comprehensive review of behavior associated with dairy antimicrobial use, one of the recommendations was to improve "ability and confidence to implement prudent AMU practices while maintaining animal-welfare standards" (18). Reporting therapy outcomes provides at least some context of animal welfare implications for changes in antimicrobial use. The simultaneous monitoring of outcomes along with use could provide needed confidence for stakeholders to make changes in use patterns by demonstrating that outcomes are important and are being tracked along with antimicrobial use. Additionally, it sets a precedent that true stewardship means use optimization first by disease incidence reduction, combined with optimizing case definitions for antimicrobial treatment. This should all occur within the context of therapeutic outcomes. Consideration of outcomes supports antimicrobial stewardship step 5. However, the authors wish to make it clear that outcomes determined in this manner should not be construed as representing the effect of the interventions in place. Rather, they may be considered in light of other inputs such as clinical trial outcomes.

Additionally, outcomes provide context for interpreting TE/100CY by offering some additional information which may drive questions about case definitions or diagnosis. For example, consider that mastitis on Red Dairy falls high into the upper right quadrant in years 2016 and 2017, indicating that both TE/100CY and REG/100CY were high compared to other study dairies (Figure 1, and dark red background in Figure 4). However, they fall well into the central gray zone in 2018 and 2019. Did they decrease both antimicrobial use and disease incidence by simply failing to detect or record disease? Their treatment outcomes in Figure 5 indicate that this is not likely to be the case. Their % success actually improved in 2018 and 2019 compared to previous years. This would decrease the likelihood that previously detected disease is now being ignored, and indicate that some other variable changed, such as improved disease prevention with better overall cow immune status, or decreased disease pathogenicity of endemic pathogens (e.g., eradication of *Prototheca*?).

Blue Dairy falls on the opposite extreme with all years falling into the lower left quadrant of the scatter plot (Figure 1). Their % Success for mastitis therapy is average in 2016-17, very low in 2018 and not ranked in 2019. Do they have low disease and low use because they are ignoring or misclassifying disease? From these benchmarks alone it is impossible to determine whether this low TE/100CY and low REG/100CY are the result of very good management or very poor management. It is good management if the farm is doing a great job at disease prevention, and simply has average to low % Success because of high culling pressure for mastitis cows on this farm. It is poor management if they are simply ignoring or misclassifying disease, and therefore treating few animals very late. This would give them low TE/100CY and low REG/100CY, but poor outcomes because only the most severely affected animals are documented in the record. This is yet another example of appropriate and useful interpretation of comparative metrics between farms only being possible by investigation of reasons for the differences at the farm level.

One of the primary limitations of the benchmark metrics proposed here is that the 30-day outcomes are relatively crude measurements. Ideally, reports should offer more granular outcomes such as outcomes related to milk production and/or quality (e.g., somatic cell counts), or 120-day pregnancy rates. Although such outcomes are more complex to calculate and were well-beyond the scope of this project, their inclusion as context for antimicrobial use should be further explored. The authors strongly recommend that production efficiency measures are included in any large-scale program which benchmarks antimicrobial use, and that evolutions of existing benchmarking programs include this context.

Although others have evaluated the role treatment threshold (case definition) might play in antimicrobial stewardship (19), to the author's knowledge this study is the first to utilize detected disease as a denominator for antimicrobial use in dairy cattle. It was not described as a denominator in any of the 12 benchmarking programs described within the AACTING review paper (5). In the work reported in this manuscript, it was quickly discovered that dairies who utilized pathogen-based treatment programs could not be easily differentiated using only the standard denominators such as Cow Years or kilograms of animal treated. This challenge drove the development of the "dashed line" in the scatter plots, and the RT-ratio. Both comparison of antimicrobial REG to total TE as the RT-ratio (REG/TE) and the distance a dairy is from the dashed line in a scatter plot represent the compilation of multiple therapeutic decisions to use or not use an antimicrobial for a particular disease therapy (TE).

For example, the scatter plot of dry cow treatment (mastitis-dry, Figure 2B) demonstrates cross hairs (intersect of x and y axis medians) very near the diagonal dashed line (representing an RT-ratio of 1:1). When considering all study farms, most are very tightly grouped on this line since most farms use "blanket" dry cow therapy where an antimicrobial is included for every therapeutic event. However, there are a few (small black dots) falling far below the dashed line. Most of the dairies with very low RT-ratios were confirmed to be utilizing selective dry cow therapy rather than blanket dry cow strategies. For selective dry cow therapies, a portion of the therapeutic event included only a non-antimicrobial teat sealant rather than both a sealant and an antimicrobial or just an antimicrobial.

Large shifts in variation of a metric should raise questions about potential changes in case definitions, case management, recording practices, or disease prevention strategies. Presenting all metrics on one page in tabular format (Figures 4–6) is an attempt to provide as much context as possible for all metrics to highlight differences in case definitions or recording practices. When all information is interpreted within its context the tabular outputs are helpful to answer some initial questions generated by the scatter plots such as:

- Was low success due to a higher cull rate or more relapses?
- Is disease level classified as high just because records are more complete (i.e., "are no treats" recorded)?
- How might case definition be affecting the amount of disease reported?

Although many details are included in these tabular outputs, "boots on the ground" knowledge of farm activities is still necessary for accurate interpretations.

Figure 1 presents an example of a large shift in values for Red Dairy. This was investigated and confirmed that a significant management change occurred between years 2017 and 2018. In 2016 and 2017, there were 3 locations, each managed separately. In 2018, these locations were combined so that all fresh cows were managed at a single location. Therefore, the observed change across years was due to changes in disease incidence driven by management change, change in case definitions driven by personnel change, therapeutic decisions driven by a change in how responsibilities were allocated, and only minor changes in record keeping practices. Recognition that interpretation of each farms data requires farm level knowledge of recording practices is essential to gaining accurate incites about antimicrobial stewardship.

As demonstrated in the lameness plot (Figure 2C), sometimes observed differences are caused by differences in recording practices (i.e., recording all lameness events vs. only those treated with an antimicrobial) rather than fundamental differences in treatment protocols. Pursuing a goal of completely standardized recording practices across all farms is unrealistic. However, efforts to achieve some basic health record keeping standards are needed for farms to gain actionable insights from their health data. Examples of basic standardization include recording all disease events not just those treated with antimicrobials, and associating treatments with a disease or condition (applying a case definition) rather than only listing the drug used for treatment.

In addition to differences in recording practices, there is also farm-to-farm variation in case definitions. For some farms, the case definition of mastitis is observation of a few "flakes" in the milk or the cow tests positive on a California Mastitis Test, while the case definition of mastitis on other farms is not met until milk is significantly abnormal for multiple consecutive days. This can easily lead to long detailed debates on the most appropriate case definitions. However, that is not the goal. The main goal is that the reporting format allows for detection of changes over time, reported across multiple metrics which allows evaluation of an individual farm's progress by those familiar with what changes may have occurred.

While some might interpret the above-mentioned differences in case definitions and recording practices as detrimental, the authors contend that they are simply representative of current dairy systems and accurately describe current practices. It is unlikely that a single recording method and/or single case definition would be appropriate on every farm. Each farm has a unique set of personnel with unique skill sets, and a unique population of bacteria present on their dairy. Identification of these differences is one of the opportunities associated with this style of reporting. Reports intended to support antimicrobial stewardship applications should elicit questions of "why is...?" rather than produce statements pronouncing a particular dairy falling above or below a particular (arbitrary) target value. Interpretation of measures with as much context as possible provides more information to those actively involved in defining and providing therapy to sick animals. This offers the potential to identify multiple opportunities which can lead to a more holistic approach to antimicrobial stewardship rather than simple reduction of the use metric without the context of the associated disease incidence or therapy outcome.

Some have suggested that farm level sales data should be the gold standard for antimicrobial use measurements, as use measured by farm records has shown to underestimate use measured by sales data (20). While sales data may more accurately account for the sale of each mg of drug distributed to a farm, the authors of this manuscript believe that the farm treatment records provide more utility in identifying actionable opportunities for changes in disease diagnosis or treatment regimens. The context provided by the treatment record is necessary to direct investigations as to the appropriateness of the antimicrobial use, to identify actionable changes to improve use, and to keep track of therapeutic outcomes subsequent to alterations in antimicrobial use. Ignoring this context makes it more difficult to identify actionable changes and fails to provide for monitoring of both antimicrobial use and treatment outcomes subsequent to changes in antimicrobial use. Although documented changes in REG/100CY, TE/100CY, and RT-ratios do not directly indicate why the change occurred, efficient reporting mechanisms to observe these changes can drive reasonable and in citeful questions leading to meaningful investigations of cause.

An absolute requirement for the appropriate use of antimicrobial use metrics and benchmarks is that the interpreter is acutely aware that selected metrics describe "what happened" (with varying degrees of accuracy) and not "why it happened." Even when there is intimate knowledge of farms being evaluated by veterinarians and their clients, there are unknown factors affecting peer farms being used for comparison which are equally as important to understand, highlighting the need for a central resource which is able to further investigate when unexpected patterns or distributions of farm values occur. Developing regulatory policy, legislative initiatives, or marketing programs based on poor understanding of inadequate metrics has the potential to cause harm to animal welfare and efficiency of production with minimal to no improvement in antimicrobial resistance selection pressure.

In an effort to remain consistent with benchmarking other complex processes within the dairy industry and to accurately represent reality, this benchmarking system utilizes multiple measures of antimicrobial use. When reporting benchmarks back to the participating farms and veterinarians, the early reporting format with simple metrics required little explanation, but participants struggled to know what to do with the measures and were quick to mention that they might have more disease or question if they were more successful with their therapies. The more complex reports, as presented here, required some amount of training for interpretation but generated more engagement and excitement about having access to the benchmarks. It should be noted that some antimicrobial use monitoring systems disagree with the approach and recommend that "It might be advisable not to benchmark too many aspects, as multiple benchmarking results for a single species (category) might become confusing and end up being counterproductive, especially if the results appear contradictory." While recognizing that no single metric was sufficient, Craig et al. suggested that a single metric should be chosen to simplify the communication of the measure (21). We disagree with this approach, instead recommending that when results appear contradictory studies should be conducted to evaluate which actions might be most effective at improving antimicrobial stewardship. There is valuable information in determining why two metrics give different pictures of the same systems. Forcing a simplistic single numerical metric for antimicrobial use is an approach which may increase the simplicity of detached decision-making, but also increases the potential that the detached decisions are precisely wrong.

Conclusion

This study demonstrates that existing treatment records can be utilized to provide antimicrobial use reports containing multiple measures presented within the context of disease pressure and therapeutic outcomes. The authors are optimistic that if further advances are made in data interoperability, a similar system of reporting could be scaled up to include a larger population of dairies. Because the benchmark system outlined in this manuscript is detailed and relates directly to farm level disease management practices, there is hope that the described data structure might provide a starting point for identification of future opportunities related to the development of antimicrobial stewardship tools. If broad level antimicrobial use summaries are created at a commodity or national level, adding a description of disease pressure (despite the challenges) could allow a more contextual interpretation of antimicrobial use data.

Nomenclature

Construct definitions

Standardized treatment regimen (REG) – a standardized regimen refers to a treatment or group of treatments administered consecutively to an individual animal where the gap between administrations is never >7 days. A REG is drug (active substance) and route specific. A detailed definition of a REG can be found in Schrag et al. (12) (see Supplementary material 1.1 for more details).

Therapeutic event (**TE**) – A group of REGs where the time gap between the last administration of one REG and the first administration of another is never >7 days. These regimens may address a single disease event. A TE may also span multiple disease events (e.g., mastitis followed by metritis) if these diseases are identified in the same cow with a 7 day or less gap between regimens associated with each disease. When a TE contains multiple types of diseases it is classified as a "complex disease" therapeutic event (see Supplementary materials 1.2, 1.3 for more details).

Outcome – The outcome (Success, Sold, Died, Relapse) associated with each therapeutic event, evaluated at a maximum of 30 days after the final administration of the last REG. If used in a numeric context it is the percent of Therapeutic Events resulting in a particular outcome, i.e., % Success.

Mathematical summaries of construct frequencies

REG/100CY – numeric count of the number of *antimicrobial* regimens (REG) divided by the average yearly cow inventory (CY), then expressed in relation to 100 cow years.

TE/100CY – a numeric count of therapeutic events (TE) divided by the average yearly cow inventory (CY), then expressed in relation to 100 cow years. It is important to note that *all* therapeutic events count here, including ones which do not include an antimicrobial in any of the regimens.

RT-ratio – the ratio of count of antimicrobial regimens (REG) to count of therapeutic events (TE). This measure is an indicator of the frequency with which antimicrobials are included in therapy.

% Success – the number of therapeutic events resulting in an outcome of success divided by the total number of therapeutic events. This measure can be grouped by any number of categories but is usually reported by disease syndrome.

Data availability statement

The datasets presented in this article are not readily available because the data used in this study belongs to the farms, and sharing the data in a non-summarized format was not part of the original agreement for the study. Requests to access the datasets should be directed to mapley@k-state.edu.

Author contributions

NS, SG, and MA contributed to conception and design of the data collection. NS and DA organized the database and performed the analysis. NS wrote the first draft of the manuscript but relied heavily on all other authors to provide guidance within their areas of expertise. All authors contributed to the methods of data analysis, contributed to manuscript revision, read, and approved the submitted version.

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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/fvets.2022.1022557/full#supplementary-material

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Reporting on 16 years of laboratory capacity building while exploring the future of WOAH's Laboratory Twinning Programme

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Animal health laboratories are an increasingly important part of safeguarding animal and public health due to their role in surveillance and diagnostics of animal diseases, food safety, and in the development and production of medicinal products, vaccines, and diagnostic tools. Despite their importance, the global distribution of veterinary laboratory expertise is uneven, with greater concentration of reference laboratories in wealthier countries. To address this issue, the World Organization for Animal Health (WOAH, founded as OIE) created a Laboratory Twinning Programme in 2006. The paper will briefly review this Programme in the context of an increasingly populated global health security field, based on a literature review and on a combination of public and internal WOAH data and describe the implementation of the Programme in the past 16 years, noting the drivers for project implementation, its links with the global livestock biomass distribution and with the current distribution of veterinary laboratory expertise. There has been broad uptake and diversity in the focus of the twinning projects implemented in WOAH Member Countries. The Laboratory Twinning Programme would benefit from an evaluation that looks at its outcomes and quantifiable impact in beneficiary countries. A case is made for the development of a monitoring and evaluation system tailored to the Programme's specificities.

KEYWORDS

animal health, laboratories, veterinary laboratories, twinning, sustainability, public health

Introduction

Development assistance in the context of global health is an increasingly populous and fragmented field (1). A literature review covering the political economy of foreign aid allocation in the context of global health initiatives led to the conclusion that most aid is unequivocally motivated and still reflects old colonial relationships and western-led geopolitical relationships (2, 3). However, it has not been possible to determine whether aid that is not overtly politically driven, such as that of charities, philanthropies, and non-governmental organizations, is in any way less effective than "official development assistance" allocated by a donor country to a beneficiary country (4). Foreign aid has contributed to remarkable improvements in health, education, and poverty, during the 20th century, thereby spurring economic growth in numerous developing countries (5). Given the socioeconomic advancements experienced by countries that had previously been in the low-middle income level, new donors have arisen in the development assistance space. These include, but are not limited to, the BRICs: Brazil, Russia, India, China, and South Africa. These countries tend to have a more equalitarian relationship between donor and beneficiary, framing development assistance projects as "technical cooperation," and to allocate foreign aid irrespective of the political system of the recipient– a radical departure from the model used by Western donors, which tend to give more aid to democratizing countries and to frame development assistance as "capacity building" initiatives (2, 6–8). International and intergovernmental organizations have increasingly acted as intermediaries in foreign aid allocation, contributing to the multilateralization of the field of development assistance, especially in relation to global health (9, 10). International organizations working in this field have increased in number and in scope, as have philanthropic organizations, thereby resulting in a densely populated field, which is hard to coordinate and steer, with organizations frequently overstepping each other's mandates (1, 3).

Disease emergence at the interface between animals and humans has always been important, but is becoming increasingly more so (11). Given that the majority of infectious diseases are of zoonotic origin, animal health practitioners have a key role to play in preventing, detecting and mitigating the emergence of zoonoses at their source (12). More specifically, animal health laboratories have a critical role to play in the detection of new infectious agents and in supporting surveillance and alerting for the occurrence of new and known diseases in their geographic area. These laboratories have a broad range of competencies, from food safety to diagnostics, vaccine production and quality control, research and development, etc., ultimately impacting public health along with animal health. The capacity, reach, and resources of national veterinary services vary widely across countries, which is reflected in the global distribution of veterinary laboratory expertise (13). WOAH Reference Centers are laboratories and other types of scientific institutes which have the capacity to uphold the standards of the WOAH *Terrestrial* and *Aquatic Manual of Diagnostic Tests and Vaccines*, and to contribute for scientific progress in their fields through active research and development. As of May 2022, there were 266 WOAH Reference Centers globally. These institutes are expected to support animal health systems globally through testing of samples, to provide training and advice to other Member Countries, and to collaborate with dedicated scientific networks.

Only some developed, high-income, nations have the capacity to systematically assess the health status of their livestock and wildlife populations through surveillance and monitoring programmes and look for pathogens that could spill-over to humans. These, countries benefit from early-warning systems, which allow them to detect outbreaks sooner and to reduce the socioeconomic impact of animal diseases on animal and public health systems. This leaves poorer countries in a position where they must bear a heavier socioeconomic burden caused by animal diseases (14), in part caused by their limitations in implementing surveillance an monitoring systems. However, pathogens travel across borders and oceans through travel, trade, and migrations, and the missed opportunity of early detection results in higher likelihood of international disease spread. Therefore, countries and organizations concerned with global health security provide support to laboratories of developing countries in an effort to improve their capacity for detection and control of diseases, thereby contributing to evening out the global laboratory expertise and technical capacity, including that of animal health laboratories. One such initiative is the World Organization for Animal Health (WOAH, founded as OIE) Laboratory Twinning Programme, which was founded in 2006 with the aim to support the development of veterinary laboratory expertise in underserved regions. In these projects, one WOAH Reference Center is paired with an institute from another WOAH Member Country and together the institutes develop and implement a 2–3-year workplan focused on a disease or on a topical area. The Delegates of the countries and institute directors must confirm their agreement and support to the twinning initiative. There has been broad uptake and diversity in the focus of twinning projects implemented under the scope of this programme. Yet, a formal evaluation of the Programme's impact and the sustainability of the outcomes of individual projects has not been done. In this paper, we describe the implementation of the Programme in the past 16 years, making a connection with the global distribution of livestock biomass and the current distribution of veterinary laboratory expertise. This paper makes a case for the development of a monitoring and evaluation (M&E) tool for WOAH Laboratory Twinning projects which will inform similar capacity building initiatives in the public health space and positively impact animal health systems.

Methods

The data in this paper were sourced from a combination of public and internal WOAH records, in addition to a review of scientific literature. The map in Figure 1 was developed

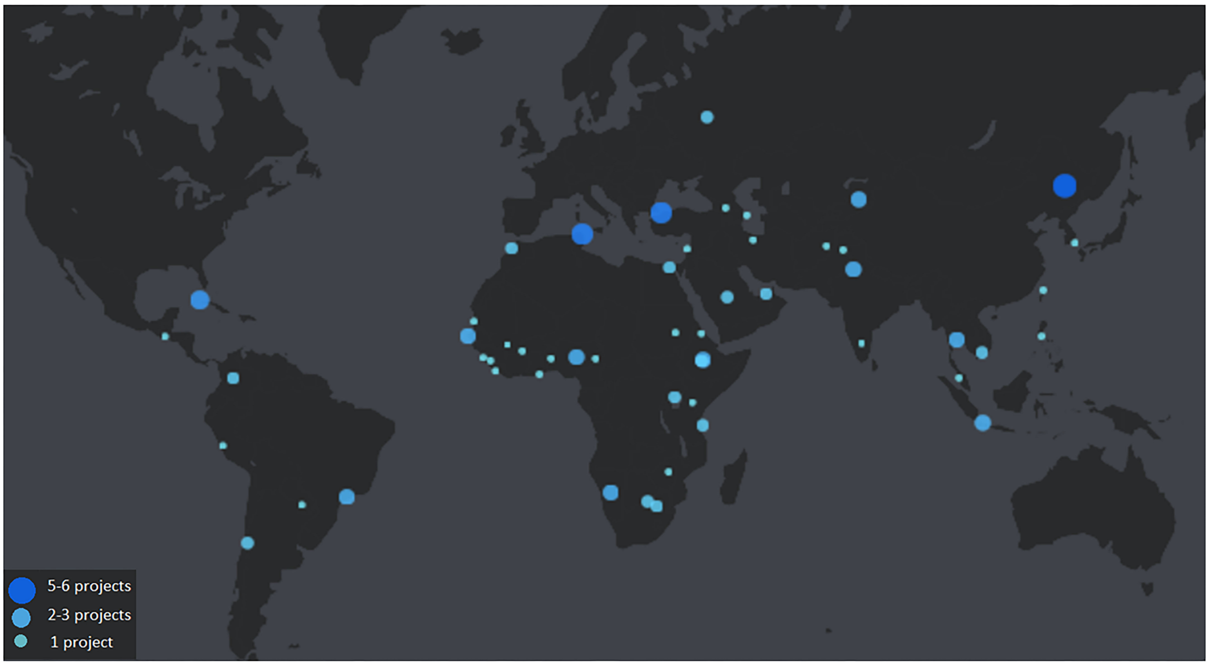

FIGURE 1
Global distribution of WOAH Laboratory Twinning projects implemented in the period 2006–2022. Larger dots indicate a higher number of projects implemented in one country.

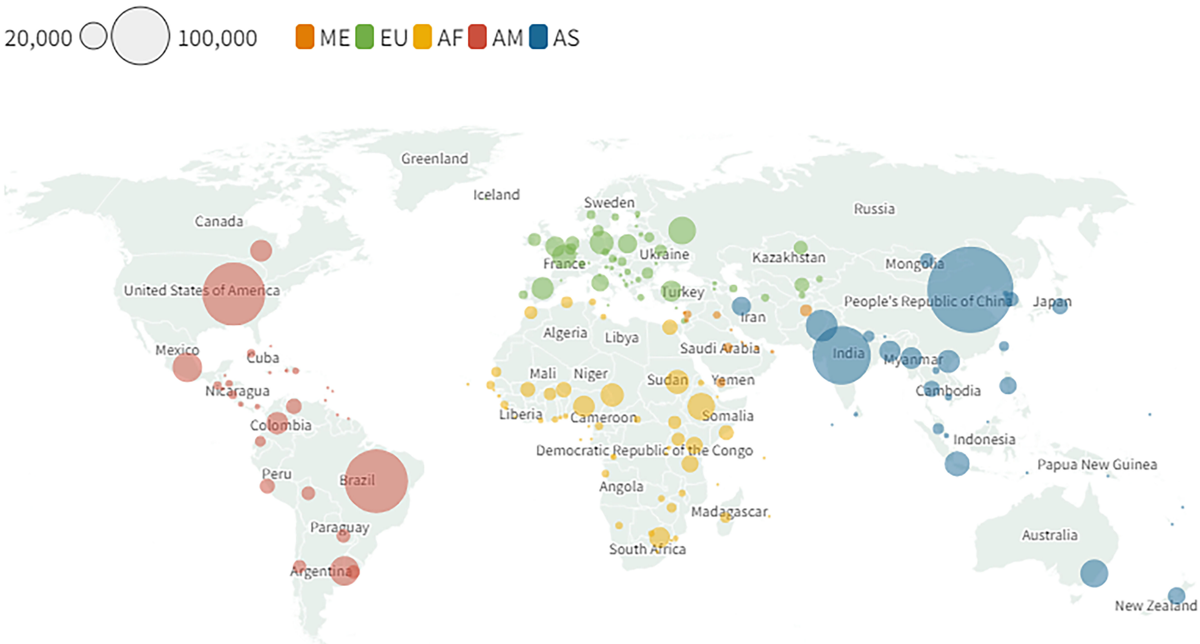

FIGURE 2
Global distribution of livestock biomass (including terrestrial and aquatic animals) in thousands of tons for the year 2018.

using the open access version of Flourish Studio (15) with historical data from project implementation publicly available in WOAH's website (16). The map in Figure 2 was created using the open access version of Flourish Studio with data from the animal biomass[1] indicator developed by WOAH's Antimicrobial Resistance & Veterinary Products Department through a methodology adapted for the annual WOAH antimicrobial use data collection survey (17). The association between the origin of funding and the achievement of Reference Center status was tested using a Chi square test in MS Excel.

Results

Between February 2008 and May 2022, 114 projects were initiated under the WOAH Laboratory Twinning Programme. Approximately two thirds (60%) of these projects have been completed, a third (30%) are underway, and 10% were canceled. The geographical location of the majority of twinnings, illustrated by Figure 1, matches the regions that were identified in 2006 as lacking laboratory expertise – Africa and Asia. African countries were the beneficiaries of 48 projects, Asia-Pacific received 29 twinnings, the Americas 16, Europe 12, and the Middle East only 7. It should nevertheless be noted that the Middle East region of WOAH is the least numerous in Member Countries (12) given its comparatively smaller geographical coverage in relation to the other four WOAH regions which have more Member Countries: Americas (31), Africa (54), Asia-Pacific (32), and Europe (53).

There is a great diversity of topics that interest twinning candidates. Although avian influenza and brucellosis were the most sought-after topics in the early years of the Programme, a cluster of new projects has favored the field of viral haemorrhagic fevers. Rabies has raised steady interest over time. Overall, Africa is the region with the highest percentage of donor-funded twinnings (98%), followed by the Americas (93%), and Europe (92%). The Asia-Pacific and the Middle East regions are the ones with the most self-funded projects[2], at 81 and 33%, respectively. In all WOAH regions combined, 11.4% of twinning projects are self-funded.

Fifteen new WOAH Reference Centers have been designated as a direct result from participation in a WOAH laboratory twinning project. These Reference Centers are located in Abu Dhabi (1), Botswana (1), Brazil (1), Chile (1), China (4), India (1), Rep. of Korea (1), Russia (1), Senegal (1), Thailand (2), and Turkey (1) – totalling two new Reference Centers in Africa, two in the Americas, eight in Asia, two in Europe, and one in the Middle East. Out of the 15 projects that have resulted in new Reference Centers, only two were self-funded by the candidate countries. It was shown that there is no association between funding origin (self-funding vs. donor funding) and the achievement of Reference Center status. At present, the regions with the greatest number of WOAH Reference Centers are Europe, Asia - Pacific, and the Americas. There is a great disproportion in the distribution of Reference Centers inside these regions, favoring higher-income countries: the majority of Reference Centers in Europe are located in members of the European Union, and the bulk Reference Centers in Asia and the Pacific is in located in four countries: Australia, China, Japan, and the Republic of Korea; the vast majority of Reference Centers in the Americas are based in Argentina, Canada, and the USA.

The global livestock biomass – including terrestrial and aquatic animals – is unevenly distributed across the world. Four countries, China, Brazil, India, and the USA, have as much as 43% of the global livestock biomass, as shown in Figure 2, while in Africa and Europe the livestock biomass is more evenly distributed among neighboring countries.

Discussion

Achievement of WOAH Reference Center designation is not a standard objective for every project. Aiming for such designation depends heavily on the starting capacity, staff commitment, equipment availability, and management engagement of the candidate institute, as WOAH twinnings are solidarity-based and no funds are spent on equipping and maintaining the laboratories. Nevertheless, at this point in the review of the Laboratory Twinning Programme, achievement of Reference Center designation is a concrete indicator of success, which is used to describe and compare progress in the regions involved in the Programme. The completion of the work plan set out in the beginning of the project as an indicator of success was considered. However, WOAH twinnings are not considered finalized until the work plan is fully implemented, which leaves all finished projects in a similar standing with regard to work plan implementation and is not a guarantee of success. That is why the review of the Twinning Programme includes the identification of success determinants and of indicators related to the sustainability of project outcomes.

Nearly half of the WOAH Laboratory Twinnings to date were implemented in Africa, with a number of African countries including Ethiopia, Namibia, Nigeria, Senegal, and Tunisia having benefitted from multiple twinnings. Nevertheless, this has not been translated into a higher ratio of new Reference Centers resulting from twinnings in the African region in relation to the other WOAH regions. This does not seem to match the original objective of the Programme, which was to even out the global distribution of veterinary laboratory

1 Biomass calculated includes the live animal population biomass.

2 Self-funded projects are usually financed by the candidate country, which manages the project's budget independently from WOAH. In donor-funded projects WOAH's financial rules for laboratory twinnings apply.

expertise. There is a number of possible explanations for this finding: (1) the baseline capacity of the laboratories chosen to participate in the Programme may have been such that the improvement acquired during the project were not sufficient to elevate it to Reference Center level; (2) the laboratories may not have enough endogenous investment (as opposed to "donor investment") or absorptive capacity to sustain and build on the work done during the project; or (3) the achievement of Reference Center status may not be a priority for many of the candidate countries, as this designation comes with its own set of bureaucracy, expenses, and responsibilities.

The origin of the funding supporting the projects has not shown to be associated the achievement of Reference Center status by the candidate laboratory. This is a reassuring finding given that nearly 90% of WOAH Laboratory Twinnings are donor funded. The location and topics of WOAH twinnings are significantly constrained by the availability of donor funding. Most commonly, these funds come with conditions related to the regions where the projects can be implemented and the topics that can be covered, according to donors' priorities and geopolitical interests. This is well illustrated by the low implementation of twinning projects in Central and South America, the high availability of funds for implementation of projects in Africa and Asia, and by the change in the popularity of certain twinning topics. In the early years of the programme, which included the 2009–2010 global swine/H1N1 flu outbreak, nearly all new projects focused on avian influenza. In the years after the Ebola crisis in West Africa, there was a significant increase in funding available for projects on viral haemorrhagic fevers. More recently, after African Swine fever started spreading in Asia, there was an uptick of funding for ASF. Wealthier countries with multiple participations in twinnings, such as China, seem to have the capacity to maintain interest and investment after the twinnings are concluded resulting in the systematic establishment of Reference Centers after twinnings.

Visual inspection of Figures 1, 2 seems to indicate that the distribution of WOAH Reference Centers and of Laboratory Twinning projects is not correlated with the global distribution of animal biomass. However, further qualitative analysis is needed to investigate the relationships between livestock distribution and animal health laboratory expertise. Nearly half of all WOAH Reference Centers are located in high-income countries in the European region, which has a relatively low concentration of animal biomass compared with China, Brazil, India, and the USA, the countries where the livestock biomass is the highest. It should be noted that the USA has a high number of WOAH Reference Centers in relation to other high-income countries, and that China, the country with the largest share of livestock biomass and the highest human population count, has taken significative advantage of the WOAH Twinning Programme, being the country that has the greatest number of new Reference Centers (4) resulting from laboratory twinnings. Interestingly, the Chinese example has also occurred in other emerging economies that belong to the BRICS, albeit at a smaller scale – Brazil, Russia and India have each one new Reference Center resulting from twinning projects. This seems to suggest that the ability of countries to take advantage of WOAH twinning projects and advance toward designation as a WOAH Reference Center is correlated to their income level and, thereby, with the capacity to leverage the investment made during the project possibly and take it forward with endogenous funds. This raises two questions: (1) What is the minimum standard that a candidate laboratory should have in order to be set up for success within the Programme; and (2) Which indicators other than Reference Center designation can be used to characterize successful projects.

Defining "success" is important to avoid settling for unstructured feedback describing successful twinning experiences. Given the solidarity-based character of WOAH twinnings, there is the risk that projects act as a band-aid for a bigger problem: the lack of investment in veterinary laboratories within public health networks. The minimum standard for candidate laboratories to benefit from a twinning project could be based on relevant sections of Chapter 1.1.1 of the WOAH Manual regarding Management of Veterinary Laboratories.

A monitoring and evaluation (M&E) tool for laboratory twinning projects is needed to better understand projects' impact, the sustainability of their outcomes in the medium to long term, and the characteristics shared by successful projects. Logically, the monitoring and evaluation components of the tools should be separated, as the indicators used to monitor projects' implementation would be different from those used to evaluate the project's outcomes, impact, and sustainability post-implementation. The process to create the M&E tool should build on the results of WOAH's recent work on laboratory sustainability (18), that analyzed data from a cohort of laboratories participating in the WOAH Performance of Veterinary Services Sustainable Laboratories (PVS Lab) missions and found that while capacity building efforts may have improved bench-top capacity in laboratories, this capacity is unsustainable. The M&E tool would not directly assess laboratory sustainability, as that is already covered by the PVS Lab tool, but rather integrate it into its indicators while focusing on the implementation and outcomes of laboratory twinning projects. Such tool would allow project implementors to learn from past experiences and better plan future projects, thereby promoting resource optimisation and improving the likelihood of success.

There is the opportunity to learn about the effectiveness of capacity building interventions in animal health laboratories from the data and experiences accumulated during the past 16 years. The factors associated with projects' success, failure and with the achievement of Reference Center status should be identified and systematized so as to ensure that resources allocated to the Programme are being well spent and that the Programme is complying with the objective of evening out the global distribution of veterinary laboratory expertise.

Conclusion

WOAH's Laboratory Twinning Programme is a well-established and recognized initiative for capacity building in animal health laboratories. Its reach is global and the regions that received the most investment were the ones lacking laboratory expertise the most. However, this has not translated in a higher ratio of Reference Centers resulting from twinnings in these regions compared to the rest of the world. There is potential from learning from the experiences of the countries that have better leveraged their participation in the Programme, becoming Reference Centers in their own right. However, there are no data concerning the impact and the sustainability of the outcomes of twinning projects after their implementation is finished. An evaluation process that covers the outcomes and impact of the twinnings would help to optimize the implementation of future projects, ultimately providing better support to national veterinary services and improving animal health systems globally. A framework for such an evaluation is being developed and will be the subject of a future publication.

Author contributions

MM conceived, designed, and performed the analysis, in addition to writing the paper. EA contributed to the analysis. MJ provided the biomass data. JR, WG, AN, and KH supervised the production of the paper. All authors contributed to the article and approved the submitted version.

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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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Use of a new antimicrobial consumption monitoring system (Vet-AMNet): Application to Dutch dairy sector over a 9-year period

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Introduction: The urgency of preventing the increase of antimicrobial resistance has been emphasized by international authorities such as the World Health Organization, European Medicines Agency, and World Organization for Animal Health. Monitoring systems capable of reporting antimicrobial consumption data are regarded as a crucial pillar of this fight. The Vet-AMNet system was developed to collect and analyze national antimicrobial consumption data in Portuguese dairy farms to support the veterinary authority in stewardship actions and to assist both veterinarians and farmers in daily decisions related to antimicrobials.

Methods: To evaluate the robustness of the system and other identified critical success factors, it was used to analyze antimicrobial consumption data available from the Dutch dairy cow sector over the period from 2012 to 2020. The data previously used for publications by the Netherlands Veterinary Medicines Institute (SDa) were imported and pre-processed by the Vet-AMNet system according to the SDa's standard operating procedure and the Dutch metrics to measure antimicrobial consumption were calculated.

Results: By comparing the outputs with the figures generated by the system established in the Netherlands, the Portuguese system was validated. Antimicrobial consumption data from the Dutch dairy sector during the 9-year period will be presented in unpublished graphs and tables, where each molecule's pharmaceutical formulation, pharmacotherapeutic group and line of choice will be related and discussed, illustrating the evolution of sectorial antimicrobial consumption against a background of a strong national antimicrobial policy initiated by public-private cooperation and supported by legislation.

KEYWORDS

antimicrobial, consumption, surveillance, stewardship, resistance, dairy (cows), monitoring

1. Introduction

To understand and control the emergence of antimicrobial resistance (AMR), it is essential to monitor the use of antimicrobials and resistance development (1). The main aim of veterinary antimicrobial consumption (AMC) surveillance programs is the promotion of prudent use of these substances, among veterinarians and farmers, which can be achieved by interpreting patterns and tendencies of use related to the emergence of AMR (2). In the Netherlands, the association between the reduction of AMC and a decrease in the prevalence of AMR genes in indicator *Escherichia coli* has been proven, in several livestock sectors (3).

AMC monitoring systems establish a foundation for evaluating the effectiveness of implemented control measures, by identifying the emergent use of certain antimicrobial (AM) substances, enabling risk evaluation and management. They also allow the comparison of AM usage at a national and international level, when the same indicators are applied, and within a given time frame (2).

The European Medicines Agency (EMA) started the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project, with the purpose of collecting data on AMC in animals to inform policy makers. National participation has been established on a voluntary basis. However, from 2023, reporting AM sales and consumption to EMA will be a legal obligation, after Regulation (EU) 2019/06 on veterinary medicinal products (4).

The Vet-AMNet project started in 2019, as a conjoint initiative of the *Instituto de Ciências Biomédicas Abel Salazar*—Porto University (ICBAS-UP), the Portuguese Veterinary Authority (DGAV) and the Portuguese Dairy Farmers Association (ANABLE). The system's main goal is to collect and analyze veterinary AMC data within Portuguese dairy farms. It will be used by DGAV in the context of acquiring information for the ESVAC project, and to respond, by January 2023, to the AMC data submission requirements stated in Regulation (EU) 2019/06. Besides this main goal, it has also been created with the aim of sharing mobile dashboards where AMC figures are presented along with associated costs, milk yield values and information captured *via* field questionnaire. Therefore, it may be used by veterinarians and farmers in their daily activities as an analysis tool to assist in AMC related decision making.

The following characteristics were identified, at this stage, as critical success factors to the systems' good performance and longevity:

- Flexibility: to allow adaptations to different output requirements and future data processing alterations.
- Universality: it should be compatible with most data input formats, so future collaborations are not restricted by input norms.
- Real time responsiveness: after establishing the system's architecture, it should be able to produce outputs in a time-efficient way, after the submission of predesigned standardized inputs.
- Customizability: as the need to guide decisions in an efficient way was identified, the system was designed to be able to provide different stakeholders with information that is relevant to their needs.

In what concerns veterinary antimicrobial consumption, the Netherlands is considered to be an international success model, where the combination between voluntary and mandatory actions (5) has led to an overall reduction of 70.8% in kilograms of antimicrobials sold in the Dutch livestock sector, since 2009. Antimicrobial usage in the Dutch dairy sector is considered to be low and acceptable by the livestock sector and by the Netherlands Veterinary Medicines Institute (SDa) expert panel, with most of the usage relating to individual animal treatments, and a very low consumption of substances of critical importance (6).

The SDa aims to promote the responsible use of antimicrobials (6), protecting public health while taking animal welfare into account. The institute receives the totality of the AMC data annually. Dutch livestock sectors receive AMC data reports from the SDa, but have their own dynamics regarding data analysis and communication with farmers and veterinarians (7). The SDa, by publishing annual reports, provides insight to the government and general public regarding national AMC figures, and establishes regularly updated consumption targets (benchmark values) (6).

This work aims to:

- Validate the Vet-AMNet system, by assessing its robustness to sustain a country level AMC monitoring program and evaluate the critical success factors identified, by recreating the data analysis produced by the AMC monitoring system that is implemented in the Netherlands and has been used to produce previously published reports.
- Provide guidance for the design of an AMC monitoring system.
- Assess the detailed consumption of AM substances in Dutch dairy farms by differentiating the sectorial figures into pharmacotherapeutic groups, pharmaceutical formulation, national line of treatment and segmenting farms into percentiles according to their AMC. The previously unpublished outputs generated, by correlating the mentioned variables, demonstrate new perspectives on the evolution of the sectorial AMC. These will be interpreted in the scope of the likely effect of restriction policies and other measures implemented, over this period, to promote a more responsible use of AM substances.

2. Materials and methods

2.1. Antimicrobial drug information

Information regarding the dosages of veterinary antibiotic drugs registered in the Netherlands is contained in a database called the "DG-standard." In this, each drug is associated with a specific "number of treated animal kilograms" which corresponds to the number of animal biomass kilograms that may be treated by using one package of the specific drug in question. The "DG-standard" database also encompasses the European Article Number (EAN), pharmacotherapeutic group, pharmaceutical formulation, package units and size for every licensed veterinary antimicrobial product since 2003 (1). Antimicrobial molecules for veterinary use have also been classified into first, second and third line of choice for animal treatments, according to national treatment guidelines published by the Veterinary Antimicrobial Policy Working Group (WVAB) of the Royal Dutch Veterinary Association (KNMvD), based on directives form Dutch Health Council (2, 3) In Supplementary Table 1, the pharmacotherapeutic groups of the veterinary antimicrobial medicines registered for use in dairy cattle can be seen, together with their line of choice classification.

2.2. Dutch antimicrobial usage indicators

To express the amount of antibiotics used in the Netherlands within a particular livestock sector, the SDa has developed two indicators that correspond to the defined daily doses animal (DDDA) consumed in a given year, the $DDDA_{NAT}$ and the $DDDA_F$ (1).

The $DDDA_{NAT}$ is used to evaluate trends in AMC at a national level and represents the average number of days/year an average animal, within a particular livestock sector, is treated with AM. It is calculated by dividing the number of treated animal kilograms times the number of days within a livestock sector for a particular year by the average number of total animal kilograms present within the livestock sector concerned, for the given year (1), as shown in Formula 1 in the Supplementary material.

The $DDDA_F$ is used to assess AMC at farm level and compare a farms' consumption with a predefined benchmark value. It represents the number of days per year an average animal is treated, at that farm. It is calculated by dividing the number of treated kilograms times the number of days on a farm for a particular year by the average number of kilograms of animals present on that farm (1), as shown in Formula 2 in the Supplementary material.

The use of the national defined daily dose animal ($DDDA_{NAT}$) units allows the standardization of country level AMC and its comparison over time by categories of choice, pharmaceutical formulation, and individual molecules. Unlike the $DDDA_F$ this method is not influenced by redefinition of population parameters, as it happened for instance in the pig sector, when the combined population of sow and piglets was split in two distinct populations, sows + suckling piglets and weaned piglets. Therefore, the DDD_{NAT} can be used to follow trends in AMC over time and to assess the impact of interventions within a livestock sector. Also, the size of the farm is not influencing the outcome since all AMC is related to all animals within a livestock sector in the country.

The consumption of individual farms, expressed in $DDDA_F$, allows the analysis of the differences between farms, and may be helpful in identifying parameters influencing AMC. This indicator is also used to benchmark the farm's AMC. It is important to realize that in the national overall average farms' defined daily dose animal ($DDDA_F$), small farms and big farms have identical impact, while the $DDDA_{NAT}$ reflects the weighted average of $DDDA_F$. This indicator also allows the assessment of the impact of interventive actions at farm level. Some farms may register 0 $DDDA_F$, over a year. This can happen if a farm identification number is associated with registered dairy cattle, but with no antimicrobial prescriptions over the given period.

2.3. Policy measures introduced and market fluctuations in the Netherlands since 2012

To facilitate the interpretation of the findings in AMC in dairy cattle, we summarize the most relevant policy measures introduced and market fluctuations in the Netherlands since 2012, in Table 1.

2.4. Data sources

To calculate the DDDA ($DDDA_F$ and $DDDA_{NAT}$) values, the SDa is supplied by the livestock sectors with information, at farm level, regarding the average number of animals present and veterinarian antimicrobials prescription registries.

In the Netherlands, veterinarians working in the veal, broiler, turkey, cattle, pig, rabbit and goat farming sectors are obliged to report all AM prescriptions into the livestock sector database, which is mostly done *via* software packages with a Practice Management System (PMS) like Animana®, Easyvet® or VIVA®. These PMS's are in place to register all interventions and dispensed medication to clients e.g., farms by veterinarians for veterinary care, logistic and financial purposes. The usage data of antimicrobial medication are then provided to the SDa by the sector quality systems, supplemented with the animal data. Data quality regarding AM prescriptions is ensured by requirements that the SDa has established and can be consulted in the standard operating procedure of the organization (4).

TABLE 1 Relevant policy and market fluctuations restricting the use of antimicrobials in the dairy sector in the Netherlands since 2012.

Year	Policy/market fluctuation	Main effect	Source
2009	Establishment of General reduction targets for the use of antibiotics in livestock set by Dutch government: - 20% reduction in overall in antibiotic use by 2011; - 50% reduction in overall antibiotic use in 2013.	A reduction in antibiotic use across all monitored livestock sectors.	SDa note on precision reduction targets (8)
Before 2012	Lack of products with first line AM substances for treating mastitis, and limited popularity of these to be used in dry cow therapy	Mainly second line intramammary (imm) products were used, both in dry cow and lactating cow treatment	2012–2013 SDa report
2013	Royal Dutch Society for Veterinary Medicine (KNMvD) published its guideline on the application of selective dry-cow therapy ("Selectief droogzetten")	Decrease on the overall usage of dry-cow imm products overall and a shift from products with combinations of antibiotics toward products with first line penicillins	2014 SDa report
2013	Introduction of more strict legislation allowing only first-line AMs for individual treatment to be available in small amounts for the farmer	Important reduction of AM's stock at farms	Regulation of the state secretary for economic affairs of 15 August 2013, no. WJZ/13031524 (9)
2017	Introduction of first-line mastitis injectors in NL	Shift from lactating cow second-line products to first-line imm products	2017 SDa report

Records from 2012 to 2020 containing farm-level prescriptions, animal population and relevant drug characteristics, such as the Dutch national defined dose-based unit of measurement "DDDA," active ingredient, substance group, and pharmacotherapeutic formulation, as previously used for publications by the Netherlands Veterinary Medicines Institute (SDa) were imported in and analyzed by the Vet-AMNet system. The analyzed dataset comprises all the Dutch farms that registered at least one dairy cow in the covered years, encompassing a yearly average of around 17,000 farms and 495,000 treatment registries.

The animal sector quality systems also provide the average number of animals present over the period of a given year and these figures are either collected by inspection visits or extracted from the national mandatory "Identification and Registration System (I&R)" for animal registration (4).

To assess the average number of live animal kilograms on a farm, which represents the animal population at risk of being treated with antimicrobials, a standardization of the animal biomass denominator had to be made, to make the estimates feasible and more precise. Therefore, in the case of dairy cattle herds, animals are split into 4 categories, with divisions related to age. Each of these categories is associated with a previously estimated and defined standardized weight per animal (1). The analyzed dataset amounts for a yearly average of around 1.6 million animals and the animal categories and associated weights can be found in Supplementary Table 2.

2.5. Data analysis using the Vet-AMNet system

All the data gathered was systematically pre-processed using the Vet-AMNet system; mainly done by aggregating entries from the original source and by shaping data to the desired structure, designed to harmonize all inputs. Herd data inputs were aggregated by farm and year and then multiplied by the standard weight values defined by the SDa. AMC inputs were aggregated by year, farm, active ingredient, and pharmaceutical formulation. Drugs without defined antimicrobial use in cattle in DG-Standard were removed from the analysis.

After pre-processing all data inputs, these were modeled into an adapted version of the Vet-AMNet data architecture, illustrated in Figure 1. Components including milk yield data provided by the dairy cooperatives and the costs of antimicrobial drugs are also part of the original Vet-AMNet system, together with farm assessment questionnaires collected by veterinarians on topics such as biosecurity. However, these were not encompassed in the present analysis since the main aim of this work was to validate the use of the Vet-AMNet system to report national AMC monitoring figures alone.

The European article number (EAN) was used to connect the drugs prescribed with the specified product characteristics present in the official list of licensed AM drugs (DG-standard), and each farm's AM sales data was related to its respective animal data based on each farm's unique identification number. The indicators described above ($DDDA_F$ and $DDDA_{NAT}$) were

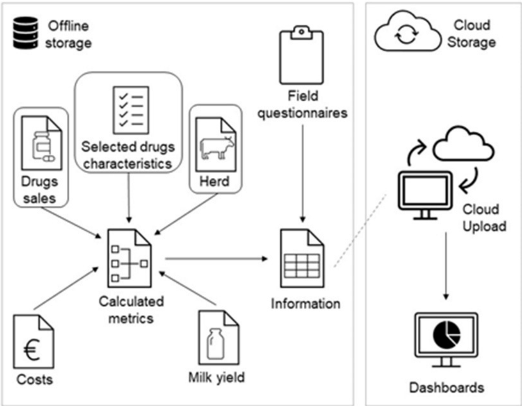

FIGURE 1
Vet-AMNet's adapted data architecture. The metrics calculated in this analysis required "drug sales," "herd data," and "selected drugs characteristics" as highlighted in the model.

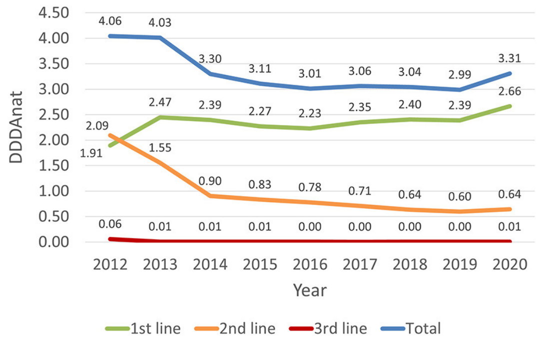

FIGURE 2
Antimicrobial consumption in the Dutch dairy sector, in DDDANAT units, from 2012 to 2020, divided in total, 1st, 2nd and 3rd line products.

calculated, per each of the analyzed years, also using the Vet-AMNet system. The components of the Vet-AMNet system that were used in this analysis were built on Microsoft Power BI Version: 2.103.881.0.

3. Results

3.1. Validation of the Vet-AMNet system

The Dutch indicators used to measure AMC were recreated using the same source data and achieving the same figures as previously generated and published by the SDa, in their yearly reports on the usage of antibiotics in agricultural livestock in the Netherlands (5–14). A compilation of these figures published by the SDa may also be found in Supplementary Table 3, by comparison, it can be verified that the figures presented in the graphs and tables produced by the Vet-AMNet system and presented in the next sections are accurate recreations of the overall figures produced by the Dutch system.

The information produced by the Vet-AMNet system, regarding the last 9 years of AMC in the Dutch dairy sector, was compiled and converted into dashboards composed of different visuals such as the tables and graphs presented in this segment, that were developed to meet the SDa's expert panel data visualization requirements.

3.2. Overall AMC in the Dutch dairy sector

AMC in the Dutch dairy cattle sector can be considered low over the entire study period. An average cow received antimicrobial treatment for <5 days per year (5 $DDDA_{NAT}$) in all the years AMC was recorded. As depicted in Figure 2, the overall consumption of antimicrobial substances, between 2012 and 2013, was similar. From 2013 to 2014, there was a 24% decrease in consumption, and a further 6% reduction from 2014 to 2015. The sector then achieved equilibrium from 2016 to 2019, registering a consistently low yearly consumption of ∼3 $DDDA_{NAT}$, with the SDa expert panel reporting that these small percentual variations were expected and not a concern (5). From 2019 to 2020 overall AMC increased 11%.

Figure 2 shows that the overall reduction in AMC was mainly due to a marked decline in the use of second-line products between 2012 and 2014 (−57%) which then continued until 2019, although in a much less abrupt way. In the 9 years covered, the use of second line AM reduced from 2.09 to 0.64 $DDDA_{NAT}$, representing an overall reduction of ∼69%. The use of third-line products was already residual since 2013, and therefore fluctuations in the use of these substances did not have a notable impact in the overall consumption. In contrast, the use of first line AM products grew from 1.91 to 2.45 $DDDA_{NAT}$, increasing almost 23%, from 2012 to 2013, resulting from a shift from second-line products. After this increase, the use of these molecules remained almost constant, in the 6 years that followed, and increased by 11% from 2019 to 2020.

In 2012, the first year of full coverage AMC monitoring in the dairy cattle sector, first line antimicrobials were not the most used products, accounting for 47% of the treatments. They became the most used line in the following year, with a 29% increase in the consumption of these substances at the cost of second line products, which can be seen in Table 2. In 2014, the consumption of first line products stabilized, representing over 70% of the treatments, with a steady relative growth each year and reaching more than 80% in the 2020. The relative consumption of second line products decreased sharply from 2012 to 2014, and gradually from 2014 to 2020, representing less than 20% of the usage registered in 2020. The consumption of

TABLE 2 Yearly antimicrobial consumption, in $DDDA_{NAT}$ units, from 2012 to 2020 by pharmaceutical form and by line, in consumption and overall variation (Total Var).

Pharmaceutical forms	2012	2013	2014	2015	2016	2017	2018	2019	2020	Average	Total Var
Imm dry	1.87	1.97	1.40	1.29	1.30	1.3	1.30	1.24	1.33	1.45	−29%
1st line	0.98	1.41	1.36	1.26	1.26	1.3	1.28	1.22	1.30	1.26	33%
2nd line	0.89	0.56	0.04	0.03	0.03	0.0	0.03	0.03	0.03	0.19	−97%
3rd line	0.00	x	x	x	x	x	x	x	x	x	x
Imm lactating	0.79	0.82	0.76	0.71	0.65	0.7	0.70	0.70	0.80	0.74	2%
1st line	0.00	0.01	0.01	0.01	0.00	0.2	0.21	0.25	0.34	0.11	8300%
2nd line	0.77	0.81	0.75	0.70	0.64	0.6	0.49	0.45	0.47	0.63	−39%
3rd line	0.02	0.00	0.00	0.00	0.00	x	x	x	x	x	x
Intra-uterine	0.15	0.14	0.13	0.11	0.10	0.1	0.09	0.08	0.08	0.11	−47%
1st line	0.12	0.12	0.11	0.10	0.09	0.1	0.08	0.07	0.07	0.09	−41%
2nd line	0.03	0.03	0.02	0.02	0.01	0.0	0.01	0.01	0.01	0.02	−68%
Oral	0.11	0.06	0.04	0.04	0.04	0.0	0.03	0.02	0.02	0.04	−78%
1st line	0.05	0.05	0.04	0.03	0.02	0.0	0.02	0.01	0.01	0.03	−71%
2nd line	0.06	0.02	0.01	0.01	0.01	0.0	0.01	0.01	0.01	0.02	−84%
Parenteral	1.13	1.02	0.97	0.96	0.94	0.9	0.93	0.95	1.07	0.98	−5%
1st line	0.74	0.87	0.89	0.88	0.85	0.8	0.83	0.84	0.94	0.85	27%
2nd line	0.35	0.14	0.08	0.08	0.08	0.1	0.10	0.10	0.13	0.13	−64%
3rd line	0.04	0.01	0.00	0.00	0.00	0.0	0.00	0.00	0.01	0.01	−86%

TABLE 3 Total number of farms, average farm's antimicrobial consumption, percentage of zero consumption farms, and percentiles 5, 25, 50, 75 and 95 from 2012 to 2020, in $DDDA_F$ units.

Year	No of farms	Zero consumption farms	Average DDDaf	DDDaf percentile 5	DDDaf percentile 25	Median DDDaf	DDDaf percentile 75	DDDaf percentile 95
2012	18,053	394	2.9	0.23	1.63	2.72	3.75	5.6
2013	18,005	296	2.79	0.3	1.78	2.78	3.7	5.29
2014	17,747	229	2.27	0.29	1.37	2.19	3.04	4.47
2015	17,737	227	2.16	0.27	1.29	2.08	2.91	4.24
2016	17,529	244	2.11	0.25	1.24	2.06	2.87	4.16
2017	17,121	369	2.14	0.18	1.21	2.07	2.94	4.31
2018	16,499	305	2.14	0.2	1.19	2.05	2.95	4.39
2019	15,871	300	2.2	0.2	1.23	2.1	3.03	4.53
2020	15,522	296	2.39	0.23	1.36	2.26	3.26	4.95

third line products decreased from 2012 to 2013 and represents a minor fraction of total AMC.

3.3. Farm level AMC in the Dutch dairy sector

In Table 3, the average consumption pattern of the sector farms ($DDDA_F$) is split in quartiles, and the range between them and their respective yearly tendencies are shown, highlighting the response of the sector segmented into different AMC categories. On average, the interquartile range between the second and third quartiles is 1 $DDDA_F$. The average range between the 5th and the 25th percentile is 1.13 and between 75th and the 95th is 1.5, showing a slight right skewness. Average consumption of farms on the 5th percentile remains relatively constant across the whole period. In percentile 25th, no significant move is visible in the second year, but a strong reduction can be seen from 2013 to 2014, with low reductions in median farms and below, until 2019. A general increase is noticeable in 2020. From 2016 onwards, farms from 95th and

75th percentile diverge from the median. A visual representation of the percentiles described in Table 3 may be found in Supplementary Figure 1. The number and proportion of farms that registered zero AMC in the analyzed period remains stable around 2%.

3.4. Consumption by pharmaceutical formulation in DDDANAT

Figure 3 represents the evolution of the sectorial AMC in $DDDA_{NAT}$ along with the relative weight of the consumption of different pharmaceutical formulations, from 2012 to 2020. Intramammary forms, both for lactating and dry cows, constituted more than 60% of the annual consumption over this period. However, the relative weight of intramammary formulations for the dry period in the yearly consumption dropped from 46 to 40% and the intramammary treatments for cows in lactation remained constant in $DDDA_{NAT}$ units, but increased relatively from nearly 20–24%, due to the overall reduction registered. The consumption of intramammary formulations for dry cows dropped from 2013 to 2014 after the introduction of the guideline for selective dry-cow treatment, and after this marked reduction, it stayed roughly constant until 2020. The overall consumption of lactating intramammary formulations stayed approximately the same, with slightly lower $DDDA_{NAT}$ levels between 2015 and 2019, but increased again in 2020. Oral treatments represent only a small part of the overall usage weighing about 2.7% in 2012 and 0.6% of the treatments in 2020 and intra-uterine formulations decreased from 3.7 to 2.4%. The usage of parenteral AM has remained approximately constant over the years, in number of $DDDA_{NAT}$, but the relative weight increased from 28 to 32%.

3.5. Dynamics of combined pharmaceutical formulation and national line of choice

Table 2 breaks down the consumption of antimicrobial substances by both line of choice and pharmaceutical formulation and shows trends in AMC over the 2012–2020 period. Overall, during the 9 years covered, there was a reduction of 18% in the consumption of all pharmaceutical forms (Figure 2), with a reduced consumption of most pharmaceutical formulations, except for intramammary treatments for cows in lactation that remained almost constant, with only a 2% change from 2012 to 2020, and showing a percentual increase from 20 to 24% of the overall treatments, in Figure 3. Parenteral overall usage was reduced by 5%, followed by reduction in intramammary formulations for the dry period of 29%. Oral and intrauterine consumption showed marked reductions of 78 and 47% respectively.

Table 2 presents the calculation of annual variations in AMC, allowing us to dissect the sudden rise from 2019 to 2020. Firstly, as already identified in Figure 2, it shows that all AM products showed an increase from 2019 to 2020, higher for first line products (11%), and (8%) in second line formulations. Additionally, it can be seen that parenteral, intramammary lactation and intramammary dry forms are mainly responsible, in absolute terms, for the increase, respectively 0.12, 0.1, and 0.09 $DDDA_{NAT}$. The relative changes were 13.3% for parenteral, 14.7% for lactating IM and 7.1% for dry cow IM formulations.

The reduction in second line AMC might be partially attributed to a shift in the consumption of both intramammary formulations to first line. After a 3-year reduction in AMC of second line parenteral formulations, there was an increase from 2016 to 2020 of 0.045 $DDDA_{NAT,}$ however, over the studied period AMC still decreased by 64% from 0.351 $DDDA_{NAT}$ to 0.128 $DDDA_{NAT}$.

Regarding first line products, the use of dry cow formulations increased in 2013 to 1.406 $DDDA_{NAT}$ (44%) after which, it remained at around 1.2–1.4 $DDDA_{NAT}$. In 2017, first line intramammary antimicrobials for use during lactation were introduced in the market.

The use of first line parenteral products first rose in 2013 (19%), then it remained steady until 2019 and it grew 12% in 2020.

Oral and intrauterine formulations consumption was markedly reduced for both first and second line products. The use of third line products was below 0.01 $DDDA_{NAT}$ from 2013 onwards and only shows apparently arbitrary fluctuations.

3.6. Consumption of antimicrobial active ingredients by pharmaceutical formulation and national line of choice

In Figure 4, the consumption of first line molecules is detailed by pharmaceutical formulation. Penicillins usage grew substantially from 2012 to 2013, decreased from 2013 to 2015, then from 2015 to 2016 grew slightly, and from 2017 to 2020, with the introduction of intramammaries for lactating cows, it increased significantly. In penicillins, the parenteral use remained stable as well as the intramammary dry-cow therapy.

The use of trimethoprim/sulphonamides increased 30% from 2012 to 2020, and the use of tetracyclines, dominantly in the parenteral and intrauterine forms was reduced 23%. The usage of other first line products remained roughly constant and are not presented in the Figure 4, because these molecules represent <5% of the use in this line of choice.

Second line products showed an overall decrease of 70% in usage. Figure 5 details consumption by pharmaceutical formulation and molecule of this category. The usage of aminopenicillins and substance combinations remained relevant, despite following the same decreasing trend. Regarding

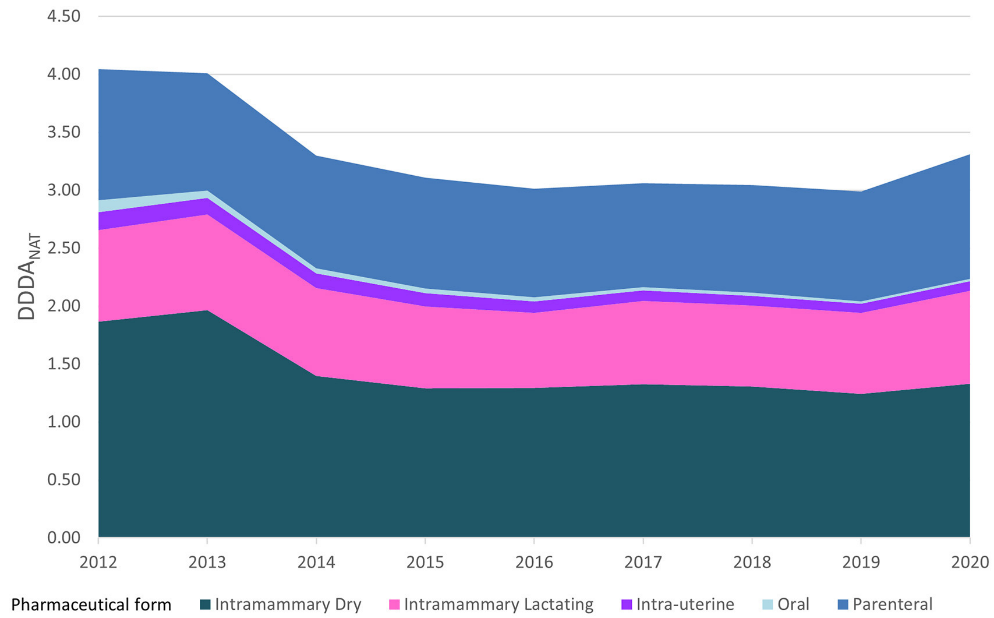

FIGURE 3
Antimicrobial consumption in the Dutch dairy sector, in DDDA$_{NAT}$ units, on a yearly basis from 2012 to 2020, segmented into the different pharmaceutical formulations and the respective weight proportion in each year.

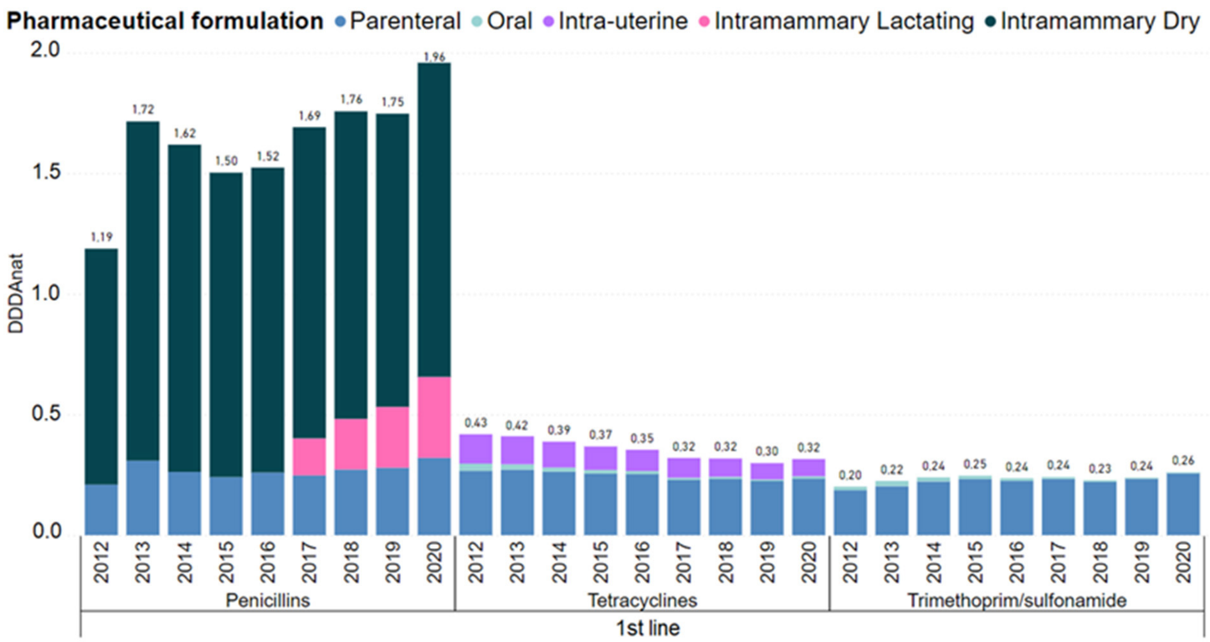

FIGURE 4
Antimicrobial consumption in the Dutch dairy sector of 1st line products, in DDDANAT units, from 2012 to 2020, segmented into the different pharmaceutical formulation. Amphenicols, macrolides/lincosamides were left out because each group represented <5% of the overall use in this line of choice. Full graph can be consulted in the Supplementary material.

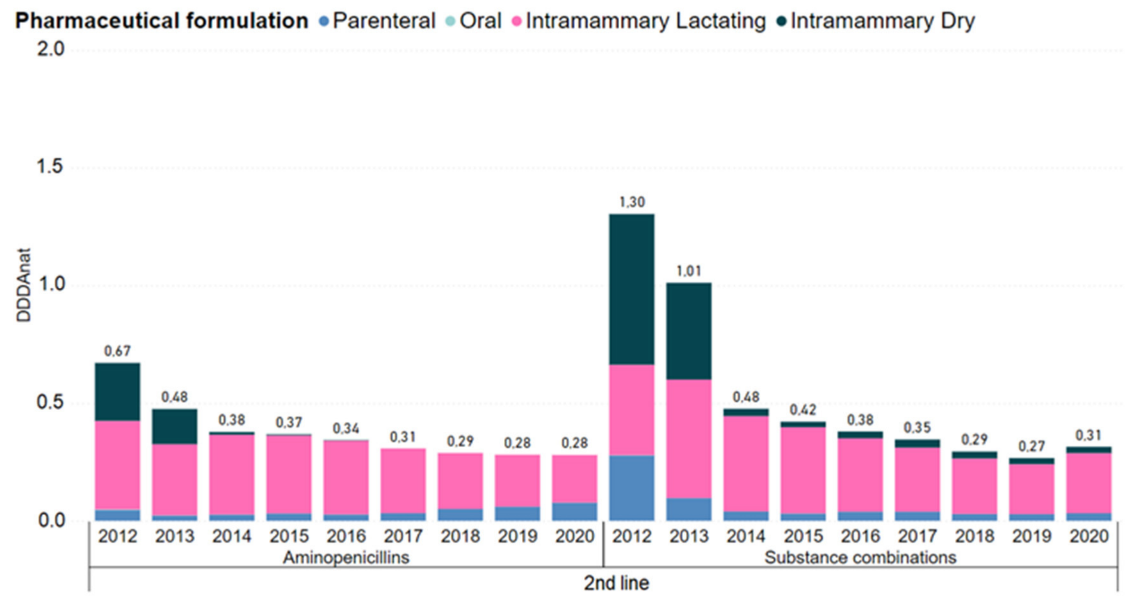

FIGURE 5
Antimicrobial consumption in the Dutch dairy sector of 2nd line products, in $DDDA_{NAT}$ units, from 2012 to 2020, segmented into the different pharmaceutical formulations. Aminoglycosides, cephalosporins 1st and 2nd gen, long-acting macrolides, polymyxins and quinolones were left out because each group represented <5% of the use in this line of choice. Full graph can be consulted in the Supplementary material.

aminopenicillins, use of intramammary forms for dry cows disappeared in 2015 and lactation IM forms although reduced, remained with a considerable level of usage. The use of parenteral substance combinations was also reduced to very low consumption levels.

3.7. Use of critical molecules

Even though the use of third line products was already very low, in 2012, around 0.058 $DDDA_{NAT}$, Table 2, over the last 9 years, there was a substantial reduction in the use of critical molecules such as cephalosporins of 3rd and 4th generation, fluoroquinolones and polymyxins. There is an absolute reduction in the proportion of farms using these groups of molecules, as presented in Table 4. Currently almost no farms are using cephalosporins of 3rd and 4th generation or polymyxins and only about 6% of farms remain attached to the use of fluoroquinolones. However, the proportion of farms using fluoroquinolones remains approximately stable since 2013.

4. Discussion

4.1. Validation of Vet-AMNet system

By accurately recreating the Dutch indicators and achieving the same figures as previously generated and published by the SDa that were compiled in this analysis, the Vet-AMNet system was successfully validated, demonstrating robustness to manage and relate nation wide antimicrobial prescription data with the respective drug characteristics and animal registry datasets, as will be demanded to produce national reports. The system's flexibility was evidenced by the adaptation of the original Vet-AMNet data model to include only the necessary information to allow the calculation of Dutch specific AMC indicators ($DDDA_{NAT}$ and $DDDA_F$). The original Vet-AMNet data architecture also includes information related to AM costs, milk yield values or field questionnaires, because it is intended to also be used as an analysis tool to assist farmers and veterinarians in AMC related decision making. These parameters were not included in this analysis because in the Netherlands, communication with individual farmers and veterinarians is the livestock sector's responsibility. Scientific and technological advancements together with new health and societal challenges may justify changes in a surveillance system. So, it is relevant to frequently evaluate the system's performance in meeting the proposed objectives, while operating under a budget (6). Other critical success factors will need to be evaluated by the Vet-AMNet management team once it is fully implemented. These are, among others, the user friendliness and acceptability of the system, cost efficiency, and the safety and quality of the data according to the FAIR (findability, accessibility, interoperability, and reusability) principle.

Microsoft Power BI®, the software of choice, is very versatile in what concerns data sources: it can be connected

TABLE 4 Number of farms that consumed 3rd line products and polymyxins, from 2012 to 2020, their yearly variation and percentage in the whole sector.

Year	3rd and 4th gen Cephalosporines	%Total farms	Fluoroquinolones	%Total farms	Polymyxins	%Total farms
2012	2,838	16%	2,554	14%	4,474	25%
2013	606	3%	1,335	7%	2,252	13%
2014	327	2%	1,244	7%	1,192	7%
2015	332	2%	1,321	7%	871	5%
2016	273	2%	1,238	7%	698	4%
2017	201	1%	899	5%	354	2%
2018	177	1%	900	5%	349	2%
2019	139	1%	898	6%	301	2%
2020	34	0%	945	6%	308	2%

to Excel®,.csv files and SQL servers, among many others (7), providing the necessary universal compatibility mentioned which was very relevant for the implementation of the system in Portugal, given the fact that the partner dairy cooperatives, veterinary authorities and European institutions store data relevant for processing in a non-harmonized way. However, there is a 1 gigabyte limit to the data sets imported using the free version of the software (10).

Microsoft Power BI® allows the creation and automatic update of dashboards and visuals, in real time, such as the graphs and tables presented in this paper. Even though the software also supports the creation of data visualizations using R and Python language, these can be built using the native Microsoft Power BI® reporting interface in an intuitive drag and drop process that does not require programming skills to produce, once the relevant variables are set. This ease of use was very relevant to the initial stakeholder engagement process in Portugal. This makes the tool user-friendly for a broad scope of users and facilitates the customization of outputs. The SDa's expert panel found the reporting component of the Vet-AMNet system intuitive and a good tool to have in live discussions, where there is a need to quickly produce exploratory graphical outputs and tables.

The Vet-AMNet was developed to process data from the Portuguese dairy sector and used to analyze data from the Dutch dairy sector. However, the system's data architecture and data pre-processing procedures should be easy to adapt to other animal species and countries, providing that animal population data, antimicrobial sales and a national antimicrobial registry database are organized in a similar structure to the scheme in Figure 1 and there are interoperable codes that allow the establishment of connections between the different information sources.

During this work, several differences between the Portuguese and the Dutch systems were identified. To make a comparison between the two systems was not an objective of this paper, given that they have different overall aims. The Dutch system is mainly focused on producing annual reports, detailed information to transmit to the animal sectors and is a basis for the development of national antimicrobial stewardship measures. The aim of the Portuguese system is the communication of results to different actors, with data visualizations tailored to their needs. This highlighted the need to develop and conduct a structured and detailed framework analysis of the different systems in place to report national veterinary AMC information, to identify the best practices in the design and management of such systems to serve as a starting guide for newly developing countries and identify further possible improvements in already established ones.

4.2. Consumption of antimicrobials at sectoral level

The 9 years analysis of the AMC reveals an overall reduction of 18% in the use of AM in the dairy Dutch sector. Antimicrobial usage in the dairy sector is considered to be low and acceptable by the SDa, with narrow average $DDDA_F$ distributions and only minor differences being observed between individual farms. AMC in the sector was stable from 2014 to 2019. From 2019 to 2020, although there was an 11% increase in the average consumption, in absolute terms it increased only around 0.3 $DDDA_{NAT}$.

The decrease in consumption was pronounced in the first years after the implementation of the AMC monitoring and benchmarking system and stabilized after. From 2012 onwards the AMC of all dairy cattle farms was recorded, and benchmark values were set by the SDa. A signaling value of 3 $DDDA_F$ and an action value of 6 $DDDA_F$ were set originally (12). These values are subject to adjustments according to changes in the distribution of the $DDDA_F$ of the dairy cattle farms. A farm that exceeds the action value needs to take immediate action to reduce their AMC. Farms with a usage level above the signaling value, but below action value require additional attention to reduce their usage, but no immediate measures must be taken. As the registered AMC of most dairy cattle farms at the start of the monitoring was considered relatively low, benchmark values aimed primarily at reducing the use of the persistently high users. When analyzing the percentile distribution of AMC registered by each farm, in $DDDA_F$ units, it can be seen that more than 95% of the farms stayed below the action value during the whole period and around 75% stayed below the signaling value from 2014 to 2019, reflecting a consistent and sustainable level of control of the sectors consumption at farm level.

The introduction of more strict legislation allowing only first-line AMs for individual treatment to be available in small amounts for the farmer is likely connected with a shift of almost 15% in dry cow treatment from second line to first line products in 2013 (Figures 4, 5). Also, in 2017, the guideline for selective dry-cow treatment was introduced (8). The combination of these two measures, likely resulted in a 20% reduction of dry cow treatments, from 50 to 40% of total treatments (Figure 3). With the introduction on the market of first choice AMs for lactating cows in 2017, an increase in the overall national consumption of this pharmaceutical form is noted. In Table 2, the specific changes in lactating cow treatment are shown. In contrast to the dry-cow treatment, where a shift from second line to first line was noted, it looks like the introduction of first line injectors in 2017 (+0.151 $DDDA_{NAT}$) only resulted in a small reduction in second line products (−0.078 $DDDA_{NAT}$) resulting in an overall increase in 2017. In 2020, first line lactating cow injectors accounted for 0.336 $DDDA_{NAT}$ (from 0.003 $DDDA_{NAT}$ in 2016), while the second line lactating injectors accounted 0.467 $DDDA_{NAT}$ (coming from 0.642 $DDDA_{NAT}$ in 2016); an overall increase with 0.158 $DDDA_{NAT}$. One of the confounding factors is the authorization of the new products. Products with cloxacillin are authorized for a treatment of 6 days, while the older second line products are authorized for a treatment of 1.5 days. When applied in concordance with the authorization, substitution of a second line treatment with the newly introduced first line treatment, might increase the number of DDDA with a factor 4. It is known from practice that the older products were sometimes applied for longer periods, and the new products won't be applied for the 6 days in all cases, so the impact of the new introduction won't be a factor 4 but might account for some increase in total consumption. When expressed in defined course doses (DCD's), which correspond to standardized units to represent a full course treatment, one would probably not even notice a decrease, if it is the case that DCD's are defined accordingly to the authorization (so 1 DCD for the older products would be 1.5 DDD, and 1 DCD for the new product would be equivalent with 6 DDD).

The registered AMC changes in the years 2017–2020, with overall values being stable from 2017 to 2019 and slightly increasing from 2019 to 2020, is for more than 50% attributable to the shift of second line lactating cow injectors to first choice ones. Additionally, between 2019 and 2020, an increase in parenteral treatments of 0.126 $DDDA_{NAT}$, with 80% of this increase being first line AM's, is noticed and can't be explained by a change of products. However, given the low absolute usage levels no immediate action is required (9).

4.3. Consumption categorized by recommendation of 1st, 2nd or 3rd line

A strong shift from second line to first line AM products was shown in the first years, from 2012 to 2014, mostly connected with the intramammary treatments for dry cows, and this tendency was kept in subsequent years. Third line AM products do not play a relevant role in the sector, given the overall negligible level of consumption.

5. Conclusions

The Vet-AMNet system demonstrated to be sufficiently robust to encompass a country-wide AM monitoring program and to meet the critical success factors identified. Starting in 2023, it is projected that it will be used by the Portuguese veterinary authority to analyze national AMC trends and on a sample number of farms it will be implemented as a decision tool, where AMC figures will be presented with other relevant data. The antimicrobial stewardship initiatives adopted in the Netherlands demonstrated to be successful in the Dutch dairy sector, given the sector's low and acceptable AMC.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Dutch dairy sector farmers own the data. Results can be consulted in the yearly SDa reports. Requests to access these datasets should be directed to sanders@autoriteitdiergeneesmiddelen.nl.

Ethics statement

Ethical review and approval was not required for the animal study because we analyzed medicines sales data. All the treatments were independent from the study. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

PM and JN-R designed the original system used for the data analysis. PM, PS, and IVG adapted the original system to process Dutch data. PM and IVG crafted the original study that was later expanded by PM and JN-R. All authors read and commented on the manuscript and agreed on the final version.

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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

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SWOT analysis of risk factors associated with introduction of African Swine Fever through vehicles returning after export of pigs

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Denmark is a major pig exporter and applies a high level of biosecurity, with washing and disinfecting stations for returning livestock vehicles. The introduction of African Swine Fever (ASF) would have significant economic consequences related to loss of export of live pigs and products thereof. In this study, we focused on the role of empty livestock vehicles returning after exports of pigs for the introduction of ASF. Initially, the current components and measures related to export of livestock were described. Next, analyses of strengths, weaknesses, opportunities, and threats (SWOT) were conducted, covering the components and measures identified. Then, export of pigs was described either through assembly centers or directly from farms. Washing and disinfection, as required and undertaken at the designated stations, constitutes the most important among all risk-reducing measures identified. Recommendations are to: (1) ensure the quality of washing and disinfection through staff training; (2) find new, safe, and more efficient disinfectants; (3) ensure the required temperature, and therefore effect, of the disinfectant and water. It was impossible to assess, the influence of export through assembly centers compared to direct transport. However, through SWOT analyses we identified the strengths and weaknesses of the two pathways. Moreover, components/measures with risks of unknown sizes are also discussed, such as vehicles undertaking cabotage and the current vehicle quarantine periods.

KEYWORDS

qualitative analysis, risk assessment, disease introduction, ASF, Denmark

1. Introduction

Denmark, mostly surrounded by sea, has only 68 km of land border with Germany, which eases the ability to establish high levels of biosecurity at the borders to protect against introduction of exotic livestock hazards. Denmark is one of the largest pig exporters in Europe (1). In 2021, Denmark exported 14.5 million live pigs, whereas another 17.4 million finishing pigs were slaughtered inside the country. Moreover, 79% (1,597,359/2,029,000 tons) of the pig meat produced was exported (2). Therefore, the introduction of a notifiable disease in pigs would have huge economic consequences due to loss of export of live pigs and pig meat (3).

African Swine Fever (ASF) constitutes a threat to the global pig industry. ASF, caused by African Swine Fever Virus (ASFV), is not zoonotic but can be transmitted between domestic pigs and wild boars of all ages. Despite the transmission rate for ASFV is lower than observed for Classical Swine Fever Virus and Foot and Mouth Disease Virus (4, 5), ASFV is still infamous because of its high mortality rate (6, 7), multiple transmission routes including direct and indirect contact (8), long-term viability in the environment due to persistence of the virus in various materials and animal tissues (9–12), lack of effective vaccines (13), and last but not least, huge economic consequences. Since the outbreak of ASF genotype II in Georgia in 2007, the area in Europe affected by this genotype has gradually expanded. The main markets for Danish pig exports are Poland, with an ongoing epidemic since 2014, and Germany, which has been affected since September 2020 and has registered seven domestic outbreaks of ASF since the beginning of 2021. Therefore, precautionary measures related to export of pigs are very important for the Danish pig industry and the Danish Veterinary and Food Administration (DVFA).

There are many potential pathways that could lead to introduction of ASF to a naïve pig population, including import of pigs/pork, human-related activities (swill feeding, visits by veterinarians, hunting tourism, etc.), wild boar movements, and returning livestock vehicles (14, 15). Indirect exposure to contaminated environments was identified by ESFA as a likely route of ASF infection in domestic pigs and wild boar (16). In Denmark, empty contaminated livestock vehicles returning after export and not well-cleaned and disinfected are considered one of the main risks (17), because: (1) annual pig imports into Denmark are low, e.g., 47 pigs were imported in 2017–2021; (2) large numbers of vehicles export live pigs, e.g., 26,918 vehicles exported livestock in 2021, among which 25,252 were pig exports (18), and; (3) there are almost no free-living wild boar (19). In 2019, a fence was erected along the border with Germany, reducing the risk of migrating wild boars entering from northern Germany (20). Combined with Denmark's active culling policy on wild boars, the probability of introducing ASF through wild boars is considered very low (21).

In this study, with respect to the risk of introduction of ASF, we focused on the role of empty livestock vehicles returning after export. We firstly examined how the export system is arranged and run. Secondly, we focused on the type of export: (1) directly from pig farms or (2) indirectly *via* assembly centers, as these two options could differ with respect to the probability of introducing ASF into Denmark. The aims were to:

- Analyze the strengths, weaknesses, opportunities, and threats (SWOT) of all components and measures identified in the Danish pig transportation system.
- Compare the risk of ASF introduction through returning vehicles exporting pigs from assembly centers and directly from farms.

An additional aim was to identify which more detailed studies to initiate. The outcome of the study targets public and private risk managers, in and outside Denmark, who are interested in effective measures to reduce the risk of introducing ASF *via* returning livestock vehicles.

2. Materials and methods

2.1. Data collection

Between November and December 2021, two of us (YG & LN) visited one organic, two conventional pig farms, and two assembly centers. These farms and assembly centers were selected based on their representation of the main types of farms in Denmark (Farm size, export size, geographical location, partnerships, etc.). During the farm visits, the entire structure of the farm was inspected from the perspective of external biosecurity. Focus was on entrance of humans and pigs including the delivery facilities. The owner and the daily manager participated in these visits and discussions were taken about the procedures in place on the farm. Additionally, all three existing washing and disinfecting stations approved by the Danish Agriculture and Food Council (DAFC) were visited. Here, the author LN is the expert, which facilitated the systematic inspection from the arrival of a vehicle at the station until its departure after washing and disinfection. Relevant details were inspected such as measures in place to ensure the required temperature of the disinfectant agent and the photo control of each vehicle to document compliance with the rules. This was followed by a visit to two assembly centers located in two different parts of the country. Again, a systematic inspection was undertaken, following the pigs as they moved from the Danish side to the export side. The owner and the daily manager participated in the discussions taking place during the visits. Focus was on how cross-contamination could happen between vehicles and people in the assembly centers. Moreover, published reports and scientific papers, expert opinions, and various statistics were obtained. Finally, information was retrieved about the three private standards applied to Danish pig production [DANISH Product Standard (22), DANISH Transport Standard (23), and Global Red Meat Standard (24)].

2.2. SWOT analysis

SWOT analysis is qualitative, fact-based, structured, and provides realistic descriptions of business planning and functioning (25). SWOT analysis was performed to elucidate the roles of key components and measures in the Danish pig export transportation system to identify their strengths (S), weaknesses (W), opportunities (O), and threats (T). Here, S and O refer to factors that could be helpful in achieving the purpose, and W and T refer to those that could be obstacles to achieving the purpose. From the analytical source perspective, S and W can be considered as having internal origins and O and T as having exogenous origins (26). SWOT analysis was chosen, because it is an adequate tool for how to develop comprehensive and suitable strategies based on the reality of the situation.

The SWOT analysis was conducted by all authors, and the results were subsequently discussed and updated separately with representatives from the pig industry and the Danish Veterinary and Food Administration (DVFA) in two rounds, with preliminary discussions in December 2021, followed by final discussions in May 2022.

2.3. Comparison of two different routes of pig export

Danish pig producers can either export their pigs directly from the farm or move the pigs to an assembly center from where they are exported. The purpose of assembly centers is to separate export vehicles from vehicles used nationally. To illustrate the differences in these processes, two simplified mappings were constructed to enable comparison of the risk of ASFV introduction. The comparison utilized data from the DAFC, the current Danish standards, and the SWOT analysis.

3. Results and discussion

3.1. Description of the Danish pig export system

3.1.1. Danish pig herds

Between 2011 and 2021, the number of Danish pig farms decreased from 9,069 to 8,117 (2). Specialization is increasing, so e.g., some farms only have sows, which produce piglets up to 7 or 30 kg, whereas other farms specialize in buying either 7 or 30 kg piglets and raise these to the finisher stage. Moreover, the number of outdoor farms is increasing. By 24 August 2022, there were 468 farms with outdoor pigs, including organic farms and farms holding fenced-in wild boars, whereas in 2011, there were 314 outdoor pig farms. Furthermore, there are hobby farms with pigs and farms with pet pigs. Danish legislation states outdoor farms must be entirely double-fenced. In 2021, 68% of Danish pig farms, covering 97% of pigs produced, were part of the DANISH Product Standard, implying the farms comply with housing and management rules (27). Moreover, most sow farms are specific pathogen free (SPF) farms, so comply with external biosecurity requirements. Under this, the herd's health status is monitored routinely for the presence of infections, such as *Mycoplasma* lung disease, *Actinobacillus pleuropneumoniae*, swine dysentery, Porcine Reproductive and Respiratory Syndrome (PRRS) virus, atrophic rhinitis, scabies, and lice. According to SPF-Sund, 78% of all pigs born in Denmark are from a sow herd with SPF status (28).

Livestock vehicles enter the pig farm area to load or unload pigs for breeding, raising, slaughter, or export. Most farms have a special area for loading that is separated from the other farm facilities, and the area is cleaned and disinfected after use. Such safe delivery facilities are highly recommended by the Danish SPF system. If this is not established, the pig producers are advised to place the pigs in a trailer that is moved away from the farm before the pigs are loaded onto the livestock vehicle.

3.1.2. EU requirements for livestock movement

In accordance with European Union (EU) Regulation (29), all livestock vehicles should be cleaned and disinfected immediately after every transport of animals. This is conducive to the prevention and control of infectious diseases. However, the legislation does not require control of the vehicles regarding the quality of the washing and disinfection (30). The DVFA studied the effectiveness of washing and disinfection for Danish pig export vehicles. In 2018, 42% of the vehicles were inadequately cleaned and disinfected; this reduced in 2020–2021 to 15% of vehicles being unsatisfactorily cleaned and disinfected (31, 32).

Cabotage road transport constitutes another potential source of contamination by pigs that may result in increased risk of ASF introduction. Cabotage means that vehicle drivers have the right to carry out three transport services within the EU Member State the vehicle has gone to (33). Although the livestock vehicle registers the countries to which pig export is destined, there is a lack of knowledge in the Danish system, regarding additional destinations, because the TRACES system is set up to only share information about movements from one's own country.

3.1.3. Washing and disinfecting stations

In addition to the washing and disinfection required by the EU after unloading, three washing and disinfecting stations have been set up in Denmark. Two are located in the western part of the country, i.e., southern Jutland close to the Danish/German border, whereas one is located in the eastern part of the country, close to a ferry with a connection to Germany. These privately-run stations are financed and supervised by DAFC.

A series of washing and disinfection procedures have been set up based upon the following considerations: a room temperature of 20°C and disinfection solution temperatures of 20–25°C are enquired to ensure effectiveness. The vehicles to be treated are not necessarily washed with soap. Therefore, organic material may be present before disinfection takes place. This needs to be considered when assessing the effect of the disinfection product. When washing at the DANISH approved cleaning and disinfecting sites, the contact time is short - around 10 min - and disinfection may take place on partially wet surfaces, which may limit the effect of disinfection. The ambient temperature on the vehicles is low during a large part of the year, again potentially lowering the effect.

Moreover, the product is used in closed indoor environments, where plenty of staff is working at all times of the day. Therefore, the product must comply with the Danish Working Environment Regulation, which prescribes use of products that are non-harmful for humans and the environment. The following issues must be complied with before a disinfection product will be approved by the DAFC: Data from laboratory tests must be provided because scientific articles are not accepted as documentation. Such laboratory tests may be performed at accredited laboratories. Test results must be provided, which should show correlation between concentration, contact time and temperature relating to African Swine Fever, Foot-and-Mouth Disease, and Classical Swine Fever. The laboratory tests must be performed at different temperatures down to 5°C. Moreover, the product safety data sheet must be provided. Finally, documentation must be provided that the product is registered on the list of relevant substances and the respective substance and product suppliers, in accordance with Article 95 of the EU Biocidal Products Regulation No. 528/212.

For example, glutaraldehyde is a high-level disinfectant, but it cannot be used for the disinfection of vehicles. Because according to the safety datasheet for glutaraldehyde (g5882, sigmaaldrich.com), this substance is harmful if inhaled or swallowed and toxic by inhalation, skin contact and ingestion. In general, there are only three disinfection products left to use in the DANISH system: Virkon S, Kiemkill, and Vanodox.

All vehicles in the DANISH Transport Standard system are required to be washed and disinfected after entering Denmark, and as stated above, the service is free of charge for farmers and transport companies. According to DAFC, there is full compliance with the rules regarding washing and disinfection of all vehicles. Washing certificates are issued based on, among other things, the vehicle's GPS data. Data covering 4 weeks in each of spring and autumn, 2021, showed that around 40% of export vehicle drivers upload their vehicle's GPS data to DAFC's webserver (Unpublished data from DAFC). The remaining drivers could have various reasons for not uploading GPS data—see below.

3.1.4. ASF risk zones

Risk zones regarding ASF are defined by DAFC based on evaluations covering outbreak conditions, proximity to outbreak zones, and ocean currents. The risk zones are updated whenever the epidemiological situation changes, and new risk zones are placed on the DAFC website (23). The risks related to neighboring countries are described by colors; black, red, and green, in decreasing order of risk. The color of the zone, from which a vehicle returns, determines the type of certificate and the quarantine policy required for the vehicle. Black certificates are issued for vehicles unable to or uninterested in submitting GPS data, and for vehicles returning from black zones. If a vehicle has been in a black zone, a 7-day quarantine is imposed before a new transport can be done directly from farms. In contrast, green certificates impose the minimum 2-day quarantine when exporting directly from a farm. Quarantine rules are explained in detail on DAFC's website (23). The type of certificate is considered by drivers when they plan their next transport: in-country transport, export transport *via* an assembly center, or export transport with direct access to a Danish farm.

3.1.5. Assembly centers

Altogether, 29 DANISH-approved, privately owned assembly centers operate in Denmark. The services in these centers are paid for by the exporter. Arriving vehicles are foreign vehicles or Danish export vehicles. Each center is divided into two sides: a Danish side, open only to vehicles arriving from Danish farms to unload pigs, and an export side, open only to vehicles arriving to load pigs. The middle part of the center has tunnels that connect the two sides and several pig pens. The pigs to be exported are inspected by official veterinarians, focusing on health conditions as part of the fit-for-transport assessment, undertaken as the pigs pass through a tunnel. To limit the spread of infection, tunnels are used in one direction only, so pigs go from the Danish side to the export side. Moving pigs from an assembly center instead of directly from a Danish pig farm is preferred by most vehicle drivers with black certificates to avoid the 7-day quarantine period.

3.2. SWOT analyses

The results of SWOT analyses of the washing and disinfecting stations, quarantine period, ASF risk zone identification, and cabotage driving are shown (Tables 1, 2). SWOT analyses for the two different routes of exporting pigs from Denmark are shown (Table 3).

Based on the results of the SWOT analysis, the washing and disinfection conducted at the three stations seem to constitute the most effective way of preventing introduction of ASF compared to the other measures identified (quarantine period, assembly center/direct to farm, and cabotage/national

10.3389/fvets.2022.1049940

TABLE 1 SWOT analysis of components and measures at washing and disinfecting stations for returning livestock vehicles.

Washing and disinfecting stations for livestock vehicles returning from outside Denmark	
Strengths (S)	**Weaknesses (W)**
The use of the washing and disinfecting stations is required by the Danish livestock industry, effectively implying that all livestock vehicles must be washed and disinfected when entering Denmark Three washing stations are available, and use is free of charge implying that returning vehicles (and utensils) are cleaned and disinfected outside and inside At an initial 100% visual check, vehicles with visible dirt are sent back to further cleaning and disinfection. The vehicle is cleaned on the outside, followed by disinfection in- and outside for at least 20 min with disinfectant kept at 25°C, along with random bacteriological sampling $\geq$ disinfection effect is secured. In 2021 a total number of 3,523 so-called Hygicult E/β – GUR samples were taken of 543 different vehicles. This random test is an indicator of how effective the disinfection is at the DANISH approved washing and disinfecting stations.	The washing effect greatly depends on the washing staff. In rare cases, visible dirt can still be found after washing and disinfection Boots in the cabin are inspected but not washed, and the vehicle cabin is not inspected or washed The use of the washing and disinfecting stations is required by the Danish livestock industry, but it is not a legal requirement, and it costs a lot of money to run these facilities. The results of samples taken from the vehicles after washing and disinfection show variation. A preliminary analysis of these data indicate that a substantial part of the variation may be related to the cleaning status of the vehicle when it arrives in Denmark
Opportunities (O)	**Threats (T)**
The current system creates awareness among vehicle drivers regarding the importance of having clean vehicles entering Denmark New disinfectants, which are more efficient than the currently used, which meet the safety standards of the working environment of staff, could be identified	The low temperature in winter can cool the disinfectant, reducing its effectiveness and allowing ASFV to remain viable After crossing the border, the vehicle can go elsewhere before going to the washing station The waste water arising after washing is not allowed to be reused, but the current disinfection process and effectiveness of wastewater treatment are unknown
Recommendations	
1. Provide station staff with continued education to maintain their understanding of the importance of washing and disinfection 2. Improve temperature control of both disinfectant and water, especially in cold seasons 3. Investigate new disinfection systems regarding safety, effect and costs	
Quarantine period for livestock vehicles	
Strengths (S)	**Weaknesses (W)**
The quarantine system encourages vehicles from risky zones to load pigs at assembly centers, likely reducing the number of such vehicles entering Danish farms Weekly tracking of vehicle compliance with quarantine periods is performed by DAFC	ASFV is very persistent in the environment especially at low temperature; ASFV's viability in the current quarantine periods during cold months is considered inadequate
Opportunities (O)	**Threats (T)**
In warmer months ($>20^{\circ}$C), a quarantine period shorter than 7-day is sufficient, even if the vehicle was contaminated and not effectively cleaned and disinfected	Where and how vehicles spend the quarantine period is unknown and uncontrolled. If a dirty vehicle is close to an outdoor farm, indirect spread could occur
Recommendation	
1. Use GPS data from all vehicles for the last 7 days before entering Denmark to classify vehicles more correctly according to risk zones	
Identification of ASF risk zones	
Strengths (S)	**Weaknesses (W)**
Denmark identifies, and updates weekly, ASFV risk zones wider than those published by EUROSTAT and OIE Washing certificates are mainly based on the risk zones, which are strictly distinguished. When the vehicle provides a complete GPS record covering the last 7 days, an appropriate certificate is issued. Otherwise, a black certificate is issued	Green zones in the DANISH Transport Standard could contain undetected ASF-infected pigs/wild boars, especially if translocations over long distances occur. The risk zone classification is not fully evidence-based, but is a management tool where confidence in the veterinary system is included in a non-specific way
Opportunities (O)	**Threats (T)**
Pig producers need only check the vehicle's washing certificate, which is clear and straightforward	Domestic transport vehicles as part of cabotage do not require a washing certificate from a DANISH washing and disinfecting station, and so are a risk for ASFV spread
Recommendation	
1. Identify and implement timely information collection regarding new ASF outbreaks	

TABLE 2 SWOT analysis of livestock vehicles used for cabotage.

National transport/cabotage	
Strengths (S)	**Weaknesses (W)**
National transport/cabotage results in efficient use of vehicles, possibly enabling cost-effective transportation	A returned vehicle, after washing, disinfection, and quarantine, can move pigs inside Denmark. If ASFV in a contaminated vehicle remains viable during these procedures, the virus could spread to pigs being moved from one Danish farm to another
Opportunities (O)	**Threats (T)**
Carrying out national transport/cabotage requires additional quarantine time for vehicles coming from risky zones, which will reduce the number of vehicles undertaking national transport/cabotage, thereby reducing the risk	National transport/cabotage in areas with detected/undetected ASF is riskier than similar transports within Denmark. Pigs from potentially infected zones could be loaded onto vehicles. Once a vehicle is contaminated, ASFV could be introduced to many pig farms before the first case is detected
Recommendation	
1. Run awareness campaigns regarding the importance of vehicle washing and disinfection 2. Open the TRACES system to share information about the movements of all pigs, because the TRACES system is currently set up to only share information about movements from one's own country	

transport). This is because washing and disinfection takes place at the first stop after the vehicle enters Denmark. Moreover, there is some uncertainty regarding the appropriate length of the quarantine period in the cold months.

In summary, the recommendations that seem most feasible are related to the washing and disinfection. ASFV, as a complex enveloped virus, is susceptible to detergents such as soaps, as well as to several disinfectants and dehydration (34). However, many disinfectants are unsuitable for use in practice, because of safety issues: the cleaning staff are using disinfectants in a confined space and, therefore, any disinfectant that may cause skin or eye irritation or be suspect of carcinogenic effect is not permitted to be used in Denmark. This rules out e.g., glutaraldehyde. Many factors need to be taken into account, when setting up a robust system, e.g., if organic material is present when disinfected, the efficiency of disinfectants like chlorine compounds and oxidizing agents will be reduced (34). The search for new, safe and more efficient disinfectants that can be applied is important. But for the present, focus should be on how to ensure properly performed cleaning and disinfection, using the required temperature of the disinfectant and water. This involves staff training, where a future study of knowledge, attitudes and practices (KAP) may add valuable information to further ensure effectiveness of the system in place.

3.3. Comparison of different routes of exporting pigs

In 2021, about 60% of annual pig exports were *via* assembly centers, while 40% were exported directly from farms. When the vehicle is outside Denmark, there is no difference between the two export methods (Figure 1). Differences lie in two aspects. (1) Before export, the vehicle can load pigs directly from one or more farms and head directly to the receiving farm located in another country. Alternatively, the vehicle with pigs from a Danish farm can head to an assembly center and unload all pigs for veterinary control. Thereafter, the pigs are reloaded in the same or another vehicle. (2) Vehicles returning to Denmark are assigned differing quarantine periods, e.g., if a vehicle with a black certificate is scheduled to enter a Danish farm, a 7-day quarantine period is applied, but no quarantine is required if the vehicle goes to an assembly center. See detailed explanation on the DAFC website (https://pigresearchcentre.dk/DANISH-quality-assurance-scheme/The-Danish-Transport-Standard).

For direct export, we found the 7-day quarantine period is sufficient for ASFV, which naturally decays when the temperature is above 20°C. This is supported by Olesen et al. (35), reporting short ASFV viability times in non-cleaned experimental facilities at 20°C. However, our preliminary results show ASFV can remain viable longer at lower temperatures (36). Hence, the 7-day quarantine period before visiting a Danish pig farm could be insufficient especially when the temperature is lower than 10°C. However, the increased risk of longer ASFV viability time in cold months will also greatly increase the risk related to assembly centers, and therefore, the comparison of risk between the two routes of exporting pigs is difficult and deserves further attention.

Still, export *via* assembly centers is considered by the Danish pig production sector to be associated with a high level of prevention of introduction of ASFV into Denmark, because foreign vehicles do not have contact with Danish pig farms. However, we were unable to assess the relative risk related to export through assembly centers compared to direct export from farms. More research is needed, e.g., assessing the risk of cross-contamination between vehicles and people in the assembly centers. In line, the environmental transmission rates in the assembly center are unknown, and this area deserves further

TABLE 3 SWOT analysis of two methods for exporting pigs from Denmark; through assembly centers or directly from farms.

Export through assembly centers	
Strengths (S)	**Weaknesses (W)**
Centers have a Danish side and an export side, reducing the probability of domestic vehicles coming into contact with vehicles from high-risk zones Vehicles returning from outside Denmark do not come in close proximity to Danish farms	Drivers, official veterinarians, and staff can walk back and forth between Danish and export sides, facilitating cross-contamination The centers' pens, aisles, and tunnels are only washed intermittently, and not between all batches of pigs
Opportunities (O)	**Threats (T)**
It may be easier to prevent introduction of ASFV at a low number pf assembly centers compared to at a high number of pig farms undertaking direct export	If the vehicle is not or only ineffectively washed and disinfected Multiple vehicles congregate at the assembly centers simultaneously. ASFV from one vehicle could spread to other vehicles ASFV can enter the center if vehicles are insufficiently washed and disinfected
Recommendations	
1. Implement continued education for assembly center staff and other persons on preventing ASFV 2. Instigate random controls of cleanliness and procedures	
Export through direct transport from farms	
Strengths (S)	**Weaknesses (W)**
Pigs stay in the same vehicle during the whole export process, reducing the risk of contracting other infections A minority of the vehicles have a high level of biosecurity, e.g., SPF vehicles for export of breeding pigs. These vehicles have air filters to prevent airborne transmission of pig pathogens	Vehicles returning from ASF risk zones can go to Danish pig farms after having been cleaned, disinfected, and quarantined. If the regulations regarding washing, disinfection, and quarantine are not followed, a risk of ASFV spread could occur Recommendations regarding pig loading are not always followed. Occasionally: (i) drivers enter the inside of the farm area; (ii) employees leave the farm area, and; (iii) the delivery area is not fully washed and disinfected after loading
Opportunities (O)	**Threats (T)**
Pig producers could be more aware of ASF prevention measures	ASFV can be introduced to Danish farms if: (i) If the vehicle is not or only ineffectively washed and disinfected; (ii) quarantine rules are not followed, and; (iii) farm delivery facilities are inadequate
Recommendation	
1. Instigate continued education for pig producers using direct export regarding preventing ASF and other hazards every time a vehicle arrives 2. Increase the frequency of random controls of cleanliness	

attention. A next step could be to undertake a KAP study among the persons involved in the different areas of the system, to understand in more details, where the limitations are: in the knowledge of the persons working in the system, their attitudes, or the practices which result from the system.

Pig producers who prefer to export directly from their farm are advised to ensure that each incoming vehicle is clean and has a valid washing certificate, implying the driver has complied with vehicle quarantine rules. Moreover, proper use of safe delivery facilities is recommended to prevent hazards on/in vehicles from entering farms. However, more knowledge is needed regarding compliance with these recommendations.

In view of these considerations, this study recommends the continuation of washing and disinfection at the assembly centers, as this is an important activity preventing ASFV from entering Denmark, as stated by Bronsvoort et al. (17), who also pointed to issues regarding the quality of washing and disinfecting transport vehicles.

4. Conclusion

This study characterized the current components and measures related to export of pigs from Denmark. The SWOT analysis contributed to better understanding of maintaining a low probability of introducing ASF. Denmark already has a high level of biosecurity preparedness. However, there are some areas that might constitute potential risks.

The main recommendations concern washing and disinfecting undertaken at the designated three stations. Focus should be on continuously ensuring the effect of washing and disinfection, which is of paramount importance, particularly in cold months, when the 7-day quarantine is likely insufficient for ASFV to decay enough to avoid transmission. This involves offering staff training and controlling sufficiently high temperatures of wash water and disinfectant during the year, in particular

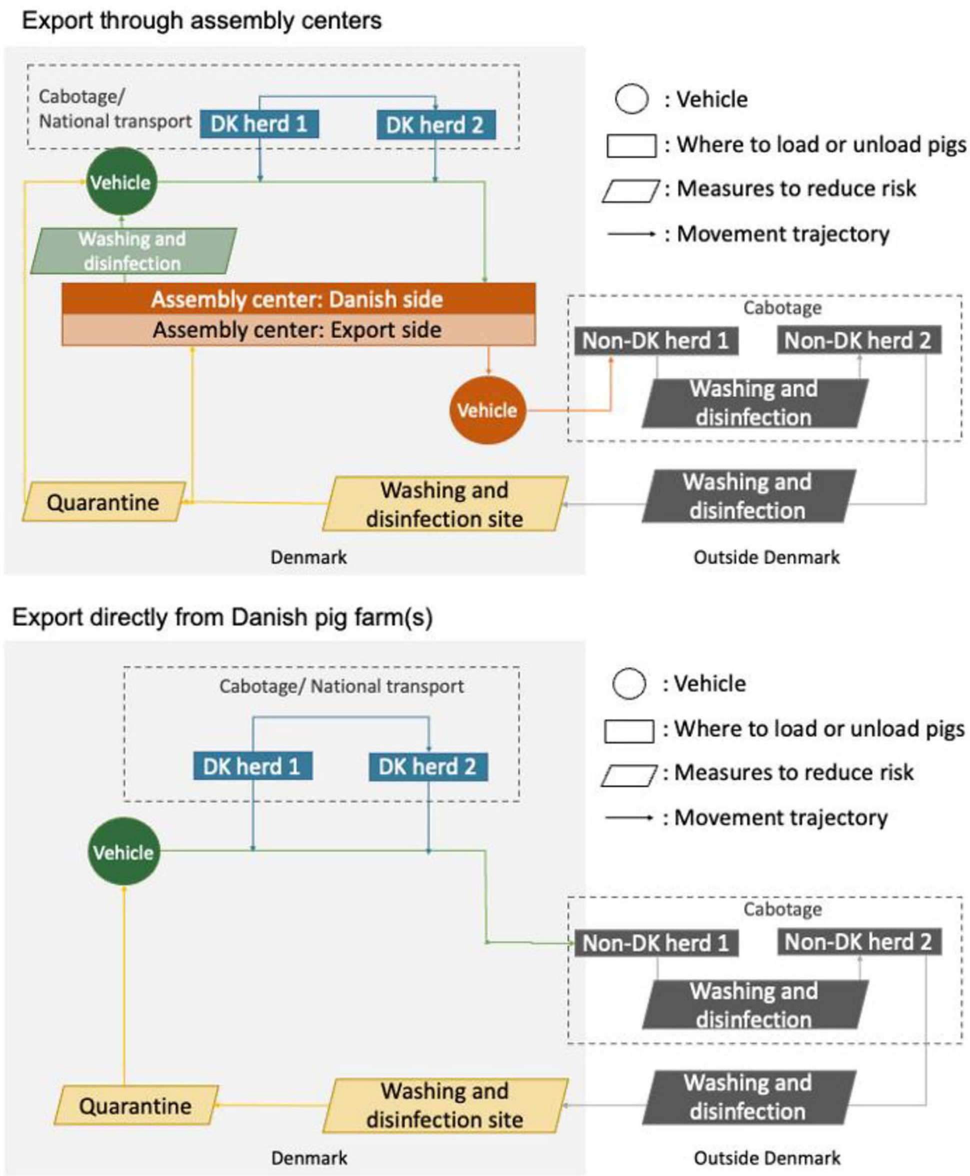

FIGURE 1
Simplified mappings of the Danish pig transportation system. The **upper** panel depicts pig export through assembly centers, while the **lower** panel depicts pig export directly from Danish pig farm(s).

from September to March, to ensure the efficacy of washing and the correct application of the disinfectants. Moreover, DAFC should search for new, safe and more effective disinfectants.

We were unable to assess relative risks of export from assembly centers compared to direct export from farms. Further research is needed, including a KAP study among personnel in assembly centers as well as related to direct transport. Both export routes have their advantages and disadvantages. Hence, pig farmers and other persons involved need to follow best practices when applying any of the two ways of exporting.

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

LA, MJ, LN, and AB contributed to the conception and design of the study. LN and YG carried out the farm visits and collection of information. LA, LN, AB, and YG brainstormed during the SWOT analysis and wrote parts of the manuscript. YG wrote the first draft of the manuscript. All authors participated in the revision of the manuscript, read, and approved the submitted version.

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Conflict of interest

LA and LN are employed by Danish Agriculture and Food Council.

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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Data-fed, needs-driven: Designing analytical workflows fit for disease surveillance

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Syndromic surveillance has been an important driver for the incorporation of "big data analytics" into animal disease surveillance systems over the past decade. As the range of data sources to which automated data digitalization can be applied continues to grow, we discuss how to move beyond questions around the means to handle volume, variety and velocity, so as to ensure that the information generated is fit for disease surveillance purposes. We make the case that the value of data-driven surveillance depends on a "needs-driven" design approach to data digitalization and information delivery and highlight some of the current challenges and research frontiers in syndromic surveillance.

KEYWORDS

big data, epidemiology, decision support system, syndromic surveillance, data-driven surveillance

1. Introduction

The continuous and systematic collection and analysis of health-related data–a practice coined syndromic surveillance (SyS)–has gained momentum in public health since the turn of the century, buoyed by the putative benefit that SyS will allow detection of disease outbreaks or other public health trends earlier than traditional surveillance which relies on laboratory test results or clinical diagnoses.

"Big data analytics" is now recognized as a term referring not to the size of the data handled, but to the development in technologies needed to extract information from raw data, in an evolving and complex context (1). In animal health surveillance, this means specifically being able to convert data into actionable information for decision-makers tasked with disease prevention, detection and control. In 2011, Fricker (2) provided a broad overview of the issues related to the use of (digital) biosurveillance in practice. We highlight here his emphasis on the need to give more attention to system design, to ensure that the right information is available at the right time and in the right place to inform animal health actions.

By 2011, the idea of incorporating SyS methods into animal health surveillance systems were being more widely discussed (3). An intensive exploration of various sources of data ensued, as documented in reviews in 2013 (4), 2015 (5) and 2016 (6). The various initiatives documented in these reviews tended to share a focus on specific, individual streams of data. In these early stages, exploration focused on the methodological aspects of converting health events and other data streams into time-series that could be subjected to temporal aberration detection algorithms (TADA), and on validating the statistical analyses.

Ten years later, research into what Fricker had called the "operational challenge of biosurveillance" (ensuring statistical performance) has developed extensively across a range of veterinary SyS initiatives. But how close are we to achieving his view of a surveillance system that is designed to take into account stakeholders' needs and that produces the actionable information needed to support decision-making in practice?

2. Materials and methods

Power (7) identified three main characteristics of a decision support system (DSS): it should facilitate the decision-making process; it should *support* rather than *automate* decision making; and be able to respond quickly to the changing needs of decision-makers. The typical components of a DSS are the same as those we previously outlined for a data-driven surveillance framework (8)–data acquisition, analyses models and user interface.

There is no specific technical description for a DSS, which should be "defined in terms of the context and use" (9). System development can therefore only be successful if the users are explicitly involved. Sprague (10) argues, however, that not even the decision makers can anticipate the functional requirements of the system, as their needs are constantly changing, and the process of decision making itself can be altered by the system. He suggested that a DSS cannot be developed using the traditional "analysis, design, construction, implementation" cycle. Instead, these steps should be combined into a single step, which is iteratively repeated. The simplest system is built and delivered to users, and their feedback is continuously captured and incorporated into the DSS.

We reflect on some open research questions and the associated challenges these bring to SyS implementation, and suggest how some of those could be addressed using this simple DSS approach, which asks a single question: "how can this information improve the decision process of the final user?".

In line with our view that early disease detection is too narrow a goal for data digitalization (8), we borrow the term syndromic surveillance for its established connotation as the "continuous monitoring of health data," though our discussion considers surveillance of both exotic and endemic diseases.

We anchor our discussion around three main complementary examples:

(A) time-series of laboratory tests submissions, representing "typical" SyS;

(B) on-farm records relating to reproductive events in pigs, as an example of the still under-explored use of production data within SyS;

(C) food-borne surveillance as a One-Health example; specifically, the monitoring of gastrointestinal illness in humans and Campylobacter positive slaughter batches of chickens.

3. Results and discussion

3.1. Data acquisition

Most of the early SyS work was data-driven, i.e., focused on data that was relatively easy for system developers to access. Working example (A) is a typical case, where data owners, analysts and decision-makers all sit within the same organization (e.g., a national veterinary service). When data access is treated as a main impediment to further system development, only the needs of a subset of animal health stakeholders are considered. However, the majority of health-related data is collected by entities within the "animal health" network (e.g., industry groups) whose interests are different from those who are trying to draw actionable inferences from those data. For example, reproductive inefficiency will primarily be considered from an economic profitability standpoint by the manager of a dairy operation. That same increase in the number of abortions may be perceived by the veterinarian as an indicator of some underlying health issue in the herd. The regional veterinary services, which have received notifications in the previous 2 weeks of a large number of calves born with congenital deformities, may interpret this further as additional suspicion of a regional Bovine Viral Diarrhea outbreak which may require the enactment of an eradication scheme.

As a result, the field has started to move away from the pre-conception that data centralization is necessary to conduct population level surveillance. The technology of data federation allows the distribution of queries and models from a central location/body to the data nodes in a stakeholder network, rather than data having to flow in the opposite direction. In this "code to data" scenario (as opposed to the traditional "data to code"), data interoperability is prioritized over data harmonization. We have previously addressed this discussion and highlighted the importance of ontologies as a research priority (8).

Some surveillance systems may need to fulfill the decision support needs of the individual data providers themselves as well as those of the (non-data generating but policy-making) central node in the network. System design for implementation in the case of example (B) will require in the first instance the elicitation of the farmers'/associations' management requirements (i.e., their motivation to join the DSS). More research will subsequently be required around the technology available to deliver a system that analyses and delivers information at source, while sending only limited signals back to the network. Finding a balance between keeping farmers data as private as they wish, while collecting enough information to add value to decisions at a broader population level will require further discussions, with active farmer involvement.

The One Health example (C) represents yet another complex network of stakeholders. In this case, it is typical for separate central governmental bodies to have access to different data sets at the population level. The obligations of animal health and public health agencies to safeguard the identity of animal owners and individuals, respectively, may prevent data sharing between agencies at a high level. These data sources may be accessible, but can rarely be readily integrated. This is not an issue to solve with data management technology, but rather a feature to incorporate explicitly in DSS implementation, and we address this in the data analyses section below.

3.2. Syndromic indicators

SyS is mainly based on time-series analyses. The creation of a time-series is straightforward when data providers record the health events of interest in discrete time slots (commonly, days or weeks), as in examples (A) and (C): number of tests, number of cases, etc. per time unit.

Production data are recorded continuously on farms during normal activities, and events recorded are not necessarily associated with any health hazard. As such the events of "syndromic" interest must be defined, and metrics to determine their occurrence developed (11). Some production data may lose value if aggregated according to different time unit. In example (B), consider for instance the recording of the date of farrowing for each individual sow. The analyses may aggregate the number of farrowings per week in a particular farm, or report the average number of farrowings per sow per year. However, reproductive health may be better monitored by length of pregnancy, and farm management may thus be more interested in the time between two farrowings. A series where every farrowing is a new observation, and the value of the observation corresponds to the "number of days between farrowings," is a continuous time-series. Observations are not grouped in any particular unit of time, as in the discrete time-series that SyS are typically designed to handle. Control charts, commonly used in SyS, were originally designed to monitor industrial processes that more closely resemble continuous time-series, so the application of TADA to these types of series is not a bottleneck. The challenges for their incorporation into automated monitoring systems are rather related to the definition and interpretation of outputs, and the large number of potential time-series that must be evaluated. We address system outputs in more detail below.

3.3. Data analyses

Aberration detection within single time-series has been intensively explored in SyS. When TADA are applied individually to time-series that represent counts of one type of syndrome, from one source, as is typical in (A), their use in practice will depend on resolving two main questions: how should we interpret alarms, that is, how to decide when an alarm deserves action?; and how can we best combine the evidence from multiple series? The answer to the first question almost certainly depends on the second, as single alarms are likely meaningless until placed within their broader context.

The need to combine evidence from multiple data streams has been addressed and reviewed before in both human (12) and animal health surveillance (13). However, the statistical solutions to monitoring multiple parallel time-series only solve a limited part of the problem. They are applicable in typical cases such as example (A), when a same source can produce multiple time-series aligned in time, or data for the same syndrome is coming from multiple sources, such as multiple regions (14).

In example (C), evidence combination may be primarily a question of system design. If the SyS aim is to monitor cases in humans, using the chicken cases as a predictor will actually explain a lot of the variability in the number of observations, reducing the chances of an alarm. It is a good explanatory statistical model, but a poor fit for SyS goals. A better option might be to develop a predictive model that uses the chicken data to foresee when human cases are likely to start increasing. This will however depend on having access to both of these data sources continuously and in a timely manner. When data sharing is not possible, alternatives can be sought by considering this explicitly as a DSS problem. What is the main decision we are trying to support? If this is preparedness to act in the case of a human outbreak, it may be enough to monitor the chicken time-series independently. Results from this monitoring process would then be continuously transmitted to public health officials.

Consider now example (B). As noted earlier, the farm-level indicators will be a combination of discrete and continuous time-series. Statistically, this poses a challenge to parallel monitoring. As the number of potential indicators at the farm level is high, we must find a way to combine their evidence; otherwise, users are left with a myriad of daily/weekly alarms that they will find difficult to interpret. To add complexity, statistical analyses must take into account predictors at different levels. In a single farm, monitoring an indicator of reproductive performance, for instance, may demand consideration of the age/parity of sows. This is not trivial, as typical syndromic indicators are grouped by unit of time, and therefore TADA can typically only handle variables that can be summarized per time point.

Making sense of multiple sources of evidence, all of which contribute to situational awareness around the same problem, remains an open area of research. If surveillance is framed as a problem of DSS design, the solution may not (only) be statistical. Rather, it involves a better understanding of the decisions we aim to support, and how each of the pieces of information generated can be used in that decision process. This will require intensive social research involving all stakeholders in the network; or, in DSS implementation terms, several rounds of iteration with users.

3.4. Interpreting alarms—the decision-making process

In order to start involving stakeholders in rounds of system implementation in practice we are missing one essential component of a DSS: the user interface. Discussions around dashboards for visualization of times-series analyses often stumble on a disconnection between the expertise of those who perform the analyses, and understand their outputs, and the experience of decision-makers.

The DSS approach suggests that the solution is to construct the simplest dashboard we can, and be prepared to iterate through the entire continuum, from data ingestion to output visualization, continuously, with direct user involvement. Decision-makers are not invited to design the system abstractly, but to use the system and give feedback based on one simple question: "how could this better support your decision-making process?" (15).

This approach assumes that implementation is context-based, which then leaves one main question–what is the decision-making process that we are primarily trying to support? "Early disease warning" may be too vague a goal to inform concrete design and implementation choices. As Fricker cautioned in his seminal paper in 2011 (2): "Looking for everything means it is harder to find any one thing."

Phrasing decisions in a common language which both system designers and users are familiar with will likely require narrowing down to concrete threats. This may mean that we design systems not to "detect emerging diseases" but which can, for instance, "provide an early signal of the introduction of PRRS (Porcine Reproductive and Respiratory Syndrome) in this specific region." While the focus on specific diseases seems to go against the general preparedness that SyS was intended to address, it enables us to move forward with the practical implementation of real-world applications, which support surveillance in practice. It will bring stakeholders together and establish collaborative practices that can be used to gradually expand system goals, and address an increasing number of decision scenarios.

4. Conclusion

The data-driven focus of SyS to date has resulted in times-series analyses being applied to the data at hand, without sufficient consideration being given as to the key questions such analyses should be attempting to answer. Implementation in practice will require that we define the following: who are the decision makers?; what specific problems they are trying to handle?; and how will information that supports their decisions can be delivered in consumable ways? The field of decision support systems design suggests that the main goal should not simply be, "getting the right information to the right person at the right time," but that "the ultimate objective must be viewed in terms of the ability of information systems to support the improved performance of people in organizations" (7). We suggest that a DSS approach to SyS system design will help solve many of the current methodological challenges, in particular those associated with combining numerous and varied sources of evidence as well as assisting users to make sense of complex system outputs.

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

FD wrote a first article abstract, which all authors reviewed and approved. All authors contributed to the thoughts developed in this article and actively engaged in discussions to mature the ideas proposed.

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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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Semi-quantitative risk assessment of African swine fever virus introduction in pig farms

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A semi-quantitative risk assessment was developed to classify pig farms in terms of the probability of introduction of African swine fever virus (ASFV). Following on-farm data collection *via* a specific checklist, we applied a modified failure mode and effect analysis (FMEA) to calculate the risk priority codes (RPC's), indicating increasing risk levels ranging from 1 to 5. The importance of biosecurity measures was attributed by experts. To consider geographic risk factors, we classified pig farms based on local density of farmed pigs, and on the estimated wild boar population density. The combination of RPC's with geographical risk factors resulted into a final ranking of pig farms in terms of the risk of ASFV introduction. Furthermore, the estimation of frequency and levels of non-compliance with biosecurity measures was used to identify weak points in risk prevention at farm level. The outcome of the risk assessment was affected by choices in assigning non-compliance scores and importance to specific components of biosecurity. The method was applied in 60 commercial farms in major pig production areas in Italy. Furthermore, we applied a reduced version of our checklist in 12 non-commercial/small commercial ($\leq$20 pigs) farms in the northern Apennines. In commercial farms, highest RPC's were obtained for biosecurity measures associated with personnel practices and farm buildings/planimetry. Intervention should be addressed to training of personnel on biosecurity and ASF, to avoid contacts with other pig herds, and to improve practices in the entrance into the farm. Sharing trucks with other farms, and loading/unloading of pigs were other weak points. Fencing was classified as insufficient in 70% of the commercial farms. Among these farms, breeding units were characterised by the lowest risk of ASFV introduction (although differences among median ranks were not statistically significant: P-value = 0.07; Kruskal–Wallis test), and increasing herd size was not significantly correlated with a higher risk (Kendall's $\tau = -0.13$; P-value = 0.14). Density of farmed pig was greatest in the main pig production area in northern Italy. Conversely, exposure to wild boars was greatest for non-commercial/small commercial farms on the Apennines, which were also characterised by non-compliance with critical biosecurity measures.

KEYWORDS

African swine fever, semi-quantitative risk assessment, biosecurity, pig, failure mode and effect analysis

1. Introduction

African swine fever (ASF) is an infectious haemorrhagic and severe disease in domestic and wild pigs caused by the African swine fever virus (ASFV). The clinical syndromes vary from hyperacute, acute, and subacute to chronic, depending on the virulence of the virus. ASF is a notifiable disease to the World Organization for Animal Health (WOAH) and is one of the major threats to the swine industry worldwide. Its spread into new countries leads to devastating socio-economic losses in the entire swine production sector among others owing to the trade restrictions on animals and animal products (1).

The first report of ASF outside Africa came from in Portugal, in 1957. Epidemics occurred in European and American countries in the following decades. After the eradication of ASF from the Iberian Peninsula in 1995, for several years, the Italian island of Sardinia was the only non-African region where the infection was present (2). The unexpected introduction of ASFV genotype II into the Caucasus in 2007 resulted in an unprecedented geographical spread of the disease. The number of countries or territories reporting the presence of the disease has increased in the last few years, and ASF has officially been notified to the World Organization for Animal Health (WOAH) by member countries from sub-Saharan Africa, Europe, Asia, and the archipelago of the Caribbean region (3). On 7 January 2022, ASFV was confirmed in a wild boar in the province of Alessandria (Piedmont region, northwest Italy), followed by several other cases in the wild boars population up to now, mainly between the Piedmont and Liguria regions[1] (4) (Figure 1).

The relative importance of different transmission routes and the possible duration of the persistence of ASFV vary across habitats and pig husbandry methods (1, 5, 6). The virus is mainly transmitted by direct contact between infected and susceptible pigs (*via* infectious body fluids and aerosols over short distances between pens) or through the ingestion by susceptible suids of ASFV-contaminated carcasses or pork. The illegal movement of live pigs and pork is considered to be important for the long-distance spread of ASFV (1). Other disease transmission pathways include vehicles and other fomites, such as clothing, footwear, surgical equipment, workers and visitors, slurries, and genetic materials. In certain areas, soft ticks of the genus *Ornithodoros* play a role in transmitting the disease. Wild boars are susceptible to ASF, and in the current European scenario, the disease is endemic in wild boars in several countries and, in the affected areas, they represent a constant threat to domestic pigs.

Since there is no effective vaccine available, the prevention and control of ASF is based on biosecurity and the early detection of the infection by effective surveillance. Recent studies have indicated that insufficient biosecurity measures and ineffective surveillance contribute to virus introduction and spread (7). Biosecurity can be defined as a set of structural, logistical-managerial, and behavioural measures aimed to eliminate or reduce the risk of introduction, establishment, and spread of disease-causing agents in a population (8). Biosecurity measures should be adapted to each disease and farming system. Over the years, checklists were developed to evaluate biosecurity at the farm level. These are based on an objective assessment of measures and may include weighting coefficients, reflecting the relative importance of the assessed parameters. Among these checklists, Biocheck.UGent™ is a risk-based scoring system, which considers the relative importance of all different biosecurity measures to quantify the on-farm internal and external biosecurity (9). Other checklists have been developed, such as the Italian ClassyFarm biosecurity checklist[2], which extends the collection of information on biosecurity according to Biocheck.UGent™ to include also animal welfare, veterinary antimicrobial use (AMU), antimicrobial stewardship in farms, and inspections at slaughterhouses. Data processing results into a final score for each area of interest, allowing a comparison of the farm results with the average at the national, regional, or local level. Other checklists are the APIQ√® –Australian Pork Industry Quality Assurance Programme[3], and Japanese BioAsseT (10).

Whereas, the checklists listed above are targeted to general farm biosecurity, disease-specific checklists have been developed for pig farms to evaluate the risk of introduction and spread of *Streptococcus suis* (11) and porcine reproductive and respiratory syndrome virus (PRRSV) (12–14). Two ASF-specific tools are available: the webpage of Vechta University, which allows German pig farmers to perform a self-evaluation[4], and the outcome-based checklist for ASF-free compartments from WOAH (15). Both checklists, however, include limited information on geographical risk factors. The densities of wild boars, and of farmed pigs at local level were shown as important risk factors for ASFV introduction into farms (16–19). Therefore, the development of a biosecurity checklist, considering ASF-specific transmission routes, as well as risk factors at the geographical level, is necessary for risk assessment and for disease prevention (20, 21).

The evaluation of biosecurity is part of the animal health risk assessment, which is a transparent process for estimating the probability and consequences of the introduction of infectious diseases in free populations. It is based on the reconstruction of phases leading to adverse health outcomes using the best available scientific evidence (22). Important issues related to ASF and biosecurity have been illustrated by several authors. For example, qualitative risk assessment of the introduction of ASFV was applied at the country or continent level when information was limited, and the identification of gaps in knowledge was part of the study's objectives (23). Other authors used a quantitative risk assessment to predict the probability of ASF, and uncertainties in the parameters were included using probability distributions (24). Moreover, a semi-quantitative risk assessment of ASF resulted in the ranking of routes of ASF introduction from wild boars into pig farms by expert elicitation, providing the basis for prioritisation in prevention (25).

The collection of information at the farm level has most often been used to estimate the association between the risk factors and ASF occurrence in the analysis of past epidemics (26–28). Results from these studies provided scientific evidence supporting the adoption of criteria, when assessing the risk of pig farms before ASF occurrence. Such a farm-level risk assessment can be useful for identifying the critical points in biosecurity measures as targets for intervention, and for the classification of establishments for the risk of disease introduction, as provided by the European Animal Health Law as the basis for prevention and control (8).

In this study, we applied a semi-quantitative risk assessment method to classify and rank pig farms in terms of the risk of introduction of ASFV, which takes into account the relative importance of the different transmission pathways. We developed an *ad hoc* checklist for the collection of data on the potential routes of ASFV introduction into pig farms, which were filled during farm visits. To consider the geographical risk factors, we classified the

1 https://storymaps.arcgis.com/stories/7f16f51731654a4ea7ec54d6bc1f90d4, accessed on 02 January 2023.

2 www.classyfarm.it, accessed on 08 November 2022.

3 available at www.apiq.com.au, accessed on 24 November 2022.

4 available at www.risikoampel.uni-vechta.de, accessed on 24 November 2022.

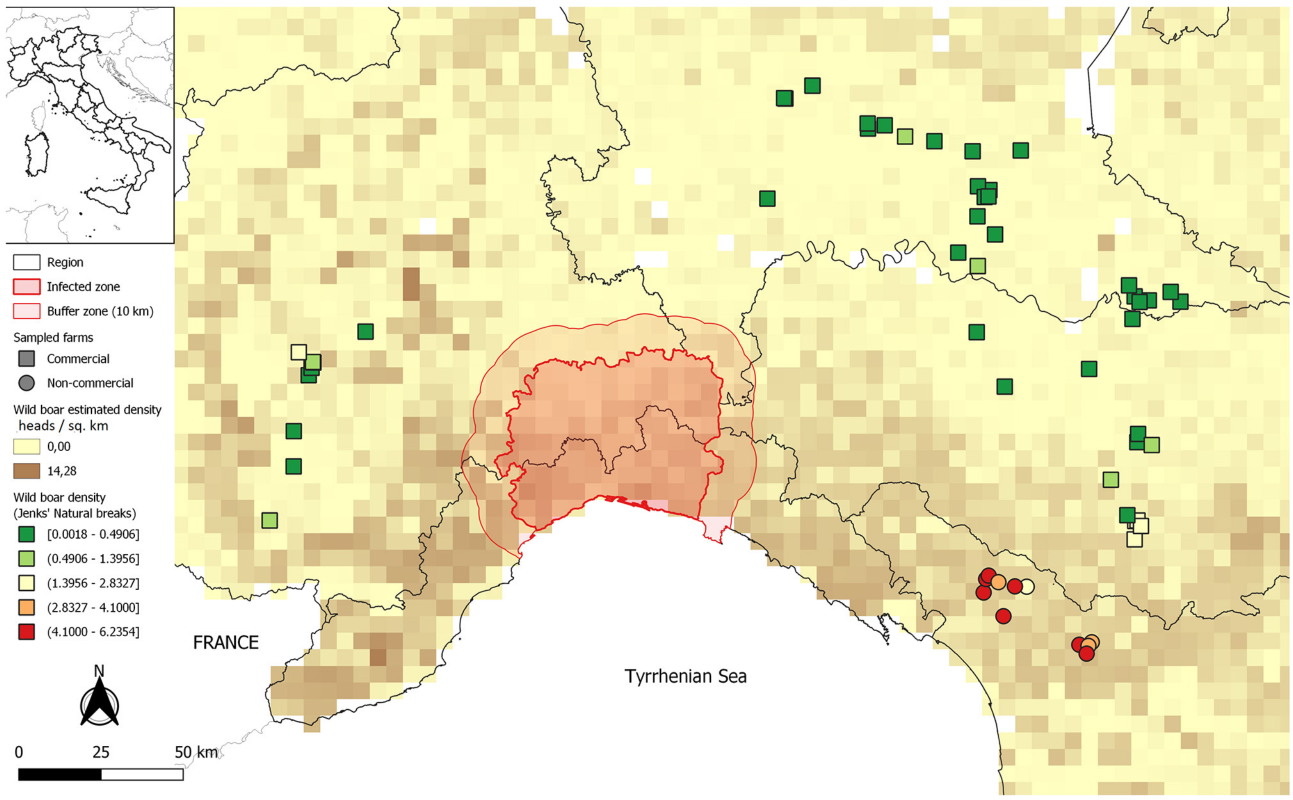

FIGURE 1
Geographic distribution of visited 72 pig farms, and of the population density of wild boars in Italy. Commercial farms are identified by squares. Non-commercial and small commercial farms in north-western Tuscany are identified by circles. Farm location colour is based on Jenks natural breaks classification method, of all farm types combined, to better visualize variations in wild boar density. The infected area for ASF (4) is reported.

pig farms based on the estimated wild boar population density in the surrounding area. Furthermore, we used an index of local spatial clustering to classify the farms in terms of the domestic pig population density. The data were analysed using a modified failure mode and effect analysis (FMEA) which was previously used to provide a rank of failure modes in the manufacturing industry (29). We adapted such a modified FMEA to identify potential points of failure in the prevention of ASFV introduction in pig farms, taking into account the ordinal properties of biosecurity scores, and their importance. As a result, risk priority codes were obtained for main biosecurity criteria. Their combination with geographical risk factors resulted into a final ranking of pig farms in terms of the risk of ASFV introduction. Furthermore, the estimation of frequency and levels of non-compliance with biosecurity measures was used to identify weak points in risk prevention at farm level. An example of application of the checklist to pig farms in northern Italy is presented.

2. Materials and methods

2.1. Development of a biosecurity scoring system

The ASF-specific questionnaire developed in the present study aims to describe the complete biosecurity situation in a pig herd. Its development was based on the main biosecurity principles listed in Dewulf and Immerseel (7), Biocheck.UGent™ (9), and the ClassyFarm biosecurity checklist for the Italian Veterinary Authority.[5] Other biosecurity principles more specific for ASF were introduced from the prescriptions listed in the European Commission working document SANTE/7113/2015—Rev 12[6] and Commission Implementing Regulation (EU) 2021/605 (30). All the ASFV transmission routes were considered, such as direct contact transmission, movements of animals, semen, ova, embryos, food-borne transmission (e.g., water hygiene, swill feeding), indirect transmission (e.g., personnel, wild birds, insects, environmental enrichments, equipment, rodents, or pets), and environment (e.g., cleaning and disinfecting the barn) (1, 26–28). The final checklist consisted of 98 questions (items) with dichotomous answers. The objective of a checklist with dichotomous answers was the collection of factual observations, excluding subjective opinions. The 98 items included in the checklist were grouped into 24 sub-criteria and, subsequently, into six main biosecurity criteria. The number of sub-criteria contributing to each main criterion varied from three to five (Table 1).

5 www.classyfarm.it, accessed on 08 November 2022.

6 Directorate General for Health and Food Safety Strategic Approach to the Management of African Swine Fever for the EU 2020. Available online: https://ec.europa.eu/food/sites/food/files/animals/docs/ad_control-measures_asf_wrk-doc-sante-2015-7113.pdf (accessed on 24 November 2022).

TABLE 1 Main criteria and sub-criteria of the ASF specific checklist and the importance score of each sub-criterion assigned by the experts' opinion.

	Main criteria	Sub-criteria	Importance score (95% CL's)
A	Personnel	A1 Entrance of personnel into the farm	4 (3, 5)
		A2 Contact of personnel with other pigs and wild boar hunting	5 (5, 5)
		A3 Food introduction by personnel	2 (2, 3)
		A4 Personnel training	3 (2, 5)
B	Animal introduction and management	B1 Health/feeding/breeding status of introduced pigs	5 (5, 5)
		B2 Number of farms of origin of the introduced pigs	4 (3, 5)
		B3 Management of animals with an impaired growth	3 (3, 4)
C	Animal shelters management	C1 Quarantine	3 (2, 5)
		C2 Internal animal flow and cleaning procedures	4 (3, 5)
		C3 Vaccine prophylaxis and treatments for other infectious diseases	1 (1, 1)
		C4 Structure and buildings	5 (4, 5)
		C5 Dead pigs' management	2 (2, 3)
D	Animal transport vehicles	D1 Live animal transport vehicles	5 (3, 5)
		D2 Live animal unloading/loading	3 (2, 5)
		D3 Carcass disposal	5 (3, 5)
		D4 Equipment and tools for loading/unloading live animals	4 (2, 5)
E	Material management: feed, slurry, and other vehicles	E1 Procedures for loading/unloading of feed and materials	4 (3, 5)
		E2 Feed and materials storage	3 (2, 5)
		E3 Slurry management	2 (2, 5)
		E4 Vehicles for loading/unloading feed and materials	5 (4, 5)
F	Buildings and farm planimetry	F1 Farm perimeter barriers	5 (4, 5)
		F2 Other animals and disinfection procedures	5 (4, 5)
		F3 Pest and rodent control	3 (2, 3)
		F4 Visitors	4 (3, 4)

The importance ranges from 1 (very low) to 5 (very high). 95% confidence limits (CL's) were obtained by bootstrapping.

2.2. Farm categorization method

2.2.1. The importance score: Assignment of importance to different biosecurity sub-criteria

Given that not every ASFV transmission pathway has the same efficiency, biosecurity measures are not equally important in protecting the health of farm animals. For example, it is well known that direct contact between animals (e.g., purchase of live animals, possibility of free range of pigs) poses a higher risk, whereas indirect contact (e.g., transmission of pathogens by fomites, contact with infected material) is less efficient in the transmission of pathogens (31). To establish a hierarchy of importance of the 24 sub-criteria within the six main criteria, the Borda method was used (32). Eight experts from countries affected by ASF, with experience in pig management and ASF control, assigned an importance score ranging from 1 (least important) to 5 (most important) to each of the sub-criteria within each of the six main criteria with respect to its relevance in reducing the risk of ASF introduction into the farm. A modified Borda method was used to obtain a summary importance score for each sub-criterion as the sum of the scores assigned by each expert:

$$\boldsymbol{I_b(x) = \Sigma_{i=1}^{m} I_i(x)}$$

Where:

$I_i(x)$ is the importance score assigned to sub-criterion x by the i-th expert, and m is the number of experts (in this case, $m = 8$). The most important sub-criterion x^* is that with the highest Borda score, as shown below:

$$\boldsymbol{I_b}\left(\boldsymbol{x}^*\right) = \max_{\boldsymbol{x \in S}} \{\boldsymbol{I_b(x)}\}$$

where S is the set of compared sub-criteria, which are part of each of the main criteria. The most important sub-criterion was assigned a score of 5; the scores of the other sub-criteria were subsequently calculated in decreasing order, until a score of 1, which was assigned to the least important sub-criterion. In our application, the Borda method was modified to allow for ties in the importance scores, which were assigned by experts to each sub-criterion. To report variability in the attribution of importance scores to sub-criteria by the eight experts, and its consequences on the summary importance score I_b, as estimated by the modified Borda method, we obtained 95% confidence limits by a bootstrap approach. In particular, for each sub-criterion, we randomly sampled, for 10^4 times, the eight importance score assigned by the experts (*sample* function in the R software, specifying "size = 8", and "replace = TRUE") and calculated I_b. By sampling with replacement, the importance score assigned by a

TABLE 2 List of the 9 "Critical items" selected by the expert's panel, with reference to the sub-criterion in which they are included.

Critical item	Critical item (sub-criterion)	N (and %) of non-compliant commercial farms ($n = 60$)	N (and %) of non-compliant non-commercial and small commercial farms ($n = 12$)
1	Change of clothes and footwear is carried out (A1)	2 (3.3)	11 (91.7)
2	The staff has no other pigs (A2)	14 (23.3)	10 (83.3)
3	Staff has no contact with other pig farms (A2)	38 (63.3)	12 (100.0)
4	Staff does not engage in wild boar hunting activities (A2)	6 (10.0)	10 (83.3)
5	Animals are not fed catering waste, canteen waste, or household leftovers (swill feeding) (B1)	0 (0.0)	2 (16.7)
6	While loading animals, transporters help inside the truck, but never enter any clean farm area, which is clearly demarcated (D4)	2 (3.3)	
7	Clothing provided to transporters is company or freshly laundered, and boots are company issued (D4)	7 (11.7)	
8	There is an external fence for the entire farm perimeter that prevents the entrance of wild animals and visitors (F1)	42 (70.0)	12 (100.0)
9	Disinfectants with proven efficacy against ASF are available (F2)	0 (0.0)	12 (100.0)

particular expert could be selected more than once to be part of each random sample of scores. As a consequence, a greater variability of scores resulted into more variables I_b estimates. The 2.5th, and 97.5th percentiles of the distribution of those 10^4 I_b estimates were used as the lower, and upper limits of the 95% confidence interval.

During the evaluation of the sub-criteria by the expert panel, some of the 98 items were considered of crucial importance for biosecurity against the introduction of ASFV in pig farms, and those were defined as *critical items* (Table 2).

2.2.2. The non-compliance score

Each of the 24 sub-criteria was assigned a non-compliance score, ranging from 1 (high compliance) to 5 (low compliance), based on the application of the checklist during the on-farm visits. Several items contributed to the score of each sub-criterion, and each of them allowed two possible answers: "yes", indicating compliance with biosecurity; "no", indicating non-compliance. The increasing non-compliance score of each sub-criterion was calculated based on the decreasing proportion of "yes" answers to the items in that sub-criterion, as shown in Table 3. In few cases, a sub-criterion included items allowing five mutually exclusive answers, corresponding to an increasing order of non-compliance levels (e.g., sub-criteria B2, and B3, see Supplementary material). In these cases, it was possible to respond only one of these answers, and the corresponding level, from 1 to 5, was assigned as the non-compliance score for that sub-criterion.

If one of the critical items was not satisfied, the corresponding sub-criterion was assigned the maximum non-compliance score of 5, regardless of the answer to the other items belonging to the same sub criterion.

2.2.3. Calculation of the risk priority codes by failure modes and effect analysis

The importance and non-compliance scores of the sub-criteria were used to calculate a risk priority code (RPC) for each of the six main criteria for each pig farm, using modified failure modes and

TABLE 3 Description of the sub-criterion non-compliance scoring system.

Sub-criterion non-compliance score	Description
1	All items are satisfied
2	Between 62.6 and 99.9% of the items are satisfied
3	Between 37.6 and 62.5% of the items are satisfied
4	Between 0.1 and 37.5% of the items are satisfied
5	No items are satisfied, or at least one "critical item" is not satisfied

effect analysis (FMEA), as shown below:

$$RPC\,(a_i) = Max_j\ \{Min\ [(Ig_j)\,,\ g_j\,(a_i)]\}$$

Where:

$RPC(a_i)$ is the Risk Priority Code for the criterion a_i (with $i = 1,\ldots, 6$);

$g_j\,(a_i)$ is the non-compliance score for each sub-criterion j (with $j = 1,\ldots,$ n) included in the criterion a_i (calculated as in Table 3);

$I(g_j)$ is the importance score of each sub-criterion g_j, included in criterion a_i, as estimated using the Borda method;

Max_j is the maximum of the minimum (*Min*) between the non-compliance score for sub-criterion g_j (resulting from the checklist's results) and the importance score that was assigned to sub-criterion g_j.

This equation corresponds to the second analysis model described by Franceschini and Galetto (29). The aim is to assign a high RPC for a given criterion (a_i) to those farms that had the highest non-compliance score (corresponding to low biosecurity) on the most important sub-criteria. As an example, a sub-criterion which has been assigned a non-compliance score of 4 and a low importance score (i.e., 2) would be considered of value 2, as the minimum between 4 and 2. Therefore, the contribution of this sub-criterion to the RPC will be limited. In contrast, if the importance of the sub-criterion was 5, a value of 4 would have been chosen (being

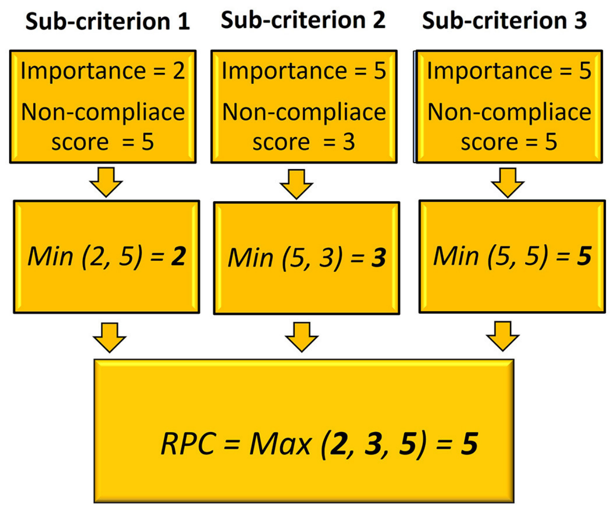

FIGURE 2
Example of calculating the Risk Priority Code (RPC) for one of the main criteria, in a hypothetical pig farm, using the modified failure mode effect analysis. Sub-criterion 1 was assigned an importance score = 2 by the 8 experts. While, in the assessment of biosecurity on the farm by completing the checklist, the veterinarians assigned a high non-compliance score to this sub-criterion (score = 5), indicating low biosecurity. In the calculation, sub-criterion 1 contributed to the overall RPC with a value of 2, which is the minimum between 5 and 2. Sub-criterion 2, on the other hand, was assigned a high importance (5) and a moderate non-compliance score (3). Therefore, according to the procedure just described sub-criterion 2 was assigned a score of 3. Finally, sub-criterion 3 was assigned a high importance = 5 and a high non-compliance score (5). Therefore, sub-criterion 3 contributed 5 to the final result. In fact, the overall RPC for the main criterion, including sub-criteria 1 to 3, was equal to 5, which was obtained as the maximum of the three minimums: 2, 3 and 5.

the minimum between 5 and 4), and this sub-criterion would have contributed more to the RPC. Indeed, the final RPC of each of the six main criteria was calculated as the maximum value among the scores of all sub-criteria. A graphical description of the calculation of RPC is shown in Figure 2.

2.2.4. Geographical risk factors

Given the major role of wild boars in maintenance and transmission of ASFV in Europe (16), the pig farms were classified based on the estimated wild boar population density at the farm locations. A high-resolution raster map of predicted wild boar densities across most of Eurasia was obtained (33), and imported into the R software (*raster* function, *raster* package). Predicted wild boar densities, corresponding to the locations of examined pig farms, was obtained by the *extract* function. Subsequently, farms were classified by Jenks natural breaks of the estimate, to obtain five ordinal levels of increasing risk of exposure to wild boars, for consistency with the five levels of RPC.

Previous research showed that population density of farmed pig was associated with the occurrence of ASF (17–19). The risk of transmission of ASFV between domestic pigs is a function of the distance between farms and can be modelled by transmission kernels (34). To classify farms also in terms of pig population density, we calculated a modified G statistic (35), as an index of local spatial density as shown in the equation below:

$$G_i = \frac{\sum_j w_{ij} x_j}{\sum_j x_j}$$

where G_i is an index of local density of pigs around the visited farm i; x_j is the number of pigs in each of the other pig farms j; and w_{ij} is a distance kernel (equation below):

$$w_{ij} = \frac{k_0}{1 + \left(\frac{h_{ij}}{r_0}\right)^{\alpha}}$$

h_{ij} is the distance between the sampled farm i and each of the other near farms $_j$; k_0 is the value of w_{ij} when $h = 0$; r_0 is the distance at which $w_{ij} = 0.5\ k_0$; and α is the kernel shape parameter. To obtain a smooth decay of local density with increasing distance from other pig farms, we assigned the values of $k_0 = 1$, $r_0 = 0.55$ m, and $\alpha = 2.27$. Such kernel parameters were previously estimated by Boender et al. (36) during the classical swine fever epidemic in the Netherlands, in 1998, and subsequently proposed for ASF by EFSA (34). Farms were subsequently classified by Jenks natural breaks of G_i, to obtain five ordinal levels of increasing risk of local density of domestic pigs. All the pig farms were included in the kernel calculation, although, due to the specific kernel shape, only farms within a certain distance influenced the density weight. Spatial analysis was performed by the R software, version 4.1.2, whereas geographic representation, and Jenks natural breaks of the estimates were obtained using QGIS 3.16.2 Hannover Edition.

2.2.5. Overall risk ranking of pig farms

Each examined pig farm was attributed ordered scores (from 1 to 5) for a total of eight indicators: six RPC's for criteria, which were estimated from the on-farm checklist, and two geographical risk indicators, corresponding to wild boar population density, and local density of domestic pigs. To obtain an aggregated risk index, we calculated, for each farm, the counts of decreasing values of those eight risk indicators, from counts of 5 to counts of 1. Subsequently, a risk rank was assigned to each farm, by sorting them in a decreasing order. In this way, highest risk was attributed to those farms which were characterised by the greatest frequency of RPC's = 5. Then, among farms with the same number of 5 s, the one with the greatest number of 4 s was classified at the highest risk. Then, the counts of 3 s, and of 2 s were considered. An overall ranking of farms, ordered from the farm at the highest risk of ASFV introduction (rank = 1) to the lowest risk was obtained.

Limited to commercial farms in major pig production areas in Italy, non-parametric correlation of risk rank and herd size was estimated by Kendall's τ, using the *KendallTauB* function of the *DescTools* package in the R software. Kendall's τ value is appropriate for estimating the correlation between ordinal variables in the presence of ties. Differences among median risk ranks for different production phases were tested by Kruskal Wallis test (*kruskal.wallis* function, R software). See below for definition of herd size and production phases.

2.3. Data collection

The farm data collection was carried out mainly in Lombardy, Emilia-Romagna, and Piedmont, the three regions where 77.2% of Italian commercial pig farms are located.[7] Moreover, non-commercial and small commercial pig farms (pig farms with a maximum of 20 animals) were also visited in Tuscany, in a Northern Apennine area, ∼150 km from the Italian ASF-infected area (4). In this second sample of farms, we applied a reduced version of our checklist, not including items which are typical of commercial pig production (Table 2, Supplementary Table S1). The inclusion of non-commercial/small commercial farms in the study must, therefore, be considered as preliminary to more in-depth investigations on these types of pig farms.

We selected pig farms based on the farmers' availability from a list provided by seven pig veterinary practitioners and two official veterinarians. Prior to the assessment, we provided all farmers with a comprehensive explanation of the aims and procedures of the study and obtained informed consent.

Three trained veterinarians who participated in the creation of the checklist carried out the farm visits from March through December 2021. To improve the harmonisation of data collection, the three assessors previously discussed all 98 items and agreed upon written guidelines for filling the checklist. Moreover, if any doubt emerged, the three assessors collectively discussed and took decisions on answers to any specific item.

For each farm, the following general information was collected prior to the biosecurity assessment: geographical coordinates, type of farm (commercial, non-commercial/small commercial), production phase (breeding; post-weaning—from weaning to ∼30 kg of body weight; fattening—from ∼30 kg of body weight to slaughter; not specialized—more than one production phase on the same farm), production cycle (closed, open, and semi-closed), and herd size (in case of post-weaning and fattening sites: number of farmed pigs present the day of the visit; in case of breeding farms: number of productive sows present the day of the visit). The data were collected through direct observation and face-to-face interviews with the farmers. As suggested by Dewulf and Immerseel (7), it was decided to first visit the farm in order to make a visual assessment of the situation, and then fill the questionnaire with the farmer to simplify and speed up the assessment. Depending on the farm type, it generally took 30 min to 1 h to complete the checklist. During the on-farm biosecurity assessments, the assessors always acted according to good biosecurity practices.

3. Results

The checklist was filled in for 60 commercial pig farms and 12 non-commercial and small commercial (≤20 heads) farms. Among the commercial farms, 53 (88.3%) were in Lombardy, Emilia-Romagna, and Piedmont. A limited number of commercial farms were also visited in Umbria ($n = 3$), Abruzzo ($n = 2$), Apulia ($n = 1$), and Veneto ($n = 1$). These additional farms belonged to companies involved in Lombardy and Emilia Romagna. The 12 non-commercial and small commercial pig farms involved in this study were located in northern Tuscany. Thirty-three (55.0%) of commercial farms were fattening farms, six (10.0%) were post-weaning sites, whereas 11 (18.3%) were breeding sites. The other 10 farms (16.7%) were not specialized in a specific productive phase, and included both the post-weaning and fattening phases. The median of heads reared in the commercial farms was 1,915 (minimum = 50 heads; Q1 = 1122; Q3 = 3631; maximum = 42,000).

3.1. The importance scores

The importance scores assigned by the experts' panel to each sub-criterion, together with 95% C.L.s, are reported in Table 1. The list of the items that the experts considered as critical for specific biosecurity in the case of ASF consisted of nine of the 98 items, as shown in Table 2.

3.2. The non-compliance scores

Details of the non-compliance scores of each of the 98 items are reported in the Supplementary Table S1.

3.2.1. Main criterion A: Personnel

Four critical items were identified by the expert panel among the main criterion A (Table 2). In commercial farms, negative answers to critical items 2 (the staff had no other pigs, in 23.3% of farms) and 3 (the staff had no contact with other pig farms, 63.3%) resulted into a relatively frequent non-compliance score = 5 for sub-criterion A2 (Figure 3). Non-compliance to sub-criterion A3, was associated to 36.7% of the farms where the answer was "staff may introduce food in the farm, and there is no indication to the area in which it must or may be consumed". Regarding sub-criterion A4, in 28.3% of the farms a non-compliance score of 5 resulted from the selection of the option "the farmer and staff are either not trained at all on biosecurity and the risks of introducing ASF, or there is no clear evidence of courses". In 30.0% of the farms, non-compliance score was 3 for the item "only a portion of the staff working on the farm is trained in biosecurity and the risks of introducing ASF during the last year". Overall, 71.7% of the farms had some workers who did not receive any training on biosecurity and the risk of introducing ASF. A high frequency of non-compliance score of 3 for sub-criterion A1 was the result of 70% of the farms having an improper access area, with overlapping clean and dirty areas. Moreover, personnel do not take a shower before entering in 90% the farm, and 93.3% do not have a Danish entry (i.e., a bench or other physical barrier that totally separates the dirty and clean areas and remember the personnel the threshold).

Considering non-commercial and small commercial farms, most of the farms were non-compliant to all four critical items belonging to main criterion A (Table 2). The non-compliance score for sub-criteria A1, A3, and A4 was 5 for the majority of the farms (>75.0%).

3.2.2. Main criterion B: Animal introduction and management

Only one critical item was identified by the expert panel for the main criterion B (animals were not fed by swill feeding),

7 VetInfo, Italian National Zootechnical Registry, updated 31 December 2021; https://www.vetinfo.it/j6_statistiche/#/report-pbi/31, accessed on 02 October 2022.

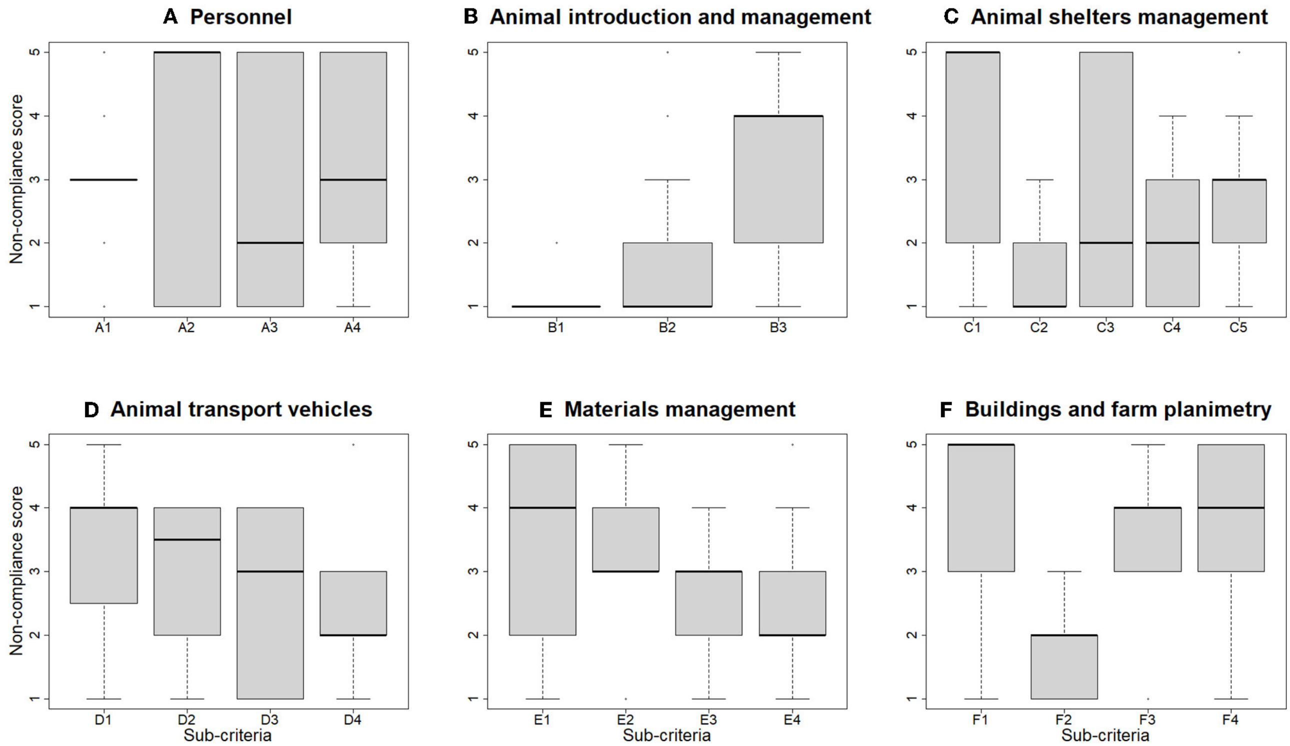

FIGURE 3
Boxplot of the distribution of non-compliance score for each sub-criterion, obtained by evaluation of 60 commercial pig farms in Italy. In the boxes, the thick horizontal line represents the median non-compliance score; the base and the top of the boxes are the first and third quartiles, respectively. Whiskers extend to a maximum of 1.5 times the interquartile range. Points represent extreme values.

and all commercial farms were compliant, whereas two non-commercial/small commercial farms were non-compliant (16.7%, Table 2). In general, biosecurity associated with the introduction of animals and related management showed a good level of compliance for both commercial and non-commercial/small commercial farms. Considering commercial farms (Figure 3), the most frequent non-compliance score for the sub-criterion B1 and B2 was 1 (i.e., the lowest risk of introduction of ASF); within sub-criterion B1, the totality of the commercial farms knew the health status for ASF of all animals prior to their introduction into the herd, correctly identified all the animals on the farm, accurately registered all animal movements both in/out and within the holding structures, and banned swill feeding. Regarding the sub-criterion B2, the majority of the commercial farms (65.0%) always introduced animals from the same farm of origin during the year. Considering non-commercial and small commercial farms, the frequency of non-compliance score 1 was greater for all three sub-criteria.

3.2.3. Main criterion C: Management of animal shelters

No critical items were identified by the expert panel in main criterion C. The assessment of biosecurity practices associated with quarantine of newly introduced animals (sub-criterion C1) was applicable only to 20 commercial farms, as the remaining 40 farms applied all-in/all-out practices. A non-compliance score of 5 was assigned to 55.0% of commercial farms (Figure 3). Cleaning and disinfection practices were carried out satisfactorily (sub-criterion C2). The importance of proper carcass management and disposal appeared to be, in general, understood by farmers, and a non-compliance score of 5 was recorded in only six farms (10.0%, C5). All the non-commercial/small commercial farms were assigned a non-compliance score of 4 to sub-criterion C3 and a non-compliance score of 2 to sub-criterion C4, whereas sub-criteria C1, C2, C5 were not included in the checklist for these types of farms.

3.2.4. Main criterion D: Animal transport vehicles

Two critical items were identified by the expert panel in main criterion D, and a few commercial farms were non-compliant (Table 2). Biosecurity practices during the transport of live animals through vehicles (sub-criterion D1) and their loading and unloading (sub-criterion D2) were not optimal, resulting in a non-compliance score of 4 for both in at least 30 (50.0 %) commercial farms (Figure 3, see Supplementary material for details). In 84.7% of the farms, vehicles were shared with other pig farms, in 75.0% a loading/unloading bay was not present, and in 69.5% no special gates were in place to prevent animals from returning to the barn. Notably, in non-commercial/small commercial farms, the majority of the farms showed a non-compliance score of 5 regarding sub-criterion D1, and of 4 to sub-criterion D2. Carcass disposal (sub-criterion D3) showed heterogeneous results in commercial farms; in 50.0% of these, the truck for the removal of carcasses entered the farm area (Figure 3). For sub criterion D4 (equipment and tools for loading/unloading live animals) compliance was generally greater than for other sub criteria of criterion D, even though disinfection of animal loading bay was not carried out after every usage in 71.1% of farms. Sub-criteria D3, D4 were not included in the checklist for non-commercial/small commercial farms.

3.2.5. Main criterion E: Material management (feed, slurry, and other vehicles)

No critical items were identified by the expert panel in main criterion E. The practices in loading and unloading feed and other materials (sub criterion E1) were unsatisfactory in 40.0% of commercial farms (median score was 4, Figure 3). In particular, the most frequent negative answer was recorded for the item associated to the common treading of the material loading/unloading bays by internal personnel and external operators without dedicated clothing and footwear (90.0% of the commercial farms, Supplementary Table S1). Feed and material storage (sub criterion E2) resulted in non-compliance score ≥3; 81.4% of the farms were non-compliant with the storage of feed, forage, bedding, or environmental materials for at least 30 days before use. Regarding sub-criterion E3, the entrance for slurry transport operations was not separated from the access of the pigs' area in 65.5% of commercial farms (Supplementary Table S1), and the median non-compliance score was equal to 3 (Figure 3). The management of vehicles transporting materials (sub criterion E4) was satisfactory in the majority of commercial farms, with a median non-compliance score of 2; however, in 54.5% of farms, the vehicles were not disinfected on a dedicated area before access to the farm. In contrast, 100.0% of non-commercial/small commercial farms had a non-compliance score of 5 for sub-criterion E4, whereas sub-criteria E1, E2, E3 were not included in the checklist for these types of farms.

3.2.6. Main criterion F: Buildings and farm planimetry

The panel of experts identified two critical items belonging to the main criterion F (Table 2). In 70.0% of commercial farms and 100.0% of non-commercial/small commercial farms the fencing was incomplete (critical item n. 8, Figure 3, sub-criterion F1). Furthermore, the farming area of 71.7% of commercial farms was not surrounded by an asphalted zone (Supplementary Table S1). Conversely, critical item 9 ("disinfectants with proven efficacy against ASF are available", part of sub criterion F2) was always satisfied. Pets were present in 60.0% of the commercial farms. Pest management in commercial farms was often suboptimal (sub-criterion F3), rodent control was usually self-managed (85.0% of farms), and no farm had insect and bird control plans in place (median non-compliance score = 4, Figure 3). In non-commercial and small commercial farms, sub-criteria F2, F3, and F4 showed a non-compliance score of 4 or greater in all farms.

3.3. The RPC's

The distribution of RPC's resulting from the FMEA calculation in each individual commercial farm, considering the non-compliance and importance scores of the sub-criteria, are shown in Figure 4. The highest RPC score (median = 5) was obtained for biosecurity measures associated with personnel practices (main criterion A) and for buildings and farm planimetry (main criterion F). Median RPC = 4 was obtained for biosecurity measures associated with the management of animal transport vehicles (main criterion D) and

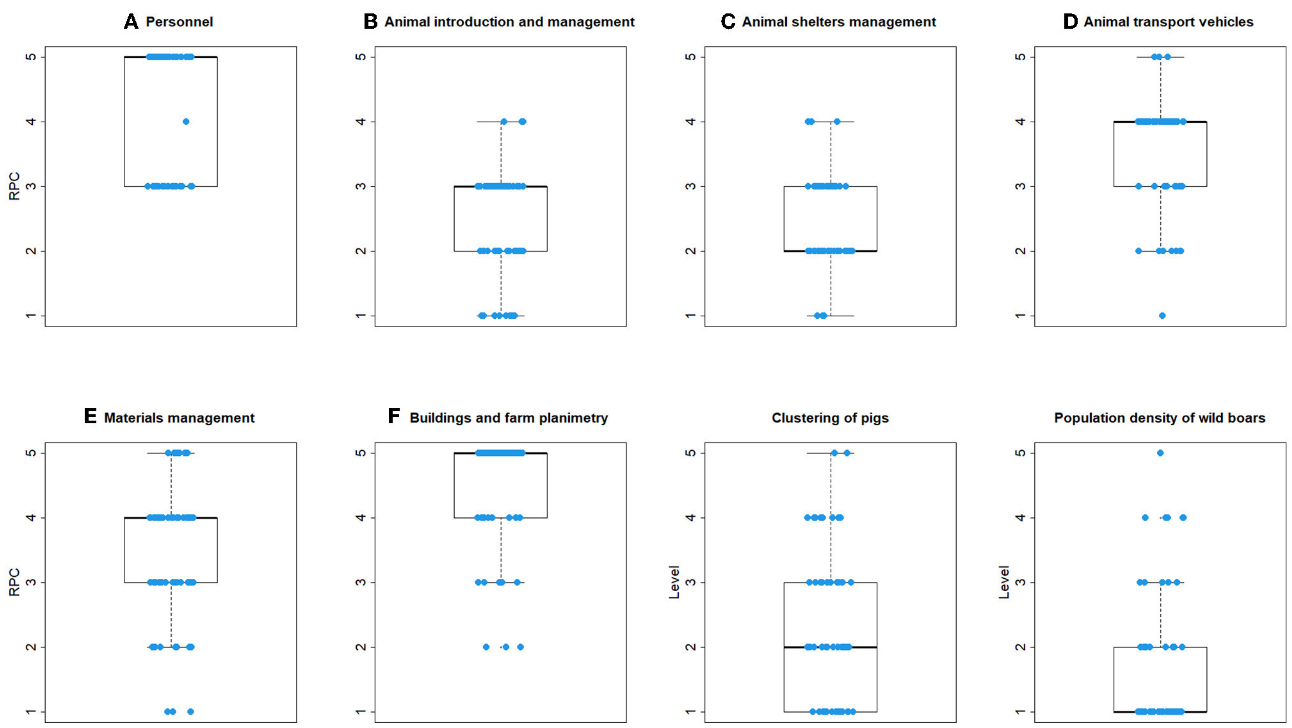

FIGURE 4
Boxplot of the distribution of risk priority codes (RPC) for each criterion, obtained by the application of FMEA, local density of pigs, and wild boar population density, estimated in 60 commercial pig farms in Italy. In the boxes, the thick horizontal line represents the median risk priority code; the base and the top of the boxes are the first, and third quartiles, respectively. Whiskers extend to a maximum of 1.5 times the interquartile range. Points represent extreme values. Blue circles represent individual farm's RPC's.

with material management (i.e., feed, slurry, other vehicles; main criterion E). Lower, and upper limits of RPC's, which were obtained by adopting the corresponding 95% confidence limits of importance of sub-criteria in calculation, are shown in Supplementary Table S2. The effect of the variability of the importance was more limited for RPC's for the main criterion A, which were most consistently high.

In non-commercial and small commercial farms, a median RPC of 5 was observed for criteria D, E, and F (animal transport vehicles; material management: feed, slurry, other vehicles, buildings, and farm planimetry). Criteria A showed median RPC = 4 (personnel). Better results were obtained for criteria C (animal shelter management), B (animal introduction and management) (Figure 5). Lower, and upper limits of RPC's (Supplementary Table S3) indicated some degree of variability, as the consequence of variable importance assignment, except for criteria B, and C.

3.4. Geographic risk factors

Median wild boar density (first, third quartile) at the location of non-commercial and small commercial farms was 5.2 (4.1, 5.6) heads/km^2, based upon 5 km resolution raster maps (33). It was greater than at locations of commercial farms, where it was 0.13 (0.02, 0.61) heads/km^2 (Figure 1). On the other hand, the visited commercial farms in the Po River Valley were in densely populated livestock areas. The local density of pigs, as estimated by G statistics, was highest in Lombardy (Figure 6). The median (first, third quartile) number of farms, surrounding each commercial farm, within a 3 km distance, was 7.5 (3.75, 12.0), whereas the number of pigs was 13,234 (5,889, 28,216). Spatial density was very low for non-commercial/small commercial farms, with 1.0 (0.0, 2.0) farm, and 11.0 (0.0, 37.8) pigs, within 3 km from each farm.

3.5. Overall ranking for the risk of introduction of ASFV in pig farms

The ranking of commercial and of non-commercial/small commercial farms, in terms of the risk of ASFV introduction, based upon combination of on-farm criteria, population density of wild boars, and local density of domestic pigs are shown in Supplementary Tables S2, S3. Separate ranking of these farm types are presented, following the indications reported on DG SANTE working document.[8] Among commercial farms, median (first, third quartile) rank was lowest in post-weaning units: 21.5 (9.2, 33.0), indicating a relatively high risk of ASFV introduction (Supplementary Table S2). Farms that were not specialized in a specific rearing phase (e.g., both post-weaning and fattening in the same farm), were characterised by a median rank of 29.0 (17.5, 50.5), whereas for fattening units: median = 30.0 (13.0, 42.0). Breeding units, median rank = 51.5 (31.8, 57.8), were at a relatively low risk of ASFV introduction. However, the observed differences among median ranks in different production

8 DG SANTE, Directorate General for Health and Food Safety. Strategic Approach to the Management of African Swine Fever for the EU. 2015. SANTE/7113/2015-Rev 12. Available online: https://ec.europa.eu/food/sites/food/files/animals/docs/ad_control-measures_asf_wrk-doc-sante-2015-7113.pdf (accessed on 27 September 2022).

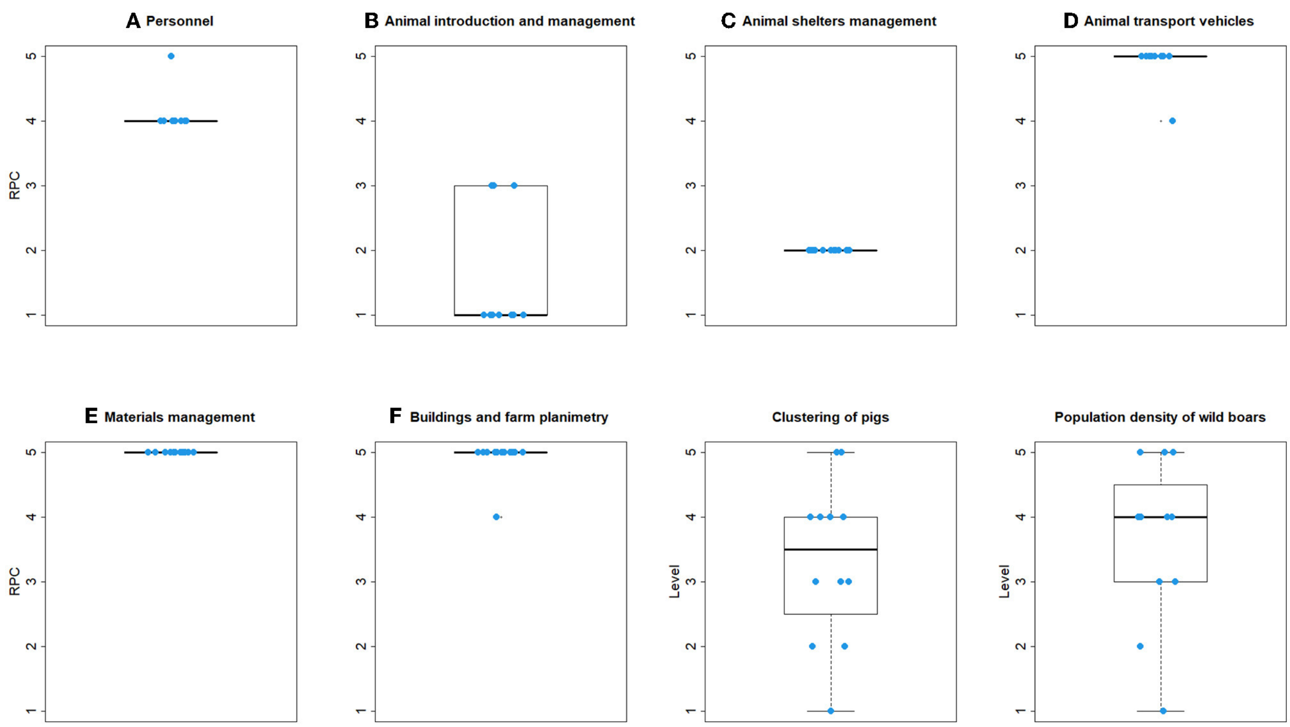

FIGURE 5
Boxplot of the distribution of risk priority codes (RPC) for each criterion, obtained by the application of FMEA, local density of pigs, and wild boar population density, estimated in 12 non-commercial and small commercial pig farms in Italy. In the boxes, the thick horizontal line represents the median risk priority code, whereas the base and the top of the boxes are the first, and third quartiles, respectively. Whiskers extend to a maximum of 1.5 times the interquartile range. Points represent extreme values. Blue circles represent individual farm's RPC's.

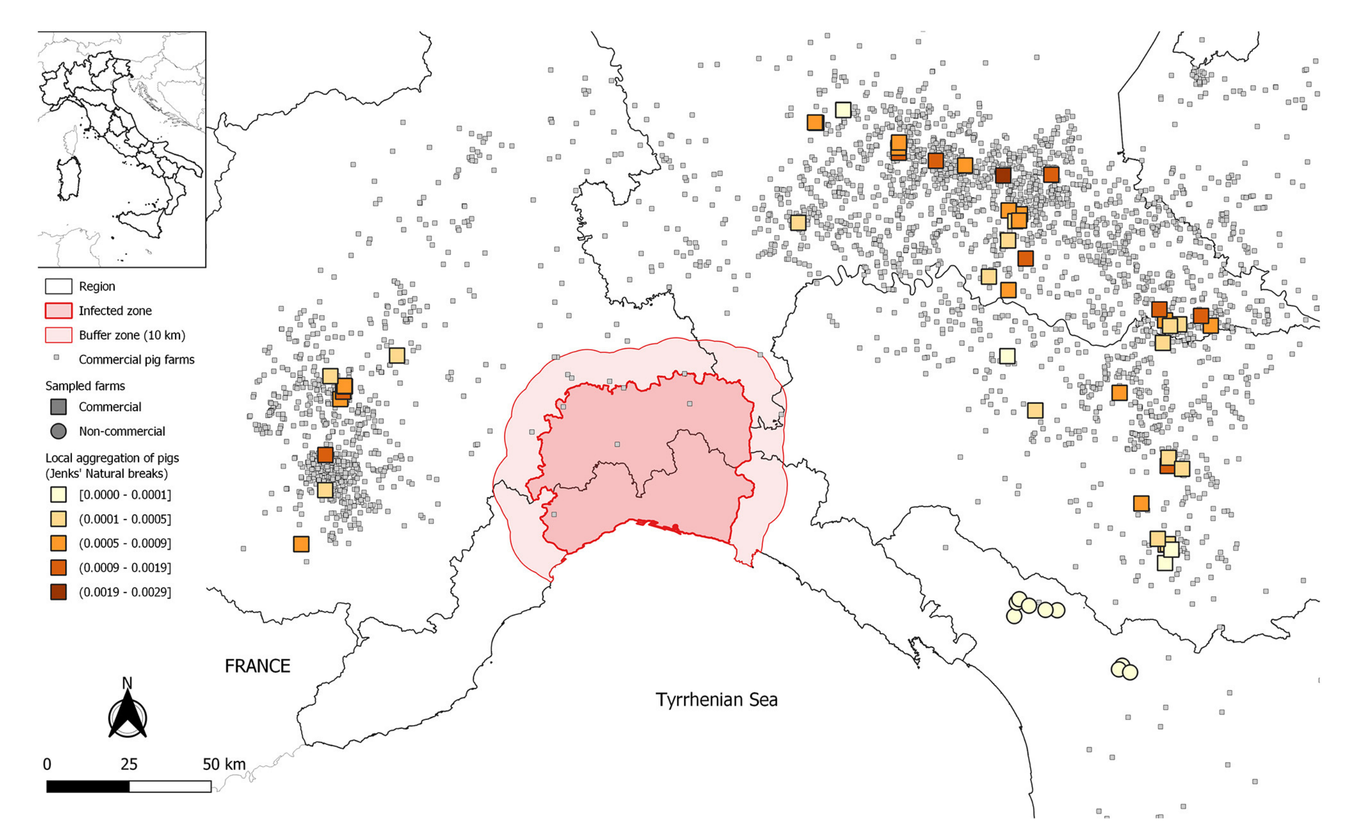

FIGURE 6
Geographic distribution of commercial pig farms in Italy, and of 72 visited farms, which were classified by G statistics, as an index of local density of farmed pigs. Commercial farms are identified by squares. Non-commercial and small commercial farms in north western Tuscany are identified by circles. Farm location colour is based on Jenks natural breaks classification method, of all farm types combined. The infected area for ASF (4) is reported.

phases, in commercial farms, was not statistically significant at the 0.05 significance level (P-value = 0.07). A weak, negative non-parametric correlation between risk rank and herd size of commercial farms, indicating a slight increase in risk with increasing herd size of commercial farms, was not statistically significant (Kendall's $\tau = -0.13$, $p = 0.14$).

4. Discussion

This study was planned in the context of a larger research program on ASF (Defend European project), and focused on the development of a semi-quantitative risk assessment tool to estimate the biosecurity level of pig farms in a standardized and reproducible manner. The scoring system includes the most relevant aspects of biosecurity, which are specifically connected to ASF prevention and control. The aim was not to replace the other existing tools for the assessment of farm biosecurity (e.g., Biocheck.UGent™ or ClassyFarm), but to address specific risk of ASFV introduction in pig farms. In contrast to questionnaires in which no weights are given to the different measures and only the number of compliant items is considered, the modified FMEA includes an importance score, which was based upon opinions by a panel of expert (37). In our approach, the most dangerous failure mode of a biosecurity criterion is obtained by the highest evaluation of non-compliance on the most important sub-criterion (29). *Via* this process, the modified FMEA identifies weak points in biosecurity, and sets the basis to prioritize intervention on those specific biosecurity measures in individual farms, which are associated with high RPC's. Furthermore, in FMEA we combined data of different types and sources, taking into account the ordinal properties of five-level scales. By integrating RPC's (as obtained by FMEA of data collected in visited farms) and geographic risk factors (population density of wild boars and local density of farmed pigs), we obtained an overall ranking of pig farms, in terms of the risk of introduction of ASFV. Based upon such a transparent and easily communicable process, surveillance and intervention resources can be primarily dedicated to farm categories, production phases and geographical areas where the risk of infection is greatest (38).

In our approach, risk-ranking of pig farms corresponded to a decreasing ordering of counts of high RPC's and scores of geographical risk factors. In this way, farms with the highest frequency of scores = 5 were considered at greatest risk. According to this approach, farms with high non-compliance levels to important biosecurity components were considered at the greatest risk of ASFV introduction. An alternative approach could be adopted by ranking farms based on the overall sum of RPC's and geographical risk factors. However, in this way, the same sum could be obtained by different combinations of results, and more farms would be assigned the same risk rank, with a lesser weight of non-compliance to the most important sub-criteria.

It is important to highlight that the present study was entirely performed before the ASF occurrence in northern Italy. Indeed, several authors have suggested that the risk perception of a disease and its consequences on the farm is the main factor leading to the application of biosecurity measures. The greater application of biosecurity measures has been observed after outbreaks of diseases such as porcine reproductive and respiratory syndrome or influenza, as well as in densely populated areas of pigs, probably due to a higher perception of the transmission risk between neighbours (39, 40).

In the ranking of the sampled commercial farms, which we obtained by the application of FMEA and of geographical risk factors, the risk of ASFV introduction was lower in breeding herds than in other production phases, although difference among median ranks was not statistically significant at the 0.05 level. Silva et al. (14) also found that breeding herds were the ones with best biosecurity scores, since these were most likely to undergo certification and annual monitoring by the official veterinary service. Moreover, breeding farms are the top of the sanitary pyramid in pig production, they have a high sanitary status and a reduced risk of introduction of pathogens (41). Herd size was not correlated with risk in our sample of 60 commercial farms. Although most of the ASF outbreaks in Europe have occurred in small pig holdings (1), it should also be noted that smaller commercial farms require lower investment to implement biosecurity measures (14).

In the ranking of commercial farms, the first seven positions (i.e., the farms with highest risk of introduction of ASFV) were occupied by farms having RPC's = 5 in the main criteria A (personnel practices) and F (buildings and farm planimetry). Such criteria are under the direct responsibility of farmers, and they are influenced by the farm manager's decisions and investments. On the other hand, exposure to geographic risk factors, such as local density of farmed pigs, which is greatest in the main pig production areas, and wild boar population density, is beyond control by individual farmers, and it should be the object of national or regional disease prevention programs.

RPC = 5 for criterion A can be explained by the presence of several critical items. In fact, the experts selected 9 of the 98 items as *conditio sine qua non* to achieve a sufficient biosecurity level in the farm, and four of these were included in criterion A. In 63.3% of commercial farms, employees were involved in activities in other pig farms, often belonging to the same ownership. Personnel working on the same farm most of the time, occasionally worked in different farms to carry out tasks, such as loading/unloading live animals, or cleaning and disinfecting procedures. Furthermore, priorities for intervention were identified in 70% of the farms, which were characterised by improper access area, with overlapping clean and dirty areas. A shower was mandatory before entering only in 10% of farms, and a Danish entry was present in just 6–7% of farms. The combination of these non-compliances amplifies the seriousness of the risk of introducing the disease and provides clear indications on the priorities of intervention (42). The risk related to the personnel in the visited farms can be worsened by another priority intervention: the lack of specific training on biosecurity and the risk of introducing ASF, observed in 71.7% of the farms. Some authors reported that, in breeders' view, training on biosecurity, is not useful because often it is not well understood or adequately explained, despite it is a low-cost intervention (43). The results of the modified-FMEA also suggest the need for training on practices regarding the introduction and the consumption of food in the farm, as 36.7% of the establishments did not provide any specific indication. Indeed, waste food has frequently been implicated in the spread of ASFV (44, 45). However, swill feeding was not performed in any of the visited commercial farms, while it was carried out in two of 12 non-commercial and small commercial farms. Based on the work of Olševskis et al. (46), swill feeding is one of the most likely routes of transmission of ASFV to domestic pig farms, and it was selected among the critical items by the experts' panel in the present study. Our results identified a better situation in comparison with that reported by Boklund et al. (26) from Romania, a country which is known for having thousands of backyard farms and for using swill feeding as one of the few countries in Europe: the authors found that several farms fed swill to pigs (especially backyard farms), despite its total ban in the EU since 2002 (47).

The main criterion of building and farm planimetry (F) was identified as another priority for intervention in both commercial and non-commercial/small commercial farms. In particular, non-compliance in 70.0% of commercial farms and 100.0% of non-commercial/small commercial farms emerged in relation to the critical item of fencing, which were often incomplete. A perimeter fence with a permanently closed door that can only be opened from inside the farm was suggested by Alarcón et al. (48) as the crucial requirement for an efficient division between "inside" and "outside" the farm. Fences have also been tackled in the recent EFSA report on ASF in outdoor farms (49): the authors were 66–90% certain that if single solid or double fences were fully and properly implemented, in all outdoor pig farms in ASF affected areas of the EU, this would reduce the number of new outbreaks within a year by more than 50%, without requiring any other outdoor-specific control measures. Moreover, our prioritization indicated that around the farming area of 71.7% of commercial farms, an asphalted zone was missing. Debris and grass around the barns are considered as a risk because they allow the breeding of insects and rodents as a vehicle of infection, and attract wild animals (50). Domestic and wild animals were present in 60.0% of commercial farms. Furthermore, 85.0% of the farmers declared that they self-managed the rodent control plan (i.e., no external professional rodent control company was involved), and no farms had insect and bird control plans in place. Moreover, biosecurity practices during the transport of live animals through vehicles and their loading and unloading were not optimal. Notably, the vehicles used to transport animals between farms, slaughterhouses, and drivers can play an important role in the transmission of pathogens, as described by Alarcón et al. (48).

As expected, non-compliance with biosecurity measures was frequent in non-commercial and small commercial farms, where three critical items were never satisfied, and other three were only rarely satisfied, out of seven for which information was collected. Staff always had contact with other pig farms, external fences were absent, and no disinfectant was available. Procedures related to material management (feed, slurry, other vehicles; main criterion E) were also unsatisfactory. The results obtained in non-commercial/small commercial farms are in agreement with the EFSA report published in 2021 (51), which described small-scale farms as often characterised by little, if any, investment in farm infrastructure and equipment. In the literature, non-commercial farms are described as one of the weakest parts of the biosecurity chain and the biggest risk factor for ASF introduction in domestic pig populations (46, 52). Although non-commercial farms can be a dead-end in terms of the disease spread, units that sell animals at the local or regional levels can play a role in the spread of diseases (1). As a consequence, these farms must adopt the necessary control measures to mitigate the risk. On the small sample of non-commercial/small commercial farms, we applied a reduced version of our checklist, and the obtained risk ranking is not directly comparable with that of the 60 commercial farms. Nevertheless, the inclusion of these results in the present study is important due to the location of farms on the northern Apennines, where an ASF-infected area has been identified at ∼ 150 km distance, and where the risk of ASFV diffusion by abundant wild boars was particularly high. These preliminary results set the basis for further larger scale studies on non-commercial, and outdoor pig farms in Apennine areas.

The implementation of certain biosecurity measure in farms requires considerable economical investments (e.g., building a proper perimetral fence around the farm). Conversely, non-compliance with other measures, which was observed in the present study, could not be attributed to economic constraints, but it was most likely due to established habits, and to a negative attitude toward biosecurity practices, which might be considered as time-consuming and not perceived as useful by the farmers (53). In fact, training of farm personnel, and good communication among all stakeholders can play a central role in ASF prevention. Worth mentioning that with the introduction of EU legislation 2021/605 (9), swine farmers are required to put into place a biosecurity plan against ASF. In this context, the information of farmers on weak points in biosecurity and on preventive measures may enhance their proactive role in the fight against ASF.

We selected commercial farms with the collaboration of a limited number of veterinarians and based upon the voluntary inclusion of farm owners. Results could, therefore, be affected by a bias. The application of our method to representative samples of farms would allow drawing more solid conclusions at the populations level. Further studies might be needed to validate this approach, possibly by evaluation of reproducibility of scoring on the same farms by multiple assessors. Moreover, the integration with up-to-date information on animal movements among farms, as described in Bellini et al. (54) would be useful for network-modelling the spread of the disease.

Results of FMEA were affected by choices in assigning non-compliance scores,. Furthermore, scores of several sub-criteria were arbitrarily grouped into main criteria. Importance was assigned, to each sub-criterion, by a limited number of experts, and the variability of estimates, as expressed by 95% confidence limits, could affect RPC's of the main criteria (Supplementary Tables S2, S3). Such an effect was reduced for criterion A, for which non-compliance was generally high; this was in part due to the presence of critical items which, if not satisfied, invariably led to non-compliance score = 5. It is, therefore, evident that choices in assigning non-compliance and importance scores, and the selection of critical items can affect FMEA results. This must be taken into account when applying the method to different pig farming systems. In the case of geographic risk factors, different methods of classification into five ordered exposure levels (e.g., Jenks natural breaks vs. quintiles) also affect the overall ranking of pig farms. Moreover, boundaries of exposure levels were relative to the examined sample of farms, and will change when a different sample is assessed, especially if in areas with different wild boar and domestic pig densities. Ranking of farms in term of the risk of ASFV introduction must, therefore, be referred to the population at hand, and generalization should be considered with caution.

The adoption of our semi-quantitative risk assessment method might be useful to identify farms eligible to be part of a compartment. The compartment, following indications provided by the WOAH, is one or more establishments, separated from other susceptible populations by a common biosecurity management system, and with a specific animal health status with respect to one or more infections or infestations, for which the necessary surveillance, biosecurity, and control measures have been applied for the purposes of international trade or disease prevention and control in a country or zone. It is worth mentioning that the compartment concept was initially developed by the WOAH. However, recently, the possibility of implementing a compartment has also been established under the Animal Health Law, which means that compartmentalisation is now a disease control option applicable to the European Union.

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

AM and SB conceived of and designed the study. AS, FV, AR, GF, and AB designed the checklist and collected and compiled the data. GF, AM, and AB performed statistical analyses. AS, FV, GF, AR, SC, VC, AB, AM, GA, and SB drafted and edited the manuscript. All authors have contributed to the manuscript and approved the submitted version.

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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/fvets.2023.1017001/full#supplementary-material

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A systematic approach toward progressive improvement of national antimicrobial resistance surveillance systems in food and agriculture sectors

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The first Food and Agriculture Organization of the United Nations (FAO) Action Plan on antimicrobial resistance (AMR), published in 2016, identified the need to develop capacity for AMR surveillance and monitoring in food and agriculture sectors. As part of this effort, FAO has developed the "Assessment Tool for Laboratories and AMR Surveillance Systems" (FAO-ATLASS) to assist countries in systematically assessing their AMR surveillance system in food and agriculture. FAO-ATLASS includes two different modules for surveillance and laboratory assessment. Each module includes two questionnaires that collect either qualitative or semi-quantitative data to describe and score the performance of national AMR surveillance system data production network, data collection and analysis, governance, communication and overall sustainability in a standardized manner. Based on information captured in the questionnaire by trained assessors (1) tables and figures describing the outputs of the surveillance system are automatically generated (2) a Progressive Improvement Pathway (PIP) stage, ranging from "1-limited" to "5-sustainable", is assigned to each laboratory assessed in the country, each area of the surveillance system and also to the overarching national AMR surveillance system. FAO-ATLASS allows national authorities to implement a strategic stepwise approach to improving their AMR surveillance systems *via* the FAO-ATLASS PIP system and provides an evidence base for actions and advocacy. The implementation of FAO-ATLASS at regional and global levels can contribute to harmonize and better coordinate strategies aimed at implementing an integrated AMR surveillance system under the One Health approach.

KEYWORDS

FAO-ATLASS, antimicrobial resistance, surveillance, laboratory, assessment, food, agriculture, One Health

Introduction

Antimicrobial resistance (AMR) is a major global human health concern causing potential increase in treatment failures, loss of treatment options and increased likelihood and severity of infectious disease. A recent publication on the global burden of antimicrobial resistance studying health consequences attributable to bacterial AMR for 23 pathogens in 204 countries and territories in 2019 estimated that 1.27 million deaths were directly attributed to resistance in 88 pathogen-drug combinations evaluated (1). Besides human health, AMR is also a concern for animal health and can consequently have serious impact by limiting the possibilities of treatment or increasing the treatment costs in agriculture and animal productions. In these sectors, the global consumption of antibiotics will likely increase in the future because of the growth in consumer demand for livestock products in middle-income countries and a shift to large-scale farms (2). The risk due to AMR appear particularly high in countries where legislation, consumer pressure, surveillance systems, and the prevention and control of infectious diseases are weak or inadequate.

The current AMR crisis can only be addressed by adopting a One Health approach globally, meaning that veterinary medicine, agriculture, and environment sectors will play key roles in cooperation with the human health sector. In that context the World Health Organization (WHO) adopted during its 68th Assembly in May 2015 a Global Action Plan to combat AMR (3), highlighting the need to address the AMR crisis using One Health approach with the involvement of human health and veterinary authorities, food and agriculture sectors, financial planners, environmental specialists, and consumers. FAO's Thirty-ninth Conference adopted Resolution 4/2015 in June 2015, and published two consecutive FAO Action Plans on AMR to support the Global Action Plan (4, 5). The FAO action plan on AMR 2021–2025 addresses five major focus areas in food and agriculture sectors: (1) Increasing stakeholder awareness and engagement to foster change, (2) Strengthening surveillance and research to support evidence-based decisions, (3) Enabling good practices to prevent infections and control the spread of resistant microbes, (4) Promoting responsible use to keep antimicrobials working and (5) Strengthening governance and allocating resources to accelerate and sustain progress.

Among these areas, AMR surveillance is the cornerstone for assessing and monitoring the emergence and the spread of AMR and for providing evidence for action. A sound surveillance system implemented for continuous monitoring of AMR helps to reduce and control AMR and Antimicrobial use (AMU) by providing information for targeted regulation, advocacy, awareness raising, and tailored interventions to address the development and transmission of resistance. At the global level, AMR surveillance provides early warning of emerging threats and data to identify long-term trends. At the national level it guides policy makers and helps them to apply appropriate and timely interventions. At the local level it allows actors in the field (veterinarians, para-veterinarians, farmers, crop producers) to take better decisions for the treatment of animal and plant infectious diseases. To achieve these goals, an AMR surveillance system must generate up-to-date, comparable, representative, high quality data on pathogens or indicators of concern from the target populations.

Several challenges for the establishment of AMR surveillance networks have been particularly identified in low- and middle-income countries, especially because AMR surveillance in the animal sector is still in its infancy (6). On human health side, WHO developed assessment tools for laboratories (7) as well as a checklist for AMR surveillance system (8). On the Food and Agriculture side, FAO developed an Assessment Tool for Laboratories and Antimicrobial Resistance Surveillance Systems (FAO-ATLASS) to support countries in strengthening the generation of high quality AMR data for evidence. This paper presents the structure of the tool, the methodology of the assessment and the outputs and recommendations of a national assessment using FAO-ATLASS.

Scope and development of FAO-ATLASS

FAO-ATLASS aims to assist countries in systematically assessing their AMR surveillance systems in the food and agriculture sectors by (1) mapping laboratory networks and activities to detect AMR, as well as the national AMR surveillance system (2) measuring in a standardized way the capacities and capabilities of laboratories and the AMR surveillance activities. FAO-ATLASS was developed in alignment with international standards set by the World Organization for Animal Health (WOAH), and the Codex Alimentarius Commission (CAC) as the body responsible for all matters regarding the implementation of the Joint FAO/WHO Food Standards Programme (9, 10).

The tool is currently focused on surveillance of antibiotic resistance in bacteria isolated from animals, food and feed products, plants and environment samples, sources that are considered highest risk, both from human health and animal health point of view. In FAO-ATLASS and in this article, Food and Agriculture sectors include terrestrial and aquatic animal health, food and feed safety, plant health and environment.

Although some information on AMU and residues surveillance are included in FAO-ATLASS to provide basic information, the tool is currently focused on AMR surveillance and associated laboratory activities in bacteriology. Antimicrobial use, antimicrobial residues in food products, and AMR are linked and should be evaluated in a complementary way by different methods.

To ensure consistency with the major assessment tools used at the international level, the development of FAO-ATLASS was built on existing tools and materials:

- The FAO tool dedicated to the evaluation of national surveillance in animal health (11)–FAO-SET–by selecting and adapting some questions relevant to AMR surveillance,
- The FAO Laboratory Mapping Tool (12)–FAO-LMT–for questions related to laboratory functionality, completed with some specific questions regarding bacterial identification, and AMR detection,
- The Joint External Evaluation tool, especially the specific chapter on the prevention of AMR (13),
- The questionnaire from the tripartite AMR country self-assessment survey (14),
- International guidelines for the implementation of an AMR surveillance system (15–18),
- International technical guidelines or standards on antimicrobial susceptibility testing, especially from the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute,
- Opinion from experts in the field of antimicrobial susceptibility testing and AMR surveillance.

A FAO expert team developed the first version of the tool in 2015. The tool was then reviewed in two rounds of consultations (2016 and 2019) by more than 20 reviewers with multidisciplinary expertise (epidemiology, public health, laboratory management, policy measures and development) and working in different national agencies as well as international organizations (WOAH and WHO). These reviews consisted in a qualitative assessment of the tool to check exhaustively terminology, feasibility, and agreement with other assessment tools and available AMR surveillance standards. All comments were considered and discussed with the reviewers when necessary.

Between the two rounds of consultations, the first pilots in country testing were conducted in 2016 in Senegal and Kenya, then followed by several missions in Asia and Africa in 2017. The outcomes of those reviews, in-country pilot tests and feedback from users after missions, were used to progressively refine the tool to obtain a finalized version in 2021.

Structure of FAO-ATLASS

The tool is divided into two modules (two different Microsoft Excel® files), each consisting of a descriptive questionnaire and a semi-quantitative questionnaire:

1) The surveillance module which requires answers from respondents working in different institutions involved or supposed to be included in the surveillance system and is completed at nationwide level. Questions cover five main areas of the AMR surveillance system: governance, data production network (laboratories), data collection and analysis, communication, and sustainability. This module is composed of two questionnaires:

    - "Surv": The descriptive questionnaire is composed of 85 questions organized into the five main areas of the AMR surveillance system and nine categories. Besides those questions focusing specifically on AMR, 15 ancillary questions concern the collection of basic information of the surveillance for antimicrobial use and antimicrobial residues. This questionnaire depicts the organization and outputs of the surveillance system: general national multi-sectoral framework, linkages with human health, actors involved and their roles, modalities of the AMR surveillance implementation in the different sectors (including the sample types, methods for AMR detection, indicators under surveillance, and AMR testing funding), organization of the laboratory network on AMR, upstream and downstream communication, sustainability and continuous improvement.
    - "SET-AMR" (Surveillance Evaluation Tool for AMR): The semi-quantitative questionnaire is composed of 36 questions organized according to the five areas of the AMR surveillance system (Table 1).

2) The laboratory module is completed individually for each laboratory assessed. The number of assessed laboratories may vary among countries. Laboratories to be assessed are those included, or intended to be, in the AMR national surveillance system for the food and agriculture sectors. The assessment of the laboratories covers four areas: activity, technical practices, management of data and biological material, and quality assurance. These laboratory assessments can be considered as complementary to a normative evaluation, for example according to ISO 17025 standard. Indeed they tackle in a very broad (e.g., antibiotics which are tested, modalities of data and biological storage etc.) the organization of laboratories with a view to their participation in an AMR surveillance system. This module is composed of two questionnaires:

    - "Lab": The descriptive questionnaire is composed of 70 questions organized into the four main areas and 16 categories. This questionnaire depicts the activity of the laboratory in the field of bacteriology and antimicrobial susceptibility testing: number of samples tested, resources, technical practices in bacteriology (isolation, identification) and antimicrobial susceptibility testing (methods used, antibiotics tested, standards for results interpretation), management of data and biological material, quality assurance (use of Standard Operating Procedures, use of reference strains, participation to proficiency testing).

TABLE 1 Information collected in FAO-ATLASS SET-AMR questionnaire.

Area	Subcategory
Governance	Existence of an operational structure representative of the stakeholders involved in AMR surveillance under One Health approach (multi-sectoral working group(s) or coordination committee on AMR)
	Development of a National Action Plan on AMR involving the food and agriculture sectors
	Relevance of AMR surveillance objectives and AMR indicators in food and agriculture sectors
	Regulations on AMR surveillance organization in the food and agriculture sectors
Data collection and analysis	Existence of an operational management structure (central epidemiology unit) in food and agriculture sectors
	Frequency of coordination meetings between central epidemiology unit with local units
	Representativeness of the surveillance sampling scheme in food and agriculture sectors including environment
	Adequate skill level in AMR epidemiology of members of the central unit
	Adequacy of the data management system for the needs of the AMR surveillance system (database, etc.)
	Data input interval in accordance with the objectives and use of AMR surveillance system results
	AMR data verification and validation procedures formalized and operational
	Analysis of AMR data against system requirements
Data production network	Effective integration of competent laboratories in the AMR surveillance system
	Level of the standardization of work between different laboratories involved in the AMR surveillance system
	Relevance of laboratory diagnostic techniques
	Technical level of AMR data management of the laboratory network
	Frequency of data transmission to the epidemiology unit
	Harmonization of data transmitted to the epidemiology unit
Communication	External policy for communication with decision makers and other stakeholders
	Identification and coverage of key stakeholders' expectations about the results of the surveillance system
	Existence of awareness building AMR programs for surveillance actors
	Communication of risk assessment outcomes to relevant parties
	Regular release of reports on AMR surveillance results
	Systematic distribution of AMR surveillance results to field actors (outside of a report)
	Presence of a communication system organized between field actors (mail, websites, telephone…)
Sustainability	Adequacy of material and financial resources for the multi-sectoral working group(s) or coordination committee on AMR
	Adequacy of financial resources for the implementation of the National AMR action plan
	Adequacy of human, material, and financial resources for AMR data production (laboratory network) needs
	Adequacy of human, material, and financial resources for AMR data collection and analysis (epidemiology) needs
	Adequacy of human, material, and financial resources for communication needs
	Regular advanced training for actors of the surveillance
	Adequacy of material and financial resources for training
	Development and validation of performance indicators for AMR surveillance system
	Regular measurement, interpretation, and dissemination of performance indicators
	External assessment carried out
	Implementation of corrective measures

- "LMT-AMR" (Laboratory Mapping Tool for AMR): The semi-quantitative questionnaire is composed of 42 questions organized in 12 categories (Table 2). Based on the LMT-AMR, a specific sheet (LMT-BACT: 31 questions) has been developed to assess the laboratories which are not conducting antimicrobial susceptibility testing but are conducting bacterial isolation and providing isolates to the network for AMR surveillance purpose.

In both descriptive questionnaires the results are recorded by selecting standardized answers from checkboxes in the Excel® files. For each question, the assessor can provide additional information or comments in a free field box.

In both semi-quantitative questionnaires, the assessors can select a scenario that best describes the situation assessed. Each scenario is related to a score, ranging from 1 (weakest) to 4 (best) per question (Table 3). The Excel® files allow data to be recorded from three different assessments in order to monitor progress over time.

A short manual with application guidelines is provided to the assessors directly in the Excel® files, as well as additional information to complete each question. The tool is currently available in four languages (English, French, Portuguese, and Spanish).

ATLASS assessors and ATLASS community

The ATLASS assessors are either international or national experts with experience in bacteriology and antimicrobial susceptibility testing, and/or applied epidemiology for AMR surveillance in the field of the food and agriculture sectors. The national experts are selected and nominated by countries and attend theoretical and practical training organized by FAO to become ATLASS assessors. This training process ensures standardized assessments from one country to another and from one assessment to another over time. The process to become an ATLASS assessor includes: (1) attending an initial training session (with theoretical lectures and practical exercises) dedicated to the use of the tool as well as the implementation of assessment missions, (2) participation in a mentored FAO-ATLASS mission with an experienced ATLASS assessor and contribution to the drafting of the assessment report, and (3) conducting a FAO-ATLASS mission in autonomy including writing of the report, which is validated according to a defined process. Once completed the three steps, the ATLASS assessor is considered fully trained. Between March 2017 and December 2019, six training sessions have been organized by FAO in Rome (Italy), Singapore (Republic of Singapore), Moscow (Russia), Lusaka (Zambia), Kochi (India) and Dakar (Senegal), gathering 118 trainees from 48 countries.

Besides the short manual available in the Excel® files, the ATLASS assessors are provided an assessor kit that includes generic presentations to be used during the assessment, information material, and guidelines. The guidelines present the structure of the tool, explain how to prepare and conduct assessment missions, and give indications about the expected recommendations, including a standardized report template. They include information about approaches to AMR surveillance and concepts used in FAO-ATLASS. The kit includes a report template to present the assessment results in a standardized manner.

Since March 2017, as an outcome of the training sessions, FAO has been developing and maintaining several ATLASS communities worldwide, enrolling assessors working in government agencies, laboratories, multilateral organizations and academic institutions from different regions/countries in Africa, Asia, and Europe. The ATLASS community serves as regional and national technical resource to monitor and sustain the momentum toward the enhancement of AMR surveillance in the food and agriculture sectors. All ATLASS assessors should regularly conduct FAO-ATLASS assessments, including in their own country, and actively participate in the ATLASS assessors' community in order to ensure a common approach to applying the tool, offer suggestions for possible improvement of the tool and keep up to date with new developments. The participation to the ATLASS community also engages the assessors to participate to information exchange *via* social networking applications or regular coordination/refresher meetings.

In 2019 FAO initiated the training for ATLASS laboratory focal points in each laboratory to ensure familiarity with FAO-ATLASS laboratory module. These ATLASS laboratory focal points are requested to collaborate with the ATLASS assessors during the FAO-ATLASS assessments and to follow up on the recommendations provided to each laboratory. The ATLASS laboratory focal points also complete the regular laboratory self-assessment and share the results with the national ATLASS assessor for the compilation of assessment results from each laboratory within the national network.

FAO-ATLASS assessment process

The tool was designed to be used through a standardized process either for external assessment of an AMR surveillance system, or by any country as a self-assessment tool when applied by a trained national ATLASS assessor. The suggested approach is to perform an initial external assessment, and then conduct follow up assessments considering the needs of the country either through external or a self-assessment.

Countries can request, on a voluntary basis, an FAO-ATLASS external assessment through their FAO representation. Once the mission is confirmed, a team of two ATLASS assessors is set up with complementary profiles (laboratory and applied

TABLE 2 Information collected in FAO-ATLASS LMT-AMR questionnaire.

Area	Category	Subcategory
Activity	Sustainability	Financial capacity (allocation of funds)
		Management
	Workflow organization	Quality of samples submitted
		Sharing of results with customers
		Sample acceptation criteria
	Collaborations	Training about antimicrobial resistance
		Scientific publications
		Collaboration with other laboratories in the country
		Collaboration with laboratories outside the country
Technical practices	Resources for bacteriology testing	Biosafety of bacteriology laboratory
		Equipment for bacteriology and AST
		Animal diseases–media and consumable-
		Food safety–media and consumable
		Water and environment–media and consumable
		Plant health–media and consumable
		Reagents availability for AST
	Bacteriology- technical practices	Bacteriology methods
		Bacterial identification
	Antimicrobial susceptibility testing (AST) methods	Standard for AST
		Bacterial inoculum calibration for AST
		Panels definition
		Revision of panels of antibiotics
		Method for reading disk diffusion results
		Method for reading MIC results
		Standard for interpretation of disk diffusion results
		Standard for interpretation of MIC results
	Molecular tools	Molecular characterization (resistance gene confirmation or typing)
		Sequencing of resistant strains
Management of data and biological material	Management of biological material	Sample identification and follow-up
		Proportion of isolates archived in a library
		Method for bacterial preservation
		Inventory of archived isolates
		Duration of bacterial isolates archiving
	Data management	Individual reports on AMR data to the customers
		Data archiving
		AMR data transmission to a dedicated epidemiology unit
Quality assurance	Documentation	SOPs on AMR detection implemented
		SOPs on AMR detection updating

(Continued)

TABLE 2 (Continued)

Area	Category	Subcategory
	AMR detection	Reference strains for AST quality control
		Proficiency testing for AST
	Staff	Initial training in AMR testing
		Staff skills validation and continuous proficiency

TABLE 3 Example of scoring with the semi-quantitative questionnaires.

Subcategory	4	3	2	1
Existence of an operational management structure in food and agriculture sectors	A clearly recognized structure exists, its organization is in coherence with the needs of the AMR surveillance system and activities are actively conducted	A clearly recognized structure exists, its organization fits the needs of AMR surveillance but activities are partially conducted	A clearly recognized structure exists but its organization does not fit the needs of AMR surveillance activities OR an epidemiology unit is functional in other fields of food and agriculture (e.g. zoonosis or animal health surveillance) but not involved in AMR surveillance	No dedicated structure OR no structure officially designated for AMR surveillance purpose

In all semi-quantitative questionnaires (LMT-AMR, LMT-BACT and SET-AMR) the assessors can select a scenario that best describes the situation assessed. Each scenario is related to a score, ranging from 1 (weakest) to 4 (best). The example given in that table is taken from the SET-AMR questionnaire, Area: Data production network, Subcategory: Effective integration of competent laboratories in the AMR surveillance system.

epidemiology on AMR). The ATLASS assessors then start to collate information in advance with the assistance of the local FAO office, in particular regarding (i) the main animal (terrestrial or aquatic) and plant production or importation, (ii) the national action plan to combat AMR in the country, the policy and legal frameworks on AMR, (iii) reports and scientific publications about the AMR situation in the country, (iv) results from other previous assessments (e.g., Performance of Veterinary Services (WOAH), Joint External Evaluation (WHO), etc.). Information regarding the national AMR surveillance laboratory network is also necessary to define the laboratories to be assessed in the food and agriculture sector and thus the mission's agenda.

During the assessment mission, which lasts up to 1 week depending on the country situation, an initial briefing meeting is held with all relevant stakeholders involved or to be involved in the national AMR surveillance system. The aim of this meeting is to present the objectives of the mission and gather information on the country's organization through a participatory and multisectoral approach. Although the tool is designed to assess AMR surveillance in the food and agriculture sectors (including environment), key representatives and stakeholders from human health are invited in order to describe and assess the cooperation between all sectors. Additional bilateral meetings may be organized during the week with the main actors of the surveillance system to better detail the information collected during the first meeting, and to cross check information recorded. The information is then recorded using the FAO-ATLASS surveillance module.

During the week, the team visits the selected laboratories which are those included, or intended to be, in the AMR national surveillance system for the food and agriculture sectors (e.g., terrestrial and aquatic animal health, food safety, plant health and environment). Evaluations concern not only each selected laboratory to be assessed but also the functioning of the laboratory network, including the role of the national reference laboratory if existing. These laboratories can either perform antimicrobial susceptibility testing or only provide isolates to be tested by the network, and either be central or district ones. The usual process for each visit includes a first meeting with the laboratory managers to gather information on laboratory's organization and role in the AMR national laboratory network, followed by a technical visit in the bacteriology laboratory. The findings are recorded using the FAO-ATLASS laboratory module (and FAO-ATLASS surveillance module regarding the functionality of the laboratory network).

On the last day of the mission, a restitution meeting is held, ideally with the same stakeholders present on the first day to share information collected during the mission and discuss and agree on a summary of key recommendations. This meeting is also an opportunity to generate a discussion among stakeholders from different sectors about gaps that may be identified in the implementation of the surveillance system in an integrated manner, according to a One Health approach. After the mission, a report is written by the ATLASS assessors, and transmitted to the country for review and clearance. Once cleared, the mission report is officially transmitted to and owned by the country.

Outputs and recommendations of a national assessment using FAO-ATLASS

The tool can be used to generate a baseline, to monitor progress, and to support countries in building their AMR surveillance system. The results of the assessment are presented in a narrative report that includes figures and tables with semi-quantitative analyses.

Descriptive information addresses the organization of the AMR surveillance system in the country, as well as the capacities of each laboratory visited. A summary of information collected in the descriptive questionnaires allows to automatically generate tables presenting:

- For each laboratory and for the network the activities and AST methods used for each sector,
- The AMR indicators monitored by the country for each sector (type of surveillance, animal species, type of production, sample type, bacterial species, antimicrobial panels).

Besides those specific tables, numerous information can be extracted from the database about the organization of the surveillance (e.g., linkages with AMR surveillance in human health, actors and their roles in the surveillance system), and capacities of the laboratories (equipment, personnel, data management etc.).

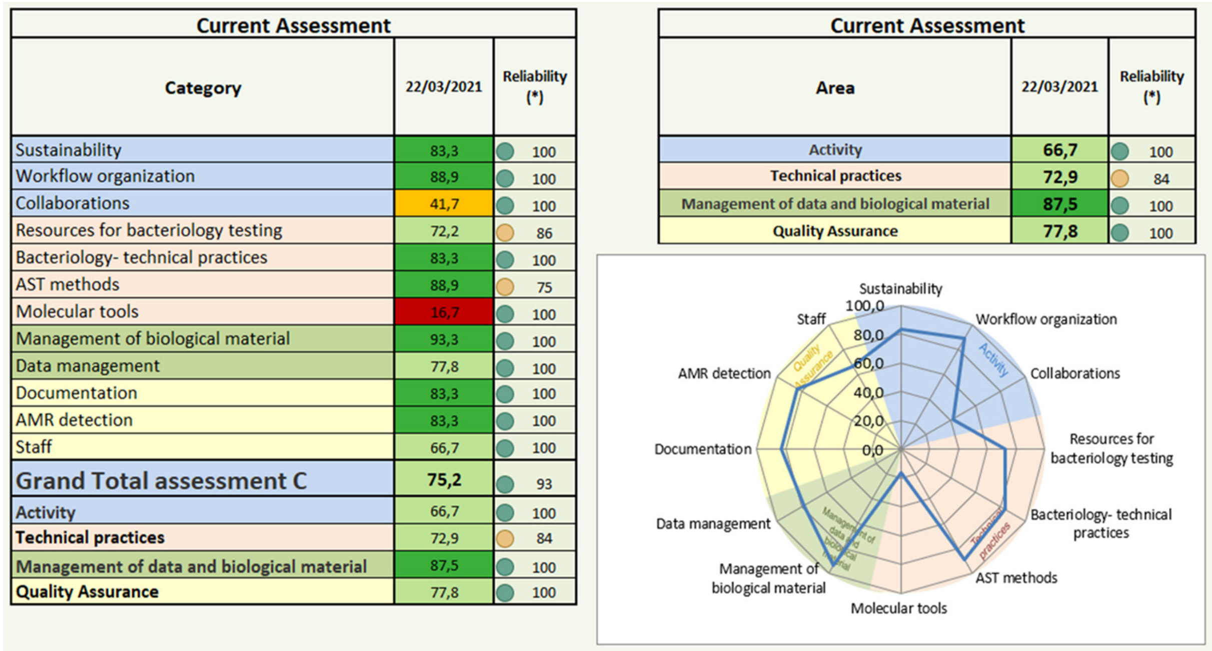
Quantified results are obtained using the scores (from 1 to 4) from the semi-quantitative questionnaires for each of the laboratories visited and for the surveillance system. The combination of these results allows to:

1) Automatically generate a table and a spider web in the FAO-ATLASS laboratory module to easily summarize strengths and gaps (Figure 1) for each laboratory assessed. LMT-AMR can be also used to compare the results of the current assessment with the two last previous assessments, where such information is available, in order to monitor progress of the laboratory over time.
2) Assign the FAO-ATLASS Progressive Improvement Pathway (PIP) stage ranging from "1-limited" to "5-sustainable" to each of the assessed laboratory and to the surveillance system. The stage "3-developed" is considered as the threshold for claiming reliable activities (data production by laboratories or data use by the surveillance system). As for the Joint External Evaluation (19) scoring process, the laboratory or the surveillance system moves to the next PIP stage only when it has achieved all the attributes of its current PIP. For example, to reach "3-developed" capacity, it has to meet all the attributes of "1-limited" and "2-moderate" stages.

a. For each laboratory, the level of fulfillment of the attributes are expressed as a minimum score to be reached for each question of the LMT-AMR. A summary of the minimum requirements that the laboratory should meet for each specific PIP laboratory stage is presented in Table 4. The same process (using LMT-BACT data) is done for determining the PIP stage of laboratories which role is to conduct bacterial isolation and provide isolates to the network for AMR surveillance purpose.
b. For each area of the AMR surveillance system (governance, data collection and analysis, data production network, communication, and sustainability), the assignment of the PIP stage is based on the fulfillment of essential attributes as assessed by the SET-AMR questions for each area of the surveillance system. An example is given in Table 5 for the PIP stage determination of the "data collection and analysis" area.
c. The overall FAO-ATLASS PIP stage of the national AMR surveillance system is determined by combining the PIP stages of the five main areas. A summary of the minimum requirements that each area of the national AMR surveillance system in the food and agriculture sectors should meet for each FAO-ATLASS PIP surveillance stage is presented in Supplementary Table 1.

Based on these results the assessors make recommendations adapted to each laboratory, and to each of the five areas, leading to prioritize actions for the improvement of the AMR surveillance system of the country. In that sense, they are presented in a standardized report template making the distinction between first-line priorities which are advised to be implemented within one to two years after publication of the official version of the report, and second line priorities which are advised to be implemented within 3–5 years after publication of the report. The recommendations can address: 1) the governance of the surveillance system related to the national action plan and the AMR coordination committee/working group(s), the strategy for gradual implementation of the national surveillance system, including the identification of possible sampling schemes, the guidance on indicators to be monitored, the modalities of data collection, interpretation, and reporting to authorities and users, etc. and 2) the organization of the laboratory network, including the possible roles and coordination by the National Reference Laboratory, and capacity building required for the laboratories. For each laboratory assessed, a summary table presents the strengths and weaknesses, as well as the recommendations for reaching the next PIP stage.

Missions and workshops ("Post-ATLASS" support) can be organized as a follow up to the ATLASS assessment at



Current Assessment		
Category	22/03/2021	Reliability (*)
Sustainability	83,3	100
Workflow organization	88,9	100
Collaborations	41,7	100
Resources for bacteriology testing	72,2	86
Bacteriology- technical practices	83,3	100
AST methods	88,9	75
Molecular tools	16,7	100
Management of biological material	93,3	100
Data management	77,8	100
Documentation	83,3	100
AMR detection	83,3	100
Staff	66,7	100
Grand Total assessment C	**75,2**	93
Activity	66,7	100
Technical practices	72,9	84
Management of data and biological material	87,5	100
Quality Assurance	77,8	100

Current Assessment		
Area	22/03/2021	Reliability (*)
Activity	66,7	100
Technical practices	72,9	84
Management of data and biological material	87,5	100
Quality Assurance	77,8	100

FIGURE 1
Graphical representation of the laboratory assessment results using the Laboratory Mapping Tool for Antimicrobial Resistance (LMT-AMR). The scoring in this table is based on the ideal situation, with 100% being the score for an ideal laboratory. The number in each cell is the percentage achieved by the laboratory assessed, compared to the ideal. Numbers displayed in percentage; numbers in each cell represent the achieved percentage compared to the optimum (100% being the ideal laboratory). Color coding: 0–20% (dark red), 20–40% (light red), 40–60% (orange), 60–80% (light green, red), 80–100% (dark green). (*) Reliability of the result depends on the percentage of questions filled or left blank per category in the LMT questionnaire. From 100 to 90%, the LMT scoring is reliable (green dot). From 90 to 70%, reliability of the scoring is medium (orange dot), from 70 to 0%, reliability is low (red dot).

TABLE 4 Overview of the main characteristics of a laboratory according to the FAO-ATLASS PIP laboratory stage.

Stage 1 Limited	Stage 2 Moderate	Stage 3 Developed	Stage 4 Demonstrated	Stage 5 Sustainable
Very weak workflow organization and financial autonomy No or very weak capacities in AST No or weak quality assurance in the field of bacteriology/AST	Capacity of testing some samples for AST on few pathogens, Weak quality assurance system and/or unstandardized methods for AST and/or gaps in the management of biological material or data	Capacity to test in a standardized manner some samples for AST on few pathogens and to manage biological material and data with basic quality assurance procedures. Challenges may exist for the financial autonomy or the management	Capacity to test in a standardized manner a wide range of bacterial species and to manage biological material and data with robust and sustainable quality assurance procedures AMR data are shared irregularly or partially for surveillance	High-capacity laboratory able to test with a national/international standard a wide range of bacterial species, including fastidious species and to share the results regularly for surveillance or decision making *+ For reference laboratories: able to characterize isolates with molecular tools, and to publish research*

This table summarize the minimum requirements that the laboratory should meet for each of the specific FAO-ATLASS PIP laboratory stages.

country or regional level to gather national authorities and experts to review FAO-ATLASS mission findings, prioritize actions and develop plans for progressive improvement of AMR surveillance. Regional analysis of PIP stages of the surveillance system/laboratories on the countries help to define shared capacity building programs to improve data standardization, such as common AMR indicators and surveillance protocols.

Discussion

Globally, knowledge of existing AMR surveillance networks in the food and agriculture sector is weak, especially in the low- and middle-income countries (6). The published analysis report of the second round of results of AMR country self-assessment survey showed a sharp contrast between the

TABLE 5 Approach for the determination of the FAO-ATLASS PIP stage of the "data collection and analysis" area.

	Stage 1 Limited	Stage 2 Moderate	Stage 3 Developed	Stage 4 Demonstrated	Stage 5 Sustainable
Existence of an operational management structure (central epidemiology unit) in food and agriculture sectors	≥1	≥2	≥3	≥4	≥4
Frequency of coordination meetings between central epidemiology unit with local units	≥1	≥2	≥3	≥4	≥4
Representativeness of the AMR active surveillance sampling scheme in food and agriculture sectors including environment	≥1	≥1	≥2	≥3	≥4
Representativeness of the sampling of AMR passive surveillance in food and agriculture sectors including environment	≥1	≥2	≥3	≥3	≥4
Adequate skill level in AMR epidemiology of members of the central unit	≥1	≥1	≥3	≥3	≥4
Adequacy of the data management system for the needs of the AMR surveillance system (database, etc.)	≥1	≥2	≥2	≥3	≥4
Data input interval in accordance with the objectives and use of AMR surveillance system results	≥1	≥2	≥2	≥3	≥4
AMR data verification and validation procedures formalized and operational	≥1	≥2	≥2	≥3	≥4
Analysis of AMR data fits the needs of the system	≥1	≥2	≥2	≥3	≥4

The level of fulfillment of the attributes are expressed as a minimum score to be reached for each question of the SET-AMR according to a progressive approach based on each attribute's importance for this area. The same approach is used for the other areas of the surveillance system (governance, data production network, communication, and sustainability). The intensity of the color shades increases with the value of the minimum score to be reached for each question of the SET-AMR questionnaire.

non-human sectors and the human health sector where most countries have established an AMR surveillance system for common bacterial pathogens (20). On the non-human side, 67 countries (43.5%) collect some data from animal and 60 (38.9%) from food, whereas in the environment and plant sectors most countries have no system in place for surveillance. On the other hand, some countries developed sophisticated surveillance systems, leading sometimes to overlap between national and international systems and the duplication of efforts and economic resources (21). Furthermore, AMR surveillance and monitoring systems vary substantially between sectors and across countries in the type of data collected and reported, as well as laboratory methods. More generally, health care professionals and policy-makers may feel the need to raise awareness of data availability and the potential value of this data, and to ensure that data systems are more accessible (22). Thus, FAO-ATLASS, by mapping and assessing the national AMR surveillance system including all sectors of the food and agriculture and the linkage with AMR surveillance in human health, can be a powerful tool to assist countries in identifying their needs for a robust AMR surveillance system in non-human sectors and thus to make progress in accordance with their AMR national action plans. In that sense, FAO-ATLASS repository allows to share data on the laboratory capacities, as well as the organization and the outputs of the surveillance and plan for harmonized AMR surveillance.

Although some international standards or guidelines on surveillance exist for aspects of food safety and animal health (15–18, 23) significant gaps remain concerning common standards for methods, data-sharing and coordination at local, national, regional and global levels. In that context, the FAO-ATLASS objective is to provide countries a method to assess their AMR surveillance systems in the food and agriculture sectors in a systematic and standardized manner. Assessment data are automatically compiled to assign a Progressive Improvement Pathway (PIP) stage to each laboratory, to each major areas of the AMR surveillance system, and to the overall national AMR surveillance system. The PIP stage is determined on the basis of an internal guideline summarized in Table 4, Supplementary Table 1, which offers countries a progressive development scheme for the organization of the surveillance system and laboratory capacities. This guideline has been reviewed by a multidisciplinary team as part of the revision process and has been adjusted as far as possible with international recommendations. This allows the provision of practical recommendations for laboratory capacity building and surveillance strengthening, which can be prioritized and adapted to the country to ensure a progressive and achievable approach. This also facilitates reaching common and standardized objectives for the implementation of AMR surveillance systems in the food and agriculture sectors worldwide. To assure the standardization of assessments, efforts have also been made to design a formatted and easy to use tool, for the collection and analysis of assessment data. In their surveillance systems evaluation, Calba et al. (24) considered that some of the main limitations of the evaluated approaches were the level of details provided to evaluators for the practical implementation. For FAO-ATLASS we have developed a detailed method with ready-to-use questionnaires designed to assess defined attributes of the AMR surveillance system and produce automatic compilations with graphical representation and scoring. The training process for FAO-ATLASS assessors also contributes to ensure the standardization of the assessments.

Countries around the world are increasingly committed to taking a multisectoral approach to address complex health threats such as AMR at the human-animal-environment interface. As practical implementation of this approach can be challenging, many organizations have provided technical and financial support to countries, using available tools to promote the operationalization of a multisectoral approach. For human medicine, WHO has developed tools to help countries identifying gaps and challenges that relate specifically to participation in the Global Antimicrobial Resistance Surveillance System (GLASS) which fosters standardized AMR surveillance globally, by collecting and reporting data on AMR rates aggregated at national level (25). Some authors developed a roadmap to help low-income countries to participate in this system (26). But although some aspects can be comparable between human and food and agriculture sectors, others, for example sample sources, target organisms, sampling design and laboratory testing, can be quite different. Pelican et al. (27) built a conceptual model representing a consensus on the links and synergies between 12 tools (including FAO-ATLASS, LMT and SET) for advancing One Health implementation, to highlight a potential approach to linking and coordinating the implementation of these tools. In this view, efforts were made to align FAO-ATLASS with other tools, such the Joint External Evaluation (JEE) tool which was developed by WHO for a global multisectoral evaluation process, including the country's capacity to prevent AMR in zoonotic diseases (9). Other tools can be used to describe and evaluate AMR surveillance systems in the food and agriculture and in human health. Recently, some authors provided an overview of what three available tools offer and require from the evaluators, showing that each of them had their strengths and weaknesses in evaluating the different areas and levels of the surveillance systems (28). This study included FAO-Progressive Management Pathway (29), which assesses the progress in the implementation of the country National Action Plan through different focus areas and stages of development for informed decision-making at country level but not meant for comparison between countries. A recent study on the assessment of evaluation tools for integrated surveillance of antimicrobial resistance showed that PMP-AMR and ATLASS seemed to be the most user-friendly tools, particularly designed for risk managers (30). FAO-ATLASS provides deeper insight into the organization of the national AMR surveillance system specifically in the food and agriculture sectors, including assessments of laboratories which are the main data producers for AMR surveillance. Besides the organizational and technical aspects of the AMR surveillance systems, the assessment also concerns governance and funding which are central issues to be considered for assessing the sustainability of a system. Communication and feedback to stakeholders to ensure their awareness and the acceptability of the surveillance system are also taken into account. The semi-quantitative questionnaire developed for the assessment of the surveillance with FAO-ATLASS was based on a previously published tool called OASIS (31) which was also used by FAO as a basis to develop the FAO-Surveillance Evaluation Tool (FAO-SET) which provides countries with a comprehensive and standardized way to evaluate animal disease surveillance systems, including zoonoses. Simultaneously, the semi-quantitative questionnaires developed for the assessment of laboratories involved in the AMR surveillance system were developed on the basis of the FAO-LMT-Core module (32) which can also be used during the assessments to add additional value through describing the functionality and capacity of the laboratory in a more comprehensive way (management of personnel skills, equipment, premises, etc.).

A One Health approach to combat AMR requires the collaboration of multiple sectors. Regarding AMR surveillance, it appears that beyond data integration, the concept of One Health needs to be applied to different tasks, including data collection and analysis, interpretation and dissemination of results (33). FAO, WOAH, UNEP and WHO, also known as the Quadripartite, have a key role in supporting multisectoral responses to AMR. However, there are significant challenges in data sharing and harmonization across sectors to support a One Health response. As reflected in the AMR and AMU surveillance and monitoring information note of the Global Leaders Group on AMR, most data are currently only available in the human health sector and somewhat available in the animal sector, while there is a paucity of data in the plant sector and the environment. More financial resources, more technical capacity, and better infrastructure are needed for AMR/AMU integrated surveillance - particularly in low and middle-income countries. More efforts are needed to use the data generated for informing actions against AMR; and surveillance efforts at all levels, global, regional, and country, need to be coordinated and aligned in data sharing. The Quadripartite organizations are making great efforts to support the generation of information and evidence on AMR and AMU globally. Following the agreement between the organizations to create synergies, WOAH and WHO have established global systems for collecting and analyzing AMU data in terrestrial and aquatic animals and AMR/AMU data in humans, respectively. The collection of data on AMR in animals and food commodities and data on the use of antimicrobial pesticides in crops is under the mandate of FAO and the Organization is currently developing the International FAO Antimicrobial Resistance Monitoring (InFARM) system to cover this information gap in agri-food systems.

Different surveillance approaches and designs can be followed to generate AMR evidence. Building up or strengthening passive laboratory sample-based surveillance, as proposed by GLASS, is a good means to generate AMR data, although it has several limitations in the perspective of clinical decision making, public health practice and epidemiological research, which could be compensated by case-based surveillance (34). In the food and agriculture sectors, active surveillance which involves AMR surveillance in healthy animals entering the food chain, contributes to the pool of information intended to protect human health. Moreover, the availability of standards and possibilities for practical implementation make easier the standardization of surveillance in healthy animals. On the other hand, AMR surveillance in bacterial pathogens from diseased (terrestrial and aquatic) animals also provides a basis for developing evidence-based treatment guidelines that contributes to better antimicrobial stewardship in animals. AMR passive surveillance could also have other advantages: (1) conduct integrated analysis of data obtained under comparable conditions in human and food and agriculture sectors, as surveillance is passive in human medicine; (2) obtaining clinical data to provide the necessary feedback to antimicrobial users in order to improve their practices in the use of antimicrobial; (3) obtaining data for animal species that are harder to reach through active surveillance; (4) strengthening the diagnostic capabilities of bacterial diseases, which is a prerequisite for better use of antibiotics. Standardization and harmonization will allow more meaningful analyses of AMR surveillance under a One Health approach. In that sense, FAO-ATLASS should allow to provide information on the different aspects of the surveillance that should be integrated and ensure the quality and reliability of the data (linked to the PIP stage) used in the surveillance system. FAO-ATLASS will be an essential tool in support of the InFARM system and of the global AMR/U surveillance architecture that is being developed and coordinated by the Quadripartite.

The demand for FAO-ATLASS missions worldwide during the period from 2017 to 2022 demonstrates the interest by FAO member nations in strengthening their capacities for AMR surveillance. Systematic feedback from the FAO-ATLASS community on the tool helps to continuously refine it and address any question associated with interpretation and scoring. Consequently, certain elements of the tool may progressively evolve to take into account this feedback.

Conclusion

As a global multidisciplinary organization for food and agriculture, FAO plays a key role in providing integrated and coherent support to countries in preventing and minimizing the emergence and spread of AMR across all sectors. Through FAO-ATLASS, FAO provides a valuable tool to help and encourage countries in improving their national AMR surveillance, share reliable AMR data at national level and plan for harmonized regional and global AMR surveillance and data compilations for food and agriculture sectors. The use of FAO-ATLASS is also creating opportunities for laboratory capacity building and increased awareness in countries and regions, which is critical in assuring success in the global fight against AMR under 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

GD, MT, BM, and NK developed the tool. NK drafted the manuscript which was reviewed by co-authors. EK, OI, and MGor implemented its expansion in Africa (EK and OI) and Asia (MGor). All authors provided their feedback on the tool.

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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/fvets.2022.1057040/full#supplementary-material

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Government veterinarians' perceptions of routine biosecurity focused on dairy cattle farms in north-western and north-eastern Spain

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The implementation of biosecurity measures in livestock production systems can be affected by the psychosocial factors of its stakeholders, which can be observed through their knowledge, attitudes and perceptions/practices. In Spain, there are no regulations *per se* to promote biosecurity. Of all stakeholders, farmers and veterinarians have been addressed in previous biosecurity studies, but not veterinarians belonging specifically to the government services. This study explores this particular group's perceptions of routine biosecurity in livestock production systems in north-western and north-eastern Spain, an understanding of which could help to improve the implementation of biosecurity measures on farms. Eleven interviews were conducted with veterinarians from different levels of the government services in Galicia and Catalonia, and were analyzed through content analysis. Dairy cattle farms were considered as the reference livestock production systems. The respondents stress the limited availability of staff and time resources for biosecurity. The advisory role of government veterinarians is not well recognized among farmers, who feel that their services prioritize their sanctioning role. In fact, government veterinarians consider that farmers only implement biosecurity measures to avoid being sanctioned, and not because they are aware of the importance of biosecurity. Meanwhile, the participants comment that biosecurity regulations should be flexible and need to consider the contexts of the farms where biosecurity measures are implemented. Finally, government veterinarians are willing to attend biosecurity meetings together with all farm stakeholders, at which the government services could be informed about biosecurity issues on farms. The person who could take on the biosecurity advisory role should be defined, along with further discussion of such matters as the responsibilities of each stakeholder. Government veterinary services need to be considered in studies of biosecurity operations in order to improve their implementation. It is therefore concluded that government veterinarians are seeking to balance their own institutional perspective with that of farmers and veterinarians in the routine implementation of biosecurity measures.

KEYWORDS

biosecurity, content analysis, dairy cattle farm, interview, government veterinarian, perception

1. Introduction

In livestock production systems, biosecurity can be defined as "*a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of animal diseases, infections or infestations to, from and within an animal population*" (1). Biosecurity can benefit animal health and, consequently, the performance of livestock production (2). The implementation of biosecurity measures by farm stakeholders, such as farmers and veterinarians, can be influenced by individual, collective, local and general psychosocial factors. Individual factors include age and gender, whereby older farmers are stricter about the entry of animals of unknown health status and women have a higher level of education (3); information sources, for which purpose farmers can turn to veterinarians, magazines and media, other professionals, and the government (4, 5); education and knowledge, whereby farmers and veterinarians with higher levels in this regard are more willing to promote biosecurity and to invest money and time in it (3, 6–9); and risk-benefit perception, whereby more perceptive farmers and veterinarians prevent animals from interacting with others that are at risk of infectious disease, and less perceptive veterinarians in this regard do not consider themselves a risk, and do not organize their visits in consideration of the risk of a farm having an infectious disease (10–12). Collective factors include communication dynamics, whereby poor communication between veterinarians and farmers can negatively affect biosecurity (13, 14); and interpersonal relationships, where a trusting relationship between veterinarians and farmers encourages collaboration to improve biosecurity (15–17). Local factors include the location, size and infrastructure of farms, whereby family farms (which are smaller and older) implement fewer biosecurity measures (18–21). General factors include economics, whereby farmers who do not see the short-term economic benefits of biosecurity measures do not implement them (3, 9, 18, 22, 23); and legislation and government actions, where the absence of legislation and abundance of government bureaucracy makes farmers reluctant to implement biosecurity (24, 25). It is therefore plain to see that there are psychosocial factors that can *positively* or *negatively* affect the implementation of biosecurity measures on farms, and which can be observed through the knowledge, attitudes and perceptions/practices of stakeholders.

The European Union (EU) Animal Health Law (Regulation 2016/429 on transmissible animal diseases, 26) stresses the importance of biosecurity, not only in case of outbreaks of exotic diseases but also in day-to-day routines. This legislation encourages the development and establishment of biosecurity plans that are flexible and adaptable to different types of animal production, mainly considering local factors. In this sense, the Member States are encouraged to promote more detailed biosecurity regulations. In Spain, there are no current regulations *per se* on the implementation of biosecurity measures in livestock production systems. However, a regulation on minimum biosecurity measures is expected in the near future (27), which will give more competencies in animal health to the veterinarians belonging to government veterinary services.

Previous studies have shown that veterinarians are the main source of information on biosecurity for farmers and can influence their decision-making in a *positive way*, but also in a *negative way*. This is especially true with regard to government veterinarians (6, 18, 28–31), who farmers often conceive as "bad policemen" (18, 32). Together with distrust of the government services (33, 34), this has led farmers to not view these veterinarians as a source of advice (10). It has been pointed out that government veterinarians are unaware of the realities and problems of farms, and acquire a mainly sanctioning role, while their advisory role is only secondary. This might explain why the government veterinarians' advice may not be fully adapted to farms and why some farmers may not take this advice into account in their biosecurity decision-making (32).

Pig and poultry farms tend to have a high level of biosecurity due to mandatory measures, while cattle farms tend to have a low level due to such measures being voluntary and hence poorly implemented. Improved biosecurity in cattle farming could help prevent the transmission of zoonotic diseases that are such a threat to public health (35). In making these improvements, all stakeholders should be considered, including not just farmers and veterinarians, but also government veterinarians, for example. However, the biosecurity studies that have been carried out to date do not include government veterinarians as crucial actors in biosecurity, as they have mainly focused on the farmers and/or veterinarians that most frequently work on farms (6, 10, 18, 28–34). Knowledge of the regulations concerning government veterinary services and the farmers' opinions about government veterinarians in relation to biosecurity is important for animal health interventions on farms. It is also crucial to know more about government veterinarians' own opinions on farm biosecurity and the psychosocial factors that might affect the implementation of biosecurity measures in order to improve the services that they offer. The aim of this study was to explore perceptions of the implementation of routine biosecurity measures on livestock farms by government veterinarians in the Autonomous Communities (AC) of Galicia (north-west) and Catalonia (north-east) in Spain. In particular, it sought their opinion about government biosecurity services and their sanctioning and advisory actions; and on the government services' knowledge of the reality and problems of routine biosecurity on farms. This study is one of the first to consider and involve government veterinary services.

Eleven remote interviews were conducted with government veterinarians, which focused on dairy cattle farms in Galicia and Catalonia. The main findings were grouped into constraints on biosecurity implementation on farms, roles of government veterinary services, biosecurity awareness, biosecurity training, knowledge about farms, biosecurity meetings on livestock production, and mandatory biosecurity measures on farms.

2. Materials and methods

2.1. Study area

Spain has a total of 17 AC, which are regional entities with their own institutions and representatives and certain legislative, executive and administrative competencies, including animal health competencies. Each AC is divided into provinces, and each province into counties. This study was carried out in the ACs of Galicia (north-west) and Catalonia (north-east), mainly because the different types of dairy cattle farms in each region were considered to offer a good comparative framework, for the former are mainly family farms and the latter are mainly industrial (18, 19, 32), and so the similarities and differences between the participants' responses can be highlighted.

2.2. Participant recruitment

In Spain there are three types of veterinarians who usually work on livestock farms. First, there are private veterinarians, who advise farmers in technical areas related to herd health management. Second, there are veterinarians employed by the Health Defense Associations (HDA), which support farm associations with disease control programs. HDAs are managed by farmers and receive financial subsidies from the government services. Third, there are veterinarians belonging to the government veterinary services, who make sure that farmers and veterinarians carry out certain mandatory practices. This study focuses on this third type of veterinarian, who work on four different levels that are linked to each of the territorial divisions of Spain, the national, autonomous community, provincial and county levels. The national and autonomous community levels take a political-administrative approach with legislative implications, while the operations at the provincial and county levels are sanctioning-advisory through direct contact with farmers in their public offices or on farm visits.

For the purposes of the study, government veterinarians were considered to be the key informants. This is partly due to their knowledge and experience (36, 37), but also enabled the researchers to collect quality data in a short amount of time (38). Key informants were identified through an initial exploration of all structural and organizational levels of the government services according to the territoriality of Spain to ensure that the key informants offered a good representation of all these different levels.

Government veterinarians with knowledge and experience of biosecurity on livestock farms and who were known to be willing to participate in the study were contacted and recruited through purposive sampling. The authors used their research networks to contact government veterinarians with whom they had worked on other projects or studies. JC and AA contacted government veterinarians at the national and autonomous community levels in Catalonia, while EY and FD contacted other potential respondents at the autonomous community, provincial and county levels in Galicia. However, veterinarians at the autonomous community level also helped the authors to contact veterinarians from the provincial and county government services. These potential respondents were presented with a fact sheet that informed them about the objectives and characteristics of the study, stating that the topic was biosecurity with specific reference to Spanish dairy cattle farms, and particularly those in Galicia and Catalonia.

Eleven government veterinarians finally issued their informed consent to participate in the study, representing both the higher (national and autonomous community) and lower (provincial and county) levels. There were two from the national level (labeled N1 and N2), two from the autonomous community level (labeled GA and CA), three from the provincial level (labeled GP1, CP1, and CP2), and four from the county level (labeled GC1, GC2, CC1, and CC2) in both Galicia and Catalonia.

2.3. Data collection

Data was collected during the COVID-19 pandemic. Europe had one of the highest rates of positive cases in the world from early 2020 until early 2021, and Spain established containment policies, with a significant restriction of mobility between and within its territories (39, 40). Hence the data was collected by means of remote interviews *via* an online conference program, as in other fields of study (41, 42), which also offered advantages in terms of less displacement in the field and more flexibility in participants' schedules (43).

The remote interviews were conducted and recorded between 19 March 2020 and 19 October 2020. They were conducted by SM, who was at the time a PhD candidate and had conducted similar studies on dairy cattle farms with farmers and veterinarians (18, 19, 32). The interviews involved only the participants and SM and lasted between 45 and 120 min. They were semi-structured, which allowed the participants to express their views through their knowledge and experiences without following an established order (44). Two pilot interviews were conducted with government veterinarians at the national level, which were included in the study. The thematic guide included general and specific questions (Table 1). The former was related to the levels of government veterinary services and their resources, priorities, actions, proposals, and constraints in terms of biosecurity; and the specific questions were related to the reality and problems of farms, mandatory and minimum regulations, sanctioning and advisory roles, and participatory meetings. The interviewees were also able to add any additional information that they could offer. The interviews were subsequently transcribed and analyzed in their entirety by SM.

2.4. Data analysis

The interviews were analyzed through content analysis using the qualitative ATLAS.ti software. The approach proposed by Elo and Kyngas (45) and Elo et al. (46) was considered. As biosecurity was being explored through a limited number of participants, a mainly deductive logic was used (47). The transcriptions were read for a general understanding of their content, and then established codes (i.e., deductive approach) were created along with their meaning units (Table 2), which are sections of text that are related to the objective of the study. Each meaning unit was coded according to its content in the established codes, but emerging codes (i.e., inductive approach) were also created as the texts were analyzed. Finally, all codes were compared, and similar codes were grouped and labeled into categories. Preliminary categories were discussed and agreed between SM and AA.

For validity and reliability of findings, sampling adequacy, positionality, data triangulation, peer debriefing and methodological consistency were included (48, 49). Sampling adequacy was evidenced through data saturation (50), which is the point when participants' statements are merely repeated without providing new findings. Regarding positionality, the authors adopted a critical stance in relation to the participants' statements, which involves questioning the reasoning and making judgements based solely on these responses. Regarding data triangulation, previous studies on biosecurity on dairy cattle farms were used to compare the opinions of farmers and veterinarians with those of government veterinarians. For peer debriefing, meetings were held with all authors, specifically between SM and each of the authors separately. At these meetings the authors shared their ideas and interpretations with regard to the final categories. For the purposes of methodological consistency,

TABLE 1 Thematic guide on perceptions of government veterinary services in relation to biosecurity measures.

General questions - What was your previous profession? What is your current position? How long have you been in this position? What are your routine tasks in relation to animal health? - What resources do the government services have available to implement biosecurity measures? What resources do you use or have you used? Why? How are these resources managed? - What biosecurity measures are currently a priority in your position? Why? - What is the current position of government services on improving the implementation of biosecurity measures? What position should it take? What actions are being (or should be) taken to achieve this improvement? How are these actions being (or should these actions be) applied? What proposals exist (or should exist) to achieve this improvement? How are these proposals being (or should these proposals be) applied? Why? - What constraints exist (or could exist) to improve the implementation of biosecurity measures? Why? How relevant is the regulatory framework to these constraints? How can (or could) biosecurity measures be improved within this framework?
Specific questions - In previous studies, farmers and veterinarians have pointed out that the government services do not know the reality on farms, as the government services promote biosecurity measures that are difficult to implement. What is your opinion on this lack of knowledge? Why? What is the reality, or the problems, on farms in relation to biosecurity? How could the reality and problems on farms be better known? - In previous studies, farmers and veterinarians have pointed out that biosecurity measures from the government services are perceived with reluctance, due to the few arguments to justify its implementation. What is your opinion on this situation? What is your position on the mandatory nature of some biosecurity measures? What biosecurity measures should be mandatory? What biosecurity measures should be minimum? How could these measures be implemented? What position should the government services take on these mandatory and minimum measures? Why? - In previous studies, farmers and veterinarians have pointed out that the government services have mainly a sanctioning role but also an advisory role. What is your position on the sanctioning role of the government services as perceived by farmers and veterinarians? What is your position on the advisory role that the government services should have with farmers? What actions should be taken to achieve this role? How? - Finally, farmers and veterinarians share the idea that there should be biosecurity meetings between all actors. What is your opinion on these meetings? Why? What constraints are there (or could there be) to holding these meetings? How could these meetings be held? What is your position on binding participatory processes?

congruencies were found between the research questions, the materials and methods used, and the research results.

Finally, it is important to note that only the codes with their respective quotations linked to the study objectives were incorporated in the results. Representative quotations were included for illustrative purposes. These quotations were selected by SM and AA and translated from Spanish into English. The quotations were presented in relation to the four existing levels of government veterinary services.

2.5. Ethics statement

The Ethics Committee of the Universitat Autònoma de Barcelona approved the study proposal (CEEAH 4055) and helped design the informed consent for participants, which explained the objective of the study and the conditions and guarantees of its participants. It also stated that the data collected would be confidential and anonymous, that there would be no financial benefit for participating, and that the interviews would be audio and/or text recorded. The decision to participate in the study was entirely voluntary, and participants could stop and leave the interview at any time they wanted. The informed consent was then signed by the participants and SM, with both parties receiving a copy.

TABLE 2 Codes and meaning units in content analysis.

Codes	Meaning units
Measures	Biosecurity measures that are implemented on farms according to government veterinarians.
Constraints	Constraints to implement biosecurity measures according to government veterinarians.
Resources	Resources available to government veterinary services to lead the implementation of biosecurity measures according to government veterinarians.
Roles	Sanctioning and advisory role of government veterinary services and its approaches and consequences according to government veterinarians.
Importance	Importance of biosecurity measures for government veterinary services and farmers according to government veterinarians.
Awareness and training	Awareness and training programs for farmers, veterinarians, and government veterinarians according to government veterinarians.
Knowledge	Knowledge of the reality and problems of farms according to government veterinarians.
Meetings	Feasibility of holding meetings between different stakeholders and government veterinary services according to government veterinarians.
Mandatory	Mandatory biosecurity measures on farms according to government veterinarians.

3. Results

3.1. Constraints on biosecurity implementation on farms

The provincial and county levels pointed out that farmers' economic resources may be a constraint for the implementation of biosecurity measures, such as wheeled rains, footbaths, changing rooms, animal quarantine, animal unloading and loading areas, and perimeter fencing. However, the national levels commented that farmers sometimes used this as an excuse to skirt their responsibilities, for they also fail to implement measures that do not require a major financial investment, such as records of the entry and exit of people and vehicles, transit from clean to dirty areas, and non-shared tools in animal handling. Furthermore, government veterinary services were aware of other constraints on the implementation of biosecurity measures, such as small farms with small herds, which are usually family-run, and farms with an atomised infrastructure or which share the same roads with other farms. However, the autonomous community levels believed that if the sector were to request incentives for biosecurity, the government services could set up subsidization programs for this purpose.

All levels of government veterinary services agreed on the limited budgetary resources allocated to biosecurity, with the exception of those associated with disease eradication programs (e.g., cleaning and disinfection procedures in positive cases). However, the provincial levels noted the existence of budgets for biosecurity training programs, mainly through agricultural schools, while the autonomous community levels considered that the staff of government veterinary services working in biosecurity were also a resource. However, the autonomous community representatives pointed out that these staff are limited in number and the time available to perform these tasks due to overwork in other areas.

> CC2: "(. . .) There is a limitation on staff to monitor more directly on a day-to-day basis (...). The only problem is that we cannot reach all the farms. If we could inspect all the farms every year, it would raise the quality of the sector (. . .)"

County levels mentioned that the existence of regional administrative divisions was a constraint. They felt that government veterinarians from different levels of the organizational structure could have different criteria for the implementation of some biosecurity measures and that this generates comparative grievances.

3.2. Roles of government veterinary services

Government veterinary services recognized that they could serve a sanctioning or advisory role, but that they should not be paternalistic toward farmers. The national levels did not agree with the sanctioning role either, as it left aside actions focused on ensuring public health in terms of preventing the entry and spread of infectious diseases. Furthermore, the autonomous community levels stressed that farmers should be made aware of the importance of biosecurity measures and not only implement them to avoid being sanctioned.

> CA: "(. . .) I don't think that the government services should be seen as a sanctioning entity (...). If you implement biosecurity measures, your animals are healthy. However, biosecurity has to be here [inside your head], not in files (...). It is a change of thinking, not to avoid a fine (. . .)"

On the contrary, government veterinary services commented that sanctions allow biosecurity measures to be implemented. In spite of this, the county levels highlighted that the resources that farms possess should be considered before applying sanctions, as some farms are limited in this regard. Besides, the consideration of these resources could help to further adapt biosecurity measures on farms through enforceable legislation for farmers.

In relation to the advisory role toward farmers, the autonomous community levels pointed out that the government services should mainly enforce compliance with regulations, generally applying sanctions. Meanwhile, the provincial and county levels commented that whenever possible government veterinarians should try to inform and train farmers on legislative and practical aspects prior to the issue of a sanction, through courses or visits by farmers to their services, for instance.

The autonomous community levels recognized that the sanctioning role of government veterinarians can be a drawback as it means they tend to be perceived in a negative light. In contrast, the autonomous community representatives wish to be positively perceived for their advisory role. To address this drawback, the provincial levels suggested that it would be ideal to have two different teams, one of inspectors and the other of advisors, to fully develop these functions.

> CP2: "(. . .) It is difficult to give advice when you carry out inspections (...). It would not be a bad idea to create a body of inspectors, one the person for inspection. If you get it wrong, you get it wrong (...). And then there should be other veterinarians [who would also be part of government veterinary services as inspectors] who, without being inspectors, can give advice to the farms (. . .)"

The provincial representatives criticized the fact that the government services have been prioritizing their sanctioning role over their advisory one, as this advice was previously offered by the agricultural extension services, which do not exist anymore.

3.3. Biosecurity awareness

The government veterinary services want all livestock farms to implement the different biosecurity measures adequately. However, according to its veterinarians, farmers implemented different measures in a heterogeneous manner. For example, at the provincial levels, measures related to animal movements were efficiently and effectively implemented, as the farmers were aware of their importance, but this was not the case with measures related to records of the entry and exit of visitors (both persons and vehicles) to and from farms. The provincial representatives believed that inadequate implementation of biosecurity measures was mainly due to traditions and acquired farm routines, which are difficult to change.

> GP1: "(. . .) The sector tries to do things well here (...), but very often they have acquired biosecurity routines. It is difficult to change these routines because their parents and grandparents already implemented them (. . .)"

For county levels, farmers were not aware of the importance of biosecurity due to overwork in other areas, such as managerial duties. This was evidenced by the fact that farmers only implemented biosecurity measures to obtain subsidies.

On the other hand, government veterinary services also had different degrees of awareness of biosecurity measures, both between different levels and within the same level of the organizational structure of the government services. For instance, the county levels considered farm records of entry and exit of visitors to be important, as these records allow efficient traceability, and may establish comprehensive disinfection points or restrict entry to and exit from farms. Meanwhile, the autonomous community and other county levels considered animal movements to be crucial. However, the county representatives recognized that these movements were often not audited, as these biosecurity measures depended on the awareness of farmers. Despite the above, government veterinary services thought that new dairy cattle farms should comply with a set of minimum biosecurity measures, while old farms should also comply, but with certain deadlines; although for the provincial levels these biosecurity measures might already be established in the autonomous community legislation. Nevertheless, the government services believed that the implementation of minimum biosecurity measures should be flexible and consider the conditions of farmers and their farms.

> CP2: "(...) A program with some minimums and then you adapt it. However, the minimums must be mandatory (...). Three or four minimum biosecurity measures if necessary, and then you adapt them to the conditions of the farmer and the farm (...)"

3.4. Biosecurity training

For the national levels, farmers did not recognize their own responsibility for implementing biosecurity measures due to a lack of training and, consequently, a lack of awareness of their importance. In this regard, the provincial levels stressed that there should also be mandatory training for all farmers to understand the rationale behind biosecurity implementation.

> CP1: "(...) We try to provide training so that people try to understand what they are obliged to do (...). I believe that the function of the government services is to legislate and try to train people voluntarily or mandatorily so that they understand the regulations (...)"

Government veterinary services pointed out that farmers not only had a lack of training due to time restrictions, but also that farmers were reluctant and unwilling to understand the rationale of biosecurity. For example, farmers did not agree with some biosecurity measures, such as perimeter fencing, because of the feasibility issues. In contrast, the provincial levels commented that although government veterinarians constantly try to train farmers on infectious diseases, farmers were already aware of their consequences and, therefore, of the objectives of biosecurity measures.

On the other hand, government veterinarians commented that other training tools should be developed to make farmers aware of the importance of biosecurity. In this respect, the national levels highlighted the challenges associated with increased biosecurity awareness among farmers through training. These challenges were due to the absence of outbreaks of exotic diseases in the country for several years and veterinarians prioritizing fields other than prevention, such as nutrition and reproduction. In this regard, the national representatives mentioned that government veterinary services try to train veterinarians in animal health (including infectious diseases and biosecurity) to transmit a unified message to farmers. And in turn, the national levels believed that the government services should not only be responsible for the technical training of veterinarians, but also for raising awareness among sectorial associations, as the government services had limited resources for this.

> N2: "(...) Veterinarians are a group in which we invest a lot of resources, perhaps a little more technical than those dedicated to awareness-raising (...). Awareness-raising in my opinion should be approached from the point of view of the sectorial associations (...)"

The national representatives commented that all government veterinary services were also trying to raise biosecurity awareness, not just among farmers, but also among veterinarians.

3.5. Knowledge about farms

Government veterinarians were aware of the reality and problems of farms. However, while the county representatives were constantly in contact with farms, they acknowledged that the higher levels might have inaccurate knowledge about them.

> CC1: "(...) People indeed have the perspective that those who legislate [higher levels] do not know what the reality is. However, people who are at the lower level like me (...), I know the farmers perfectly well, I know not only their way of working but also their problems (...)"

For government veterinary services, the higher levels should have more contact with farms and their farmers and veterinarians. They also acknowledged that spending most of their time in the office was a constraint to understanding the problems on farms, and that the different levels of government veterinary services, together with their respective tasks, seems to influence and affect the flow of information about what really happens on farms.

> N2: "(...) There are many levels of government veterinary services with many people, there are many realities (...). We try to be very aware of the reality of the field. The problem is that sometimes in the flow of information, the information does not arrive or arrives badly or incompletely (...)"

In this respect, the government veterinary services wished to hold meetings to learn more details about the reality and problems of farms, where there would be a reciprocal flow of information between both parties (the government services and farms).

3.6. Biosecurity meetings on livestock production

In relation to the organization of meetings on biosecurity with all stakeholders (farmers, representatives of farm associations, private veterinarians, veterinarians employed by the HDA and government veterinarians), the government veterinary services proposed voluntary attendance. In addition, while the national levels addressed the importance of biosecurity whenever possible in meetings on other topics, government veterinarians commented that time was the main constraint on attendance of biosecurity meetings. The autonomous community and provincial levels pointed out that these meetings could lead to consensual advice developed by all stakeholders to facilitate awareness-raising among them. In fact, for the government veterinary services, these meetings could allow farmers to learn from each other, but they should strengthen their social networks beforehand in order to present their collective demands to the government services.

> GA: "(...) Farmers do not move anything either, they do not get together for anything. The logical thing would be for them to get together among themselves and to make an effort to solve the problems, or to raise these problems with the government services (...). This should be the starting point for farmers (...)"

On the contrary, the autonomous community levels sense the need to explore the scope of consensual advice in accordance with legislation. The government veterinary services pointed out that these meetings should be managed by the farmers' unions, which should propose initiatives on biosecurity, and not by the government services, who lacked the means to convene them.

Regarding the participation of farmers in biosecurity meetings, the autonomous community levels sense drawbacks, as farmers prioritize their political positions over possible solutions to their biosecurity problems. Similarly, for government veterinarians, another drawback was the time spent at biosecurity meetings, as farmers could get tired of them and not attend, even if they have a voice at them. Furthermore, the provincial levels pointed out that the viability of holding these meetings with farmers and veterinarians was low, as the two groups were worlds apart, with their own languages, understandings, and interests. The provincial representatives pointed out that there might be areas that should not be dealt with at these meetings, such as purely legislative issues. Despite the above, the provincial levels commented on the existence of sectoral round tables, with representation of the different groups in the sector, in which various issues, such as biosecurity, were discussed. Indeed, the national levels highlighted the so-called "local sanitation commissions" that still existed in some places, where representatives of the different groups in the sector meet to coordinate actions on an annual basis, although these commissions were disappearing due to the limited availability of staff.

> N1: "(...) The legislation described the so-called "local sanitation commissions" (...). Before starting an action in a municipality, we called together all the farmers, the representatives of the municipality, and the veterinarians in the area. And the government services would explain what we were going to do (...) so that these people could tell us what problems they saw with that action (...)"

3.7. Mandatory biosecurity measures on farms

The county levels thought that mandatory biosecurity measures would allow all actors in the sector to implement them to higher biosecurity standards, which would also boost their public image.

> CC2: "(...) There would be no problem with making biosecurity mandatory, with high standards, because farmers are also interested in having a good public image (...), a good image of good biosecurity (...)"

On the other hand, the national levels pointed out that considering biosecurity measures as an imposition is a mistake, as the sector should really be made aware of the need to achieve these standards. Besides, according to the national representatives, farmers conceived government veterinary services as an enemy because of the mandatory biosecurity measures that its veterinarians had to enforce. However, the government veterinary services also felt that this conception was changing because they were getting closer to the farmers. In addition, the national levels also commented that these measures had been permissive despite their mandatory nature.

4. Discussion

The results of the present study have presented the perceptions of government veterinarians of the implementation of routine biosecurity measures in Galicia and Catalonia. In particular, these results revealed their opinions of government veterinary services with regard to biosecurity and their sanctioning and advisory actions, and of their own knowledge of the realities and problems of routine biosecurity on farms.

Government veterinary services have limited resources for biosecurity, which tend to be focused on animal health programs, such as bovine tuberculosis (TB) (51) based on Royal Decree 2611/1996 (52), or infectious bovine rhinotracheitis (IBR) based on Royal Decree 554/2019 (53). However, the government veterinarians also mention the possibility that farmers could apply for subsidies for certain biosecurity measures. In fact, these subsidies could be beneficial as incentives for farmers to implement biosecurity measures and, in turn, make the government services aware of farmers' biosecurity needs (54, 55). However, they must be accompanied by awareness-raising to ensure that the routine implementation of biosecurity measures is efficient and effective.

Regarding the roles of government veterinary services, their veterinarians feel that the sanctioning and advisory roles perceived by farmers and veterinarians were equally necessary, even though the former is more recognized than the latter. One of the possible reasons for this recognition may be the limited resources available to the government services in terms of staff and time. This may also affect the training and advice that government veterinary services can offer to farmers, which is not viewed as efficient or effective in terms of their impact on farmers, a situation that could be evidenced by the advisory role being under-recognized (32).

The advisory role of government veterinary services was previously served by the agricultural extension services in the form of technical advice on efficient and effective practices, organization of training, refresher programs and technical-scientific dissemination events (56). These objectives could also be taken up by government veterinary services. However, there is an interest in reinforcing this advisory role toward farmers, possibly though the veterinarian responsible for each farm [Regulation 2016/429 (26); Royal Decree 993/2014 (57); Law 8/2003 (58)], or by a veterinarian belonging to a HDA. The latter also serve an advisory role in the routine implementation of biosecurity measures for TB and bovine viral diarrhea (BVD). In this respect, irrespective of who takes on this advisory role, this person could not only complement the government veterinary services, but also private veterinarians, who need to improve their biosecurity and infectious disease awareness (59–62).

Some government veterinary services highlighted a lack of biosecurity awareness among farmers, which could be the fault of government veterinarians, or of private veterinarians, to whom farmers often turn to for reliable information (63). Hence it could be interesting to evaluate the impact of the sanctioning and advisory roles served mainly by the government veterinary services, but also by private veterinarians, regarding the ultimate day-to-day implementation of biosecurity measures by farmers. Indeed, both roles could be rethought and new awareness strategies could be proposed. For example, in relation to the advisory role, the government veterinarians did not mention their own training in teaching skills, which some levels of the government veterinary services might not be receiving. This could in turn affect the training that government veterinarians provide to farmers. The government veterinary services should therefore not only offer training on biosecurity and infectious diseases, but also on teaching skills, at least for the county levels that have the most contact with farms. This training should not only consider routine biosecurity, but also outbreaks of exotic diseases, for which the sector is often unprepared.

The biosecurity training that farmers receive could also suffer from other drawbacks. For example, it might not consider the particular conditions of farmers and their farms (20), and different biosecurity materials might offer contradictory advice, leading farmers not to implement biosecurity measures out of confusion (64). Therefore, the training that farmers receive should consider their particular contexts, as well as provide unified advice based on scientific evidence, which the government veterinarians are aware of from their day-to-day routines.

According to the participants, certain biosecurity measures on dairy cattle farms are often not audited. We could note here that private veterinarians are not entitled to audit biosecurity measures in these production systems, but they can give advice and negotiate with farmers on biosecurity implementation. In contrast, veterinarians employed by HDAs can audit biosecurity measures linked to control programs for TB and BVD, although many farms are not members of a farmers' association and therefore have no contact with any HDA veterinarians. Hence, in order for biosecurity measures to be fully audited by these HDA veterinarians, all farms would need to join a farmers' association. Alternatively, HDA veterinarians could be a support for the government veterinary services when the legislation comes into force, which will endow them with greater competencies in terms of animal health.

The government veterinarians were aware that family farms face more constraints on the implementation of biosecurity measures than industrial farms. They did not offer details about the differential treatment of family farms, possibly to avoid causing grievances, but they did mention that older farms (which tend to be family farms) should have more time to implement minimum biosecurity measures than newer farms. It would be interesting to look further into the different treatment of family and industrial farms by government veterinarians in relation to particular biosecurity measures.

In previous studies on biosecurity on dairy cattle farms, farmers and veterinarians pointed out that the government services were unaware of the reality and problems on farms, as they generated biosecurity regulations and legislation that were complicated to implement on a day-to-day basis (18, 32). However, the government veterinary services agreed that only the higher levels, such as the national and autonomous community ones, were unaware of the reality and problems that farms face. To address this, the government veterinarians have agreed to hold meetings specifically on biosecurity that all actors in the sector could attend on a voluntary basis. However, although similar meetings already exist, they mainly deal with management issues and only some farm stakeholders are involved in them. But all stakeholders need to have a voice and be aware of the biosecurity problems on farms, and all levels of the government services need to take these problems into consideration. Moreover, the agreements that could be reached at these meetings could be binding, as long as they do not affect or are not affected by existing legislation.

Biosecurity meetings were not fully discussed and clarified in terms of who attends them and how, or what their aims were, a situation that should be further discussed among all farm stakeholders in view of their respective responsibilities. Furthermore, it is important to consider that the responsibility for routine biosecurity does not only lie with farmers, but also with other actors, including the dairy industry and transport companies, as well as the government services (65). In this regard, biosecurity meetings could be designed on the basis of participatory methods with consensus-based decisions across the board (66). Similar initiatives could be considered for this design, such as those carried out by Bugeza et al. (67) and Vaarst et al. (68), or those evidenced through AgriLink (69) and LIVERUR (70). These meetings and their decisions could generate greater awareness and commitment among their participants, as already occurs, for instance, in healthcare with patients, thus generating realistic and informed expectations about healthcare and increasing satisfaction and trust in it (71, 72); or in corporate organizations with employees, who are able to contribute to the organisation's productivity (73).

It is important to note that there are practically no studies involving government veterinary services on the issue of biosecurity (24, 25). Hence this is the first contribution to offer evidence from the perspective of government veterinarians through their discourses on biosecurity on livestock farms, and specifically dairy cattle farms in Galicia and Catalonia. Government veterinarians may share the opinions of farmers and private veterinarians on the intervention of government veterinary services in the implementation of biosecurity measures, but they may be constrained by the regulations that they have to enforce. Despite this, biosecurity regulations could be viewed as an advantage for government veterinary services, as they are generally absent on dairy cattle farms, only a few of which are linked to TB and IBR. This would increase the scope for dialogue between government veterinary services and farmers and private veterinarians to agree on and adjust biosecurity measures in accordance with the real context of the affected farms.

Government veterinarians are aware that the services they represent prioritize a sanctioning rather than an advisory role. This situation can be a drawback for the implementation of biosecurity. Therefore, the interventions carried out by government veterinary services should consider and reinforce their interaction with other actors in order to gain a better understanding of the reality of the farms and to adapt their advisory role to these contexts, and thus support the implementation of biosecurity measures. In addition, these interventions could also assist the transition toward the implementation of mandatory biosecurity measures that could be enacted in future regulations.

Therefore, it could be interesting to incorporate government veterinary services in future studies on biosecurity or related topics, as there is a tendency for farmers and veterinarians to question them, and this would give them the chance to share their own perspective. Better knowledge of all stakeholders' perspectives would also mean a better understanding of the psychosocial dynamics involved in such matters as heterogeneity in the routine implementation of biosecurity measures (9, 74–76). Furthermore, from a health perspective, stakeholders could also include public health professionals, especially in cases of zoonotic infectious diseases.

In conclusion, government veterinary services have a similar perspective to that of farmers and veterinarians. They generally disagree with actions and initiatives that prioritize sanctions over advice, and claim to be willing to visit farms to learn about farmers' realities and their problems with routine biosecurity, thus leading to greater flexibility of certain regulations that may be complicated for farmers to carry out. Government veterinary services do try to balance their own institutional perspectives with those of the sector, such as those of farmers and veterinarians, to ensure that biosecurity measures are implemented efficiently and effectively on a day-to-day basis. Thus, this study is useful for the generation and improvement of biosecurity interventions and regulations involving government veterinarians, which will help to regain farmers' trust in the government services by reinforcing their interaction with other stakeholders and their advisory role in the implementation of biosecurity measures. Hence, the government veterinary services and the dairy cattle sector will benefit from this study. Government veterinary services could intervene internally at their different levels to improve the performance of their government veterinarians in the institution and in the field, while the dairy sector could improve the implementation of routine biosecurity measures. Therefore, it is relevant to consider the perceptions of government veterinary services in biosecurity studies. This in turn makes it possible to appreciate the small differences within the different levels of the same institution.

5. Limitations

Eleven interviews were conducted with government veterinarians belonging to the four territorial levels of the government veterinary services in two ACs. However, the population sample is only representative of Galicia and Catalonia and not of all 17 ACs in Spain. Government veterinarians belonging to the other ACs may have different opinions from those in Galicia and Catalonia, even though the dairy cattle farms present in other ACs may be similar to those of Galicia and Catalonia. These results are framed within a particular context with its own legislations and regulations, and it is hard to compare them with other results obtained with the same methodology, both in other regions of Spain and in different countries.

Also, only government veterinarians with knowledge and experience of livestock farm biosecurity were considered. Government veterinarians could have knowledge and experience not only in dairy cattle farming, but also in other production systems, such as pig and poultry farming. The methodology could therefore be replicated in other farming sectors. The results related to these livestock production systems and their regulations could be different from those of this study, but the results linked to the government services, roles of government veterinarians and participatory processes could be similar. This is because dairy cattle farms are managed differently from pig and poultry farms, which tend to have higher levels of biosecurity due to the stricter mandatory regulations on the latter. For example, pig farms are governed by Royal Decree 324/2000 (77), Royal Decree 3483/2000 (78), and Royal Decree 1221/2009 (79) mainly related to management, and poultry farms are affected by Order APA/2442/2006 (80) and Royal Decree 445/2007 (81) mainly related to avian influenza. There are no regulations on mandatory measures for dairy cattle farms other than the stipulations of Regulation 2016/429 (26). These more stringent measures for pig and poultry farms have mainly come about as a result of recent outbreaks of African swine fever and avian influenza (82, 83).

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Universitat Autònoma de Barcelona (CEEAH 4055). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SM conducted the study, wrote the article as part of his doctoral thesis (2017–2021), collected, analyzed, and presented the data, along with its critical aspects. AA was SM's supervisor and was extensively involved throughout the study and draft of this article. JC and AA recruited government veterinarians at the national and autonomous community levels in Catalonia. EY and FD recruited government veterinarians at the autonomous community, provincial and county levels in Galicia. All authors approved the article submitted for publication.

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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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Risk perceptions of avian influenza among poultry farmers on smallholder farms along border areas of Thailand

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Introduction: Thailand has not reported any cases of avian influenza since 2008. However, avian influenza viruses circulating in poultry in neighboring countries may have potential for transmission to humans. The aim of this study was to assess risk perceptions of poultry farmers and traders in three border provinces of Thailand adjacent to Laos.

Materials and methods: Poultry farmers and traders were interviewed in-person during October–December 2021 by health and livestock officials using a standardized questionnaire to collect demographics, job histories, knowledge, and practices related to avian influenza. Knowledge and practices were scored using 22 questions with a 5-point scale. Exploratory data analysis scores above and below the 25th percentile was used as the cut-off point for perception scores. The cut-off point was used to describe perceptions of respondent characteristics in order to compare differences between groups with more or <10 years of experience. Age adjusted perceptions of disease risk were analyzed by multivariable logistic regression.

Results: Of the 346 respondents, the median risk perception score was 77.3% (22 questions with a 5-point scale, so the total score was 110). Having more than 10 years of experience in poultry farming was significantly associated with an increased perception of the risk of avian influenza (adjusted odds ratio 3.9, 95% confidence interval 1.1–15.1). Thirty-two percent of participants perceived avian influenza as a risk only during the winter season, and more than one-third of the participants (34.4%) had not received recent information about new viral strains of avian influenza.

Discussion: Participants did not perceive some key information on the risks associated with avian influenza. Regular training on the risks of avian influenza could be provided by national, provincial and/or local officials and they, in turn, could share what they learn with their communities. Participants who had greater experience in poultry farming were associated with greater risk perception. Experienced poultry farmers and traders working on poultry farms can be a part of the community mentorship program to share their experiences and knowledge on avian influenza with new poultry producers to improve their perception of disease risk.

KEYWORDS

avian influenza, risk perception, community, Thailand, poultry farmers on small farms

Introduction

Avian influenza is an infectious disease caused by influenza type A viruses in the Orthomyxoviridae family, which cause infections in both humans and many kinds of animals such as horses, pigs, cats, birds, and chickens. The disease in animals, especially in poultry, has been detected for >100 years, with occasional outbreaks in countries such as England, Canada, Australia, the United States, Mexico and Italy (1). Avian influenza viruses are generally not highly contagious to humans (2). The first evidence of animal-to-human transmission was reported when a highly pathogenic avian influenza (HPAI) A(H5N1) virus was transmitted to humans in the Hong Kong Special Administrative Region in 1997 (3). Humans are mainly infected with avian influenza A(H5N1) through poultry according to available epidemiological data (4, 5). In late 2003–2004, avian influenza detected in Thailand and neighboring countries including Cambodia, Laos, and Malaysia (1). The Division of Epidemiology in the Thai Ministry of Public Health received reports of and investigated 25 human cases of influenza A(H5N1) virus infection, including 17 deaths, from 2004 to 2006. In 2006, the year that the last avian influenza A(H5N1) virus outbreak among humans in Thailand was reported, there were three persons with confirmed infection, and all of three died (2). With respect to the high case fatality rate of avian influenza A(H5N1) virus, this zoonosis continues to be a priority for disease prevention in Thailand.

During the influenza A(H5N1) outbreaks in Thailand during 2004–2006, the Thai Department of Livestock Development implemented measures that included culling flocks that had infected birds by the veterinary authorities. Nationwide surveillance program of HPAI infections and active surveillance for avian influenza virus in poultry to control avian influenza outbreaks and to monitor the situation of avian influenza in Thailand has been implemented continuously since 2006 (4, 6). Although Thailand has not reported avian influenza A (H5N1) in poultry for more than 16 years, there is continued risk of avian influenza outbreaks. The World Organization for Animal Health (WOAH, formerly called OIE) has reported that cases of severe and mild avian influenza infections have occurred in avian populations and in people living in countries in the same region as Thailand (7).

Live poultry traders and poultry farmers may be at increased risk for avian influenza infections due to factors including the duration of time working in close contact with poultry and behaviors that may pose a risk of exposure to pathogens (8). A study by Dikky et al. (9) supported using data obtained from surveys on the behavior of personnel in the poultry industry to inform disease control measures. Identifying which people influence attitudes, knowledge and beliefs regarding avian influenza control in the community can help reduce the spread of avian influenza in the area (10). The aim of this study was to assess the risk perceptions of traders and farmers in the poultry trade network along border provinces of Thailand. Knowledge on the perceived risk of avian influenza infection in live poultry traders and poultry farmers in the study areas can inform risk communication guidelines and facilitate effective avian influenza prevention practices in the context of the country and the region.

Methods

Target provinces are located along borders with countries that have reported avian influenza outbreaks in recent years. The study was conducted in all sub-districts of three districts in Nakhon Phanom, Mukdahan and Ubon Ratchathani provinces (one district was selected in each of the three provinces). There was a registered population of chickens and ducks with a number of poultry farmers in three provinces under the Department of Livestock Development; 3,065,744 chickens and ducks, and 7,780 farmers in Ubon Ratchathani, 320,141 chickens and ducks, and 4,403 farmers in Nakhon Phanom, 143,661 chickens and ducks, and 1,097 farmers in Mukdahan. The majority of participants were small-scale poultry operations. Mixed-type poultry means raising several types of poultry on the same farm, such as broiler chickens, layer chickens, fighting cocks and ducks on the same farm. These three provinces were chosen because of the on-going active surveillance of poultry farms and trades in the areas. In addition, international movements of poultry were reported in these areas. Persons targeted to participate were poultry farmers and poultry traders who raised and contacted (for example, holding, feeding, culling) at least one bird on their farms or backyards. The inclusion criteria were poultry traders and farmers aged 18 years and older with the ability to listen, speak and read the Thai language. The study population was required to have lived in the study area for at least 1 year prior to participating in the study. The exclusion criteria for participation were not having been involved in poultry operations for >1 month before being enrolled in the study and not being included in the Provincial Livestock Office's registration database in 2019. Due to the limited number of poultry traders in the study areas, all poultry traders listed in the Livestock District Office database in each district were eligible to participate. The sample size of poultry farmers was calculated using the formula from the Tool 5 value chain sampling guidelines (11). The previous study in Karachi, Pakistan revealed that the prevalence of avian influenza viruses in commercial layers was 26.45% and prevalence of H9 virus was 40.16% (12). With respect to avian influenza, including high pathology and low pathology, however, we had no accurate data on low pathological avian influenza viruses in Thailand. The risk of avian influenza has therefore been estimated at 50% of the total population. Precision was set at 7.5% with a z-score of 1.645. The sample size was calculated to be 112 poultry farmers per district using random sampling in each sub-district. A simple random sampling of the poultry farm addresses and the poultry trader addresses from the livestock district office database was conducted based on the 2019 District Livestock Office database of poultry farmers in the three provinces using Epi Info (13). All eligible participants selected from the random sampling were invited to participate in the study through a letter informing them of the requirements. All participants were asked to sign a written informed consent document. All study procedures were reviewed and approved by the Ethics Committee for Research in Human Subjects, Department of Disease Control, Ministry of Public Health, Thailand (number FWA 00013622) on November 2, 2021.

After written informed consent was obtained, all participants answered a standardized questionnaire. The questions consisted of demographics, knowledge, attitude and practices on avian influenza for example, knowledge about severity of symptoms of avian influenza, zoonotic strain of avian influenza, route of transmission, importance of spraying disinfectant on vehicles going across the farms, practices while moving in and out the poultry in the areas, practices of separation of diseased poultry from healthy poultry in the herd, practices of raising poultry on the farms, destroying the unknown cause of death of poultry, wearing a mask while working on a poultry farm, and notification to the relevant authorities about unusually sick or dead birds. All information were recorded in the face-to-face interview. The interview process was conducted by trained health and veterinarian officers.

Exploratory data analysis and statistical analysis were performed using Epi Info (13). Two-sided $p < 0.05$ were considered to be statistically significant. Knowledge and practices were scored using 22 questions with a 5-point scale, so the maximum possible score was 110. The exploratory analysis of the data assessed the scores above and below the 25th percentile as the cut-off point for the risk perception scores. A reason for the 25th percentile was the data distribution during exploratory data analysis that the distribution was much left skewed. Simple tabulation was used to describe proportions of risk perception scores (cut-off scores at ≥25th percentile or <25th percentile) in each category of exposure variables for example, respondents were divided into two groups based on years of experience in poultry farming/trading (<1 year vs. >1 year's experience).

Univariate analysis was performed by calculating odds ratios (ORs) and 95% confidence intervals (CIs) to evaluate each risk factor for the risk perceptions. In order to account for confounding factors, multiple logistic regression analysis was performed. Backward elimination procedure was used in the model. Changes of 10% in coefficients were considered evidence of possible confounding. Any variables that remained significant were kept in the model. Adjusted ORs and 95% CIs were also calculated.

Results

There were 346 participants, all of whom were classified as domestic breeders or poultry farmers on small-scale poultry farms primarily for their own domestic use in local areas, including 338 farmers (97.7%) and eight persons (2.3%) who worked in both poultry farming and poultry trading jobs. The proportions of females and males were almost equal (184 females [53.2%] and 162 males [46.8%]). The ages of participants ranged from 18 to 78 years old. The mean and median ages were 50 years. Most of the participants (96.8%) did not have a bachelor's degree. The participants' monthly incomes were mostly under 10,000 Baht (i.e., under 300 USD). In Table 1, most poultry farmers were classified on small farms that had less than 10% of poultry farmers with over 100 poultry on their farms. Among the poultry farmers, 120 (34.7%) raised mixed-type poultry. In mixed-type poultry, there were mixed backyard poultry on the farm in a number of 103 farms, 54 farms had duck or geese mixed on the farm and 45 farms had fighting cocks on the farm. There were 171 farms that raised only backyard poultry, 47 farms with only fighting cocks, and 8 farms with only ducks.

Most farmers (>75%) in three provinces have a good understanding of good practices aimed at reducing the risk of avian influenza, for example; obtaining permission to move the poultry with the livestock agent before moving the poultry out of the area, the importance of quarantine new poultry before they

TABLE 1 Husbandry practices of poultry farmers along border areas in three provinces of Thailand.

Characteristics/husbandry practices	Nakhon Phanom	Mukdahan	Ubon Ratchathani
Total number of poultry on the farm			
2–40	54 (48.2)	71 (60.2)	59 (51.3)
41–100	46 (41.0)	37 (31.4)	46 (40.0)
101–530	12 (10.7)	10 (8.5)	10 (8.7)
Farmers agree to obtain permission to move the poultry with the livestock agent before moving the poultry out of the area			
No	28 (25.0)	14 (11.9)	72 (20.9)
Yes	84 (75.0)	104 (88.1)	273 (79.1)
Farmers understand the importance of quarantine new poultry before they are raised with the herd			
No	13 (11.7)	10 (8.5)	12 (10.4)
Yes	98 (88.3)	108 (91.5)	103 (89.6)
Farmers understand that it is important to notify the relevant authorities if they detect abnormally sick or dead birds			
No	58 (51.8)	63 (53.4)	34 (29.6)
Yes	54 (48.2)	55 (46.7)	81 (70.4)
Farmers understand that it is important not to destroy the unknown cause of death of poultry by pouring them into rivers			
No	8 (7.1)	23 (19.5)	11 (9.6)
Yes	104 (92.9)	95 (80.5)	104 (90.4)
Farmers agree to wear a mask while working on a poultry farm to reduce the risk of avian influenza infection			
No	15 (13.4)	12 (10.2)	8 (7.0)
Yes	97 (86.6)	106 (89.8)	107 (93.0)
Farmers understand that they are not selling abnormally sick and dead poultry as usual			
No	3 (2.7)	6 (5.1)	3 (2.6)
Yes	109 (97.3)	112 (94.9)	112 (97.4)

TABLE 2 Risk perception scores on avian influenza by participant characteristics among live-poultry traders and farmers along border areas in three provinces of Thailand.

Characteristics	No. (%) under 25th percentile of risk perception scores	No. (%) above 25th percentile of risk perception scores	p-value
Location (province)			0.80
Nakhon Phanom	34 (33.0)	69 (66.9)	
Mukdahan	35 (31.3)	77 (68.8)	
Ubon Ratchathani	38 (35.2)	70 (64.8)	
Sex			0.19
Female	63 (36.2)	111 (63.8)	
Male	44 (29.3)	106 (70.7)	
Occupation			0.79
Poultry farmer	104 (32.9)	212 (67.1)	
Both poultry farming and poultry trading jobs	3 (37.5)	5 (62.5)	
Age (years)			0.04
18–43	28 (30.1)	65 (69.9)	
44–58	46 (29.1)	112 (70.9)	
59–78	33 (45.2)	40 (54.8)	
Educational level			
Below bachelor's degree	104 (33.2)	209 (66.8)	0.68
Bachelor's degree or higher	3 (27.3)	8 (72.7)	
Monthly income (Baht)			0.08
<10,000	91 (14.3)	163 (85.7)	
10,001–20,000	14 (25.0)	42 (75.0)	
>20,000	2 (14.3)	12 (85.7)	
Years of experience in poultry farming/trading			0.16
<1	6 (60.0)	4 (40.0)	
1–10	45 (31.5)	98 (68.5)	
>10	56 (32.8)	115 (67.3)	

are raised with the herd, the importance of not destroying the unknown cause of death of poultry by dumping them into rivers, the importance of wearing a mask while working on a poultry farm and understand not to sell abnormally sick and dead poultry as shown in Table 1. However, less than 50% of farmers in Nakhon Phanom and Mukdahan provinces did not see the importance of informing the relevant authorities if they detected abnormally sick or dead poultry (Table 1).

A total of 183 (52.9%) participants stated they had over 10 years of experience in poultry farming or trading. There were only 10 participants who had been in the poultry industry for <1 year. Most participants (72.5%) had <1 h of contact with poultry per day.

The scores for correct responses about perceived risk on avian influenza among live-poultry traders and farmers on the questionnaire ranged from 51.1 to 96.6%. The average and median risk perception scores were 76.7 and 77.3%, respectively. Participant characteristics were classified into two levels of risk perception scores, those above and those below the 25th percentile, as shown in Table 2.

There were no significant differences in risk perception scores in different study areas or between poultry farmers and traders. The number of women with risk perception scores below the 25th percentile was higher than men and those who did not hold a bachelor's degree. However, after adjusting for potential factors, no differences were found in the risk perception scores for the variables of sex and educational level. The number of study participants who had risk perception scores below the 25th percentile was observed to be higher among older adults, but this association was not significant. There was no difference in risk perception scores based on monthly income. Having >10 years of experience in poultry farming/trading was independently associated with increased risk perception scores (OR = 3.89, 95% CI = 1.09–15.07) (Table 3).

The measures for which the most frequent respondents (over 95%) responded correctly to prevent and control avian influenza were as follows: understanding the need to avoid the consumption, sale and sudden feeding of dead poultry on farms to other animals, due to the risk of avian influenza. In addition, the measures for which the participants most commonly provided incorrect responses to

TABLE 3 Multivariate logistic regression analysis of factors associated with risk perception scores on avian influenza among live-poultry traders and farmers along border areas in three provinces of Thailand.

Variable	Crude odds ratio (95%CI)	*p*-value	Adjusted odds ratio (95%CI)	*p*-value
Age group (years)				
18–43	Reference			
44–58	1.05 (0.60–1.84)	0.87	1.05 (0.58–1.90)	0.88
≥59	0.53 (0.28–0.99)	0.05	0.51 (0.25–1.06)	0.07
Experience in poultry farming/trading				
<1 year	Reference			
1–10 years of experience in poultry farming/trading	3.08 (0.84–11.4)	0.09	3.70 (0.95–14.43)	0.05
>10 years of experience in poultry farming/trading	3.27 (0.88–12.15)	0.08	3.89 (1.09–15.07)	0.04

prevent and control avian influenza were: unclear threat from of avian influenza, avian influenza high risk only in wintertime, and lack of knowledge of new viral strains.

Discussion

In this study population, the median risk perception score was high. This result may be because of heightened knowledge and awareness following the 2004–2006 avian influenza A(H5N1) outbreak in Thailand, in which there were in 25 human cases recorded with 17 deaths (9). After the outbreaks during 2004–2006, many organizations launched public awareness campaigns about the impact of avian influenza. Therefore, people, farmers and traders in Thailand may have had increased access to information and become more aware of the risk of avian influenza.

Risk perception scores indicate that the study population had reasonably good awareness of avian influenza. Their awareness may have resulted from the experience of the previous avian influenza outbreak (10) and information from the avian influenza surveillance network along the border between Thailand and Laos (14). The information obtained through surveillance has enabled poultry farmers to receive the current avian influenza situation that they may be aware of to prevent avian influenza and keep their poultry safe. In 2005, the results of a European and Asian avian influenza found only moderate perceptions of risk compared to this study (10). A total of 3,436 respondents were interviewed participation in the study of a European and Asian avian influenza risk perception. The perception varied from 32% in Denmark and Singapore to 61% in Poland and Spain. Higher scores were observed in Europe than in Asia.

Huge impact of economic loss during avian flu epidemics in Thailand from the mass culling of over 1-billion-baht poultry as compensation to affected owners (15). According to data from the United States Department of Agriculture (USDA) in April 2022, Thailand is the world's sixth largest chicken producer and the world's third largest chicken exporter (16). This may be one reason why the public-private partnership continues to promote a higher perception of the risk of avian influenza in Thailand. The public and private sectors need to continually support collection of information and sharing of knowledge to enhance public relations on avian influenza prevention. This would help ensure that poultry farmers have better understanding of the disease and a higher level of perceived risk. The continued engagement of government and private organizations is a key factor in maintaining awareness of avian influenza in communities (6). Knowledge, attitudes and best practices among poultry farmers and traders are critical to preventing the spread of avian influenza in humans and animals.

This study found that sex and age were not associated with perceived risk and avian influenza prevention and control. That is consistent with the study by Vityakom and Chayyaphong (17). In Table 3, few women were aware of risk, compared to men, but this association was not significant. A study by Cui et al. (18) concluded that a high perception and awareness of the risk of a disease were positively correlated with willingness to practice protective behaviors to prevent avian influenza A(H7N9) infection. The community education program may be more targeted to women because the perception of risk among women may influence other family members in the household.

In Table 3, most subjects over 60 years old had low aggregate scores on perceived risks of avian influenza compared with other age groups. Our study revealed that, based on univariate analysis, older adults had a lower perception of avian influenza risk, similar to a study by Fielding et al. (19). Suggesting that they underestimated the hazards and consequences due to familiarity with the hazards and past experience, they viewed the current avian influenza outbreak as a low risk. The study by Chesser et al. (20) reported that older adults may have additional issues with memory and perception, which could reduce their perception of health risks. In addition, the study by Louie et al. (21) found that patients aged 50–59 years had a higher mortality rate due to respiratory diseases such as influenza A(H1N1). Appropriate self-care behaviors to prevent infection can decrease the severity and complications of respiratory diseases, like avian influenza which can have serious consequences, especially for the elderly (22).

The elderly in the study population had less awareness of the risk of avian influenza. It is possible that this age group may neglect to take care of their own health and may take care of sick poultry in the farm or around the house without taking precautions. If they contract avian influenza without knowing, they may delay seeking medical advice. Increasing the risk perceptions among older groups is important to avoid underestimating avian influenza in this group. In terms of basic hygiene, this study found that most elderly people have good knowledge and practices in hand-washing. Perhaps our study was conducted during the COVID-19 pandemic and most people became more aware of the importance of masks and hand washing practices.

The scores from poultry farmers and traders in our study showed that more experience was significantly associated with an increased risk perception of avian influenza [adjusted odds ratio (95%CI) 3.89 (1.09–15.07)] as shown in Table 3. These results suggest that the participants in this study who had more years of experience in poultry farming had a more realistic understanding of the risks of avian influenza. This is similar to a study by Asare et al. which showed that work experience affects the perception and knowledge of avian influenza in poultry workers in Ghana (23). In addition, it was in accordance with the study by Cui et al. that showed an association between risk perceptions and personal protective behaviors on poultry farms in China. The study found that the number of years of poultry farming were significantly associated to personal protection behaviors and biosecurity prevention behaviors (24). Many factors can influence the perception of disease risk, including individuals' backgrounds, past experiences, availability of the source of information, social context and individual interpretation. Education and learning new information play a significant role in improved individual health knowledge as shown in the study by Pawun et al. (25). An approach to help enhance awareness and understanding of the risks of avian influenza of new poultry producers is to have a community platform for the more experienced poultry farmers to share experiences and specific knowledge. Government officials or local livestock officials may consider implementing various forums for sharing experiences and knowledge as part of the community mentorship program.

According to the World Health Organization's Avian Influenza Situation Report, people infected with the avian influenza virus tend to have a history of contact with poultry or have visited live poultry markets (26). Selling poultry sick/dead of unknown reasons, especially in live poultry markets, is a significant risk factor for the spread of avian influenza in humans and in poultry flocks (27). This knowledge is particularly important to help reduce the spread of the avian influenza virus. It is good to know that poultry farmers and traders in this study had high risk perception scores to prevent and control avian influenza. Most of the participants understand that sick/dead poultry should not be sold and consumed and that they should not be used for animal feed. Knowledge such as the severity of the disease, the pathogenic strains of avian influenza and the seasonal variation of the disease may not be understood among poultry farmers and traders, as shown in the results. This may be because Thailand has not reported an outbreak of avian influenza for >16 years, resulting in lack of knowledge of information on avian diseases. Increasing risk communication to officials, the poultry industry and the public about avian influenza is a necessary strategy in line with strengthening surveillance, prevention and control of avian influenza.

This study has some limitation of bias, including a very small number of traders and fewer people with less than a year of farming experience to recruit into the study. This study used the Provincial Livestock Office's registration database as a sampling frame. Poultry farmers and traders who was not being in the Provincial Livestock Office's registration database in 2019 had been excluded from this study. Of the non-registered farmers and traders not included in this study, they may have different characteristics with the registered farmers and traders. Therefore, bias may be present in this study. All farmers came from a small poultry network, some of which held positions as farmers and traders. The poultry trader could not be split into one particular category. In addition, our study focused on small poultry farms so that results could not refer to large industrial farms.

In conclusion, the transfer of knowledge and practices from experienced poultry farmers to individuals newer in the poultry industry at the community level is a good strategy. The community mentorship program to share experiences and knowledge on avian influenza can increase the risk of disease perception through effective communication among farmers. An accurate information and awareness of avian influenza of poultry farmers can reduce the risk of contracting and spreading the avian influenza virus in the community.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee for Research in Human Subjects, Department of Disease Control, Ministry of Public Health, Thailand (number FWA 00013622) on November 2, 2021. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization and methodology: SH and WS. Investigation and data collection: PT, JS, CC, PS, JR, YT, and WS. Writing—original draft preparation: SH. Reviewing and editing: SH, PT, and WS. All authors have read and agreed to the published version of the manuscript.

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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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Data workflows and visualization in support of surveillance practice

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The Swedish National Veterinary Institute (SVA) is working on implementing reusable and adaptable workflows for epidemiological analysis and dynamic report generation to improve disease surveillance. Important components of this work include: data access, development environment, computational resources and cloud-based management. The development environment relies on Git for code collaboration and version control and the R language for statistical computing and data visualization. The computational resources include both local and cloud-based systems, with automatic workflows managed in the cloud. The workflows are designed to be flexible and adaptable to changing data sources and stakeholder demands, with the ultimate goal to create a robust infrastructure for the delivery of actionable epidemiological information.

KEYWORDS
animal health, epidemiology, data-driven, dashboards, digitalization, automation, reproducibility

Introduction

Prevention, detection and control of infectious diseases to safeguard animal health rely on the timely collection of evidence, and delivery of this evidence in formats that can be used for effective decision-making. In response to the growing availability of digital data sources which can be used to produce health intelligence, epidemiology progressively incorporates methods of big data analytics, developing digitalization workflows to convert a great variety of data into actionable epidemiological information.

To be useful in disease surveillance, however, these workflows need to be implemented continuously and remain true and relevant as not only data evolves, but also demands from stakeholders and knowledge itself. Hence, reusable and adaptable workflows for epidemiological analysis and dynamic report generation are required.

At the Swedish National Veterinary Institute (SVA), we strive toward automation to better fulfill our surveillance and knowledge communication responsibilities. We have a vision to move away from multiple parallel or manual workflows toward a common set of reusable tools, backed up by a robust infrastructure of cloud-based as well as local systems. The epidemiology team at SVA brings together different areas of knowledge, including epidemiology, software development and statistical modeling. We work closely with the community of internal and external users to ensure that the delivered tools and information serve the intended purpose.

Making tasks reproducible in a collaborative environment is challenging. Collaboration is a prerequisite, both to reduce person-dependence and because several areas of expertise are required to perform complex data analysis. Parallel development of analytical workflows often results in multiple solutions to similar problems. To avoid that, we aim to create common and shareable building blocks that can be reused in various applications. Here we describe a collaborative workflow centered on a joint development environment, common tools and a goal to reduce effort and improve reliability of results.

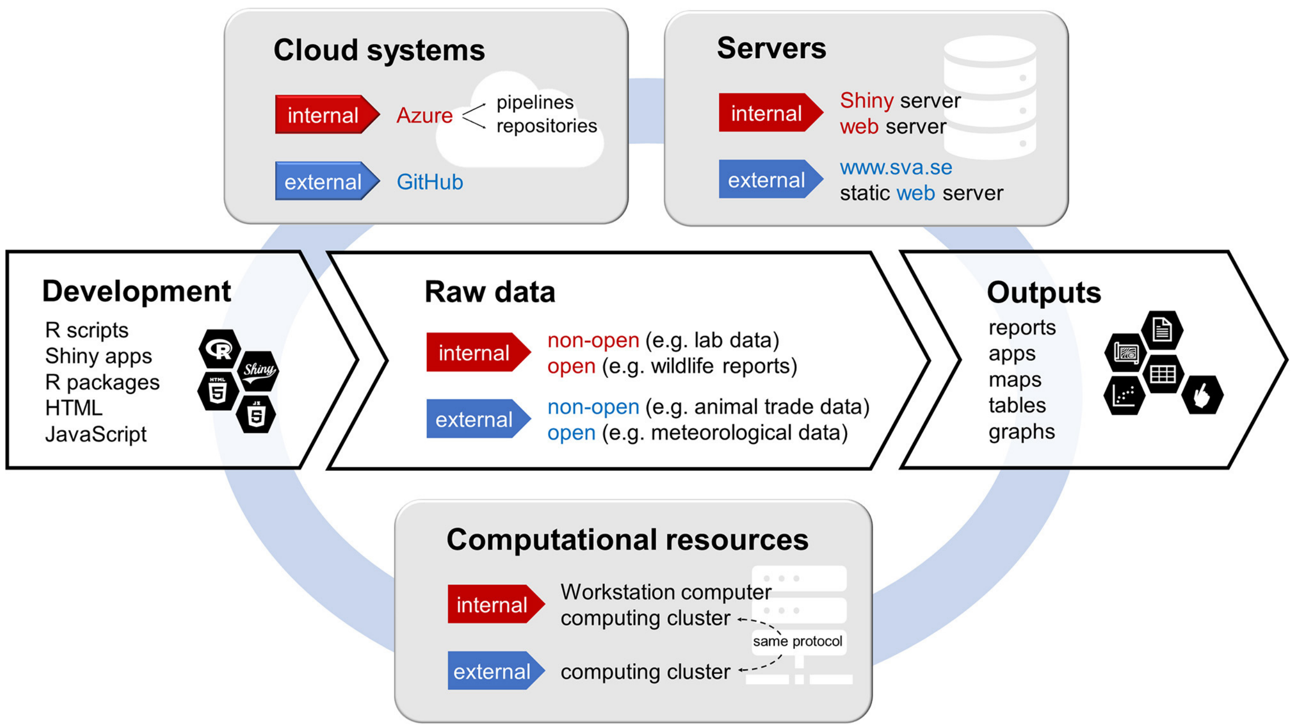

FIGURE 1
Schematic representation of the components of the described collaborative environment for data analyses. The main workflow from development to output (white background) operates through several supporting infrastructures (gray background). The supporting systems marked as "internal" are accessible by SVA only while "external" systems are publicly available. For the raw data, the distinction between internal and external instead refers to the data origin and ownership.

Workflow components

The components of our analysis and visualization workflows can be divided into four categories: (1) data access, (2) development environment, (3) servers and computational resources, and (4) cloud-based management. In general, development, version control and execution happen locally while the cloud environment is used for storage and administration of automatic workflows. See Figure 1 for an overview of the components and their interconnections.

Data access

Examinations and tests from our laboratories are entered into a laboratory information management system. Information includes analyses performed, test results, animal species and geographical origin of the samples (coordinates and/or administrative region). The data are accessed through "data dumps" from a system of curated reports which are fed by database queries.

Additionally, we use several open data sources which are accessed directly through application programming interfaces (APIs). One such source is the SVA "Rapportera Vilt" system[1] where anyone in Sweden can report findings of dead, sick or injured wild animals.

1 https://rapporteravilt.sva.se/

Development environment

Development of scripts and workflows is done locally on personal computers. Code collaboration and backup is enabled through Git, a distributed revision control system (1). Each user makes changes (*commits*) on a local copy of the code in question. When ready, the local changes are then published (*pushed*) to a remote "origin" repository from which other users can retrieve (*pull*) the new revisions. This allows several colleagues to work on the same project in parallel while maintaining a common version history and avoiding the risk of undoing each other's work.

The main programming environment that we use is the R language for statistical computing (2) due to its familiarity in the group and wide support within the fields of data science, statistics and data visualization. We have developed R packages for specific purposes, e.g., data cleaning, report production and disease spread simulation, some of which are published publicly on GitHub[2] or on the CRAN archive[3] (see (3) as an example of such a package). We use static templates written in HTML and JavaScript to produce web content including maps, graphs and tables, which can be populated with up-to-date cleaned data on demand. We also build web applications using the R Shiny package (4), which enables the development of powerful and user-friendly web-based tools in R without the need for extensive skills in web development. An R Shiny application can be extended with custom HTML, CSS and JavaScript, which makes the environment especially flexible.

2 https://github.com/sva-se/
3 https://cran.r-project.org/

Servers and computational resources

Personal computers are used for development and programming but are not ideal for execution of more computationally intensive tasks or for running automated analysis workflows. To solve this, we have two additional computational resources which are accessible for the whole department.

The first is a workstation computer running Microsoft Windows, which users can access through a remote desktop connection. This allows for the flexibility of working from a personal computer as well as the familiarity of the Windows environment while providing the user with additional processing power. The workstation is connected to all systems which any personal computer on the network can access, including network disks and internal web servers. Therefore, it is also used to run automatic workflows including the daily update of our web content on the current disease situation which requires access to internal data sources.

The second resource available is a cluster of computers running Linux, which are accessed by remote connection to a central node using the SSH protocol (5). This cluster is equipped with the SLURM Workload Manager (6), a scalable cluster management system in which the user can add jobs (scripts with instructions) to a queue. Once they are available, the requested resources will be allocated and the job is executed, without requiring the user to be actively logged in. Intensive jobs that do not include sensitive data can also be sent for execution in a similar system available at national level (Swedish National Infrastructure for Computing, SNIC) (7).

A deployment of ShinyProxy (8) on an internal server is used to host applications developed in the R Shiny framework. Each application is developed in the R package structure and subsequently built into an image which runs the application *via* the Docker (9) runtime. An image contains the application code itself and all its specific dependencies. Built application images are stored in a container registry which allows ShinyProxy to pull the latest version during the development phase and images to be tested locally for debugging.

Cloud-based management

We use the Microsoft Azure DevOps cloud environment (10) for management of code and analytical workflows. In Azure, work is divided into projects which can be managed independently of each other. Each project contains one or several Git repositories as well as *pipelines* which are sets of instructions used to execute procedures in Azure (see examples of such procedures in the "Practical examples" Section).

The Azure DevOps projects are home to the remote origins of most of our Git repositories, for storage of scripts, R packages and content templates. For data sovereignty reasons, we do not store data on the Azure platform. The Git repositories may be directly connected to pipelines, of which there are two types: *build* and *release*. Build pipelines trigger automatically, either when new changes are pushed to the corresponding repository or on a regular time schedule and produce an output called an *artifact*. Release pipelines consume these artifacts and publish their contents. The publication location is typically a static web server that can be linked to from SVA's external website. The Azure system also has a container registry for the storage of containerized application images, which contain all dependencies for a specific application and can be downloaded and run locally (e.g., on the internal ShinyProxy server).

While pipelines and code are stored and managed in the cloud, some of the pipeline processes must be run locally to access internal data sources. A *pipeline agent* has been configured to run on the workstation computer with access to the required resources. Whenever a build pipeline configured to run on this agent is triggered, the Azure system sends the pipeline instructions and code to the local workstation for execution. The resulting artifact is then sent back to the cloud for publication. In this way, we can keep the flexibility of cloud-based management while maintaining data sovereignty.

Practical examples

Below, we have highlighted several projects and activities where this environment of tools and methods has been employed in practice.

Daily surveillance and disease situation summaries

The latest surveillance results generated at the laboratories are published daily to SVA's external website.[4] The data are visualized in interactive graphs, maps and tables, and cover a range of disease agents of interest—including but not limited to chronic wasting disease in cervids, avian influenza in wild birds and African swine fever in wild boar. This workflow is managed by an R package designed specifically for this purpose, which contains HTML, JavaScript and CSS content template files as well as tools to analyze data and deploy the final content. The R package is hosted and code is executed in the Azure cloud environment. Every morning, a time-scheduled build pipeline is triggered in Azure. The pipeline instructs the department workstation to pull the latest changes from the git repository and execute the deploy scripts. Data are fetched from our internal systems, cleaned, summarized and combined with the appropriate templates to produce HTML files. These files are published back to Azure as an artifact, triggering a release pipeline which publishes them to our static web server. The latest update can be viewed on the SVA website as soon as this workflow has finished.

The disease situation webpages keep disease control experts, animal owners and the public informed about the current Swedish animal disease situation. This timely communication is important for the public who otherwise would only have access to periodic data summaries in agency reports. Continuous updates of data have also facilitated the early detection of new trends and outbreaks, which contributes to a robust surveillance.

Annual surveillance report

Surveillance of Infectious Diseases in Animals and Humans in Sweden[5] is an annual report describing the Swedish surveillance

4 https://www.sva.se/amnesomraden/smittlage/

5 https://www.sva.se/amnesomraden/smittlage/sjukdomsrapporter-om-sva-s-overvakning/

activities during the previous year, covering important animal pathogens as well as select zoonotic agents in a One Health context. It is published by SVA in collaboration with the Swedish Board of Agriculture, the Public Health Agency and the Swedish Food Agency (11).

The report is divided into chapters, one per disease agent or topic covered. The responsible authors write their chapters in word processing software and provide data for figures in spreadsheets in a cloud environment. These documents and spreadsheets are then converted to the LaTeX (12) document preparation system using a fully open-source "report engine" built as an R package that depends on the Pandoc (13) document conversion software. An Azure DevOps pipeline stitches together the chapters in a LaTeX report template and produces a PDF document, which is published to our external web server.

This system facilitates the work of the authors, as they work in a familiar collaboration-friendly environment decoupled from the final typesetting of the report. The authors, from different agencies with access to different data, can work closely to create a synthesis of the annual surveillance results without sharing raw data with each other. Additionally, everyone is involved in the design process since the latest PDF version is always available for them to view online.

SvarmIT—Interactive tool for antimicrobial resistance surveillance

SvarmIT is a tool developed to visualize up-to-date antimicrobial resistance (AMR) data from 2010 and onward for different sample types, bacterial species, antibiotics and animal species. The trends are shown in relative frequency of resistant isolates among the total tested in a year, which can be exported as a graph or table for further analysis. A daily workflow was set up to clean, analyze and aggregate susceptibility testing data using R, and attach it to an HTML/JavaScript template which is published to our external website[6]. Additionally, SvarmIT sends a notification to the laboratory personnel when there are new samples that need further investigation based on the initial phenotypic test results.

Continuous monitoring is a fundamental part of the work to stop and prevent the spread of AMR. Every year, SVA examines ∼12,000 samples from animals for the presence of antibiotic-resistant bacteria and results. SvarmIT is designed to meet the FAIR[7] principles; it is accessible to the public and facilitates the delivery and communication of daily AMR surveillance results. Previously, stakeholders would only have access to the compiled summaries published in the annual Swedres-Svarm report (14). SvarmIT also contributes to a better collaboration and a robust and more timely AMR processing workflow within SVA, thereby enhancing the quality of the surveillance.

6 https://www.sva.se/en/our-topics/antibiotics/svarm-resistance-monitoring/svarmit-interactive-resistance-monitoring-tool/

7 https://www.go-fair.org/fair-principles/

Anthrax dashboard

The Anthrax dashboard is an interactive graphical tool to visualize historical outbreaks that occurred in Sweden from 1916 to 2016 based on information from Swedish archives (15), along with relevant weather data (16). The application was built using JavaScript, particularly two major libraries: Leaflet (17) and D3 (18). The workflow to clean, analyze and convert the historical data to JSON format was written as an R package. The dashboard is hosted on SVA's internal web server.

Following an outbreak of anthrax, bacterial spores can remain dormant in the soil and can cause new infections in susceptible grazing animals for decades (19). For decision-makers, the dashboard is a useful tool to quickly evaluate the risk of anthrax in a specific area based on whether an outbreak has previously occurred nearby.

Salmonella portal

The *Salmonella* portal is a dashboard where all surveillance activities for the disease agent are collected. It presents the historical and present surveillance results of *Salmonella* in Swedish animals, including production animals, wildlife and domestic cats. The dashboard is developed in R Shiny and hosted on the internal ShinyProxy server. *Salmonella* has been selected as a pilot case and future development is planned for several other disease agents of importance. The dashboard is therefore developed with reusability in mind, with the aim to create a main template layout. It is currently designed for internal use but will eventually be public.

The daily surveillance results are visualized in maps, graphs and tables, allowing users to browse the data and potentially identify spatiotemporal patterns. The annual surveillance report (11) summaries are also presented here, along with general information about the agent and the existing surveillance programs. This resource provides decision-makers and the public with an up-to-date comprehensive view of *Salmonella* surveillance in Sweden, leading to a better understanding of the disease over time and across several species.

Applications developed in collaboration with external stakeholders

In addition to the examples mentioned above, SVA also works on the development of several applications for users in the industry, such as farmers and veterinary advisors. The development is iterative, with regular interaction with users in the form of focus groups, surveys, workshops, prototype evaluations and meetings. Hence, user requirements and tool functionality also evolve during the development phase, with scalability and flexibility being key requirements.

The applications use data from SVA's laboratory systems as well as from external sources. Data used in these applications include test requisitions, test results, health and reproductive events, and productive performance of the animals. In some applications, the sensitive nature of the data included requires different user profiles with different levels of access to the information. Applications that are used by in-house advisors are hosted in our internal ShinyProxy server. In the future, development plans include the search for hosting solutions that will allow access by users outside the institute. These development projects allow both the data owners and SVA to demonstrate how the additional value of animal health data can be captured through interactive applications.

Discussion

Data analysis processes to transform data into information that can support animal disease surveillance have often been discussed in literature, but implementation of these in automated workflows that can be employed continuously or on-demand presents several challenges. Our experiences described here show that collaboration is required to have robust and sustainable workflows and to handle the complexity in data management and analysis in the best way.

Establishing reusable functionality is important for improving efficiency and quality control. However, it has been challenging to identify core functions that should be built as reusable components in a code library that is shared between projects. An optimal solution can never be achieved but the group must continuously work toward improvement. Currently, close collaboration with frequent communication has been used to update coworkers on what core functions are implemented for reuse. An ongoing challenge is to identify if these core functions are actively being used in new projects. Code review is not a tradition in epidemiology, and as it costs substantial time it is usually not prioritized. However, our goal to use the R package structure for collecting our work allows us to take advantage of R's available tools for code testing to guide improvements and maintain quality control.

The use of centralized cloud platforms for code storage and execution of processes has helped enable collaborative development and reduce the person-dependence of recurring tasks. It has allowed the transfer of responsibilities more efficiently during vacation periods or during periods of high workloads such as disease outbreaks. However, the use and maintenance of these systems rely on the availability of human resources, and prior planning is necessary to ensure that personnel have the appropriate skills to complete the required tasks or debug problems that may occur. Proficiency in programming and tools such as version control is not normally expected of veterinary epidemiologists and has required substantial training.

The current landscape for practical work in epidemiology is changing rapidly with the introduction of new tools and strategies primarily adapted from methods used in software development. This requires education to establish these new skills and close collaboration within a team. At SVA, we have advanced our working strategies over recent years but acknowledge that continuous effort is required to maintain and continue to improve how we work. These modern approaches support the timely and accurate reporting of surveillance results to the public as well as forming a sound foundation for expert evaluation of trends or other changes in the Swedish animal disease situation.

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

WG is the main responsible for the implementation and maintenance of pipelines in Azure DevOps. JF is the group leader. FD, WG, and TR prepared a first draft of this manuscript, which was reviewed, and amended and approved by all other authors. All authors participate actively in the development, maintenance and use of data workflows at the Department of Disease Control and Epidemiology.

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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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Evaluations of the Disease Surveillance Centre network in Scotland: What parts has it reached?

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Regular evaluation is a prerequisite for systems that provide surveillance of animal populations. Scotland's Rural College Veterinary Services' Disease Surveillance Centre (DSC) network plays an integral part in surveillance to detect new and re-emerging threats within animal populations, predominantly livestock. In response to surveillance reviews and proposed changes to the network, an initial evaluation of diagnostic submissions data in 2010 to mid-2012 established a baseline "footprint," while highlighting challenges with the data. In this recent evaluation for the period 2013–2018, we developed a new denominator using a combination of agricultural census and movement data, to identify relevant holdings more accurately. Iterative discussions between those processing submissions data and those involved in collection at source took place to understand the intricacies of the data, establish the most appropriate dataset, and develop the processes required to optimise the data extraction and cleansing. The subsequent descriptive analysis identifies the number of diagnostic submissions, the number of unique holdings making submissions to the network and shows that both the surrounding geographic region of, and maximum distance to the closest DSC vary greatly between centres. Analysis of those submissions classed as farm animal post-mortems also highlights the effect of distance to the closest DSC. Whether specific differences between the time periods are due to changes in the behavior of the submitting holdings or the data extraction and cleaning processes was difficult to disentangle. However, with the improved techniques producing better data to work with, a new baseline footprint for the network has been created. This provides information that can help policy makers and surveillance providers make decisions about service provision and evaluate the impact of future changes. Additionally, the outputs of these analyses can provide feedback to those employed in the service, providing evidence of what they are achieving and why changes to data collection processes and ways of working are being made. In a different setting, other data will be available and different challenges may arise. However, the fundamental principles highlighted in these evaluations and the solutions developed should be of interest to any surveillance providers generating similar diagnostic data.

KEYWORDS

disease surveillance, evaluation, network, veterinary, farmer, passive surveillance, livestock

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Introduction

Systems that provide surveillance of animal populations can be implemented to meet either one, or more, specified objectives. Examples of such objectives could be to: allow declaration of freedom a specific disease; determine the frequency of a disease in a population or, detect a new or emerging threat.[1] Priorities for surveillance systems should be identified when the infrastructure is being built or reformed, and the success of them should be measured against set criteria. However, it is recognised that both priorities and success criteria will be subject to iterative adaptation and evolution to meet changing needs of those commissioning the system, be they industry, government state, or other stakeholders. Regular review, monitoring and evaluation is required. Such reviews should provide information about whether the surveillance system is generatits intended outputs and meeting objectives, whether these objectives appropriate at the current time and for the visible future, and therefore whether impronts or changes are required (1, 2). Evaluation of the existing system can also provide a baseline against which to measure the ef of proposed change. These reviews are, therefore, an essential step in the policy cycle (3).

A systematic review in 2015 (4), identified three evaluation approaches available in the field of animal health surveillance (5–7). These approaches provide either a general or a structured approach (6), methods (7), or a tool (5) for the evaluation process. More recently, in 2019, an additional tool for integrated evaluation has been developed, tested, and demonstrated (2). These tools and approaches are often time-consuming and resource intensive to apply in full. The idea that evaluations should be individually tailored was highlighted for public health systems by Klaucke (8), and has been recognised in the United Kingdom's approach to animal health surveillance.[1] The expectation is that each of the four administrations (England, Wales, Scotland, and Northern Ireland) will independently evaluate the performance of their own animal health surveillance systems.

Up until 2019 Scotland's Rural College (SRUC) Veterinary Services (VS) provided a network of eight Disease Surveillance Centres (DSCs) in Scotland to support livestock disease surveillance through submissions of vet and farmer-selected samples and carcasses that were submitted for diagnostic purposes and post-mortem (PM) examination. In 2019, the PM room capacity of this network was reduced,[2] although the services of the network continue to be delivered by SRUC VS, on behalf of the Scottish Government.

The submissions assist with improving animal health at the farm level, while the diagnostic information is available, and contributes to, the passive surveillance system (9, 10) both in Scotland and across Great Britain (GB).[3,4] Comparable networks exist within[5] and across other countries.[6] These networks, when enhanced and developed, have been shown to be an increasingly viable method to observe new patterns in endemic diseases and to identify new diseases (11–13).

A fundamental requirement for the effectiveness and efficiency of such networks is engagement and participation. Previous studies have shown the importance of the individual in surveillance (10, 14, 15). Whether it is the farmers themselves (11) or the veterinarians working with those farmers (16–18), these individuals act as gate-keepers and can have an impact on when and how disease is reported. An understanding of how the network is used can help policy makers determine if access to the network is appropriate and if it is providing sufficient coverage to allow conclusions to be drawn with confidence. The first step in an investigation of drivers for submission is to look at how the network is used and whether there are links between surveillance submissions and geographic location (19), or distance to a laboratory (20).[7]

Between 2011 and 2019, the British network underwent significant review and restructure.[6]

As part of background evidence for policy advisors, the organisational attributes of coverage and usage from 2010 to mid-2012 of the Scottish DSC network, by Scottish livestock holdings with any of the main target species (cattle, sheep and pigs), was evaluated.[8] However, it was challenging to provide complete analysis of the network usage and drivers of that usage because of systematic issues in the data collection that were identified.

In conjunction with Scottish Government science and policy advisors, it was decided that it would be appropriate to re-evaluate the SRUC VS diagnostic submissions data for the period 2013–2018. The primary aims were to evaluate if the initial quality, and thus utility, of data had improved therefore providing more reliable and complete geolocation data on submitting holdings, to determine coverage and usage for the new study period and to investigate the spatial pattern of usage of the DSC network by these livestock holdings. If these aims could be achieved, the outcome would be the provision of a baseline against which the restructured network could be re-evaluated and an assessment of whether the data are suitable for further analysis of factors that drive network usage. Here we present the evaluation process and results for 2013–2018; we compare the outputs to those from the 2010 to mid-2012 evaluation and provide discussion on any differences identified.

Materials and methods

The Disease Surveillance Centre network

Prior to 2019, there existed a network of eight DSCs located across Scotland. These were facilities, run by SRUC VS, for PM examination, as well as for diagnostic testing. The range of diagnostic tests run on-site differed between DSCs, but all included parasitology and bacteriology. If required, samples could be forwarded into the network for additional diagnostic testing that was not available on-site, such as serological and molecular testing as well as histopathology and biochemistry.

1 https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/869173/uksf-animal-health-surveillance.pdf

2 https://www.sruc.ac.uk/all-news/implementing-a-new-model-of-disease-surveillance/

3 http://apha.defra.gov.uk/vet-gateway/surveillance/scanning/vida.htm

4 http://apha.defra.gov.uk/vet-gateway/surveillance/diagnostic/national-network.htm

5 https://www.gov.ie/en/service/7f9f7-laboratory-services/#regional-veterinary-laboratories

6 https://www.iaea.org/services/networks/vetlab

7 https://webarchive.nrscotland.gov.uk/3000/https://www.gov.scot/Resource/Doc/362344/0122619.pdf

8 Tongue et al. (2015), *The SAC Consulting Veterinary Services Disease Surveillance Centre network - what parts does it reach?*, unpublished internal peer reviewed report for Scottish Government, available on request.

Submissions, each relating to a single animal or group of animals from a single holding, were made to a DSC, often by the consulting vet. These submissions, or samples from them (for example faeces, blood, tissue samples), could either be dealt with directly or passed on internally to another DSC within the network. The submissions included in this analysis were labelled as having been submitted for diagnostic purposes, rather than monitoring of healthy animals. A subset were identified as postmortem farm animal (PMFA).

Holdings dataset – the denominator

Before we were able to analyse how the DSC network was used, it was crucial to establish the type and location of any holdings who might potentially make a submission. The aim with the denominator dataset was to identify all holdings in Scotland that had at least one animal of any of the major livestock sector species i.e., bovine, ovine, or porcine. These are the by far the most common species submitted to the surveillance network.

There is a statutory requirement that anyone having at least one of the animals in this species list on their property is required to be registered as a "holding".

Within GB, the term "holding", when applied to livestock, usually describes the land and buildings that people use for keeping livestock,[9] including livestock kept as pets. Each holding is assigned a unique county parish holding (CPH) number. CPH numbers have the format 12/345/6789, where the first two digits represent the county, the next three the parish and the final four an individual holding within the parish. A CPH number can be temporary, or permanent and it can cover a range of land and buildings within a specified distance from a main livestock handling area. A livestock business may, however, consist of have more than one CPH. The CPH number and the term "holding" therefore approximates to, and usually is, the basic unique identification of a farm used in many British livestock recording systems. However, care is needed when handling datasets as other types of premises and/or land such as markets, lair ages, slaughterhouses, ports and showgrounds have CPH numbers. A CPH is also necessary to comply with the legislative requirements for recording and reporting of livestock movements.

To identify holdings of interest information from three separate sources were combined: the agricultural census; the Cattle Tracing System (CTS); and the ScotEID database. The agricultural census takes place every June and collates, amongst other details, the number of cattle, sheep and pigs on each livestock holding.[10] The CTS database is used to record all births, movements onto and off holdings, and deaths of cattle within the UK. It is managed by British Cattle Management Service (BCMS)[11] and access to these data is granted by the Animal and Plant Health Agency (APHA).[12] ScotEID[13] is the livestock traceability system for Scotland managed by the Scottish Agricultural Organisation Society on behalf of the Scottish Government. It was used for sheep and pig movements records in the study period, although from 2022 ScotEID it also holds the cattle tracing system. Any sheep and pig movements that contain all or part of their movement within Scotland are recorded in ScotEID. The study period was 2013–2018. All the datasets used in this study were provided to the Scottish Government's Centre of Expertise on Animal Disease Outbreaks (EPIC) and stored in the EPIC data repository, which is a centrally curated collection of data resources.

The agricultural census and movement data were extracted and cleaned separately for each species. The sheep data includes information on batch movements and individual animals, whilst the cattle data is stored solely as individual animal movements. Pig movement data is only recorded in batches. Holdings were identified as having sheep or pigs if they had animals move off their holding at any point in the study period, as by proxy they then must have had animals on that holding, which may have needed diagnostic services. To ensure all cattle holdings were identified, these were included if they had movements onto their location, as each individual cattle birth is recorded as a movement onto the holding but with no "off location" (21). Some small herds could have no movements off within a year, with the only change being births.

For all species, every attempt was made to restrict the data to locations identified as agricultural holdings, rather than another type (e.g., market or abattoir). Inclusion in the agricultural census automatically identified a location, otherwise it was dependent on species. In the cattle data, the "premises type" identifier was used and only those marked as agricultural holding were retained. For the sheep and pig data, only holdings identified as livestock units were included in the final holdings denominator dataset.

A holding was identified as having an individual species based on having a non-zero value in the agricultural census or if recorded as moving that species of animal between 2013 and 2018. The holdings identified for each species were then amalgamated to provide a dataset to represent all Scottish holdings.

Submissions records – the numerator

As with the process of identifying the holdings that could use the DSC network, it was necessary to combine multiple datasets to establish which holdings had made submissions. The first step in the process was to extract submission records from all eight DSCs. At the time of the extraction, the eight DSCs operated eight independent Laboratory Information Management Systems (LIMS). The LIMS had the same structure and interacted with each other. Each submission was given a unique identifier that was held across all samples and any internal submissions generated. Some of the tables in the individual LIMS were the same but, crucially, the clients table was different for every DSC. This meant that the same holding could be included in the clients table of multiple DSCs. Furthermore, client tables allowed multiple entries of the same farm within a particular LIMS.

Individual holdings were identified in LIMS by their CPH number. The CPH was sometimes missing from submissions and in other cases the CPH number recorded was not in the correct format. Efforts were made to both clean the data (for example correcting CPH form (e.g., 123/456/789 corrected to 12/345/6789) and to match

9 https://www.gov.uk/guidance/register-land-you-use-to-keep-livestock#holding-meaning-and-the-area-it-can-cover

10 https://www.gov.scot/collections/june-scottish-agricultural-census/

11 https://secure.services.defra.gov.uk/wps/portal/ctso

12 https://www.gov.uk/government/organisations/animal-and-plant-health-agency

13 https://www.scoteid.com/

TABLE 1 The number of Scottish livestock holdings by species in the period 2013 - 2018 inclusive, as derived from demographic and movement data.

Species on holding		Number of holdings with > 0 animals*	Per cent (%) of all Scottish livestock holdings*
Total livestock holdings		24,057	100%
Livestock holdings with named species present	Bovine/cattle	12,513	52.0
	Ovine/sheep	19,374	80.5
	Porcine/pigs	1,327	5.52

*Holdings appear more than once if they were recorded as having more than one species.

submissions to previous submissions with the same address to allow identification of the CPH (for example by using another unique identifier of a concatenation of the name and postcode on the submission). There were, however, 134 farms where the submissions had the same name and postcode but different CPHs recorded. In these cases, the CPH number in the earliest submission was used.

The submitting holdings were linked to the eligible holdings using the CPH and a single Easting and Northing was recorded with that CPH. At this point, any holdings that were out with Scotland were removed from the data set.

Finally, the straight-line distance of all the holdings to each of the DSCs was calculated. This enabled the identification of the geographically closest CPH for each holding. In turn this allowed catchment areas to be created for every DSC i.e., the group of holdings where a particular DSC was the closest centre (or entry point to the network) for each of them.

Descriptive analysis using both datasets

Having completed construction of both submission and holding datasets, the geographical distribution of all Scottish livestock holdings was visualised, as was the distribution for each individual species. The holding and submission datasets were also combined to construct density ratio maps (kernel density 10 km^2, grid cell size 1 km^2) to examine the proportion of all Scottish livestock holdings that submitted from a specific area.

The submission data from 2013 to 2018 were described in terms of the numbers and proportions (or percentage) of holdings submitting (a) to the network, (b) to their closest centre and (c) according to their distance from their closest centre. PMFA submissions were described separately as well as in conjunction with all diagnostic submissions. The descriptive outputs for this 2013–2018 evaluation were then placed in the context of those from the 2010 to mid-2012 period.

All descriptive spatial analysis was carried out using qGIS (22) with all other results calculated in R (23), using the diverse suite of packages (24) and plots created using ggplot2 (25).

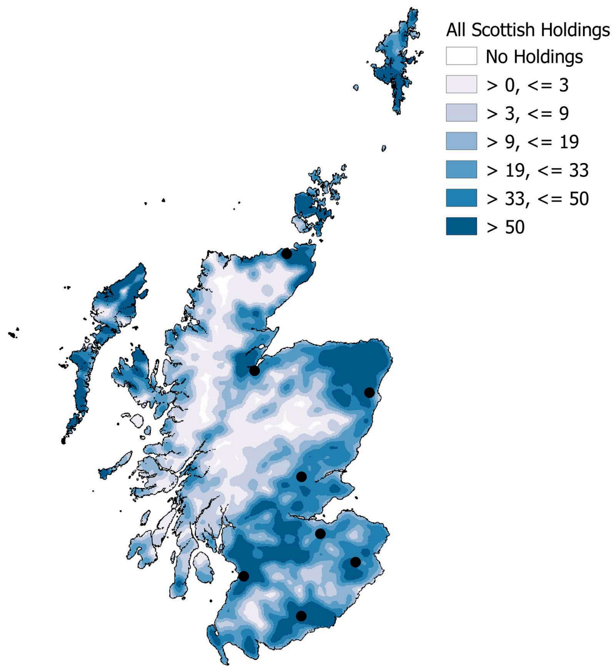

FIGURE 1
All Scottish livestock holdings 2013 - 2018 inclusive, as defined from census and movement data - Kernel density 10 km radius, number of holdings per 10 km-square - with the locations of the eight DSCs (black spots).

Results

Holdings dataset – the denominator

Having extracted and cleaned the holding dataset as described above, 24,057 livestock holdings were identified across Scotland in the period 2013–2018 inclusive (Table 1). The total number of pig holdings is of the order of a tenth of those with cattle and a sixteenth of those with sheep. It should be noted that a holding is counted identically regardless of the number of animals present, so a smallholding with one cow, sheep or pig is treated the same as a holding with 300 animals.

The spatial distribution of livestock holdings across Scotland was heterogeneous (Figure 1). The eight DSCs (marked as black dots in Figure 1) were located in livestock-holding dense areas The higher densities for all livestock holdings observed in the South of Scotland, Orkney and Shetland were due mainly to cattle (Supplementary Figure SM1) and sheep (Supplementary Figure SM2) holdings. The majority of pig holdings were located in the North-East (Supplementary Figure SM3).

The spatial distribution of holdings was not uniform across the DSC network when they were classified by their geographically closest DSC (Figure 2). The DSCs in the South of Scotland had a smaller geographical catchment area when compared to the two most northerly centres, Inverness and Thurso (furthest North).

The numerical distribution, like the spatial, was not uniform across the network, with the most northerly regions having the highest number of individual holdings (Table 2). The numbers of holdings for each DSC were consistently slightly higher in 2013–2018, compared to the corresponding values derived from the different denominator used for 2010 to mid-2012. There were slight shifts in the percentage frequency distribution (Table 2).

Across all centres, just over half (56.1%) of the holdings were within 50 km (straight line distance) of their closest centre (Figure 3). This was very similar to 2010 to mid-2012 where it was ~57% of all such holdings. Cumulative distribution plots for each centre show that the percentage of holdings located within 50 km varies widely between DSCs (Supplementary Figures SM4–11). Five of the eight DSCs had at least 75% of holdings within 50 km (Supplementary Figures SM4, 6, 7, 9, 10) and the two most northerly DSCs both had <40% of their closest holdings within 50 km (Supplementary Figures SM8, 11).

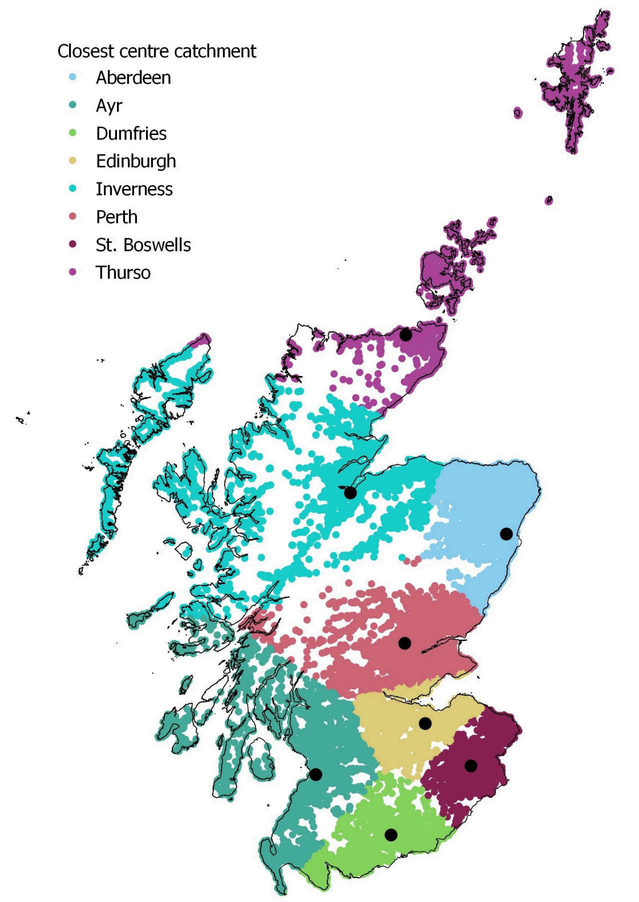

FIGURE 2
All holdings within the denominator dataset coloured by their closest DSC, which represents their closest point of entry to the network.

Submission records – the numerator

In the 2013–2018 evaluation there were 86,996 diagnostic submission records initially extracted, of which 67,360 records had CPH numbers recorded in the correct format. For seven of

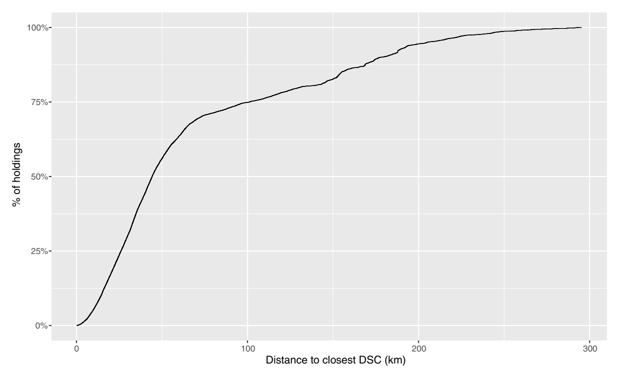

FIGURE 3
The cumulative frequency (%) of Scottish livestock holdings (2013 - 2018) by distance (km) from the existing closest network entry point (closest existing DSC) for each livestock holding.

TABLE 2 The number and percent of all the Scottish livestock holdings that have the stated DSC as their closest centre, i.e. were within its catchment area, for 2013 – 2018 and (2010 to mid-2012).

DSC name	Scottish livestock holdings for which a specific DSC is the "closest centre"	
	Number of livestock holdings 2013 - 2018 (2010 to mid-2012)	% of all Scottish livestock holdings 2013 - 2018 (2010 to mid-2012)
Inverness	6,480 (5,563)	26.9 (25.5)
Thurso	3,520 (3,315)	14.6 (15.2)
Ayr	3,429 (3,241)	14.3 (14.9)
Aberdeen	3,415 (3,203)	14.2 (14.7)
Perth	2,355 (2,112)	9.8 (9.7)
Dumfries	2,057 (1,841)	8.6 (8.5)
Edinburgh	1,665 (1,545)	6.9 (7.1)
St Boswells	1,136 (964)	4.7 (4.4)
Total	24,057 (21,784)	100 (100)

the eight DSCs this represented between 83 and 90% of their submission records. However, the overall network figure is 77.4% because of one DSC for which more than half of its records did not have valid CPH numbers. This represented a substantial improvement from the first evaluation (2010 to mid-2012), where all DSCs initially had at, or below, 70% of records with CPH in the correct format and three had <50%. In the current evaluation, after all cleaning was completed, the proportion of valid CPHs for the overall network had risen to 80.1%. This was mainly due to improvements in six DSCs (now with a range of 84.6–90.1%).

Just over one in four (26.3%) Scottish livestock holdings made at least one diagnostic submission of any type, to the network, in 2013–2018. This is a similar, but slightly higher estimate than that obtained from 2010 to mid-2012 (23.4%).

At the end of the submission records extraction and cleaning process, 40,564 individual diagnostic submissions were identified as having been submitted from at least 6,322 unique Scottish livestock holdings, during 2013–2018. This compares to 34,035 submissions from 5,095 unique Scottish holdings identified in the first evaluation (2010 to mid-2012).

Figure 4 shows the percentage of Scottish livestock holdings making a diagnostic submission to the network and those who made a submission and submitted to their closest DSC. The results are split into the catchment areas of the individual DSCs. The results from 2013 to 2018 are comparable with the earlier results from 2010 to mid-2012 with submission rates varying between the individual DSCs. Submitting livestock holdings in the Edinburgh and Ayr catchment areas were least likely to have made at least one diagnostic submission to their closest DSC in 2013–2018, whilst Aberdeen and Dumfries catchment area holdings were most likely to submit to their closest DSC. Across the entire network 10% of holdings who submitted, did not make any diagnostic submissions to their closest DSC (Supplementary Table SM1). There were some apparent changes between the studies. The percentage of livestock holdings submitting at least once to the network was higher in six of eight DSC areas. The percentage of livestock holdings in a named catchment area that submitted at least

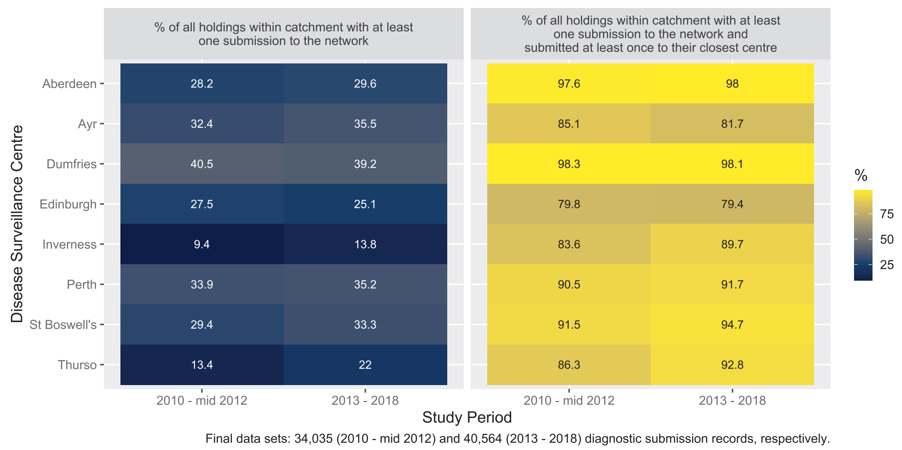

FIGURE 4
Distributions (%) of holdings by named DSC catchment area - aspects relating to all types of diagnostic submissions for 2010 to mid-2012 and 2013 - 2018.

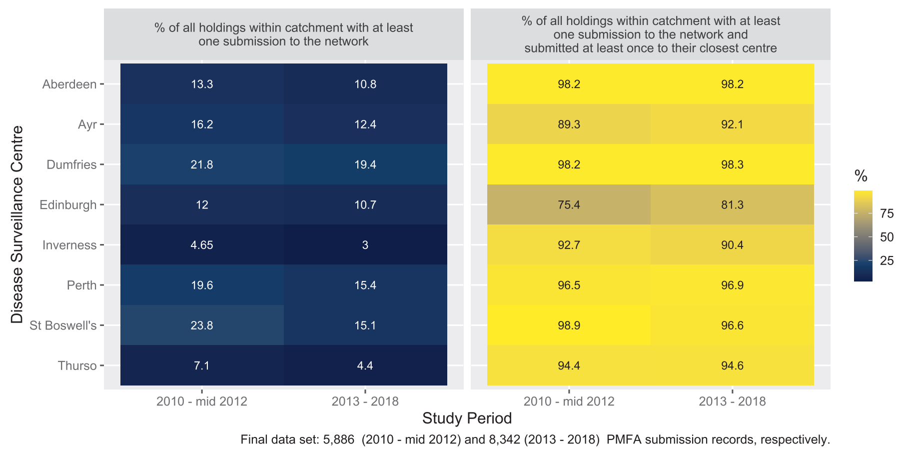

FIGURE 5
Distributions (%) of holdings by named DSC catchment area - aspects relating to PMFA submissions for 2013 - 2018 and 2010 to mid-2012.

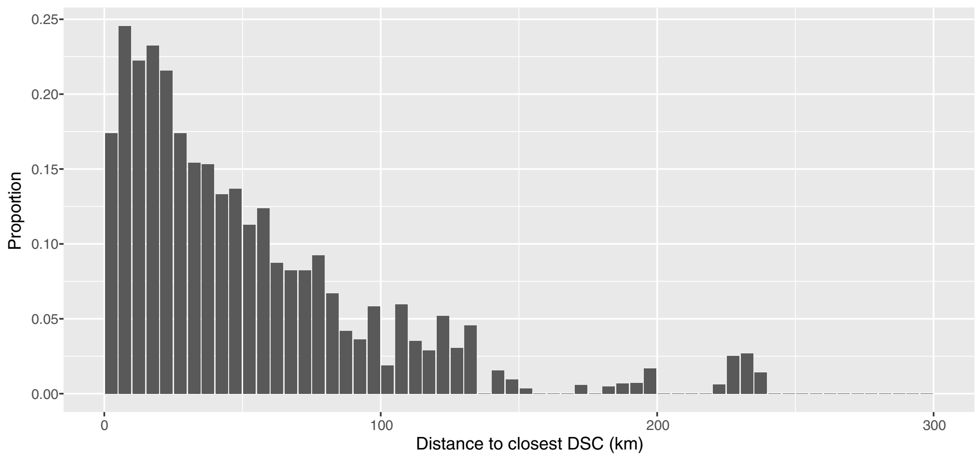

FIGURE 6
Proportion of all Scottish holdings making at least one PMFA submission, during 2013 – 2018, by the distance (in 5 km groups) to their closest DSC.

once to the network and submitted at least once to their closest centre was lower in one centre (Ayr). This value was more than 2% higher in three DSCs, with the largest increases observed for the two most northerly centres, Inverness and Thurso (Figure 4).

Of the 40,564 diagnostic submissions from 2013–2018, there were 8,342 classed as PMFA. During this period, more than one in ten holdings (12.2%) made at least one PMFA submission, to the DSC network. This is a higher estimate than the 9.4% observed in 2010 to mid-2012.

Figure 5 shows the percentage of Scottish livestock holdings making a PMFA submission to the network and those who both made a submission and submitted to their closest DSC. The results are split into the catchment areas of the individual DSCs. The complete numerical values are shown in Supplementary Table SM2.

Of the holdings that made PMFA submissions to the DSC network in 2013–2018, just over one out of 20 did not make one to their closest centre (6.1%, Supplementary Table SM2). This is slightly higher than was observed in 2010 to mid-2012 and appears to be predominantly due to apparent changes among Edinburgh catchment area PMFA submitting holdings. A lower percentage of these holdings submitted their PMFAs to Edinburgh (their closest centre) in 2013–2018 (75.4%) than they did in 2010 to mid-2012 (81.3%). This was the only centre where the percentage of holdings submitting PMFA to their closest centre, given they made a PMFA submission at all, was lower when compared to all diagnostic submissions (Figure 4). The proportion of all Scottish holdings that made at least one PMFA submission to the DSC network is negatively correlated with distance to their closest centre (Figure 6). A similar, but weaker relationship, was found when all diagnostic submissions were examined (Supplementary Figure SM12).

For all diagnostic submissions (not just PMFAs), most holdings submitted only to their closest DSC. However, there were 547 holdings that did not make any submissions to their closest DSC (Supplementary Figure SM13). These holdings were well distributed throughout the locales of the individual DSCs and are not restricted to a particular catchment area (Supplementary Table SM3).

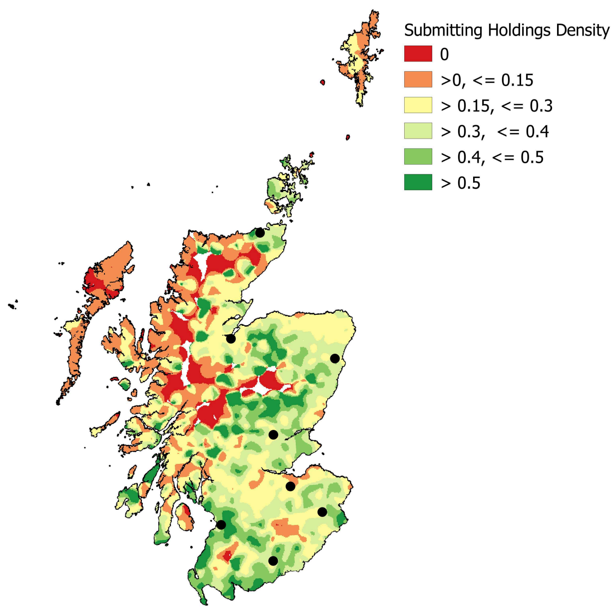

FIGURE 7
Density ratio – the proportion of Scottish livestock holdings who made at least one diagnostic submission to the network in the period 2013 - 2018 (Kernel density, 10 km radius, grid cell size 1 km^2) with the locations of the eight DSCs (black spots).

Descriptive spatial analysis of network usage

There were a few areas where livestock holdings were present but <15% made at least one diagnostic submission of any type to the DSC network in the period 2013–2018 (Figure 7). These areas were mainly along the North West coast and in the Western Isles, although such areas were scattered across other islands and remote areas, as well as a few more southern and eastern areas. Areas where more than half of the livestock holdings have made one or more submissions in the time period were not necessarily those closest to the DSCs.

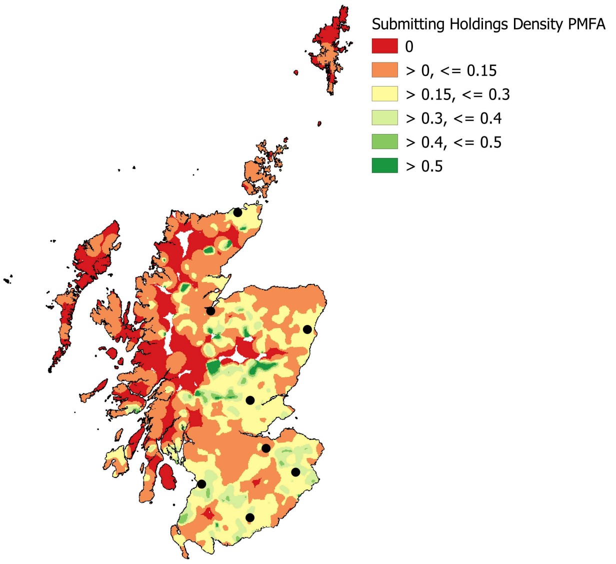

FIGURE 8
Density ratio – the proportion of Scottish livestock holdings who made at least one post-mortem farm animal (PMFA) diagnostic submission to the network in the period 2013 - 2018 (Kernel density 10 km radius, grid cell size 1 km^2) with the locations of the eight DSCs (black spots).

The under-represented areas became larger when the dataset was limited to the subset of livestock holdings that made at least one PMFA submission (Figure 8). The reduction in those areas where more than half of the holdings have submitted was also noticeable.

Discussion

We have evaluated the potential coverage and the usage of the Scottish network of disease surveillance centres for the period 2013–18 and estimated how far it reached i.e., we have described the footprint of submitting holdings during this period. During the evaluation, we developed a comprehensive extraction and cleaning process for the submission records; highlighted areas for consideration for improvement in the data collection process; developed a process for extracting a comprehensive denominator dataset for Scottish livestock holdings from existing demographic and movement datasets, and improved our confidence in the outputs compared to the earlier evaluation. This has enabled us to a produce a robust assessment of the performance of the DSC network, in terms of the attributes of coverage and data quality. It has facilitated the production of information about usage and the relationship with distance and established a baseline reference for both the surveillance provider and science-policy advisors.

With improved confidence in the data and analytical outputs, compared to the 2010 to mid-2012 evaluation, we can now start to propose potential hypotheses, with regards to drivers for submission and to propose areas that need considering, if improvements to data quality, or usage of the network, are required.

Comparison with previous study

While it is tempting to compare the results from the analysis of submissions from 2013 to 2018 with those from 2010 to mid-2012, this should be done with extreme care; several difficulties arise. Both the submission and holding datasets - numerator and denominators - in this re-evaluation were created using some of the lessons learned from the initial one. This time, with the longer period analysed, the holding dataset made use of movement data in addition to the agricultural census used earlier. The data from the census represents a snapshot of the animals on Scottish holdings. Using only a single snapshot of a single year is unlikely to produce an accurate picture of the holdings that could potentially make a submission. For example, due to their mobility and the sheep calendar year effect, the Animal and Plant Health Agency's Livestock Demographic Data Group[14] prefer to use the end of year Sheep and Goat Inventory data for sheep. This dataset was not available to us. Any change identified in the denominator could be due to an actual change in the industry sectors[15] over time, a change due to the methods, or both.

Similarly, the extraction of the submission dataset and particularly the cleaning/matching of CPH numbers went through an, in our opinion, improved process. This was due to our ability, for this re-evaluation, to bridge the gap between those collecting and managing the data and those using it. SRUC VS personnel were integrated into the evaluation team from the outset. They were able to explain some of the idiosyncrasies of the LIMS used by the DSCs and co-construct approaches, as outlined in the methods section. One outcome of the re-evaluation was an improved understanding of how submitted samples are recorded at receiving DSCs, how they are recorded if they are subsequently moved within the network for the purposes of diagnostic testing and how these processes relate to submission records. As these processes were considered in the new data cleaning protocol, we can be more confident that we have not over-estimated the effect of incorrect CPHs and double-counted samples. Any apparent change in the numerator dataset when comparing 2013–2018 and 2010 to mid-2012, could be due to several factors. These include the cleaning process, changes in propensity to submit, an increase in disease events over the period, increased days at risk, or a mixture of any of these factors and so should be interpreted with care.

The results of this re-evaluation suggest that accurate recording of CPH numbers has improved in the data from 2013 to 2018 compared with 2010 to mid-2012. The original evaluation and its outcomes may have contributed to this overall improvement in accurate CPH recording, as it did include substantial hand-cleaning of the 2010 to mid-2012 submissions data by DSC staff. Another possible contributing factor is improved completion of CPH on submission forms by submitting private veterinary practitioners. This may be an effect of the Scottish Government's Bovine Viral Diarrhea eradication scheme.[16] Launched in 2012, CPH numbers were, and still are, mandatory for submissions made for this scheme, potentially

14 http://apha.defra.gov.uk/documents/surveillance/diseases/lddg-pop-report-sheep2021.pdf

15 https://www.gov.scot/publications/results-scottish-agricultural-census-june-2021/pages/3/

16 https://www.gov.scot/publications/scottish-bvd-eradication-scheme/

leading to increased familiarity and compliance with completion of CPH numbers requested on other submission forms.

However, there was one DSC for which the new process could still not achieve as good an endpoint, in terms of the percentage of CPHs in the correct form, as in the original evaluation. This is most likely due to the receipt at this DSC of substantial numbers of submissions from holdings across Scotland and non-Scottish holdings, whereas other DSCs tend to receive submissions predominantly from the local area. On receipt of samples at these DSCs, staff are more likely to be able to use local knowledge to supplement sparsely completed submission forms. This will lead to a greater proportion of CPHs that are entered in a valid format. Similarly, it is likely that, in the original re-evaluation process, the hand-cleaning process was able to identify and delete inappropriate submission records in a way that cannot be matched by rule-based algorithms and methods. This could have led to an apparent improvement in the performance at this specific DSC. It is also possible that the phased implementation of changes to the network during the latter part of the evaluation period, and increased centralisation of diagnostic testing may have resulted in more submissions entering the network *via* a different DSC. At the entry-point DSC, the CPH could have been recorded accurately; it would not necessarily be recorded on receipt internally at the final DSC. The new cleaning process should, however, have captured these records.

Despite the improvement in correctly recorded CPHs and given all the caveats stated above about making inter-evaluation comparisons, the overall results from the two evaluations are remarkably similar. This provides some additional confidence that these missing data do not have a major effect on the answers to the questions being asked here. In an ideal world there would be little to no incomplete, or missing data. However, a balance must be found that enables operations to be conducted within the resources available, while optimising the utility of the data collected. One possible way of addressing this correct CPH issue would be for an automated cross-check between the data entered and a regularly updated master list of the CPH register. The latter is not available to the DSC network for the purpose of routine diagnostic submissions. Another option considered in the past was to provide a discount to clients where a valid CPH is provided on the submission form.

Submissions

Individual farmer or veterinarian preference over which DSC to utilise can be influenced by professional relationships (10, 28) with the SRUC VS personnel. This may be based on perceived knowledge and experience, be it local, disease or species-specific expertise and may apply particularly when the submission can be delivered by a third party e.g., posted. Location of laboratories and quality of advice were the two key features identified in a questionnaire survey, which informed the 2011 Review of Veterinary Surveillance (see text footnote 7). Although we have not explored these aspects in the current evaluation, there was some evidence of a species expertise effect associated with porcine samples in the earlier evaluation (data not shown).

As far as submission type is concerned, throughout our analysis, we have worked under the assumption, confirmed by SRUC VS staff, that PMFA submissions should require transportation to a DSC by the animal keeper, whereas non-PMFA submissions typically arrive by post. This increases the likelihood of a relationship between distance and submission rate for PMFA submissions. The differences in density of submissions and proportion of holdings submitting show that in general, as distance to the closest DSC increases, a submission becomes less likely. A similar relationship is found with all submissions, but the decrease in proportion submitting is less severe. This relationship of distance, in conjunction with holding density may help to explain the lower submission rates at the two most northerly centres, Inverness and Thurso. For example, Inverness DSC has fewer than 25% of holdings from its catchment area within 50 km of the DSC location. We used 50 km as our assessment distance of the denominator for the overall network and individual DSCs as it had been stipulated for the initial evaluation. It approximates to an hour and a half complete journey time, based on an average driving speed of 40 mph, total journey distance equal to 60 miles; radius would be 30 miles i.e., approximately 50 km. The influence of distance has been noted previously. Kinnaird[17] reported that "farmers or crofters who reported using a diagnostic laboratory were based an average of 40 miles away from the nearest SAC laboratory (DSC), compared with those who never used diagnostic laboratories, who were on average around 70 miles away."

The importance of this relationship of distance and likelihood to submit can play an important role in the policy decisions around where to locate DSCs and how to operate the network. Previous studies have highlighted the role that farmers and veterinarians can play in disease surveillance (10) and how human connections between those involved in surveillance can be critical in identifying both a new epidemic and monitoring endemic disease (28). Whilst new technologies can help with increasing testing at an individual holding (11) PMs will continue to be required and if these need to be transported, location of the PM facilities needs consideration (19, 20). Alternative initiatives can be, and have been, implemented elsewhere to reduce the effects of distance. Carcase collection services and the establishment of "a tiered surveillance network that provides 95% of holdings and animals with access to a post-mortem facility or collection point within an hour's travel time (up from the current 50%)" were advised by the Surveillance Advisory Group (2012) for the review of services in England and Wales.

There will be other factors that influence the ultimate decision to submit, as is evidenced by those areas in the density ratio plots where more than 50% of holdings present are submitting, but the areas are not located in close proximity to a DSC. Throughout this work each holding identified was treated as identical, regardless of size. Therefore, smallholdings, crofts and commercial farms are considered the same. This decision was made because the primary aim of this evaluation was not to determine drivers for submission, but to determine whether the data quality had improved, in terms of the ability to geo-locate and identify Scottish livestock holdings that had submitted to the surveillance network.

It is quite possible that the numbers of livestock on a holding play a part in the decision to submit, as flock and herd size are so often risk factors for disease occurrence and it was stated in the 2011 Review of Veterinary Surveillance (see text footnote 7) that "There was also a significant link between the size of herds or flocks and frequency of use, with larger units making greater use of laboratories.". There may also be other influences; previous studies have shown that smallholders and commercial farms move animals differently (29) and may need to be considered differently when it comes to biosecurity and surveillance (30). The implications for these different types of holding has already been envisioned for future scenarios (31) and may well need to be considered when exploring the reasons behind DSC usage. The interpersonal relationships with both their local veterinary practice and the local DSC are likely to be different for a smallholder, or crofter, compared to a commercial farm. Likewise, the financial incentives for disease investigations involving the DSC network, and this may be reflected in the likelihood to submit. Most of the areas where <15% of holdings have made at least one diagnostic submission of any type to the DSC network in 2013–2018 are areas that are traditionally associated with the Scottish croft system of livestock ownership and management, or are remote mountain and moorland areas.

For this re-evaluation we have used the term "coverage" to refer to the Scottish livestock holdings that have the potential to submit to the DSC network. If they were to experience a disease event, which led to a decision that further diagnostic support was warranted. This differs from the more usual use of this term as a surveillance attribute for the coverage achieved, which we term "usage", i.e., the holdings that did submit. Ely et al. (32) explored different measures of assessment for pig submissions in four areas of England and discussed why the values obtained varied. We have assumed that the decision to submit is made at the holding-level, while recognising that there will be multiple factors that can play a part in arriving at this decision.

Future work

For this analysis we opted to use straight-line distance to define the DSC catchment areas and the distance to the closest DSC. This does lead to some potential anomalies, most notably when this leads to livestock holdings from the same island being allocated to two different DSCs. It is more likely that the geographical and transport routes will be similar for the whole island. Remote and rural transport routes are often defined by the topography; this may be a contributing factor to the number of holdings that never submit to their closest DSC, when that closest DSC is established by straight-line distance. In addition to these topographical and transport influences, there may also be individual farmer or veterinarian preference and that of submission type. These geographical differences are likely to be one cause of the observed bimodal distributions (Figures 6 and Supplementary Figure SM12). An element of future work could include conducting a thorough route analysis (26, 27), as these techniques may enable a better understanding of any transport related differences in submission rates.

Now that we have confidence in the submissions data set, future work could also include a thorough investigation into the drivers for submission. However, there remains the difficulty of how to assign an accurate value to number of animals on each holding at each point in time and as the analysis was conducted over a five-year period, it also raises the question of how to summarise herd size over time.

Conclusions

Diagnostic services serving agricultural communities are a mainstay of many surveillance systems. However, there are questions about how these networks should be set up or existing networks modified, how representative they are of the whole population at risk and whether they indeed need to be. Outputs from such passive surveillance systems can be hard to interpret and extrapolate as it is often suspected that only a proportion of those eligible to submit do so and any potential for bias in the system is poorly described. This highlights the need for regular evaluation. With our evaluation of the DSC network, we have established a baseline reference footprint that is of use to both the surveillance provider and science-policy advisors, who fund the network for surveillance purposes. This baseline can be used in future assessments of the network. These could examine how changes in the network that were implemented from 2019 onwards, and other shocks such as the UK's exit from the European Union and pandemic restrictions, have affected usage of the DSC network. We also now have sufficient confidence in the data to investigate possible drivers for submission, if that knowledge is required. In 2022/23, a veterinary surveillance intelligence unit is being established to make improved use of additional data sources, strengthen links with users of the network and ensure that it acts as a surveillance multiplier with an overall picture of livestock disease and trends in Scotland. In addition a new LIMS is being introduced. Ideally, with these two new initiatives commencing, we would now begin to re-evaluate the footprint from the next 5 year period (2019 to 2023), as a prelude to subsequently evaluating the impact of these further changes.

Data availability statement

The data analysed in this study is subject to the following licenses/restrictions: The data generated and analysed by this study are available from APHA, ScotEID, and SRUC VS but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of APHA, ScotEID, and SRUC VS. Requests to access these datasets should be directed to ST, sue.tongue@sruc.ac.uk.

Author contributions

AD carried out the data preparation, conducted most of the analysis, and interpreted the data. JE contributed to the analysis and interpretation of the data. FB extracted the submission data and contributed to the interpretation of the data. AJ and JS extracted the holding dataset. ST conceived the idea, sourced the funding, led, managed the project including setting the direction, and contributed to the interpretation of the data. All authors contributed to revision and drafts of the manuscript and approved the final version.

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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/fvets.2023.1099057/full#supplementary-material

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Corrigendum: Evaluations of the disease surveillance centre network in Scotland: what parts has it reached?

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In the published article, there was an error. Some typographical errors were in the abstract that were not picked up during the proofing stage.

A correction has been made to **Abstract**. This section previously stated:

"Regular evaluation is a prerequisite for systems that provide surveillance of animal populations. Scotland's Rural College Veterinary vices' Disease Surveillance Centre (DSC) network plays an integral part in surveillance to detect new and re-emerging threats within animal populations, predominantly livestock. In ronse to surveillance reviews and proposed changes to the network, an initial evaluation of diagnostic submissions data in 2010 to mid-2012 established a baseline "footprint," while highlighting challenges with the data. In this recenaluation for the period 2013–2018, we developed a new denominator using a combination of agricultural census and movement data, to identify relevant holdings more accurately. Iterative discussions between those processing submissions data ahose involved in collection at source took place to understand the intricacies of the data, establish the most appropriate dataset, and develop the processes required to optimize the data extraction and cleansing. The subsequent descriptive analysis identifies the number of diatic submissions, the number of unique holdings making submissions to the network and shows that both the surrounding geographic region of, and maximum dise to the closest DSC vary greatly between centers. Analysis of those submissions classed as farm animal post-mortems also highlights the effect of distance to the closest DSC. Whether specific differences between the time periods are due to changes in the behavior of the submitting holdior the data extraction and cleaning processes was difficult to disentangle. However, with the improved techniques producing better data to work with, a new baseline foot prior the network has been created. This provides information that can help policy makers and surveillance providers make decisions about service provision and evaluate the impact of future changes. Additionally, thtputs of these analyses can provide feedback to those employed in the service, providing evidence of what they are achieving and why changes to data collection processes and ways of working are being made. In a different setting, er data will be available and different challenges may arise. However, the fundamental principles highlighted in these evaluations and the solutions developed should be of interest to any surveillance providers generating similar diagnostic data".

The corrected section appears below:

"Regular evaluation is a prerequisite for systems that provide surveillance of animal populations. Scotland's Rural College Veterinary Services' Disease Surveillance Centre (DSC) network plays an integral part in surveillance to detect new and re-emerging threats within animal populations, predominantly livestock. In response to surveillance reviews and proposed changes to the network, an initial evaluation of diagnostic submissions data in 2010 to mid-2012 established a baseline "footprint," while highlighting challenges with the data. In this recent evaluation for the period 2013–2018, we developed a new denominator using a combination of agricultural census and movement data, to identify relevant holdings more accurately. Iterative discussions between those processing submissions data and those involved in collection at source took place to understand the intricacies of the data, establish the most appropriate dataset, and develop the processes required to optimise the data extraction and cleansing. The subsequent descriptive analysis identifies the number of diagnostic submissions, the number of unique holdings making submissions to the network and shows that both the surrounding geographic region of, and maximum distance to the closest DSC vary greatly between centres. Analysis of those submissions classed as farm animal post-mortems also highlights the effect of distance to the closest DSC. Whether specific differences between the time periods are due to changes in the behavior of the submitting holdings or the data extraction and cleaning processes was difficult to disentangle. However, with the improved techniques producing better data to work with, a new baseline footprint for the network has been created. This provides information that can help policy makers and surveillance providers make decisions about service provision and evaluate the impact of future changes. Additionally, the outputs of these analyses can provide feedback to those employed in the service, providing evidence of what they are achieving and why changes to data collection processes and ways of working are being made. In a different setting, other data will be available and different challenges may arise. However, the fundamental principles highlighted in these evaluations and the solutions developed should be of interest to any surveillance providers generating similar diagnostic data."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Capturing systematically users' experience of evaluation tools for integrated AMU and AMR surveillance

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Tackling antimicrobial resistance (AMR) is a goal for many countries. Integrated surveillance of antimicrobial use (AMU) and resistance is a prerequisite for effective risk mitigation. Regular evaluation of any surveillance is needed to ensure its effectiveness and efficiency. The question is how to evaluate specifically integrated surveillance for AMU and AMR. In an international network called CoEvalAMR, we have developed guidelines for selection of the most appropriate tools for such an evaluation. Moreover, we have assessed different evaluation tools as examples using a country case format and a methodology with a focus on the user's experience. This paper describes the updated methodology, which consists of a brief introduction to the case and to the tool separately. Moreover, there are 12 functional aspects and nine content themes which should be scored using a 4-tiered scale. Additionally, four Strengths, Weaknesses, Opportunities, Threats (SWOT) questions should be addressed. Results are illustrated using radar diagrams. An example of application of the updated methodology is given using the ECoSur evaluation tool. No tool can cover all evaluation aspects comprehensively in a user-friendly manner, so the choice of tool must be based upon the specific evaluation purpose. Moreover, adequate resources, time and training are needed to obtain useful outputs from the evaluation. Our updated methodology can be used by tool users to share their experience with available tools, and hereby assist other users in identifying the most suited tool for their evaluation purpose. Additionally, tool developers can get valuable information for further improvements of their tool.

KEYWORDS

One Health assessment, integrated surveillance, evaluation, antimicrobial resistance, antimicrobial use

Introduction

It is a common goal of society to keep antimicrobials effective for the coming generations. One way of supporting this goal is to have surveillance in place for antimicrobial use (AMU) and resistance in different domains and sectors. This should preferably be done in an integrated manner, because genes coding for antimicrobial resistance (AMR) are spread within and among different human, animal and environmental domains. To ensure surveillance effectiveness and efficiency, there is a need to evaluate existing surveillance systems or components at regular intervals (1). This will help to reach the objective of surveillance which, among others, is to determine why and where action is needed to modify AMU and hereby reduce AMR.

Several tools have been developed to assist in such evaluations. Evaluation may be done by different types of professionals, who may be acting internally or externally to the surveillance system under evaluation. The users will have varying levels of experience in surveillance evaluation, access to detailed data and time to dedicate to the evaluation. Moreover, evaluation may be pursued for different purposes. This makes it necessary to choose the right tool for a given evaluation context, team and question.

During 2019–2020, an international network of scientists called "Convergence in evaluation frameworks for integrated surveillance of AMU and AMR" (CoEvalAMR) developed guidance for the evaluation of integrated surveillance for AMU and AMR (2). In this network, we defined integrated surveillance of AMU and AMR in the context of One Health as surveillance that is based on a systemic, cross-sectoral, multi-stakeholder perspective to inform mitigation decisions with the aim to keep antimicrobials effective for future generations (https://guidance.fp7-risksur.eu/welcome/evaluation-of-surveillance/). In Phase 1 of the CoEvalAMR project, a methodology was developed to gather user feedback on evaluation tools for integrated surveillance for AMU and AMR in an easy and standardized way. The focus was on compiling user subjective experience on the application of the tools; the approach chosen was partly inspired by websites using user feedback and scoring to inform decision-making of other users. The methodology consisted of four different approaches that complemented each other. The first consisted of a brief description of the case study, whereas the second covered the assessment of 11 pre-defined functional aspects of the tool including workability regarding the need for data, time and people (Table 1). The third approach covered an assessment of seven predefined content themes related to the tools' scope (Table 2). The functional aspects and content themes were scored semi-quantitatively using a scale from 1 to 4, and a comment was requested explaining the score. The fourth approach consisted of the subjective perception of the tool assessors based on an assessment of the strengths, weaknesses, opportunities, and threats (SWOT) (Table 3).

During Phase 1, six tools were assessed using the described methodology, by applying them to eight national surveillance systems as country cases. The tools were: ATLASS (The Assessment Tool for Laboratories and AMR Surveillance Systems developed by the Food and Agricultural Organization (FAO) of the United Nations), ECoSur (Evaluation of Collaboration for Surveillance tool), ISSEP (Integrated Surveillance System Evaluation Project—now called ISSE), NEOH (Developed by the EU COST Action "Network for Evaluation of One Health"), PMP-AMR (The Progressive Management Pathway tool on AMR developed by the FAO), and SURVTOOLS (Developed in the EU FP7 project RISKSUR). An overall description of this work can be found in (2)

TABLE 1 Description of the updated list of 12 functional aspects, sorted into five groups—text in bold reflects changes to the original methodology.

Group of aspects	Functional aspect	Scales and scores*
1—Ease of use	User-friendliness **related to wording, guidance and layout of the tool or framework**	**(1) Very difficult to use, (2) difficult to use, (3) manageable to use, (4) simple to use**
	Analysis and interpretation of evaluation data	**(1) Very difficult, (2) difficult, (3) manageable, (4) simple**
	Amount **and complexity** of data required, **where complexity is defined as different kinds of data from multiple sources in different formats or as primary data collection required**	**(1) High amount of complex data required, (2) moderate amount of complex data required, (3) low amount of complex data required, (4) simple kind of existing data required**
2—Scope	**Ability to address** the stated evaluation objectives	**(1) Not at all, (2) only in a limited way, (3) yes, but not fully, (4) fully compliant**
	Evaluation of One Health (OH) aspects **(collaboration across sectors/disciplines, knowledge integration, added value of OH approach, etc.)**	**(1) No OH aspects evaluated, (2) a few OH aspects evaluated, (3) many OH aspects evaluated, (4) consistens OH evaluation throughout**
3—Pre-requisites before use	**Required level of knowledge of users regarding surveillance, epidemiology and evaluation**	**(1) Specialist, (2) routine user, (3) basic, (4) no prior knowledge or experience required**
	Training to get acquainted with the tool	**(1) Impossible without, (2) highly recommended, (3) helpful but not required, (4) not necessary**
4—Time and resources	**Costs related to the access and use of the tool**	**(1) High recurring costs, (2) low recurring costs, (3) onetime costs, (4) no costs**
	Number of people in the evaluation team	**(1) >7 persons, (2) 5–7 persons, (3) 3–4 persons, (4) 1–2 person(s)**
	Number of people to be interviewed	**(1) >7 persons, (2) 5–7 persons, (3) 3–4 persons, (4) 1–2 person(s)**
	Duration of the evaluation process	(1) >2 months, (2) 1–2 months, (3) 1 week-—1 month, (4) <1 week
5—Outputs	Generation of actionable evaluation outputs	**(1) None; (2) outputs available, but not directly actionable, (3) available outputs partially actionable, (4) available outputs fully actionable**

*"Non-applicable" can also be used when scoring each aspect.

TABLE 2 Updated description of nine content themes[a].

Theme	Description (changes to the original are in bold)
AMU and AMR	Questions that are specifically addressing the case of AMR (occurrence, prevention, or response) or AMU (recording, quantification and management)
Collaboration	Questions on the organization and functioning of the collaborative framework both for governance (including the inclusive participation of stakeholders and gender balance) and implementation of surveillance activities (including data and information exchanges, resources sharing, etc.)
Resources	Questions addressing human, material, and financial resources in terms of planning, allocation and availability. Questions on the training of human resources.
Output and use of information	Questions on integrated surveillance outputs that are provided to inform public and private stakeholders, their use to inform decision making, and the benefits from this use (expected, perceived, or measured)
Integration	Questions considering three levels of integration: 1. integration of knowledge (including that of information systems across organizations), 2. integration between sectors, professions and disciplines through a shared leadership, a shared decision making and planning process, the formulation of common goals, shared activities at the different stages of the surveillance process (data collection, communication, etc.) 3. integration at all the different decision-making levels (international, regional, national and local) and with the community
Adaptivity	Questions on any structural elements allowing the surveillance system to adapt and evolve **because of** internal and external changes. This may include governance mechanisms allowing the system to adapt (such as a steering committee with an effective feedback loop), as well as supporting tools (such as continuous learning programs, internal and external evaluation, monitoring of performance indicators)
Technical operations	Questions on technical features of the surveillance operations (surveillance design, data collection, laboratory capacities management of specimens, laboratory testing methods, data storage and management, data analysis and interpretation, communication, dissemination), their quality management (SOP, traceability), and the assessment of their performance (sensitivity and specificity)
Impact	**Questions related to all immediate, intermediate and ultimate changes (e.g., in knowledge, attitudes, practices, interventions, policies and health outcomes) that can be directly or indirectly attributed to the surveillance system. These changes can be positive and negative, intentional and unintentional**
Governance[b]	**Questions related to accountability, coordination, participation, transparency and equity.**

[a]Scale to use: 1 = not covered, 2 = not well covered, 3 = more or less covered, 4 = well covered in line with Sandberg et al. (2).
[b]Governance was included as a separate theme in the case studies undertaken in Phase 1 of CoEvalAMR but is not appearing as a separate theme on the https://guidance.fp7-risksur.eu/welcome/decision-support/.

whereas (3), described the Danish case study in detail. Moreover, a description of users' experience for each country case study can be found on the website of CoEvalAMR (https://guidance.fp7-risksur.eu/welcome/case-studies/). Some of these case studies consisted of full evaluations based on the tools used. In others, the focus was mostly on the tool, and therefore, the case study only included a superficial evaluation of the surveillance system.

TABLE 3 Description of the four questions used for the SWOT-like approach, divided into the original and updated wording.

Question	Topic	Original wording	Updated wording
1	Strengths	Things that I liked, or that it covers well	The strengths of this tool are
2	Weaknesses	Things that I struggled with	The weaknesses of this tool are
3	Opportunities	Things people should be aware of when using this tool	The added value(s) of using this tool is (are)
4	Threats	Things that this tool is not covering or not good at covering	This tool might be criticized because of

We learned that some tools can be directly used to evaluate a given question, a surveillance component or a system. Such tools have a pre-defined set of steps that need to be conducted. Other tools are better described as frameworks, which provide a theoretical background and explanation as to how the evaluation should be designed. These frameworks guide users toward the most appropriate evaluation method based on the evaluation question and context. According to Calba et al. (4), a framework acts as a skeletal support for something being constructed. Hence, it is an organization of concepts that provides a focus for inquiry. In contrast, Calba et al. (4) define a tool as a process with a specific purpose. Therefore, a tool is used as a means of performing an operation or achieving an end. The ISSE is an example of a framework (5), whereas PMP-AMR is an example of a tool (6).

Among the tools and frameworks investigated, only the ISSE framework is dedicated specifically to the evaluation of integrated surveillance of AMU and AMR, outlining a logic model that can be used to conceptualize surveillance evaluations. Other tools, such as ATLASS and PMP-AMR, are designed specifically for AMU and AMR surveillance and management, NEOH for One Health initiatives in general, SURVTOOLS for surveillance in general, and ECoSur for integrated collaboration (2).

It was concluded that all tools investigated were suitable to evaluate relevant—but not necessarily all—aspects of integrated surveillance for AMU and AMR. Moreover, each tool has its specific purpose and consequently distinct advantages and drawbacks. This makes it important to define a clear evaluation question and objective to choose the right tool. We also learned that the complexity of the tool application appeared to be proportional to the comprehensiveness of the evaluation results. Moreover, governance and impacts of integrated surveillance for AMU and AMR were not fully covered by the assessment of the tools in Phase 1.

Hence, ample experience was collected regarding assessment of the tools and the developed methodology. It was concluded that the methodology worked, but the wording and definitions could be clearer, the evaluation coverage could be broadened, and the scoring system could be more standardized. It was also of interest to understand better the expectations of tool users. Moreover, we wanted to compare the CoEvalAMR methodology with the assessment process used in the newly published Surveillance and

Information Sharing Operational Tool (SISOT) (7), developed by the Tripartite (FAO/WHO/WOAH) of the United Nations (UN). These aspects have been dealt with in Phase 2 of the CoEvalAMR project, which runs from 2021 to 2023. The objective of this paper is to present the updated methodology, including an example showing the changes, as well as the considerations behind the update.

Materials and methods

In spring 2021, monthly virtual meetings began in the network, allowing members to convene and discuss how to update the methodology. A common document was set up enabling all members to provide comments and suggestions, which were subsequently discussed with the aim of obtaining consensus. This process continued until autumn 2022. Three elements were discussed: (1) lessons learned from using the initially developed methodology, (2) an analysis of expectations of tool users, and (3) the assessment process used in SISOT. Regarding lessons learned, the approach was a brainstorm in the groups' monthly meetings.

Regarding expectations of the tool users, we considered the results of a survey by Rüegg et al. (8). The survey was conducted in Phase 1 of CoEvalAMR to gather information on evaluation of existing or planned AMU and AMR surveillance systems and people's use of available evaluation tools, as well as their expectations on tools. An analysis of the 23 answers received was undertaken. We studied and discussed how we could best make use of these results to update the CoEvalAMR methodology.

Further, we looked at the list of functional aspects and content themes in SISOT and assessed if any of these would be of value for the update of the methodology. We also studied the definitions, use of scales, and visual appearance. Based on discussions in the CoEvalAMR network group, we aimed at identifying additional functional aspects, which would make the description of the individual tools more complete.

Finally, the updated methodology was tested using a case study undertaken as part of our network. Here, ECoSur was applied to the French surveillance system for AMU, AMR and antimicrobial residues in humans, animals and the environment (9). The overall objective was to evaluate the degree and quality of multisectoral collaboration within the surveillance system. In accordance with the aim of ECoSur, the focus was on evaluating the organization, functioning and functionalities of collaboration taking place in the French multi-sectoral surveillance system. The tool is available online (https://survtools.org/wiki/surveillance-evaluation/doku.php?id=quality_of_the_collaboration), for more information about ECoSur, please see Bordier et al. (10).

Results

Lessons learned from use of the initially developed evaluation methodology

The lessons learned on the methodology in Phase 1 of the network were the following:

- It takes time to make an assessment, as this requires first to get acquainted with the tool, and next to collect the necessary information and thereafter apply the tool.
- Inevitably, there is a high level of subjectivity in the assessment process, especially when it comes to developers assessing their own tools, but also to users, who are not acquainted with the tool.
- Clear definitions for all functional aspects and content themes—including the individual scores—are needed to ensure common understanding and harmonized scoring across future assessors.
- A justification is required along with the semi-quantitative scores to ensure meaningful interpretation because a specific score can be given for different reasons.
- To illustrate variation between assessors, an approach should be developed to combine the scores from different assessors/different case-studies.
- Regarding the SWOT-analysis (Table 3), the question related to opportunities was misinterpreted by some of the tool evaluators, who referred to negative aspects of the tool instead of positive aspects.

Analysis of expectations of tool users

The analysis of the 23 answers to the questionnaire undertaken by Rüegg et al. (8) showed that the respondents emphasized the following:

- The tools should provide clear results and evidence of data integration quality that can be used with confidence in research or to inform decision making.
- Standardized guidance should be available regarding which tool to use, depending on the evaluation needs.
- There should be an increased awareness of the different integrated evaluation tools available to stakeholders and in which contexts each tool could be used.
- It should be possible to undertake different levels of evaluation from superficial to deep, to enable, e.g., a rapid "general overview" evaluation with a more detailed evaluation of selected components.
- Standardized evaluation attributes and measurements across all evaluation tools would enable comparisons to be made between evaluations that use different tools.
- Standardized evaluation methods should enable evaluations that are comparable between different components.
- All tools should be free and easy to use with services available to guide users.
- Clear and easy to use tools would help to minimize bias and subjectivity of the person evaluating the system.
- There should be an opportunity to get assistance from an expert to discuss the different tools available and how and when to use them.

Essentially, people would like to see a one-stop shop with standardized tools that are flexible and easy to use. This does not sound realistic, but it puts attention to the requirement for an approach which is simple, transparent, and with clear definitions. It also means that there should be a balance between the more detailed parts of the evaluation and the general overview.

Comparison between the CoEvalAMR methodology and SISOT

The SISOT has recently been developed by the Tripartite of the UN to support national authorities in establishing or strengthening their coordinated multisectoral surveillance and information sharing for zoonotic diseases (7). SISOT can be used for identifying useful tools and resources for creating, implementing, and/or maintaining coordinated surveillance capacity, and information sharing platforms. The intention is to collect a repository of tools and resources to help users in identifying the most suitable tools and resources. Hence, the objective is like the work undertaken in CoEvalAMR which is focusing on AMU and AMR surveillance, but for a wider context as SISOT is targeting all zoonotic diseases and health threats shared between different domains.

The SISOT Evaluation Matrix describes a tool or resource using a standardized set of criteria that can be used to evaluate whether it is fit for a given purpose. The matrix can be applied to all tools and resources, which can assist in completing any step toward creation of a coordinated zoonotic surveillance system. The criteria are used to identify the strengths and weaknesses in an objective and unbiased way. There are nine categories of criteria: (1) accessibility, (2) language, (3) data needs and management, (4) data analysis and interpretation, (5) ease of use, (6) flexibility, (7) acceptability, (8) One Health, and (9) tool impact. For each category, the evaluation must address a series of pre-defined questions. There are between 3 and 10 questions per category, and for each question a scale of 1–5 is used depending on the situation observed. Radar diagrams are used to provide a graphical presentation of the results of scoring, illustrating the scores on nine different axes corresponding to each category. An evaluation criteria score is given up to a maximum of 100%. FAO has been undertaking country pilots using the SISOT Evaluation Matrix (7).

Based on the investigation of the SISOT Evaluation Matrix and discussions in the CoEvalAMR network, we identified that the addition of the following functional aspects would make the CoEvalAMR methodology more complete:

- Type of approach: framework or tool,
- Scoring-system method (quantitative, semi-quantitative or qualitative),
- Required level of knowledge of users regarding surveillance, epidemiology, etc.
- Required training to be acquainted with the tool,
- Coverage of the tools: human domain, animal domain, environmental, and food domain and combinations thereof,
- Coverage of gender aspects,
- Accessibility, and
- Languages in which the tool is available.

The updated CoEvalAMR methodology

The following updates were made on the existing CoEvalAMR methodology: First, the description of the case study was updated (Supplementary Table S1). Then, a general description of the tool, based on 10 functional aspects, was added (Supplementary Table S2). One of these aspects was gender equity. The list of functional aspects to be scored is presented in Table 1, along with the scoring system, defined in more detail than before. The functional aspects are now classified into five groups. Similarly, the updated content themes used to describe the scope of the tool are presented in Table 2, along with the original definition and the updated definition applied in Phase 2. Two new themes were included: governance and impact. The scoring system for the content themes was maintained, implying a four-tiered scale, where 1 = not covered, 2 = not well covered, 3 = more or less covered, 4 = well covered, in line with Sandberg et al. (2). The challenges related to the four SWOT questions was solved by using the words "strengths," "weaknesses," "opportunities," and "threats" (Table 3).

Visualization of the results was improved by developing radar diagrams as a way of presenting the scoring of functional aspects and content themes. An example is given in Figure 1A for the functional aspects and in Figure 1B for the content themes. Nine axes were judged as the maximum number of axes, which could be used while having a readable graphical output. Therefore, some of the functional aspects were combined. Table 3 contains the original four questions used for the SWOT-like analysis along with the revised questions. The templates are now combined in an Excel matrix, which can be found on the website of CoEvalAMR (https://guidance.fp7-risksur.eu/case-studies/).

The Excel matrix using the revised methodology was pilot tested as part of the French case study on the evaluation of collaboration within the French surveillance system for AMR, AMU and antimicrobial residues using ECoSur (11). The completed matrix can be consulted on the CoEvalAMR case studies repository (Please see Case 9 on https://guidance.fp7-risksur.eu/case-studies/). Briefly, the assessment demonstrated that despite ECoSur being somewhat difficult to use (collection of complex data and need for prior knowledge/training before use), it covered a large part of One Health aspects and generated actionable outputs (Figure 1A). In addition, most content themes identified by the CoEvalAMR consortium as relevant to the evaluation of integrated surveillance of AMU and AMR were covered by ECoSur, with the exception of AMU/AMR specific aspects (ECoSur being a generic tool) and impacts (Figure 1B).

Discussion

In Phase 1 of the CoEvalAMR network project, it was found that the users scored the individual functional aspects and content themes in a slightly subjective way. As the project progressed, a higher degree of consensus arose regarding interpretation of the methodology, including the way of scoring (2). Moreover, we discovered that the third question in the SWOT analysis was misunderstood by some of the users. We expect that with the update of the methodology, subjectivity will be reduced. Similarly, the likelihood of misunderstanding the questions will be lower.

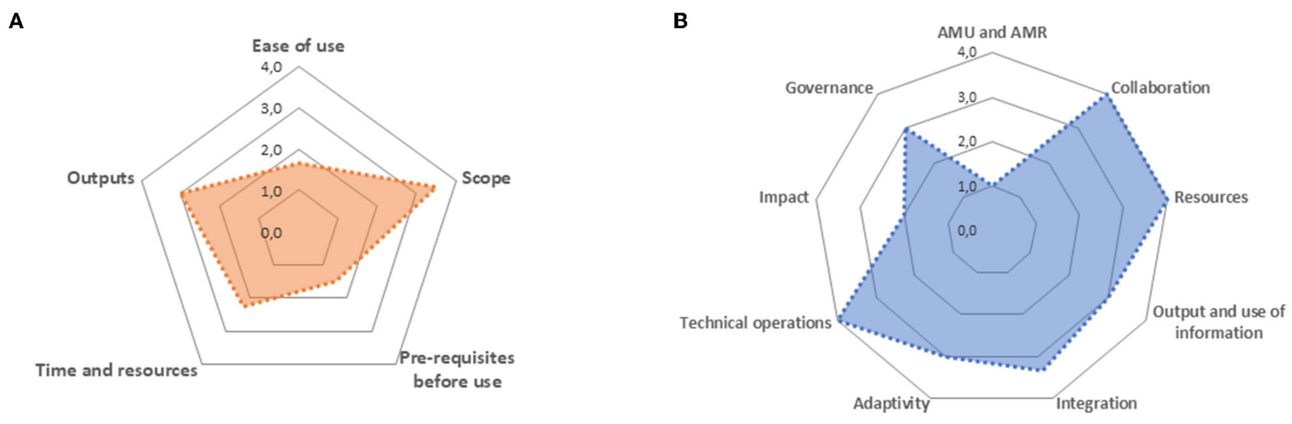

FIGURE 1
Radar diagrams depicting graphically the scoring of the functional aspects **(A)** and the content themes **(B)**, based upon a French case study using ECoSur. Source: (https://guidance.fp7-risksur.eu/case-studies/).

The importance of considering gender and equity to tackle AMR has been underlined by the WHO (12, 13) but is currently rarely integrated into surveillance system evaluation. As explained by WHO, unless we think about how AMU and AMR affect men and women and different groups in society in their day-to-day lives at home, work and in their communities, we may inadvertently design programs that fail to address what matters. Hereby, effectiveness may be reduced, and impacts lost, and we may even contribute to gaps and inequities (12). As a first step toward enhancing the inclusion of this aspect, we have added consideration of gender to the list providing a general description of the tools (Supplementary Table S2). Still, we foresee a discussion on how to assess and evaluate gender aspects and other equity issues of importance for AMU and AMR. These issues may become part of a future Phase 3 of our network. Here, chapter 4 in the Handbook for Evaluation of One Health may provide inspiration for the next steps to take (14).

The respondents of the questionnaire survey undertaken as part of Phase 1 of CoEvalAMR pointed to the need for standardization of tools (8). In response to that, we have focused on standardizing our methodology by introducing clearer definitions and scales. The question arises as to which extent further standardization of our methodology is needed. It may be argued that standardization is an essential requirement in academia, but a less important issue for persons involved with the human health and veterinary authorities, where the process initiated by the tool would be more important than the tool itself. Moreover, the intention is not to compare tools, but to describe the tools to such an extent that the future users will be guided in choosing the right tool for their purpose.

According to the survey, the users prefer tools that are easy to use, without much need for preparation or training (8). The question is how this can be operationalized. Grants are usually targeting the development of tools, whereas limited resources are available for supporting their uptake and long-term maintenance. Moreover, the results of simple evaluations may not be sufficiently valuable. Still, it is relevant to discuss the balance between required training, allocated resources, details and overview. To address this, the intended outcome of the evaluation becomes crucial. This reiterates the need for careful description of the evaluation purpose before choosing the evaluation tool.

In our updating of the methodology, we have been inspired by the SISOT matrix developed by the Tripartite. The SISOT matrix is very detailed and can be used for evaluating different kinds of tools and resources for any zoonotic risk-reducing activities. The questions and possible ways of answering show how well-developed the SISOT matrix is. Our revised CoEvalAMR tool is targeting integrated surveillance for AMU and AMR. Based upon our own experience as well as the French case study (11), the CoEvalAMR methodology appears simpler and quicker to use than the SISOT matrix, while it still contains most of the elements that form part of the SISOT matrix. In conclusion, each approach was developed for its own objectives and has its value.

The case studies reported by Sandberg et al. (2) and Nielsen et al. (3) and the French case study (9, 11) covered both multi-component and single component surveillance systems. Multisectoral means that more than one sector is working together in a joint program or response to an event. Similarly, multidisciplinary means collaboration across several disciplines. Taking a One Health approach means that all relevant sectors and disciplines are involved (15). However, it does not imply that all sectors must work together and at all stages of surveillance. The key regarding the degree of integration is relevance. For example, the Competent Authority may need AMU and AMR data in animals and humans to evaluate the effect of a ban on use of a specific kind of antimicrobial in agriculture. However, data on AMR from the environment may not be needed. In contrast, if we are trying to understand the spread of AMR in the environment, data about AMU and AMR are needed from all three sectors. The methodology we have developed is useful to provide an overview of the advantages and disadvantages of the tool investigated, irrespective of whether the tool was used for evaluation of an integrated or non-integrated surveillance system.

Evaluation of One Health surveillance is an active field, and there is a growing number of evaluation tools becoming available. The Canadian One Health Evaluation of Antimicrobial Use and Resistance Surveillance (OHE-AMURS) tool is an example of such a new tool. It has been created to evaluate progress toward integrated, One Health surveillance of AMU and AMR while focusing among others on policy and programme sustainment (16). In Sandberg et al. (2), six tools were retained for evaluation. The ambition in Phase 2 of CoEvalAMR is to apply the updated evaluation methodology to other tools, in accordance with the needs or interests of the network members. The French case study is an example of this. It showed that there is a diversity of individual surveillance programs in France (9). This makes it difficult to get an overview of the surveillance system and its level of integration (11). The ECoSur evaluation provided this overview and helped to identify recommendations, which were shared with policy makers to improve One Health collaborations within the French system for surveillance of AMR, AMU, and AM residues (11).

An ongoing common activity in WG4 of CoEvalAMR is an evaluation of the OH-EpiCap tool, which is under development by the MATRIX consortium, funded by the One Health European Joint Program (17). In a common paper about OH-EpiCap, it will be investigated how we can combine the scores of the different assessors and case studies in a way which ensures that the variation is reported.

Other persons involved in surveillance evaluation are welcome to make use of our methodology. Moreover, the tool developers can get valuable information from our case studies for further improvements of their tools.

Conclusion

The CoEvalAMR evaluation methodology is developed with a focus on the users' experience. It is free to use, simple and easy to work with. It has been updated to improve clarity, broaden the evaluation coverage, increase the standardization, and improve the visual appearance. The update was based upon experience from the CoEvalAMR network group from applying the methodology using country case studies, a questionnaire focused on the users' needs as well as a comparison with SISOT Evaluation Matrix developed by the Tripartite.

Data availability statement

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

Author contributions

LA took the initiative to update the methodology and headed the revision process through a series of meetings in WG4 of the CoEvalAMR network. Based upon the inputs from the group, LA drafted the first version of the paper, which was commented by all authors. The excel version of the CoEvalAMR tool was developed by PM. LC is responsible for the French case study. All authors read and approved the final version of the manuscript.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1107122/full#supplementary-material

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Can we use meat inspection data for animal health and welfare surveillance?

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Ante- and post-mortem inspections at abattoir were originally introduced to provide assurance that animal carcasses were fit for human consumption. However, findings at meat inspection can also represent a valuable source of information for animal health and welfare surveillance. Yet, before making secondary use of meat inspection data, it is important to assess that the same post-mortem findings get registered in a consistent way among official meat inspectors across abattoirs, so that the results are as much independent as possible from the abattoir where the inspection is performed. The most frequent findings at official meat inspections of pigs and beef cattle in Sweden were evaluated by means of variance partitioning to quantify the amount of variation in the probabilities of these findings due to abattoir and farm levels. Seven years of data (2012–2018) from 19 abattoirs were included in the study. The results showed that there was a very low variation between abattoirs for presence of liver parasites and abscesses, moderately low variation for pneumonia and greatest variation for injuries and nonspecific findings (e.g., *other lesions*). This general pattern of variation was similar for both species and implies that some post-mortem findings are consistently detected and so are a valuable source of epidemiological information for surveillance purposes. However, for those findings associated with higher variation, calibration and training activities of meat inspection staff are necessary to enable correct conclusions about the occurrence of pathological findings and for producers to experience an equivalent likelihood of deduction in payment (independent of abattoir).

KEYWORDS

meat inspection, beef cattle, finishing pigs, lesions, variance partitioning analysis, animal health surveillance

1. Introduction

In Europe, all food-producing animals are subjected to official *ante-* and *post-mortem* inspections at slaughter (1). Such activities were originally introduced to provide assurance that animal carcasses were fit for human consumption (2). However, it was subsequently recognized that such inspections also play an integral role in assessment of animal health and zoo-sanitary status, as well as in detection of certain welfare conditions (3), as they often reflect the standard of the housing and husbandry of the animal during the production period (4). Nevertheless, the information collected at meat inspection is not yet exploited to its full potential (5).

An extensive review of current practices of meat inspection in the European Union (EU) (3) reports that traditional meat inspection according to EU regulations is in principle conducted in most Member States. However, the regulation is not necessarily fulfilled with respect to all detailed requirements and inadequate ante- and post-mortem inspection still occurs. In addition, the EU legislation concerning judgment criteria at meat inspection is mostly generic, allowing flexibility on one side but also subjectivity on the other side (6). Such variability in meat inspection procedures leaves an open question about the suitability of data collected during meat inspection for animal health and welfare surveillance. In particular, it is not yet clear which inspection findings should be included in a surveillance system at the abattoir and whether data collected at meat inspection are usable for the purpose. For the information to be valuable, it is imperative that the post-mortem findings are accurately diagnosed and consistently detected, so that the result of the inspection does not strongly depend neither on the individual inspector nor the abattoir where the inspection takes place.

The accuracy of routine meat inspection of pigs has been evaluated in previous studies. Bonde et al. (7) estimated the sensitivity and specificity of post-mortem inspection performed by meat inspectors in comparison to veterinary researchers. Schleicher et al. (8) estimated the amount of variation in the recording of post-mortem findings that can be attributed to the individual official meat inspectors. In both cases, the evaluations were done at inspector level and involved one single abattoir, making it difficult to generalise the results to the common meat inspection practice. Van Staaveren et al. (9) investigated the use of carcass lesions as indicators of animal health and welfare on farm. However, the inspection at abattoir was performed by one of the authors using a pre-defined scoring system, which might not reflect the practice of meat inspection under working conditions. More recently, Klinger et al. (10) assessed the impact of farm of origin, abattoir and time of the year on the prevalence of post-mortem findings recorded in 66 Austrian abattoirs. The pathological findings were grouped into five main categories (i.e., respiratory system and heart, abdominal organs, skin and locomotor system, other pathologies, and slaughter-related lesions) which does not allow to assess the accuracy of individual lesions for their potential use in surveillance. When it comes to meat inspection in cattle, the body of literature is slimmer. Veldhuis et al. (11) quantified the associations between meat inspection findings and farm of origin characteristics in dairy cattle slaughtered in one abattoir, concluding that seven indicators provided added value to existing cattle health surveillance components, however the implementation will be challenging due to lack of standardization between abattoirs. Denwood et al. (12) estimated the farm and abattoir variation in meat inspection of beef cattle, concluding that the sensitivity of meat inspection is affected by differences in the working practices between abattoirs, resulting in biased prevalence estimates.

The aim of this study was to evaluate the quality of meat inspection data collected from several abattoirs under real working conditions, to assess which findings are currently consistently detected among abattoirs and might therefore become suitable surveillance information. To reach this objective, we estimated the amount of variation in the registered post-mortem findings of pigs and cattle that can be attributed to the abattoir where the carcasses are inspected.

2. Materials and methods

We used 7 years of data on all findings recorded in beef cattle and finishing pigs slaughtered from 2012 to 2018 in the 19 largest abattoirs in Sweden, slaughtering altogether approximately 80% of the animals in the country. Eight abattoirs slaughtered only cattle, five only pigs and six both cattle and pigs. Most farms slaughtered their animals in a single abattoir, while one-fifth supplied two abattoirs and only a few supplied three or more. More information about the number of animals slaughtered, individual batches received, and different farms served by each abattoir as well as the distribution of farms by number of abattoirs used is given in the Supplementary material (Supplementary Tables S1, S2). To reduce the variation due to animal characteristics we restricted the beef cattle population to a subcategory named *young bulls*, which identifies non-castrated male beef cattle aged 24 months. Finishing pigs included females that have never been pregnant and castrated males, aged 5–6 months and weighing around 120 kg at slaughter.

In Sweden, meat inspection is performed by official veterinarians and auxiliaries employed by the Swedish Food Agency. Official inspectors undergo specific training with regular follow ups and perform meat inspection according to EU Regulations, adopting a common national frame for condemnation (13). Meat inspectors observe carcass parts on the slaughter line before dressing and weighing. The time allowed for postmortem inspection depends on multiple factors, including the number of inspectors involved. For instance, in the case of pigs, if the speed of the slaughter line is 450 pigs per hour, four meat inspectors are present for visual control. However, if the speed is 90 pigs per hour, only one meat inspector conducts visual inspection. In situations where the speed is between 86 and 100 pigs per hour, there is a mere 36–42 s per carcass and organs available for inspection. The speed of the line, and therefore the number of inspectors, can vary significantly between abattoirs. Unfortunately, we were unable to obtain such information. At meat inspection, up to five different findings can be recorded at carcass level using a standardized coding system including 37 different lesions or abnormalities. If more than five lesions are observed, not all of them will be recorded. The full list of lesions/abnormalities and their description is provided in the Supplementary material (Supplementary Table S3).

Data were aggregated at batch level, which was defined as a group of animals delivered from an individual farm to a given abattoir on a given date. In total, 113,305 cattle and 166,658 pig batches were investigated. The mean batch size (±standard deviation) was 7.5 ± 7.5 for young bulls (min = 1, max = 121, median = 5) and 100 ± 87 for finishing pigs (min = 1, max = 758, median = 75).

Only lesions whose overall prevalence at carcass level in the study period exceeded 0.5% were included in the analysis. Their description, as provided to meat inspectors for their assessment, is given in Table 1.

In order to quantify the proportion of total variance in the outcome (i.e., presence of a given post-mortem finding in a slaughter batch) attributable to one or more effects, we fitted a logistic mixed model with cross-classified random effects for each of the lesions as proposed by Denwood et al. (12), for pigs and cattle separately.

TABLE 1 Description of the meat inspection findings investigated in this study, as provided to meat inspectors for their assessment.

Location	Diagnosis	Description
Carcass	Joint injury	Includes all types of joint injuries, both infectious and non-infectious. Acute septic arteritis is registered as "Sepsis"
	Chronic injury	Older musculoskeletal traumatic injury, for example wounds, bruises and fractures
	Acute injury	Recent musculoskeletal traumatic injury, for example wounds, bruises and fractures
	Tail lesion, pigs only	Obvious bite marks on the tail and other tail injuries where at least 50% of the tail lost. Also applies to fully healed injuries regardless of the cause
	Abscess (other than in liver)	Macroscopic finding in the carcass or organs. Liver abscesses must be registered separately
Lungs	Mycoplasma-like lesions	In swine, mycoplasma-like lesions with a minimum presence of moderate pneumonia in at least three lung lobes or high-grade pneumonia in one lobe The code is reserved for pigs only. Similar pneumonias in other animal species must be registered as "Other pneumonia"
	Fibrinous pneumonia	Pneumonia with typical changes and location suggesting the presence of *Actinobacillus pleuropneumoniae* in pigs
	Other pneumonia	The etiology of pneumonias in cattle and sheep is difficult to assess macroscopically and therefore is must be registered as "Other pneumonia." The code must also be used to register pyemic pneumonia in pigs, e.g., in connection with tail lesions
	Pleurisy/pericarditis	Presence of acute pleuritis and focal chronic adhesions when the fibrous scar(s) on chest wall are ≥3 cm. Or presence of acute, exudative pericarditis
Liver	Common liver fluke (*Fasciola hepatica*)	Presence of grey-brown, flat, flounder-shaped parasites sized 2–10 mm (juvenile) to 20–30 mm (adults)
	Lancet liver fluke (*Dicrocoelium dendriticum*)	Presence of semi-transparent, oblong (lanceolate) parasites sized 5–15 mm
	Parasitic liver damage	Refers to so-called "white spots" in pigs and parasitic granulomas in other slaughter animals as well as bile duct changes and other changes secondary to parasitic infection (without presence of parasites). Parasitic granulomas are nodular, solid nodules containing large amounts of eosinophilic granulocytes, which give the nodules a greenish color. The nodules may become tubercle-like through cheesiness and calcification
	Liver abscess	Presence of abscesses in the liver
	Pleurisy and perihepatitis	In case of pleuritic spread as described for "Pleurisy/pericarditis" and at the same time perihepatitis which causes unfitness of the whole the liver (local condemnation). Presence of perihepatitis only is recorded as "Other liver damage"
	Other liver damage	When the liver is condemned due to findings not included in any other existing code. For example: telangiectasia, perihepatitis, cirrhosis, stasis. Perihepatitis is registered as "Pleurisy and perihepatitis "when pleurisy is present at the same time. Fatty liver is registered with its own code ("Fatty liver")
Any	Other cause	In the event of pathological changes that do not have their own code and that need additional post-mortem inspection procedures by an official veterinarian for final decision (total/local condemnation)

The full list of findings is available in the Supplementary material (Supplementary Table S3).

The response variable Y_i (i.e., the number of a given post-mortem finding for batch *i*) was described using a Binomial distribution, according to the fitted probability p_i and total number of recordings N_i in the batch *i*. The model was fitted under the assumption that the probability of a specific post-mortem finding in a slaughter batch (p_i) may depend on farm characteristics and the abattoir where the meat inspection is carried out, resulting in observations being clustered between farms and/or abattoir. Therefore, the intercept and two cross-classified random effects, one for the farm and one for the abattoir, were included. Given that the post-mortem finding occurrence showed a zero-inflated distribution, we fitted an additional random effect at batch level, to correct for overdispersion as suggested by Browne et al. (14) and Harrison (15). The general form of the model is as follows:

$$Y_i \sim \text{Binomial}\left(p_i, N_i\right)$$

$$\text{Logit}\left(p_i\right) = Z + A_k + F_j + B_i$$

Where, p_i is the probability of observing the outcome in batch *i*, *Z* the common intercept, *A* the random effect of abattoir *k*, F the random effect of farm *j* and *B* the random effect of batch *i*.

All models were fitted using the *glmer* function of the "lme4" package (16) in *R* (17).

We then calculated the variance partitioning coefficients (VPCs) that quantify the extent of clustering in the meat inspection data, or in other words, the proportion of total variance in the outcome that is attributable to the random effects. The percentage of variation explained by the abattoir is given by:

$$\text{VPC}_A = \frac{\text{Var}_A}{\text{Var}_A + \text{Var}_F + \text{Var}_B + \text{Var}_\varepsilon} \times 100$$

while the percentage of variation explained by the farm is given by:

$$\text{VPC}_F = \frac{\text{Var}_F}{\text{Var}_A + \text{Var}_F + \text{Var}_B + \text{Var}_\varepsilon} \times 100$$

where, Var_A and Var_F are the variance of random effects at abattoir and farm level estimated from the model, Var_B the overdispersion parameter (i.e., variance at batch level) and Var_ε the residual variance. The latter was approximated using the latent variable method (18) assuming that every carcass has a certain *propensity* to have a given post-mortem finding, but only those whose propensity exceeds a certain threshold actually get it. This approach well suits the meat inspection process, where a particular finding is recorded only when its size/severity is big enough to note it. The unobserved (latent) individual variable follows a logistic distribution with individual level variance equal to $\pi^2/3$, which is independent of the value of any possible linear predictor (14, 19).

Ideally, there should be a minimal variation in the way carcasses are assessed among abattoirs, meaning that VPC_A should be close to zero. Besides the actual estimate of VPC_A, it is important to assess its relative magnitude compared to VPC_F. This relation is expressed by the $VPC_{A:F}$ ratio, which shows how much bigger/smaller the variation at abattoir level is compared to the variation at farm level.

3. Results

Overall, the most frequent findings at meat inspection involved lungs and liver. They were pneumonia (5.5%), pleurisy/pericarditis (5.1%) and common liver fluke (4.3%) for young bulls (Table 2) and pleurisy/pericarditis (13.6%), parasitic liver damage (4.9%) and Mycoplasma-like lesions (3.3%) for finishing pigs (Table 3). Abscesses and injuries were also reported, albeit less frequently. In general, the frequency of occurrence of abnormal findings in cattle and pig carcasses in Sweden was quite low, with only 10 lesions (in cattle) and 13 lesions (in pigs) among the 37 monitored ones showing a prevalence higher than 0.5% in the 7-year period.

3.1. Young bulls

The results of variance partitioning for young bulls are reported in Table 2. Abscesses (other than liver) and liver parasites showed nearly no variation among abattoirs ($VPC_A \approx 0$), meaning that they were consistently detected across the investigated abattoirs. It is interesting to notice that liver flukes were consistently detected among abattoirs despite the regional differences in the prevalence of *Fasciola hepatica* and *Dicrocoelium dendriticum*; differences that were captured by a high variation between farms ($VPC_F = 37–71\%$).

Traumatic peritonitis and pneumonia showed a moderately low variation at abattoir level ($VPC_A < 5\%$), which was nonetheless lower than the variation at farm level ($VPC_{A:F} < 1$). Injuries and liver damages were the lesions with the highest VPC_A (8–15%), which was also higher than VPC_F, meaning that the probability of a young bull being identified with such lesions was strongly influenced by the abattoir where the inspection took place.

3.2. Finishing pigs

The results of variance partitioning in finishing pigs are reported in Table 3. Fibrinous pneumonia and pleurisy/pericarditis showed the lowest variation among abattoirs ($VPC_A < 1\%$). Abscesses, liver damages, pneumonias and tail damages showed a moderately low variation at abattoir level ($VPC_A < 5\%$), which was nonetheless lower than the variation at farm level ($VPC_{A:F} < 1$). Injuries and unspecific lesions (i.e., "other cause") were associated with the highest VPC_A (8–20%), which was also higher than VPC_F.

4. Discussion

The probability of observing a given post-mortem finding in a slaughter batch depends on several factors that have an impact at different stages of the supply chain, such as the animal level, farm level, and/or abattoir level. Knowing the sources of variation of the post-mortem findings might help assessing the quality of meat inspection by separating individual- and farm-related factors from the accuracy of the detecting procedure (i.e., abattoir-related factors, including both detection ability of the inspectors and slaughter line conditions).

In our study, abscesses, liver parasites and pneumonia seemed to be consistently diagnosed, while injuries, liver damages and

TABLE 2 Variance partitioning for most frequent lesions recorded in 2012–2018 at meat inspection of young bulls, sorted by frequency of occurrence (*N*=113,305 slaughter batches).

Lesion	Prevalence at individual carcass level (%)	VPC_A (%)	VPC_F (%)	$VPC_{A:F}$ (ratio)
Other pneumonia	5.46	4.67	12.84	0.36
Pleurisy/pericarditis	5.06	8.21	8.39	0.98
Common liver fluke	4.30	0.04	71.63	0
Chronic injury	3.40	16.32	9.13	1.79
Other liver damage	3.20	8.59	3.23	2.66
Liver abscesses	3.03	0.01	16.81	0
Parasitic liver damage	2.55	15.19	9.58	1.59
Lancet liver fluke	1.60	0.02	37.45	0
Joint injury	0.71	10.65	10.10	1.06
Abscess	0.68	0	0	–
Traumatic peritonitis	0.64	3.78	6.15	0.61

TABLE 3 Variance partitioning for most frequent lesions recorded in 2012–2018 at meat inspection of finishing pigs, sorted by frequency of occurrence (*N*=166,658 slaughter batches).

Lesion	Prevalence at individual carcass level (%)	VPC_A (%)	VPC_F (%)	$VPC_{A:F}$ (ratio)
Pleurisy/pericarditis	13.58	0.95	19.49	0.05
Parasitic liver damage	4.92	1.34	31.03	0.04
Mycoplasma-like lesions	3.28	6.39	17.74	0.36
Tail damage	2.71	3.97	7.61	0.52
Abscess (other than liver)	1.39	1.75	2.21	0.79
Other cause	1.10	20.48	8.37	2.45
Other pneumonia	1.04	4.95	5.05	0.98
Pleurisy and perihepatitis	0.86	2.24	8.37	0.27
Other liver damage	0.82	3.33	7.3	0.46
Joint injury	0.74	6.17	7.31	0.84
Chronic injury	0.72	8.40	3.26	2.58
Fibrinous pneumonia	0.69	0	0	–
Acute injury	0.58	17.93	1.62	11.06

nonspecific findings showed a greater variation among abattoirs. It seems therefore reasonable to infer that some post-mortem findings are easier to detect and classify while others are more prone to subjective interpretation. In the latter case, the level of training and experience of the meat inspector plays a strong role in the correct identification of the findings, in conjunction with abattoir-specific slaughter line configurations (e.g., speed, accessibility of carcasses and offal for meat inspectors, luminosity of meat inspection platform, etc.). However, sometimes it is just difficult to correctly assess a specific finding. If we take injuries as an example, the distinction between acute and chronic lesions is not always clear-cut and it might require histopathological diagnostics to correctly differentiate between them. In addition, there is currently no clear description of how extensive the injury must be in order to be registered, leading to more subjective evaluations (Table 1).

Whenever a post-mortem finding is difficult to assess—either because the slaughter line conditions do not allow it, the description of the lesion is not clear enough or the finding is intrinsically difficult to assess—the probability of detection depends on the person carrying out the examination, and these findings cannot be directly translated into surveillance data.

According to Kahneman et al. (20), the sources of failure in professional judgments rely on two main components: one is the systematic error (i.e., the average error in judgments) and the other is the noise (i.e., the variability of error in judgements). The former happens across raters (e.g., because the description of a finding is not clear enough and leave room for interpretation) while the latter is more individual-dependent and arises because of personal factors such as cognitive biases, mood, group dynamics and emotional reactions. The systematic error observed in certain post-mortem findings can be reduced through specific training and calibration activities for official meat inspectors. On the other hand, reducing the noise in meat inspection might not be so straightforward, as it involves the personal reactions of each inspector. It is however reassuring to notice that, especially for pigs, the most frequent findings at meat inspection were also those most consistently detected.

Interestingly, the probability of observing abscesses (other than liver) in cattle and fibrinous pneumonia in pigs seemed to be independent from the farm of origin or the abattoir assessing the carcass. For these lesions, in fact, most of the variation (80% and 70%, respectively) was due to the batch itself (see Supplementary Tables S4, S5), suggesting that factors such as individual animal variation, failed vaccination, existing infections, stress due to transport and/or seasonality were playing a major role.

Assessment of cattle and pig lesions showed both similar and contrasting trends. In both cases, abscesses (other than liver) were consistently detected, and injuries were more subjectively evaluated. On the contrary, pleurisy/pericarditis, parasitic liver damage and other liver damage showed a high level of variation among abattoirs in cattle but a high consistency in pigs. This is partly due to different clinical manifestations in the two species (e.g., parasitic liver damage in pigs—aka milk spots—are fairly characteristic and easier to recognize than in cattle) but also to the fact that, beyond the regular training sessions, inspectors working at Swedish pig abattoirs underwent major calibration exercises in 2017 (and subsequently in 2019).

A larger body of literature is available concerning meat inspection in pigs compared to cattle, probably because the pig sector represents one of the most economically important farming sectors in the EU and worldwide (21). Previous studies on the quality of the meat inspection process in pigs reported a moderate-to-high variation both among abattoirs (10, 22) and between official veterinarians carrying out meat inspection (23), confirming that lesions such as abscesses, peritonitis, and milk spots are more consistently detected than others (e.g., skin lesions and hepatitis) (8). In addition, other authors found that pig producers experienced distrust in meat inspection due to inconsistencies among different abattoirs (24) and that a portion of the food business operators in meat, fish or dairy processing considered the food controls non-uniform (25). This highlights the importance of continuous training of meat inspectors as well as regular calibration exercises, especially for those findings that are more prone to subjective evaluation. To this regard, a limitation of this study is that it does not take into account the intra-abattoir variation. Knowing which inspector made the assessment would have enabled us to identify systematic differences among inspectors. In the same way, knowing the slaughter line configuration of each abattoir (e.g., line speed, carcass/offal accessibility, luminosity of control stations, etc.) would have helped interpreting the systematic differences among abattoirs. Unfortunately, none of the above-mentioned information was available. Nevertheless, the abattoir variation can be considered as a proxy for between-inspector variation, given that the inspectors working at a big abattoir tend to be the same. In addition, from a producer point of view, it is important to ensure a fair inspection process irrespective of which abattoir they choose.

Meat inspection data has some intrinsic limitations, not least the fact that it is impossible to identify whether the absence of a given finding is due to lack of occurrence (i.e., true absence), lack of detection (i.e., missed finding) or lack of reporting (e.g., correctly detected by not correctly registered). Nevertheless, like passive surveillance, it can still represent a valuable source of epidemiological information for cattle and pig health and welfare, given that the most frequent findings were also those most consistently reported. However, to enable correct conclusions about disease occurrence and for producers to experience an equivalent likelihood of deduction, calibration and training activities of official meat inspectors are necessary for those findings associated to higher variation within and between abattoirs. For those lesions that are undoubtedly difficult to classify, it could be hypothesized to record a lower level of detail. In case of injuries, for instance, one could just record more generically "injury," without distinguishing between acute and chronic. However, while less detailed diagnoses may be detected more consistently, they carry less information for surveillance purposes. Within the abovementioned example of injuries, it would be very important to distinguish whether an injury is acute or chronic in the context of animal welfare surveillance, but a generic diagnosis would not allow that. In addition, a generic diagnosis would not allow to identify who is liable for financial deduction for the injury (i.e., the farmer, the transporter or the abattoir, depending on how chronic or acute the injury is). To gain further insight into the issue, we calculated percentage of variation explained by the abattoir for the combination of acute and chronic injury findings in pigs, as they had been recorded as a generic injury. The VPC_A for this combined injury was found to be 8.10% ($VPC_{A:F} = 3.52$), which was lower than the VPC_A for the two lesions taken separately. However, it is worth noting that the difference with the VPC_A for chronic injuries alone (8.40%) was minor.

Looking for the balance between accuracy and precision of diagnoses at meat inspection, another option could be to support the work of meat inspectors with new digital techniques and artificial intelligence. In particular, artificial intelligence can be used to analyze and process large amounts of data from various sources, such as image processing system, chemical sensors, and microbiological tests to identify patterns and anomalies in meat quality, classify carcasses, detect potential health hazards, and provide real-time feedback to inspectors (26, 27). As a matter of fact, such options are already under development, such for instance the use of artificial intelligence for automatic detection of abattoir lesion (ADAL | F4T Lab) (28), photo artificial intelligence identification of animals (Phaid | F4T Lab) (29) and scanning animal tattoos on slaughter line for traceability (ReaDop | F4T Lab) (30).

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.

Ethics statement

Ethical review and approval were not required for this study as it did not involve any animals, animal samples nor isolates. The study only made use of data that was previously collected and stored in a private database.

Author contributions

AC: conceptualisation, methodology and data analysis, writing—original draft, review and editing, and funding acquisition. AJ, UR, AK, LK, A-KN, AL, and JF: conceptualisation, interpretation of results, and review and editing. All authors contributed to the article and approved the submitted version.

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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/fvets.2023.1129891/full#supplementary-material

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Evaluation of SARS-CoV-2 passive surveillance in Lithuanian mink farms, 2020–2021

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The newly emerged SARS-CoV-2, causing COVID-19 in humans, is also infecting American mink (*Neovison vison*), used in fur production. Since 2020, passive surveillance of SARS-CoV-2 in mink farms was implemented in Lithuania. Here, we describe data from a survey of all 57 active Lithuanian mink farms carried out during November–December 2021 to complement passive surveillance in the country. In all 57 mink farms, nasopharyngeal swab samples were collected from dead or live mink and tested by real-time RT-PCR. Dead mink samples were tested in pools of 5, while live mink samples were tested individually. In 19 mink farms, blood serum was collected and tested for antibodies to determine previous exposure to the virus. Environmental samples from 55 farms were also collected and tested in pooled samples by real-time RT-PCR. The present survey has detected 22.81% viral RNA-positive mink farms and a high number of mink farms that were exposed (84.21, 95% CI 67.81–100%) to the virus. The increasing exposure of mink farms to the virus due to growing human COVID-19 cases and limitations of passive surveillance could explain the observed epidemiological situation of SARS-CoV-2 in Lithuanian mink farms, compared to the few positive farms previously detected by passive surveillance. The unexpected widespread exposure of mink farms to SARS-CoV-2 suggests that passive surveillance is ineffective for early detection of SARS-CoV-2 in mink. Further studies are needed to reveal the present status in previously infected mink farms.

KEYWORDS

SARS-CoV-2, COVID-19, mink, *Neovison vison*, surveillance, Lithuania

1. Introduction

The newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 19 (COVID-19), was first detected in humans in December 2019 and soon became a global pandemic (1). Susceptibility to the virus was confirmed in various mammal species as a result of contact with infected humans (2). By July 2022, 35 countries have reported the infection in 24 different animal species to the World Organisation for Animal Health (3). Among these species, American mink (*Neovison vison*), used in fur production, was found to be especially susceptible to SARS-CoV-2 infection. After the first report in Dutch mink farms in April 2020 (4), the virus was reported in 12 more countries—Canada, Denmark, France, Greece, Italy, Latvia, Lithuania, Netherlands, Poland, Spain, Sweden, and USA (5). Furthermore, a few reports indicate that feral and escaped minks have also been infected (6, 7), and mink-associated SARS-CoV-2 spill-over to humans has been observed in the Netherlands, Denmark, Poland, and possibly the USA (8, 9). The virus spreads effectively through mink and thus accumulates mutations. Mink-associated virus variants with amino acid changes in the spike protein demonstrated reduced sensitivity to neutralizing antibodies (10). The risk of infection with mink-related virus strains is highest for mink farm workers (2). There is also a risk for other farm animals since cats and dogs were found infected under field conditions (11). Furthermore, there is a risk of establishing a SARS-CoV-2 reservoir in areas with a high density of mink farms or stable wild mink populations (9).

According to European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), all mink farms are at risk of infection and should be under surveillance (12). An active monitoring approach with the main objective of the early detection of the virus has been recommended by EFSA (2). Active surveillance is highly resource-demanding. Therefore, alternative passive surveillance and a risk-based approach could be implemented (13). Passive surveillance has the potential of under-reporting due to various factors, including farmers' overall disease awareness and decision-making (14).

Since 2020, the State Food and Veterinary Service of the Republic of Lithuania (SFVS) implemented mandatory passive surveillance of the virus in the country's mink farms. Mink farm owners were obligated to report higher than usual mink mortality/morbidity rates, reduced feed consumption, and confirmed COVID-19 infection in farm personnel to territorial SFVS. Furthermore, mink farms had to provide factual numbers of mink mortality and morbidity to territorial SFVS on a weekly basis. In November 2020, the SFVS carried out a sampling of dead minks in all active mink farms in the country with negative results, although relatively few nasopharyngeal swab samples were tested per farm by real-time RT-PCR (89 samples from 69 mink farms). In November and December 2020, the first two SARS-CoV-2 infected mink farms have been detected by passive surveillance in Lithuania (15). While numerous outbreaks were detected in Europe from the start of 2021, only two more mink farms were found infected through passive surveillance in Lithuania at the beginning of 2021 (15). Furthermore, in October and November 2021, SARS-CoV-2 spill-overs from mink to humans were identified by Lithuania's SARS-CoV-2 genomic surveillance program (unpublished data, reported by G. Dudas). Considering the potential of under-reporting in passive surveillance and increasing numbers of human COVID-19 cases since September 2021, a survey was performed to investigate SARS-CoV-2 in mink farms in Lithuania. We report the survey results of the SARS-CoV-2 infection and exposure in Lithuanian mink farms, performed in November–December 2021.

2. Materials and methods

In November–December 2021, according to an order of SFVS, all active mink farms (i.e., live minks present on the farm) in Lithuania had to be sampled and tested for SARS-CoV-2. Sampling on farms was done by official veterinarians. During the survey, no movement of minks was allowed in the country.

At least 30 nasopharyngeal swab samples from either dead, sick or live mink were taken at every mink farm, with the aim of detecting a 10% within-farm infection prevalence at 95% confidence. In very few cases, less than 30 samples were taken due to intensive pelting and high workload, as well as in some farms—more than 30 samples were taken, where more than one epidemiological unit was present. The priority was to take samples from dead and diseased minks. The rest of the samples were taken from minks killed for pelting or live animals. In the latter situation, samples were collected to ensure that each sector of a farm was sampled.

Nineteen mink farms out of 57 were convenience sampled for mink blood serum to determine previous exposure to the virus. Thirty mink blood samples were collected from each farm. Both adult (more than 1-year-old) and juvenile (less than 1-year-old) minks were sampled from various places of a farm. Additionally, environmental swab samples were collected from 55 mink farms using the same swabs that were used for nasopharyngeal sample collection. Each farm was divided by area size into five roughly equal parts, and one sample was taken from every area. Environmental swab samples were collected from surfaces of mink cages, walls, ceilings, and floors of open houses, as well as from household items that had contact with the minks or farm staff. Risk-based sampling was performed as samples were first collected from open houses with increased mink mortality or morbidity if present.

Information about the exact location of every taken mink or environmental sample was collected, as well as the total number of minks present on farms. Information about the age of sampled minks was collected from most of the farms. All samples from one farm were collected and delivered to the laboratory on the same day. All laboratory testing was done at the National Food and Veterinary Risk Assessment Institute in Lithuania.

2.1. Real-time RT-PCR testing

Swab samples from dead mink and the environment were tested by real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) in pools of 5 individual samples from the same mink farm. Live mink swab samples were tested individually. All samples were kept so they could be tested individually. MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Applied Biosystems, Thermo Fisher Scientific) and KingFisher Flex system (Thermo Fisher Scientific) were used for viral RNA extraction, and TaqPath™ COVID-19 RT-PCR Kit (Applied Biosystems, Thermo Fisher Scientific) was used for real-time RT-PCR reaction, according to the manufacturer's protocol.

2.2. Enzyme-linked immunosorbent assay (ELISA) testing

Mink blood serum samples were tested individually. Blood samples were centrifuged, and the serum was collected. ID Screen SARS-CoV-2 Double Antigen Multi-Species kit (ID.VET) was used to detect anti-SARS-CoV-2 antibodies in mink serum. The solutions were prepared, and testing was done according to the manufacturer's protocol.

2.3. Statistical analysis

Microsoft Excel Spreadsheet Software (Microsoft Office Standard 2019, version 1808) and Epitools software were used for statistical analysis. A 2-sample z-test for sample proportion comparison[1] was used to compare PCR-positive and antibody-positive farms, and to calculate confidence intervals. A Chi-squared test[2] was used to compare positive and negative samples taken from adult and juvenile minks.

2.4. Ethics statement

No experimental procedures were performed on animals. All animal samples were taken by official veterinarians for a compulsory animal health surveillance program. Therefore, no ethical approval was required.

3. Results

During the survey period, a total of 57 mink farms in Lithuania were tested for SARS-CoV-2, and 25 were found positive (43.86, 95% CI 30.98–56.74%) either by RT-PCR for the presence of the virus or ELISA for the presence of antibodies. None of the sampled farms reported increased mortality or morbidity during the study. The positive farms were situated across the country with no signs of obvious clustering. At the time of sampling, the number of mink present on all sampled farms varied widely and ranged from 120 to 159,916, with an average of 14,597 animals present on farms (Supplementary Table S1).

Information about infected staff in mink farms showed that in 11 farms, at least one staff was confirmed to have been infected with SARS-CoV-2 from 4 days up to 1 year before a farm was sampled. Four of these farms were viral RNA-positive, and 10 of them were positive for anti-SARS-CoV-2 antibodies. No infected staff was reported in SARS-CoV-2-negative farms (Supplementary Table S1).

3.1. RT-PCR testing of mink nasopharyngeal swabs

In total, 13 (22.81%) out of 57 tested farms were found SARS-CoV-2 RT-PCR positive (Table 1).

A total of 943 dead mink nasopharyngeal swab samples were taken from 34 mink farms. Data about the age of tested dead mink were collected from 26 farms. Seventeen pooled samples were taken and tested from adult dead mink, and one pooled sample (5.88, 95% CI 0.0–17.06%) tested positive for viral RNA. Meanwhile, 140 pooled samples from juvenile mink were tested, and 19 of them (13.57, 95% CI 7.9–19.24%) tested positive by real-time RT-PCR (Supplementary Table S2). There was no statistically significant difference between the proportions of viral RNA-positive samples taken from adult versus juvenile dead minks ($p = 0.6081$).

In total, 1,015 live mink swab samples were collected from 35 farms. Data about the age of tested live mink were collected from 22 of these farms. Samples from 76 adult live mink were collected, and 4 of them (5.26, 95% CI 0.24–10.28%) tested positive for viral RNA. Meanwhile, 565 juvenile live mink samples were collected, and 15 of them (2.65, 95% CI 1.33–3.97%) tested positive by real-time RT-PCR (Supplementary Table S3). The difference between viral RNA-positive and negative samples taken from adult versus juvenile live minks was not statistically significant ($p = 0.3689$).

3.2. RT-PCR testing of environmental samples

A total of 55 pooled environmental swab samples were collected from 55 different mink farms, five samples each. From one farm out of 55 (1.82, 95% CI 0.0–5.35%), only one of the five individual environmental swab samples tested positive for SARS-CoV-2 RNA. The positive sample was taken from the surface of a mink cage. Dead and live mink nasopharyngeal swab samples collected from this farm also tested positive for viral RNA (Supplementary Table S1), but no blood serum samples were available for testing.

3.3. Mink serum ELISA testing

Nineteen mink farms were sampled and tested for anti-SARS-CoV-2 antibodies. The limited blood serum sampling was due to intensive swab sampling and reduced resources of veterinary and farm personnel because of the COVID-19 pandemic. In total, 570 mink serum samples from 19 farms were tested by ELISA. Out of these, 298 mink samples from 16 (84.21, 95% CI 67.81–100%) farms tested positive for anti-SARS-CoV-2 antibodies (Figure 1). Only 4 of the antibody-positive farms were also viral RNA-positive.

At the time of sampling, the number of minks on the 19 serologically tested farms ranged from 1,025 to 79,300. The average number of minks on the antibody-positive farms was 14,992 and 7,298 on the antibody-negative farms (Supplementary Table S1). Data about the age of sampled mink were collected from 13 farms. Serum samples from 79 adult minks were collected, and 29 of them (36.71, 95% CI 26.08–47.34%) tested positive for anti-SARS-CoV-2 antibodies. Serum samples were collected and tested from 311 juvenile minks, and 136 of them (43.73, 95% CI 38.22–49.24%) tested positive by ELISA (Supplementary Table S4). The difference between antibody-positive and negative samples taken from adult versus juvenile minks was not statistically significant ($p = 0.3171$).

The proportion of mink farms with anti-SARS-CoV-2 antibodies was significantly ($p < 0.0001$) higher than the proportion of viral RNA-positive farms.

4. Discussion

This study was carried out to complement the ongoing passive surveillance of SARS-CoV-2 in the Lithuanian mink farm population during the COVID-19 pandemic and continuous reports of mink SARS-CoV-2 infection from various countries. The present survey, implemented in November–December 2021, has revealed considerably more SARS-CoV-2 RNA-positive mink farms (13 farms, Table 1) than was detected (4 farms) by passive surveillance in November 2020–October 2021. This survey also revealed an unexpected widespread

1 https://epitools.ausvet.com.au/ztesttwo
2 https://epitools.ausvet.com.au/chisqone

TABLE 1 Real-time RT-PCR test results of SARS-CoV-2 survey in mink farms in Lithuania in November–December 2021.

	No. of farms tested	Average no. of mink present on negative farms	Average no. of mink present on positive farms	No. of mink tested	No. of pools tested*	Positive samples**		Positive farms	
						No.	% (95% CI)	No.	% (95% CI)
Swab samples collected from only dead mink	22	24,702	10,956	647	134	21	15.67 (9.52–21.82)	6	27.27 (8.66–45.88)
Swab samples collected from only live mink	23	7,681	7,807	670	n.d.	15	2.24 (1.12–3.36)	3	13.04 (0.0–26.8)
Swab samples collected from dead and live mink	12	18,290	11,918	641 (296 dead +345 live)	60	16 (8 pools +8 individual)	3.95 (2.05–5.85)	4	33.33 (6.66–60)
Total swab samples	57	15,800	10,525	1,958	194	52	4.3 (3.16–5.44)	13	22.81

*Dead mink nasopharyngeal swab samples tested in pools of 5 by real-time RT-PCR.
**Positive samples are presented as pooled positive samples for tested dead mink, as individual positive samples for tested live mink, and as a combination of pooled and individual positive samples where both dead and live mink were tested.
n.d., swab samples from live mink were tested individually, no pooling was done.

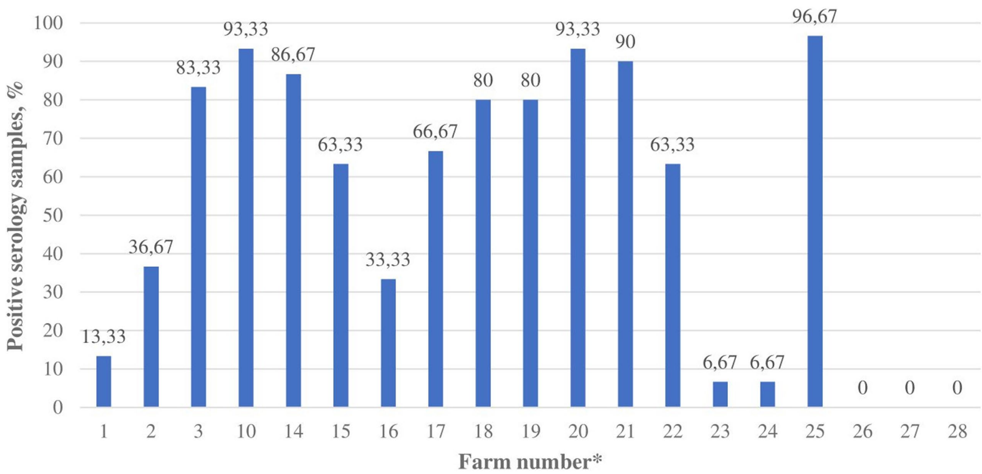

FIGURE 1
The proportion of blood serum samples with anti-SARS-CoV-2 antibodies in Lithuanian mink farms (*n*=19), November–December 2021. * Farm number corresponds to the farm numbers in Supplementary Table S1.

exposure of mink farms (84.21, 95% CI 67.81–100%) to the virus, which is evident by the presence of anti-SARS-CoV-2 antibodies in mink.

The detected epidemiological situation is the result of increasing exposure of mink farms to the virus from accumulating human COVID-19 cases in Lithuania during the second half of 2021 and limitations of passive surveillance. It has been shown in Denmark that the epidemic curve of SARS-CoV-2 in mink farms closely follows the epidemic curve of COVID-19 human cases (16). The rise of human COVID-19 cases in Lithuania has been observed in October–December 2020 and August–November 2021 (17), just before the first SARS-CoV-2 mink farm was detected by passive surveillance at the end of 2020, and before the present survey when considerably underestimated SARS-CoV-2 presence in mink farms at the end of 2021 was found, respectively. In Denmark, it has also been shown that in approx. 2 months, the number of infected farms could rise tenfold from 3 to 30 (2). The further concern is that the peak of human COVID-19 incidence in Lithuania occurred in January–February 2022, thus creating even more pressure for the virus to be transmitted to mink farms. This poses potentially dangerous possibilities for genetic mutations of the virus and a significant virus transmission risk between mink and humans in Lithuania.

COVID-19 clinical signs in mink are usually unspecific – increased mortality, mild respiratory symptoms, and decreased feed intake are observed most often, but subclinical infections also have been detected (2). In most cases, the introduction of the virus is suspected to be caused by infected humans. Therefore, the vital part of early detection monitoring should be strict periodic testing of farm personnel and other people in contact with the animals as humans are the most likely route of SARS-CoV-2 introduction into the farm (2). Once introduced in a fur farm, the virus spreads efficiently due to minks living in densely packed open houses. The contiguous cages allow for direct animal contact. SARS-CoV-2 is transmitted by direct and indirect contact (air droplets, dust particles, aerosols, and fomites). Complex biosecurity measures should be implemented on the farm to prevent the entry of the virus (2). The risk of transmission of the virus between mink and humans in Lithuania could be reduced by implementing very strict within-farm biosecurity measures (e.g., FFP respirators, goggles, hygiene, etc.), but this would be difficult to maintain at a constantly effective level for a prolonged duration as it depends on the attitude and perceptions of farm workers.

There is little data on the occurrence of SARS-CoV-2 in mink farms before the start of passive surveillance in November 2020 in Lithuania. A survey performed at the start of November 2020 has not revealed any of the active 68 farms in the country to be positive for SARS-CoV-2 infection, although only one to three nasopharyngeal swab samples were collected from dead minks. Therefore, a small sample size creates a low sensitivity of this surveillance (15). Soon after the passive surveillance started in November 2020, the first confirmed occurrence of SARS-CoV-2 in a Lithuanian mink farm has been detected. However, only this first infected mink farm was detected by passive surveillance due to the reported increased daily mortality of minks by 0.36% to SFVS. This is a little bit lower mortality than was observed (0.45%) in Denmark (16). The other infected mink farms were detected after COVID-19-infected farm workers were reported to SFVS. This information was provided from two sources – mink farmers were obligated to report to SFVS, and this information was also obtained from the National Public Health Center under the Ministry of Health, which is the official responsible authority for handling information about human COVID-19 cases.

It is not known if SARS-CoV-2 really did not cause a noticeably increased mortality and/or morbidity in Lithuanian mink farms or if it was simply not reported by the farmers, even if it was compulsory to provide the data on the mortality in mink farms on a weekly basis. The virus is known to induce subclinical infection in mink, and it has been reported in several countries like Denmark, Netherlands, France, Italy, and Greece, but clinical signs were still noticed and reported in approx. 30–42% of infected farms (2). It should be noted that clinical signs were not observed by official veterinary inspectors during the sampling in this survey. However, it could not be excluded that there was a lack of cooperation between farmers and veterinary authorities, and farmers were not willing to share information about sick animals and risk their profit and livelihood. It is also important that the passive surveillance program of SARS-CoV-2 in Lithuanian mink farms did not include any incentives that would encourage the reporting by the farmers. Meanwhile, the infected farms would have to deal with animal movement restrictions, stricter biosecurity measures, and more frequent reporting of dead and sick animals.

Underreporting is a known limitation of passive surveillance (18), and it could explain the underestimation of SARS-CoV-2 infected mink farms detected by passive surveillance in Lithuania. Another reason for this is the aforementioned lack of clinical signs and the absence of increased mortality in infected mink. The virus can cause a subclinical infection and go undetected by passive surveillance (2), which relies heavily on the observation of clinical signs (18). Most importantly, the current study was performed right after the highest spike of COVID-19 human cases was observed in Lithuania on November 6th, 2021 (over 58,000 active cases) (17). The increase of SARS-CoV-2 human cases is known to affect the virus prevalence in mink farms (16).

This investigation revealed that mink farms have been detected at various stages of SARS-CoV-2 infection. Antibodies were detected in 3.69 times more ($p < 0.0001$) mink farms than the SARS-CoV-2 RNA. SARS-CoV-2 infection was likely detected in different stages at various farms. For instance, 11 out of 16 antibody-positive mink farms tested negative by real-time RT-PCR, but most of them had a high proportion of antibody-positive samples, suggesting a long presence of SARS-CoV-2 infection in the farms. Furthermore, all pooled dead mink samples from farms No. 1 and 2 tested positive for viral RNA, but less than half of the tested mink blood samples had anti-SARS-CoV-2 antibodies, suggesting an earlier infection phase (Supplementary Table S1). Further studies are needed to explore if the virus could be eliminated from a mink farm due to the acquired immunity of minks. We found that the majority of mink farms (84.21, 95% CI 67.81–100%) could have already been exposed to the virus in Lithuania. This shows that passive surveillance has been ineffective for early detection of SARS-CoV-2 in mink. Therefore, early detection in Lithuanian mink farms might not be of major importance, but the monitoring of the virus evolution becomes the priority. Further studies are needed to reveal the present status in previously infected Lithuanian mink farms.

Research in Denmark, Poland, and Italy shows that the number of antibody-positive mink varies from 30 to 100% per farm, while viral RNA in these farms ranged from a few positive samples to 100% (16, 19–22), and we observed a similar tendency, using the same methods. Interestingly, our results show that testing of dead mink, even in the absence of reported increased mortality and morbidity, is almost two times more effective at detecting SARS-CoV-2 infected mink farms compared to only live mink sampling, although this difference is not statistically significant (Table 1). This could raise some doubts about the ability of mink farm workers to detect a disease in mink and/or the absence of proper reporting. However, the average number of animals present was greater on positive farms where only dead mink were tested compared to positive farms where only live mink were sampled. This could suggest that larger farms have more dead animals, and thus they would be more likely to do dead animal testing than smaller farms where dead minks are found less regularly. Mink age did not have an effect on the proportion of viral RNA-positive samples.

Despite the high number of viral RNA-positive or antibody-positive samples in some mink farms, only one individual sample, taken from the surface of a mink cage in one (1.82%) mink farm, tested positive by real-time RT-PCR in this study. The low number of positive environmental samples detected in our study could be due to the collection technique because swabs used in our study covered much less surface area than dust cloths used in Dutch and Greek studies (23, 24). Therefore, a method covering more surface area could have been useful to accurately evaluate the mink farm environment and compare it to the number of positive animals. Another limitation of this study was the lack of postmortem examination of dead mink. Therefore, we cannot evaluate if mink mortality could be related to SARS-CoV-2 infection. Testing a rather high number of active mink farms and restricted human resources due to the COVID-19 pandemic during the survey made it not possible to sample all farms uniformly and collect additional epidemiological information, thus limiting possibilities to investigate the course of SARS-CoV-2 in Lithuanian mink farms in more detail. Additionally, there is no data yet available about the circulating SARS-CoV-2 variants in Lithuanian mink farms, so we cannot evaluate the transmission of SARS-CoV-2 between mink farms and the human population in Lithuania in detail.

5. Conclusion

This study showed a lack of detection of SARS-CoV-2 in Lithuanian mink farms by passive surveillance. However, it remains unclear if it was caused by improper reporting or limited increase of mortality and/or morbidity of SARS-CoV-2 infected mink. The unexpected widespread exposure of mink farms (84.21, 95% CI 67.81–100%) to the virus suggests that passive surveillance is ineffective for early detection of SARS-CoV-2 in mink. Further studies are needed to reveal the present status in previously infected Lithuanian mink farms.

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 animal study because no experimental procedures were performed on animals. All animal samples were taken by official veterinarians for a compulsory animal health surveillance program. Therefore, no ethical approval was required.

Author contributions

MM, VP, PB, and AM contributed to conception and design of the study. SP and JB performed the laboratory analysis. SŽ performed the data analysis and wrote the first draft of the manuscript. AM wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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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/fvets.2023.1181826/full#supplementary-material

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Examination of critical factors influencing ruminant disease dynamics in the Black Sea Basin

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Introduction: Ruminant production in the Black Sea basin (BSB) is critical for national economies and the subsistence of rural populations. Yet, zoonoses and transboundary animal diseases (TADs) are limiting and threatening the sector. To gain a more comprehensive understanding, this study characterizes key aspects of the ruminant sector in nine countries of the BSB, including Armenia, Azerbaijan, Belarus, Bulgaria, Georgia, Moldova, Romania, Türkiye, and Ukraine.

Methods: We selected six priority ruminant diseases (anthrax, brucellosis, Crimean Congo haemorrhagic fever (CCHF), foot-and-mouth disease (FMD), lumpy skin disease (LSD), and peste des petits ruminants (PPR)) that are present or threaten to emerge in the region. Standardized questionnaires were completed by a network of focal points and supplemented with external sources. We examined country and ruminant-specific data such as demographics, economic importance, and value chains in each country. For disease-specific data, we analysed the sanitary status, management strategies, and temporal trends of the selected diseases.

Results and discussion: The shift from a centrally planned to a market economy, following the collapse of the Soviet Union, restructured the ruminant sector. This sector played a critical role in rural livelihoods within the BSB. Yet, it faced significant challenges such as the low sustainability of pastoralism, technological limitations, and unregistered farms. Additionally, ruminant health was hindered by informal animal trade as a result of economic factors, insufficient support for the development of formal trade, and socio-cultural drivers. In the Caucasus and Türkiye, where diseases were present, improvements to ruminant health were driven by access to trading opportunities. Conversely, European countries, mostly disease-free, prioritized preventing disease incursion to avoid a high economic burden. While international initiatives for disease management are underway in the BSB, there is still a need for more effective local resource allocation and international partnerships to strengthen veterinary health capacity, protect animal health and improve ruminant production.

KEYWORDS

Black Sea, surveillance and control, ruminants, transboundary animal diseases, zoonoses

1. Introduction

Livestock production is critical for the subsistence of rural populations as a source of food, income, transportation, hides, and fertilizers, contributing to 40% of the agricultural economy worldwide (1). However, in recent decades, there has been a surge and spread of endemic and exotic diseases affecting livestock (2, 3), which significantly impact the sector and threaten public health and welfare (4). This surge has been intensified by several factors, including the high increase in international trade of animals and animal products (3), rise in intensive farming driven by higher market demands for animal protein and increasing middle-class purchasing power (5–7), changes in land use (8), shifts in migration and tourism patterns (9), and the effects of climate change (9).

TADs such as foot-and-mouth disease (FMD), lumpy skin disease (LSD), and peste des petits ruminants (PPR), along with zoonoses, particularly anthrax, brucellosis (*Brucella abortus* and *Brucella melitensis*), and Crimean Congo haemorrhagic fever (CCHF) (Table 1) are diseases that are either threatening ruminants or emerging in the Black Sea Basin (BSB). Ruminant production is the most important livestock subsector in most countries in the region, ensuring food security for rural populations and contributing significantly to national economies (16–25).

Nevertheless, key aspects linked with the dynamics of these diseases in the region remain poorly understood. Knowledge gaps include disease geographic coverage and prevalence, morbidity and mortality rates, economic impact, and risk factors influencing their spread and persistence. These gaps arise from weaknesses in a country's veterinary management programmes, which can be associated with lack of human resources (authorities, veterinarians and technicians) to sustain them, inadequate government funding for agriculture or livestock sectors, limited surveillance coverage (26), insufficient legislative action, and lack of support for implementing biosecurity measures (27). As a result, disease reporting is delayed, incomplete or biased, leading to ineffective responses to disease outbreaks (28, 29). These challenges are more pronounced in rural areas of lower to middle-income countries, as the BSB, where social inequality persists. In these regions, livestock, particularly ruminants, are ubiquitous and critical for livelihoods, and animal diseases hinder food security and the sector's development.

This study characterizes ruminant production and its importance around the BSB and describes the disease status and management efforts (i.e., surveillance and control activities) for the selected ruminant diseases (Table 1) in nine countries of the region (i.e., Armenia, Azerbaijan, Belarus, Bulgaria, Georgia, Moldova, Romania, Türkiye, and Ukraine). It also explores the most relevant factors that may influence the incursion and spread of these diseases in the region.

TABLE 1 Overview of the studied diseases.

		Disease	Agent	Main domestic host (s)	Transmission	Vaccine availability
Zoonoses	Bacterial	Anthrax (10)	*Bacillus anthracis*	All mammals	Contact with *B. anthracis* spores	Yes
		Brucella (11)	*Brucella abortus*	Cattle	Direct/indirect contact	Yes
			Brucella melitensis	Sheep and goats		
	Viral	CCHF (12)	CCHF virus • g. Orthonairovirus • f. Nairoviridae	Cattle, sheep, and goats	Tick-borne	No
TADs		FMD (13)	FMD virus • g. Aphthovirus • f. Picornaviridae	Cattle, sheep, goats, and swine	Direct/indirect contact	Yes
		LSD (14)	LSD virus • g. Capripoxvirus • f. Poxviridae	Cattle	Arthropod vector	Yes
		PPR (15)	Small ruminant morbilivirus • g. Morbillivirus • f. Paramixoviridae	Sheep and goats	Direct contact	Yes

g.: genus, f.: family.

2. Materials and methods

The current paper is a component of the GCP/GLO/074/USA project, which contributes to the broader "Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs)" initiative. This project targets nine countries located around the BSB, namely Armenia, Azerbaijan, Belarus, Bulgaria, Georgia, Moldova, Romania, Türkiye, and Ukraine. Herein, Armenia, Azerbaijan, and Georgia are referred to as "Caucasus," when the statement is true for the three countries, and Türkiye is referred to as either "Thrace" or "Anatolia" when specific differences apply to each of the regions.

The primary focus of the project is on six diseases that are relevant for the region: anthrax, brucellosis, CCHF, FMD, LSD, and PPR. Consequently, this study focused on domestic ruminants (cattle, sheep and goats), which are the animal species most impacted by these diseases. These species are also interchangeably referred to as large ruminants (LR) and small ruminants (SR).

A report template was designed to collect information from each of the participating countries (Supplementary material S1). This document was developed by four authors of this paper (AA, DB-A, JC, and MA) as a semi-structured questionnaire. The selection of topics was based on the project's objectives and aimed at addressing knowledge gaps in the BSB about the ruminant sector and the impact of the selected diseases. The initial version of the document was presented and shared with respondents from the nine participating countries during a virtual meeting. The final version of the report template accounted for edits and suggestions provided by the participants.

The report template was divided into two sections. The first section focused on the ruminant demographics, types of ruminant production, national and international trade, livestock markets, slaughterhouses, seasonal movements, and value chains. The second section focused on the six targeted diseases, requesting information on disease status, recent outbreaks, surveillance and control activities, awareness campaigns, and research activities in place.

Moreover, each report template requested information in two formats: narrative answers (e.g., description of a system or production type) and quantitative data in a database format (e.g., Excel datasheet). In some cases, quantitative data could complement descriptive information. To have high-quality figures, we requested the highest level of detail (e.g., the number of smallholder farms at the smallest administrative level) and, when applicable, exact locations (e.g., georeferenced locations of a livestock market). Further instructions prompted respondents to refer to additional documents like local veterinary authority national reports and national publications (i.e., grey literature).

One focal point (FP) of each participating country was appointed by FAO to answer the report template and collect country-specific information. FPs were carefully selected based on previous collaborations, the quality of their work, their expertise in the ruminant sector and selected diseases, and access to the data necessary for further analyses. FPs were based in each respective country and were working (or had recently worked) within relevant national institutions (e.g., veterinary services, food safety authorities, or national laboratories), during data collection. All nine FPs are co-authors of this paper.

FPs received the report template via email, filled it in with preliminary information, and iteratively and upon request, added further detail, following a back-and-forth exchange of emails and virtual meetings. Data collection was carried out by the FPs in collaboration with local peers, and all activities were coordinated with national authorities to request and obtain approval for data sharing. Data collection took place between October 2020 and December 2021.

Descriptive information and quantitative data were obtained and analysed from completed report templates. Then, data were assessed, and specific topics were selected to examine in this paper. To complement data on these topics, information was sourced from national reports and websites of the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (WOAH), and the World Bank. To assess the economic importance of ruminant production for each country, we sourced data for the gross production value (GPV) of the main domestic production species from FAOSTAT (30). To find the proportional contribution of ruminant GPV to each country, we divided GPV for cattle, sheep, and goats, by the total GPV for all domestic species in 2020. Finally, ruminant distribution maps for ruminant populations (31–33) were sourced from FAO-NSAL (FAO's Livestock Information, Sector Analysis and Policy) branch.

Quantitative data was managed, cleaned, harmonized, and collated in Microsoft Office Excel (2019), RStudio® (34), and analysed and visualised in Quantum GIS (35) and RStudio® (34).

3. Results

Selected topics from the nine participating countries were organized into two sections following the structure of the report template: (1) study region and ruminant-specific information, and (2) disease-specific information.

3.1. Study region and ruminant-specific information

3.1.1. Study region

The main political changes and affiliations from countries of the study region between 1988 and 2021 are illustrated in Figure 1. Supplementary material S2 summarizes data for human and ruminant demographics, relevant economic indicators, and other characteristics of ruminant production. In 2020, most countries were classified as upper-middle-income economies, with the exceptions of Ukraine and Romania, which had a lower-middle-income economy and a high-income economy, respectively (36). The median GDP *per capita* of each region in 2020 was $4,547 USD, ranging from $3,725 USD in Ukraine to $12,896 USD in Romania. For livestock production indicators, Moldova and Bulgaria had the lowest contribution to agricultural GDP at 23%, while Belarus had the highest at 57% (30). The proportion of ruminant GPV (per total domestic production species) ranged between 23% in Moldova to 92% in Belarus (30). Further details about this indicator are supplied in Supplementary material S3.

3.1.2. Ruminant demographics

The ruminant distribution varied significantly throughout the study region, both for LR and SR. LR heads ranged from 159,000 in

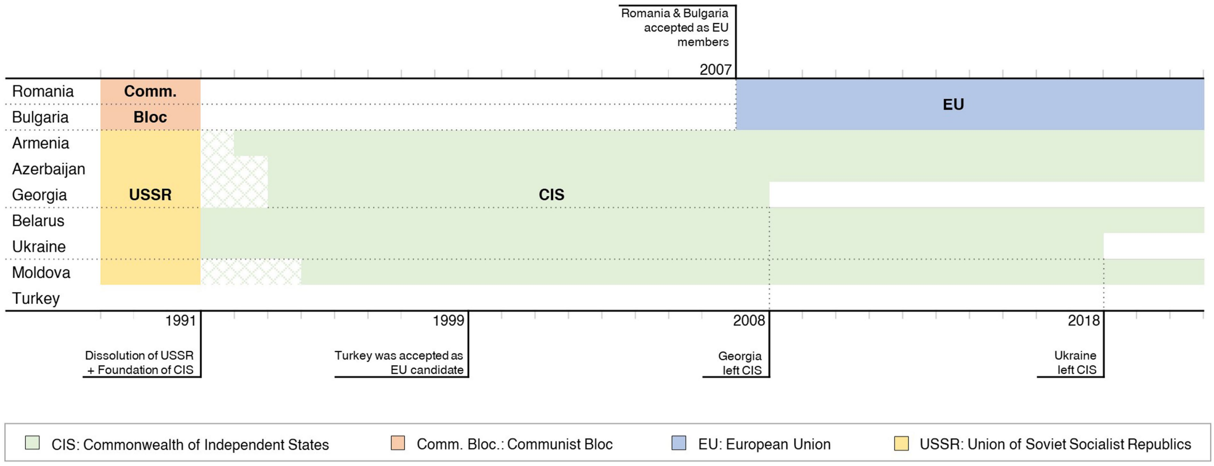

FIGURE 1
Political affiliations from 1988 to 2021 for the studied countries.

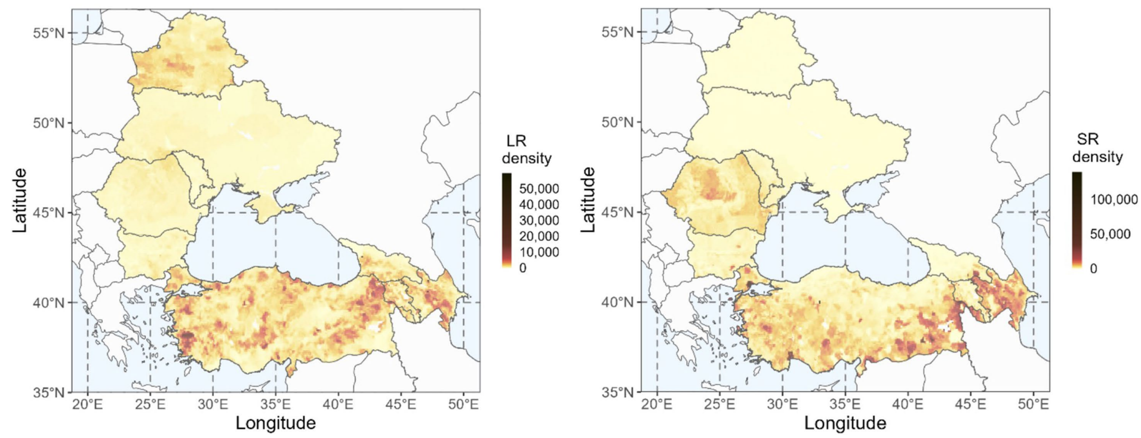

FIGURE 2
Distribution of large ruminants (LR) – cattle – and small ruminants (SR) – sheep and goats – in the study region. Source: GLW4 (Gridded Livestock of the World) data modified with countries' data and adjusted for FAOSTAT 2020 (31–33).

Moldova to 18 million in Türkiye, whereas SR heads were lowest in Belarus (148,000) and highest in Türkiye (54 million). Figure 2 illustrates the spatial distribution for LR and SR in the region and shows higher abundance of LR in Belarus, certain regions of Türkiye, western Georgia, and Azerbaijan, and higher number of SR in parts of Türkiye (Thrace and southeast Anatolia), Romania, and Azerbaijan. Additionally, the figures highlight lower LR populations in Ukraine, Moldova, southern Romania, and northern Bulgaria, and lower SR populations in Belarus, Ukraine, Moldova, and northern Bulgaria.

3.1.3. Production types

Countries classified ruminant production types using distinct terminology. To allow for comparisons, production types were grouped based on herd size and commercial purpose into smallholder and commercial farms, as defined in Supplementary material S6. In the Caucasus and Romania, over 90% of cattle farms were smallholdings, while Belarus had the highest proportion of cattle production in commercial herds. Across the entire region, more than 75% of herds keeping sheep and goats were smallholdings.

3.1.4. Animal identification and registration systems

Most countries in the BSB had established National Animal Identification and Traceability Systems (NAITS). In contrast, the Caucasus had NAITSs under development, but not yet fully implemented at the time of data collection. In Azerbaijan, this system was being developed through a European Commission (EC) framework. It entered a regional pilot stage in late 2021 and began a country-wide phased implementation over 2022 (37). In Armenia, the Centre of Agribusiness and Rural Development (CARD), with support from the Austrian Development Agency (ADA) (38, 39), developed and conducted a pilot of its NAITS in the cattle sector in January 2022. Similarly, in Georgia, after a 5-year project supported by FAO and financed by the ADA and the Swiss Agency for Development and Cooperation (SDC) (40), the system was launched nationwide in February 2022 (40).

3.1.5. National trade of live ruminants

Recordings of live animal movements were linked to the existence of a NAITS in each country. Therefore, most countries in the region recorded these movements within a national centralized database. Each registration included information regarding the individual identification of the animal and the farm of origin, the destination farm, and a veterinary health report issued by an official veterinarian.

Conversely, Georgia did not have a recording system for animal movements. In Armenia and Azerbaijan, movements between provinces were registered, but the record consisted solely of a paper-based veterinary health certificate. These records were issued by official veterinarians and archived in regional divisions. There were no centralised databases for recording live animal movements in these three countries.

Live animal movements were characterized by a seasonal pattern that is not detailed in this paper. Nevertheless, it is worth noting that these movements significantly surged during cultural-religious celebrations such as Novruz, Kurban Bayram, and Ramadan Bayram in Azerbaijan and Türkiye, in which animals are transported to cities to be ritually slaughtered. Similarly, Easter and St George's Day in Bulgaria and Romania were also preceded by an increase in live animal movement due to the traditional consumption of mutton.

Furthermore, it is important to highlight that livestock trade was closely linked to animal density in each region, the demand for animal protein in densely populated areas, the location of slaughterhouses, and specific commercial partnerships with regions or countries. For example, in Georgia, ruminant trade primarily occurred from west to east due to the high exports to Azerbaijan. As for Bulgaria, the southern regions, where LR and SR production was more intense, also had an increased movement of ruminants. Moreover, in Türkiye, ruminants were moved from small to large provinces, and more specifically from east to west and north to south of the country.

3.1.5.1. Livestock markets

The role of livestock markets in live ruminant trade varied across the region. Azerbaijan and Türkiye run ten and 150 licensed live animal markets, respectively, which played a significant role in ruminant trade. During Kurban Bayram in these two countries, markets worked exceptionally to sustain the surge in animal movements. In Armenia, Georgia, and Bulgaria, these facilities existed but were not as relevant for animal trade. In Belarus, official markets for live ruminant trade were absent, instead occasional fairs and exhibitions were held at the district level and on a small scale. In the same country, ruminant trade for breeding purposes occurred through state breeding companies. In Ukraine, smallholders used live animal markets for local ruminant trade.

3.1.5.2. Seasonal movements

Pastoralism includes seasonal movements to pastures and can be sub-classified as nomadism, transhumance, or agropastoralism (definitions provided in Supplementary material S6). These practices are key to the seasonal sourcing of graze and water for livestock and were common across the study region. In Bulgaria, the Caucasus, Romania, and Türkiye, transhumant animals were moved to summer pastures, often found in mountainous areas, in spring and summer, and to lowland pastures or stables in autumn and winter. Migrating months had slight variations yearly depending on weather and pasture conditions. Georgia, Azerbaijan, and Türkiye set up Veterinary Surveillance Points (VSP) along migration routes. These premises primarily focused on mass vaccination campaigns in Azerbaijan, but also served as rest points for supplying feed and water, as sanitary checkpoints for health status control, and anti-parasitic application in Georgia and Türkiye. The mingling of animals from various herds, regions or even neighbouring countries was common in seasonal pastures. Consequently, these animals were vaccinated either before going to pasture or during migration in VSPs. Furthermore, movements to seasonal pastures were recorded in centralized systems for movement control in Bulgaria, Romania (41) and Türkiye; however, these recordings, similarly to national movements, were not done in the Caucasus.

In Belarus, Moldova, and Ukraine, ruminants kept in smallholdings or smaller private farms in rural settings grazed seasonally in fields surrounding their holdings, in an agropastoral manner.

3.1.6. International trade of live ruminants

Partner trading countries with the BSB region are presented in the last two columns of Supplementary material S2. International trade of live animals was done based on country partnerships, contingent on the trust in the exporting country's animal health capacity and/or the sanitary status for the main contagious zoonoses and TADs (at a specific time) (42). To guarantee disease freedom on entry into a country, imported live ruminants were accompanied by a health certificate validated by a veterinarian of the exporting country's competent authority. Particularly for the importation of live animals (and animal products) into the EU, the intra-EU trade, and EU exports of live animals, TRACES (Trade Control and Expert System) (43), an EC online platform, facilitates sanitary certification required for trade and centralizes trade information. Thus, Bulgaria and Romania along with other BSB countries exporting live animals or animal products into the EU, used this platform.

Similar to national live animal movements, international trade was influenced by cultural-religious events. Therefore, a surge in live animal imports preceded Kurban Bayram and Ramadan Bayram in Azerbaijan and Türkiye, and Easter and St George's Day in Bulgaria and Romania.

3.2. Disease-specific information

3.2.1. Disease status, surveillance, and control activities

Figure 3 illustrates the country-level disease statuses for each selected disease. Countries self-classified their disease status as endemic, sporadic, or absent (definitions in Supplementary material S6). An *Absent* status was subclassified for brucellosis as "officially free" and for FMD as "officially free with or without vaccination" when WOAH officially recognised these disease statuses.

In Supplementary material S4, a table summarizes key details for the six studied diseases in each of the countries of the BSB. Moreover, temporal trends of disease outbreaks per country from 2010 to 2020 are shown in Supplementary material S5. In this subsection of the results, we review the disease status and management practices applied in the region.

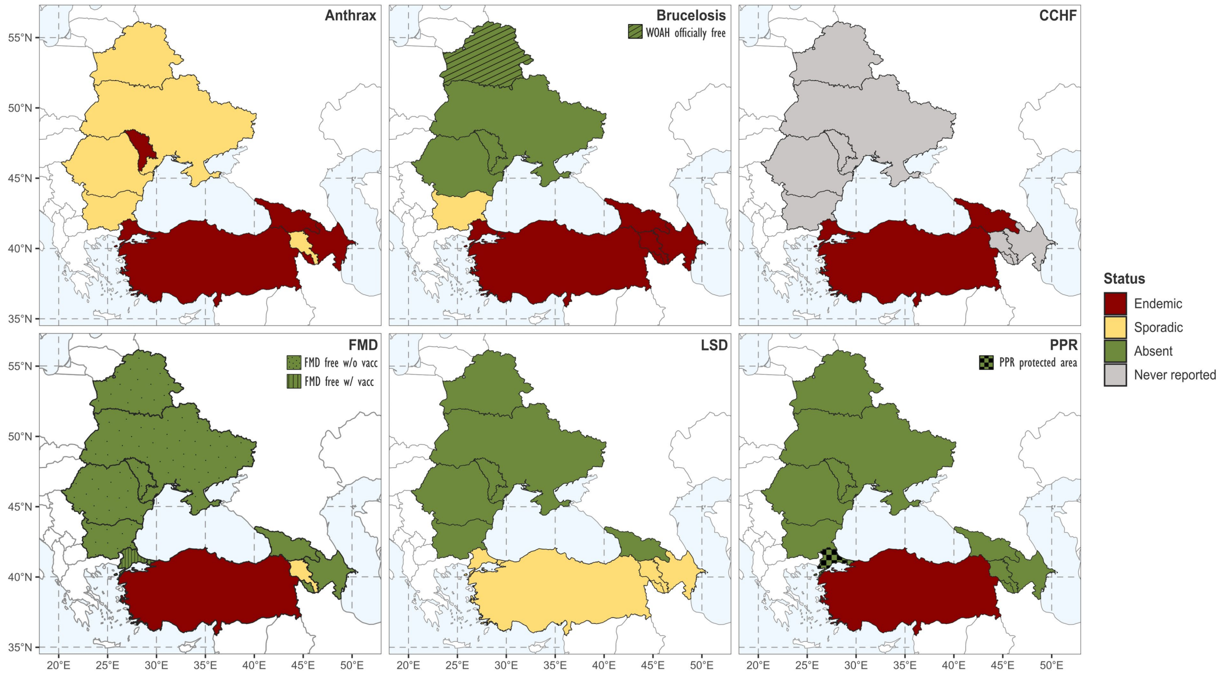

FIGURE 3
Status of target diseases in the study region. Brucellosis status refer to *Brucella abortus* and *Brucella melitensis*.

3.2.1.1. Anthrax

Anthrax was endemic or sporadic in all countries. All countries implemented passive surveillance and, upon suspicion, applied further clinical examinations, sampling, and testing. Due to the environmental nature of this disease, most national management programmes, in addition to guidelines for disease containment and carcass disposal, also included regulations for historically infected fields (e.g., signalling, fencing, digging restrictions, and awareness campaigns). Vaccination was compulsory for all ruminants in Azerbaijan, and Moldova, and all LR in Armenia. A risk-based vaccination approach was applied for all ruminants in Bulgaria, Georgia, Romania, Türkiye, and Ukraine, and exclusively for SR kept or moved to high-risk areas in Armenia. In Belarus, anthrax vaccination was not conducted.

3.2.1.2. Brucellosis

Brucellosis was endemic in the Caucasus and Türkiye, sporadic in Bulgaria, and absent in all other countries of the BSB. In 2012, Belarus was officially recognised by WOAH as brucellosis-free, and to maintain this status, serosurveillance was conducted every three years. Moreover, surveillance was exclusively passive for Georgia, and active and risk-based in all other countries. In most of the BSB, passive surveillance for brucellosis was associated with the report and investigation of abortions in ruminants, which is a syndrome of this disease, but not exclusive to *Brucella* spp. infection. In Azerbaijan, Georgia, and Türkiye, vaccination for brucellosis was mandatory for all ruminants and performed at the same time as serosurveillance. Brucellosis vaccination was not part of the national veterinary control plan in Belarus, Bulgaria, Moldova, Ukraine, or Romania.

3.2.1.3. Crimean Congo haemorrhagic fever

CCHF was endemic in Georgia and Türkiye. These countries applied control measures upon outbreak identification, focusing on tick control and community awareness campaigns. These activities comprised the application of acaricide sprays to ruminants, including during seasonal migrations from early spring to late autumn, and environmental tick elimination. Educational campaigns in Türkiye promoted contact restriction between livestock and wildlife, and tick management. These campaigns were included in the state budget at no cost to farmers. For the remaining countries, CCHF had never been reported in ruminants and there was no national surveillance programme in place. At the time of data collection, no licensed vaccine was available for CCHF in ruminants.

3.2.1.4. Foot-and-mouth disease

The WOAH official FMD status varied between the two regions of Türkiye: Anatolia was classified as endemic, and Thrace held FMD-free status with vaccination. FMD was sporadic in Armenia, absent in Georgia and Azerbaijan, while in all other countries, WOAH recognised the official status FMD-free without vaccination.

FMD surveillance was active in the Caucasus and Türkiye, as well as in regions of Bulgaria and Romania. The countries of the Caucasus were collaborating with EuFMD through the Progressive Control Pathway for Foot and Mouth Disease (PCP-FMD) to design and establish risk-based surveillance programmes. As part of these efforts, they implemented NSP (Non-Structural Protein) and SP (Structural Protein) serosurveys to evaluate the FMD virus circulation, seroconversion, and vaccination coverage. In regions bordering Thrace, Bulgaria conducted risk-based serosurveys on a sample of ruminants every three months. While in Romania, surveillance focused on clinical examination of LR and SR on high-density premises (e.g., live animal markets, exhibitions, ports, and airports), serosurveillance of all ruminants close to international borders, and SR upon their arrival from seasonal pastures.

In Türkiye, the FMD management programme in 2021 aimed to achieve FMD-free status without vaccination in Thrace and FMD-free status with vaccination in Anatolia by 2025 (44). In

Thrace, control measures comprised suspect FMD case culling, restrictions on live animal imports from Anatolia, and strict adherence to sanitary legislation. In Anatolia's southeast provinces bordering FMD-endemic countries, surveillance activities were enhanced and risk-based. Moreover, in case of an FMD outbreak, Türkiye conducted a field investigation, and vaccination, established a *cordon sanitaire*, animal quarantine, and thorough cleaning and disinfection, organized training, and awareness campaigns, and closely monitored all premises within a 10 km radius of the event.

FMD vaccination varied throughout the BSB. Türkiye vaccinated LR twice a year, and SR once a year only in Thrace. In case of an outbreak in Anatolia, SR were also vaccinated in established protection and surveillance zones. In Azerbaijan, LR were vaccinated twice a year (spring and autumn) and SR once a year, while Armenia, applied the same strategy only in high-risk areas. Since 2017, Georgia has conducted vaccination exclusively in high-risk areas, based on risk assessments, which considered seasonal migration, international borders with FMD-endemic countries, live animal markets, and informal trade.

3.2.1.5. Lumpy skin disease

LSD was sporadic in Armenia, Azerbaijan, and Türkiye, and absent in all other countries. Surveillance activities varied: clinical examination was conducted in Belarus to a sample of LR in spring and summer, and in the six regions of Bulgaria bordering Thrace monthly. Georgia had active participatory surveillance, Türkiye implemented both active and passive surveillance activities, and all other countries only applied passive surveillance. Compulsory vaccination was practised nationwide in Azerbaijan, Bulgaria, and Türkiye, and in high-risk areas of Armenia and Georgia. Vaccination was not applied in Belarus, Moldova, Romania, or Ukraine.

3.2.1.6. Peste des petits ruminants

PPR was endemic in Türkiye and absent in all other countries. In March 2021, Thrace was granted the classification of "PPR-protected area." PPR surveillance varied across the BSB: Belarus did not conduct it, Moldova, Ukraine, and Anatolia exclusively applied passive surveillance, while Thrace and all other countries applied active surveillance. In Bulgaria, areas previously affected by PPR (2018 outbreak) implemented enhanced surveillance, and regions bordering Thrace applied risk-based serosurveillance on a sample of SR every two months. In Romania, active surveillance included clinical inspection of a sample of SR herds before and after pasture season.

Vaccination was implemented in Georgia, following the first PPR occurrence in 2016. In Türkiye vaccination was conducted, yet it ceased in Thrace after the region was granted a "PPR-protected area" classification in March 2021. This measure, coupled with strict live SR movement restrictions from Anatolia to Thrace, aimed at Thrace's application for WOAH PPR zonal freedom status in 2023. In Anatolia, PPR vaccines were applied to all newborn SR and unvaccinated adults.

4. Discussion

In this paper, we summarized the ruminant production sector and reviewed the sanitary status and management of six diseases affecting ruminants (anthrax, brucellosis, CCHF, FMD, LSD, and PPR) in the BSB. Furthermore, we explored key factors contributing to the introduction and spread of these diseases in the region.

4.1. Post-Soviet Union reform

The fall of the Soviet Union caused a deterioration of public infrastructures and services across the former Soviet Union (FSU) and Communist Bloc countries, significantly affecting agricultural and livestock sectors (18, 45–48). In BSB countries, except Belarus, changes included the shift from collective and state-owned farms to private ownership, removal of government subsidies to the livestock sector (49), closure of large slaughterhouses (50), and depletion in resource allocation to veterinary services (51). Such factors left livestock production in the hands of unspecialized farmers, and unsupervised by veterinary services (52), resulting in increased disease incidence (52, 53). Thereafter, the region suffered a steep decline in the number of ruminants (49, 54) and, in some countries, as Ukraine and Belarus, a significant abandonment of agricultural lands (55). These abrupt structural changes were followed by a transition phase with gradual agricultural recovery and increasing productivity (56). Yet, rural poverty, particularly in the Caucasus and Moldova, persists and requires new and efficient policy measures that enable technological development and access to market channels and services (51). EU's farmer association model could aid smallholders of the FSU to actively engage to improve their marketing, input supplies, and support services (57).

4.2. Rural livelihoods and pastoralism

Pastoralism played a critical role in rural areas in most countries of the BSB (16–18, 20–25, 46, 58–60), creating a unique interdependence between ruminants, farmers, and the environment (61, 62). Preserving this practice is crucial, given its resilience to severe climates in arid and inhospitable areas, socio-cultural importance, and the potential opportunities brought to younger generations (62). However, its sustainability in the BSB is a matter of concern. Ageing rural farmers show reluctance to adopt new technologies and measures to improve animal production and health, and the mass migration of younger populations to urban centres leaves families without essential support for farming activities (57). Moreover, they have limited access to veterinary services also caused by ageing rural veterinarians, and difficulties in attracting young graduates due to low-income prospects and prevailing urban migration trends (57). These factors result in underperforming veterinary services (63) and high costs for disease management impeding improvements, even when advancements are made at higher levels (28). Solutions for these challenges need to be explored, as building private veterinary capacity and developing training programmes for veterinary paraprofessionals.

In addition, initiatives addressing pastoralism's limited sustainability and its associated risks to ruminant health and welfare are underway (64, 65). In Georgia (62), Türkiye, and Azerbaijan, VSPs were established along migration routes. In Armenia, the "Project Coordination Platform for Sustainable Management of Natural Grazing Lands – Pastures and Grasslands" was launched to address pasture management-related problems in the country (66). Internationally, the Pastoralist Knowledge Hub (PKH) by FAO aids the development of synergies for dialogue and pastoralist development, while an extension of the Performance of Veterinary Services (PVS) (67) evaluation tool prioritizes finding solutions to control animal diseases in pastoralist areas (63). Collectively, these initiatives aim to foster and protect pastoralism while ensuring its sustainability.

4.3. Disease management and related factors

Disease management in the Caucasus and Türkiye was often inefficient. Nonetheless, improvements were being made in the field. Particularly in the Caucasus, the full operability of the NAITSs is expected to make disease management programmes (68) and disease traceability (69) more efficient. As a result, these improvements will positively influence animal health and ruminant production, ultimately, leading to better trade opportunities and economic growth in these countries.

Sociocultural-religious events in Türkiye and Azerbaijan prompted the implementation of contingency plans and extraordinary measures, which, at times, proved inefficient in preventing disease introduction and spread. In fact, the epidemiological investigation conducted upon the PPR incursion to Bulgaria in July 2018 concluded that the high demand and resulting price difference of mutton between Bulgaria and Thrace during these festivals contributed to increased informal movements of people and animals (70).

In the BSB, only Türkiye reported the presence of all studied diseases. This can be attributed to its unique conditions, including a large ruminant population, vast geographical area with socio-economic disparities, and extensive rural regions. Moreover, its shared borders with six countries including Syria and Iraq, where social unrest leads to informal movement of people with their livestock, create a significant pathway for disease spread (71, 72). Recognising the high risk to animal and public health through this route, Türkiye introduced legislative acts for border control and supervision of the main roads (73, 74). These acts are open to amendment, and they aim to manage and identify informal/illegal trade for livestock and products of animal origin, along with enforcing animal culling. Following the implementation of these controls, a national report highlighted a significant reduction of nearly 95% and 50% of confiscated smuggled animals and animal products, respectively, caught during border controls conducted in 2011 and 2018. This demonstrates the successful impact of these actions (75).

EU countries, such as Bulgaria and Romania, had high resource allocation for disease management and prioritised the prevention of disease incursion to reduce economic losses. These countries followed harmonized live animal trade regulations set by the EC, enforcing additional control measures and trade restrictions (76) in the event of an exotic disease incursion. Therefore, responses to Bulgaria's FMD (2011), LSD (2016), and PPR (2018) outbreaks were quick and intensive (77). And LSD and FMD outbreaks resulted in an economic burden estimated at €8 million (78), and $1.5 billion USD annually (79), respectively. To prevent disease re-emergence and further economic losses, disease management activities established upon these events, were still in place as of 2021.

Moreover, Thrace's proximity to Europe and shared borders with the EU through Bulgaria and Greece, prompted the establishment of partnership programmes between the EU and Türkiye. These initiatives involved significant investments to curb disease introduction and spread into Europe, while also promoting trade opportunities (80, 81). Therefore, FMD and PPR statuses varied between Thrace and Anatolia, leading to distinct classifications by WOAH, along with distinct approaches for disease management and movement control in these two regions.

4.4. The exception of CCHF

Türkiye and Georgia were the only countries in the BSB reporting the presence of CCHF in ruminants. In spite of this, past studies identified CCHF virological or serological evidence and the presence of competent vectors in most countries of the study region, except for Belarus (12, 82–84), while CCHF human cases were also notified in Bulgaria and Türkiye (84). Non-reporting of CCHF in ruminants was linked to two factors. Firstly, its exclusion from national veterinary programmes resulted in the absence of routine official surveys, and secondly, the subclinical nature of the disease in these species allows it to circulate unnoticed (85–87). Nevertheless, domestic ruminants play an important role in the epidemiology of the disease as they are involved in its vector life cycle (83) and amplification and spread of the virus (88, 89). Moreover, ruminant CCHFV antibody titers correlate with virus presence in a region (84), as well as human disease incidence (82). Given CCHF's public health threat, including potential human incurred deaths, the prudent course of action is to include the disease in national veterinary programmes. This would ensure regular disease monitoring and prompt response to any reported cases.

4.5. Current initiatives

Achieving effective disease management requires not only efficient resource allocation for national disease preparedness and response but also promoting collaborations with other countries and unions. An initiative that strengthens regional alliances for TADs management is the GF-TADs, a joint FAO and WOAH effort, created to support capacity building and the establishment of disease management programmes based on regional priorities (77). GF-TADs' priority diseases in the BSB include brucellosis, FMD, LSD, and PPR. Under this initiative, the Global Strategy for the Control and Eradication of PPR aims to control and eradicate PPR and strengthen veterinary services (90, 91). Additionally, FAO's PCP-FMD guides endemic countries in progressively managing FMD risks and reducing its impacts and viral circulation (92–94).

4.6. Limitations

Study limitations were linked to country-specific factors and data quality issues. Absent or not fully operable NAITSs are likely to have affected data validity, and completeness is limited due to unregistered herds along with underreporting across the BSB. Underreporting is often linked to farmers' poor disease awareness, distrust in governmental authorities, risk of penalty or stigmatization, or at a higher level, lack of capacity to enforce regulations (95) and low transparency. Additionally, variability in data availability and spatial resolutions between countries led to reduced accuracy of certain indicators or made it impossible to compare and examine others. Finally, data quality might have been affected by resource reallocation during the COVID-19 pandemic, which partly coincided with the two-year data collection period.

4.7. The armed conflict in Ukraine

The armed conflict in Ukraine, starting in February 2022, had a significant impact on its livestock sector. Since its beginning, the conflict led to decreased agricultural production due to land abandonment, animal losses from death or forced slaughtering, and reduced demand for meat and milk due to mass emigration (96). It has disrupted the accessibility to veterinary services, vaccines, medication (97), and critical inputs, such as feed and fodder (96), compromising disease prevention and control, and increasing the risk of stress, malnourishment, and susceptibility to disease in livestock. Moreover, amongst security issues, unavailability of consumables and equipment, and competing urgent priorities, appropriate carcase disposal became challenging. These effects are expected to reshape ruminant demographics, its associated production sector, and value chains, particularly in front-line regions. International cooperation is vital to address the consequences on livestock health and revive the sector post-conflict. Guidelines aiming to support the livelihoods of livestock-keeping communities in humanitarian emergencies that affect livestock are in place (98, 99), being used to alleviate the consequences of the presented conflict.

5. Conclusion

This study provides a comprehensive overview of the ruminant production sector and the management of six major diseases of concern in the BSB. By examining the effects of the post-soviet reform, the importance of pastoralism, differences in disease management and countries' response to disease incursion, as well as the influence of cultural events and political affiliations on live animal trade, we have gained a valuable understanding of how these different factors work together to determine disease dynamics in the region.

Unlike the other studied diseases, CCHF was not included in veterinary management plans, and not surveyed in ruminants across the region, presenting a public health threat. Furthermore, the armed conflict in Ukraine starting after data collection will likely have a significant impact on ruminant production and animal disease emergence in this country, with potential spread to neighbouring countries.

Finally, despite recent developments in veterinary infrastructures, including the implementation of NAITSs in the Caucasus, substantial support from international agencies and targeted initiatives for ruminant disease management, the need to improve animal health persists, particularly in rural and remote regions. A thorough understanding of the primary challenges, needs, and constraints faced by smallholders in each specific country context is essential. Establishing priorities and closely assessing them in collaboration with farmers, national stakeholders, and international agencies, will aid in identifying opportunities for more effective disease management strategies contributing to alleviating and preventing future outbreak scenarios. These considerations go hand in hand with providing incentives for rural development, by seeking financial aid, efficiently allocating financial and human resources, and most importantly ensuring the sustainability of the implemented strategies.

Data availability statement

The datasets presented in this article are not readily available for public access due to restrictions imposed by the governmental entities of the nine participating countries. The data that support the findings of this study were used under license from these entities and are subject to confidentiality and legal constraints. Requests to access the datasets should be directed to margarida.decastro@uab.cat.

Ethics statement

Ethical review and approval was not required for the study of animals in accordance with the local legislation and institutional requirements.

Author contributions

AA, DB-A, JC, and MA developed the report template and contributed to the conception and design of the study. JA, TC, IK, TM, DM, AP, MP, NS, and AZ were hired as country FPs to fill in the report template and provide quantitative data and descriptive information for the project. SO wrote a document summarizing preliminary information. MA performed the data management, descriptive analysis and visualization materials, and wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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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/fvets.2023.1174560/full#supplementary-material

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