DEVELOPMENTS IN ANIMAL HEALTH SURVEILLANCE

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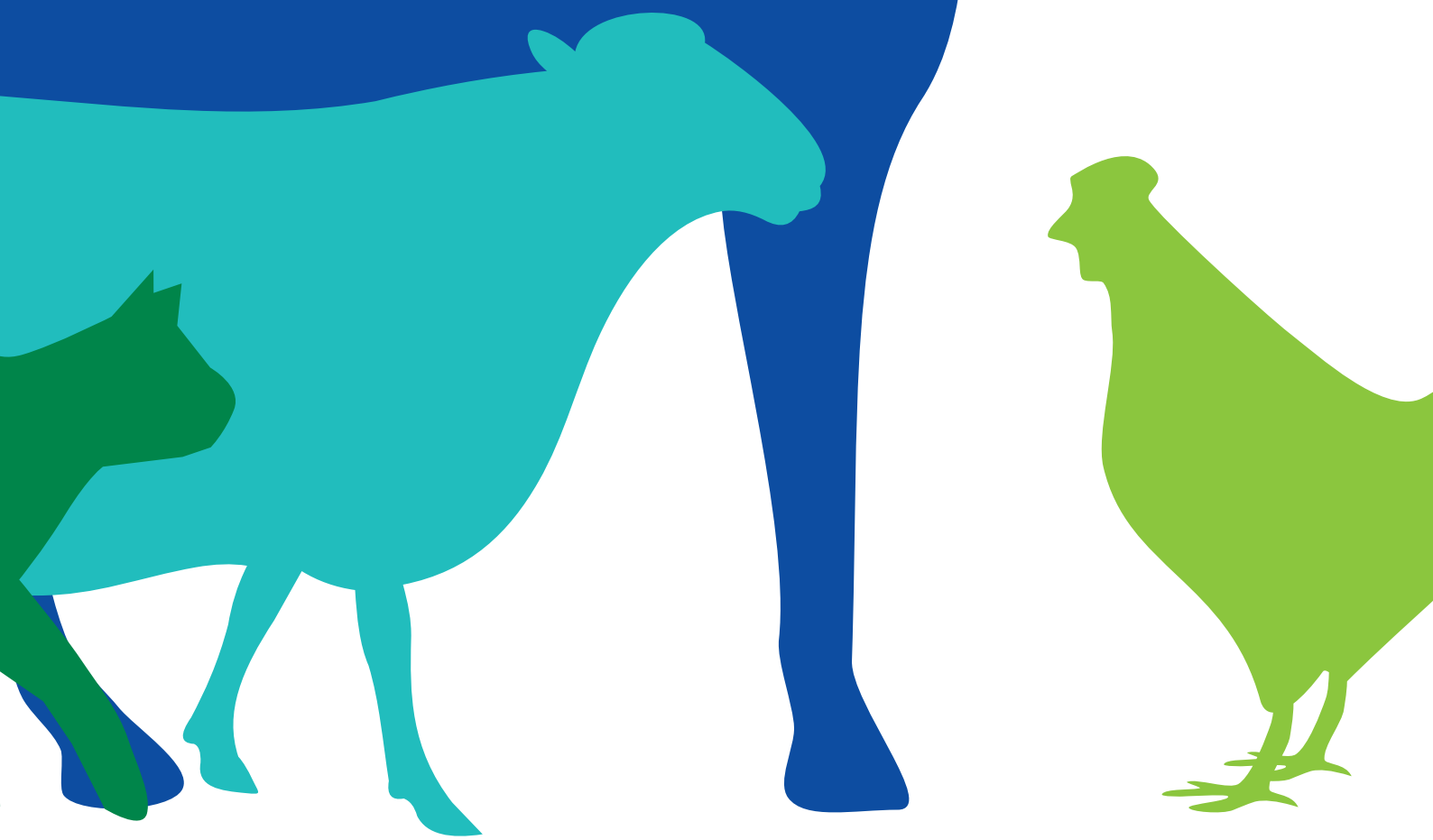

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DEVELOPMENTS IN ANIMAL HEALTH SURVEILLANCE

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EDITORIAL
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Editorial: Developments in Animal Health Surveillance

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Developments in Animal Health Surveillance

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INTRODUCTION

The emergence of new pathogens, and other threats to public and animal health, offers opportunities to develop innovative surveillance methods, particularly when implementing "One health" surveillance, an approach in which at least two sectors from human health, animal health (wildlife and domestic animals), plant health, food safety or environmental health collaborate to improve outcomes for all (1). The approach allows development of methods to drive economic sustainability of surveillance systems, and to optimize efficiency, considering resources are limited.

The broad scope and wide-ranging contributions from the animal and public health sectors in this Research Topic means readers will find animal health surveillance developments for zoonoses of ruminant, swine, poultry, companion animal, and primate origin. Altogether 145 authors contributed to 21 original research articles, one brief research report, one perspective article and two reviews, from 19 different countries of all five continents. The two reviews and two original research articles on rabies and West Nile disease describe, specifically, a "One health" approach to surveillance. Four articles focus on different aspects of syndromic surveillance, from design to analyses, interpretation and evaluation. The use of secondary data to describe risk factors and demographics for risk-based surveillance (outbreak preparedness and surveillance design) is further explored in five other articles. Six articles discuss the critical role of surveillance end-users and describe how to enhance disease reporting, engagement and surveillance output communication. Finally, five articles focus on developments in diagnostics with regards to evaluating assays, new sampling approaches, test characteristics and disease measure.

The development of new methods to support sustainable animal health surveillance systems requires accurate cost and benefit evaluation and assessment of cost-effectiveness. Although economic efficiency of surveillance is recognized as highly relevant, one of the studies (Häsler et al.) identified that there are few quantitative economic evaluations carried out in practice. This finding is supported by the lack of submissions describing economic evaluation of animal health surveillance to this Research Topic.

DEVELOPMENTS IN ONE HEALTH SURVEILLANCE

Rabies is a classical disease in which the "One health" concept is easily applied. Despite being a well-known fatal zoonosis, it is neglected in endemic countries, mostly in Africa and Asia. With a target of zero human deaths from dog-mediated rabies set by the World Health Organization (WHO), Food and Agriculture Organization (FAO), and World Organization for Animal Health (OIE) for 2030 (2), strengthening rabies surveillance and control in these countries is needed. An integrated, collaborative, "One health" approach to bite management between public health and veterinary sectors should ensure rapid testing of any animal that bites a person. Timely communication between health centers and veterinary field workers can be facilitated by mobile phone technology (Lushasi et al.). Benavides et al. used passive national surveillance data on bite patients over a 10-years period to identify areas and species with high potential risk of rabies transmission to humans across Brazil. The study reports bites by domestic dogs and cats being more prevalent than those caused by bats, primates, herbivores, and foxes, with an uneven incidence across the country and a relatively low level of adequate post-exposure prophylaxis administration.

The WHO, FAO and OIE have established that more than three quarters of the new human diseases at the beginning of the 21st Century have emerged or re-emerged from animals (3). While the origin of the current pandemic of severe acute respiratory syndrome coronavirus (SARS-CoV) infection remains to be elucidated, Devaux et al. report that most of the human pandemics to date are zoonoses, and review zoonoses that originate from non-human primates (NHP). Drivers of interspecies pathogen transmission from NHP to humans include incidents with NHP (in wildlife ecosystem or after illegal import of live NHP), contact with NHP carcasses, or NHP consumption (local consumption and illegal import of NHP meat).

Disease surveillance in wildlife populations is challenging because wild animals are not as closely monitored as domesticated. Instead, surveillance relies on voluntary, often opportunistic reporting from those in close contact with wild animals such as hunters, conservationists and enthusiastic citizens. However, the motivation to report varies among these groups and understanding the drivers behind the reporting patterns will lead to better interpretation of the surveillance data. For instance, disease clusters detected through citizen-derived data should be interpreted with human socio-demographics factors in mind (Thomas-Bachli et al.).

Hernandez et al. describe a "One health" surveillance in practice in the United States in an outbreak investigation context. *Clostridioides difficile* is regarded as a nosocomial pathogen of hospitals. However, the number and severity of *Clostridioides difficile* infections (CDI) outside the hospital environment or in individuals with onset of symptoms 48 h or less after hospital admission is increasing. This emerging public health concern in community settings has been linked to a potential household pet source, with molecular genetic screening of patients and their pets as a useful tool to inform appropriate preventive and control strategies.

DEVELOPMENTS IN DATA USE AND ANALYSIS FOR ANIMAL HEALTH SURVEILLANCE

Syndromic Surveillance

The robustness of any animal health surveillance system is enhanced by the variety of its data sources and number of components. Hence, the use of data recorded for other purposes to create additional components such as syndromic surveillance is growing. Syndromic surveillance systems are real-time systems that can contribute to enhance traditional passive surveillance systems. Syndromic surveillance aims to identify aberrations in health-related events in a population that highlight unexpected trends of endemic diseases or signal the presence of an emerging disease.

There are practical challenges in transforming data into a format appropriate for syndromic surveillance and in developing suitable analyses for aberration detection. The process often requires incremental steps in an iterative cycle. Tongue et al. investigate the use of ovine fallen stock records to detect temporal and spatial aberrations in sheep mortality in the UK. Sala et al. evaluate the use of a syndromic surveillance system that incorporates a "severity" alarm to interpret excess mortality events using cattle fallen stock records. They identified important issues for optimal functionality, such as avoiding delays in notifications to the system, the need for sufficient human, financial, and legal resources to sustain it, and the need for high coverage to obtain meaningful results. Nonetheless, the system was useful to monitor seasonal diseases, quantify absence of disease or identify atypical excess mortality events. Veldhuis et al. explore routinely collected bulk milk records to identify potential aberrations. A variety of statistical methods can be used for this purpose, but choosing the appropriate method is not trivial as they vary broadly in performance and complexity. Selection of syndromes to monitor and frequency of data collection are also important in maximizing timeliness and sensitivity of aberration detection. Faverjon et al. estimate the potential value of different time series methods for building a national syndromic surveillance system for cattle in Switzerland. They suggest that detection performance is dependent on the characteristics of the syndrome time series, the nature of the epidemic and the event detection algorithm and recommend that syndrome time series be assessed through optimal detection algorithms and detection performance evaluated, before being included in an early detection surveillance system.

Risk-Based Analytical Techniques

An in-depth understanding of animal demographics, social network structure and potential disease transmission pathways can help improve surveillance design and outbreak preparedness. By identifying populations, areas and time in which early detection of a disease outbreak is most likely to be achieved,

resources for animal disease surveillance can be appropriately deployed to yield maximum benefits. This is particularly important in countries with limited resources.

As part of disease outbreak response preparedness, social network analysis can reveal influential nodes to be targeted in limiting disease spread quickly and efficiently. This is essential for rapidly spreading diseases that impact international trade such as foot and mouth disease and African swine fever (O'Hara et al.). Equally important is to identify the most crucial strategy to prevent an epidemic. Di Pillo et al. applied a stochastic model of disease transmission and a combination of intervention strategies to find that enhancing passive surveillance in owners of backyard poultry production systems was the most crucial prevention strategy to prevent highly pathogenic avian influenza epidemics in a high-density poultry area.

The use of risk-based analysis to enhance detection is described by Tzy-yun Teng et al. through a Bayesian spatial model that considers antimicrobial resistance surveillance data on *Salmonella* in pigs together with pig farm distribution, size and management. Authors identify areas at higher risk of infection and the distribution of specific serotypes, both of which can optimize future sampling. The article by Alba et al. evaluates a real time reporting system that aims to optimize clinical diagnosis in highly industrialized swine farms by incorporating information on trends and summaries of clinical events within a geographical area, together with other important demographic information characterizing the subpopulations affected. Veterinarians gain considerable advantages by using this system, but presumptive diagnoses should be confirmed with laboratory test results particularly to discard suspicions of diseases notifiable to the OIE.

Risk-based analysis can also be used to improve the interpretation of surveillance outputs at fixed locations such slaughterhouses. Stirling et al. analyzed movement data to identify and characterize potential sources of bias in slaughterhouse-based surveillance.

DEVELOPMENTS IN REPORTING AND COMMUNICATION OF ANIMAL HEALTH SURVEILLANCE

Reporting

Surveillance is intended to produce outputs to be acted upon by decision makers and other stakeholders. Data and knowledge sharing is a key element supporting surveillance programs for disease control and eradication. For surveillance to be relevant and encourage wider disease reporting, it is important to understand the needs of its stakeholders and knowledge gaps that may exist. For example, livestock producers living in remote areas should be reached and engaged in surveillance. Again, this is particularly important in resource-deprived countries where the main surveillance stakeholders are farmers who may have limited understanding of disease risk factors and transmission.

In these circumstances, a participatory investigation could be used to gather data, improve animal disease knowledge and enhance surveillance sensitivity (Ghafar et al.). An Ethiopian participatory study (Alemu et al.) investigated small ruminant disease priorities from a smallholder perspective, aiming to inform interventions to address disease-related production loss and its impact on communities. A mixed-methods approach identified respiratory diseases, gastrointestinal parasites and neurological diseases of livestock as priorities, while gaining an understanding of why the diseases are considered important to livestock owners and the extent of their understanding of disease control. The work highlighted contrasting priorities at national and community level and the large potential positive impact of production disease interventions on smallholder households. Smallholder livestock producers exist in every country in the world and are a heterogeneous group of producers. In Hernandez-Jover et al.'s cross-sectional study followed by group interviews, the authors provide an insight into animal health management practices. A delay in reporting to veterinarians could occur because most smallholder livestock producers attempt to treat symptoms by themselves or are only concerned with endemic diseases. Importantly, animal welfare was found to be a driving motivation for disease prevention, as well as sharing experiences and information between the producers.

Finally, Perez et al. present a unique example of a self-organized voluntary data sharing system to inform disease surveillance led by the livestock industry to generate scientific-driven solutions for emerging swine health issues in North America.

Communication

Coordinated surveillance policies generally include harmonized approaches that makes the interpretation and comparison of outcomes possible. To further improve the detail, transparency, consistency and open access of surveillance reporting, Comin et al. produced a wiki that includes a provisional checklist of items that could be expanded at any time to accommodate realities different to the European context. Researchers from the European SANTERO project assessed the practical adoption of surveillance standards in Europe, and explored how to ensure innovative research reaches, and is adopted by, surveillance practitioners (Häsler et al.). They found a multiplicity of channels used to source information, and considerable heterogeneity in the adoption of recommended surveillance standards and innovative approaches among European Union, European Economic Area and Schengen countries. Although economic efficiency was considered highly relevant, few quantitative economic evaluations are carried out in practice, constraints including a skills deficit and limited resources. Recommendations included a collaborative international exchange platform for surveillance knowledge, design and dissemination of standards.

DEVELOPMENTS IN LABORATORY DETECTION OF ANIMAL HEALTH PROBLEMS

Hazard-specific surveillance requires the correct laboratory identification of pathogens to tailor specific interventions to reduce or prevent their occurrence. It is therefore fundamental to

analyse diagnostic test performance and evaluate new sampling strategies to reduce costs or increase population coverage.

Baruch et al. contributed evidence to inform a new approach to bovine brucellosis surveillance in Uruguay, using pooled-sera sampling. They estimated the analytic sensitivity of an indirect ELISA test for *B. abortus* for different pool sizes demonstrating that, in principle, the method could be applied to low-risk bovine populations.

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is endemic in most developing countries, whereas it has been eradicated from many developed countries. However, success of eradication programs differs globally, with limitations of available diagnostic tests being one of the factors impacting this success. Barandiaran et al. assessed the accuracy of a PCR-based rapid diagnostic test and compared it with that of bacteriological culture for bTB in pigs using a Bayesian approach. The study concludes that PCR could be used as an effective and rapid test for confirmation of bTB-like lesions detected at slaughtering, supporting current strategies for controlling this disease in endemic countries.

Rift Valley Fever (RFV) is an important zoonotic viral disease of livestock occurring across much of Africa; however, its epidemiology and significance in some parts of the continent are poorly understood. Bronsvoort et al. reported serological evidence of RVF in Central Africa and active circulation of the virus in the cattle population. This study also estimated the performance of a commercial RVF ELISA (ID.Vet) compared with that of a neutralization test (PRNT80), using a Bayesian no gold standard latent class analysis, concluding that the ELISA test had comparable performance and could be used as a low cost easy to use surveillance tool for the African context.

Salmonella is an important foodborne pathogen, with pork being one of the major sources of human outbreaks. Surveillance for this pathogen in pigs is important to understand prevalence and distribution. De Lucia et al. investigated the correlation of anti-*Salmonella* antibodies between serum and saliva samples, with results identifying for the first time anti-*Salmonella* antibodies in pigs' oral fluids and suggesting that saliva samples have the potential to be used for the diagnosis of *Salmonella* infection in pig farms.

Leptospirosis is one of the most widespread zoonotic bacterial diseases, and is endemic in subtropical and tropical countries. However, in some countries there is limited knowledge on the epidemiology of the disease. Alinaitwe et al. reported anti-*Leptospira* antibodies among slaughter cattle in Uganda, mainly against the Tarassovi, Sejroe and Australis serogroups, with seroprevalence being higher among older cattle. Their study compared the performance of the standard microscopic agglutination test (MAT) against a lipL32 based real time PCR (qPCR) assay and reported a higher specificity and negative predictive value for the MAT test compared to the qPCR.

CONCLUSION

This collection of papers has evidenced the current areas of interest in animal health surveillance developments, from One health to syndromic and risk-based surveillance, from public health to wildlife and livestock health, from the laboratory to epidemiological field and analytical studies. We trust readers will enjoy reading the articles in this Research Topic as much as the Editors enjoyed the process of bringing them to you.

AUTHOR CONTRIBUTIONS

MM wrote the draft of the editorial that was then revised and amended by the rest of the editors. All authors were involved in editing manuscripts, approaching reviewers and managing the review process, and in supporting each other with the editorial work.

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Animal Health Management Practices Among Smallholder Livestock Producers in Australia and Their Contribution to the Surveillance System

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The risks posed for disease introduction and spread are believed to be higher for smallholder livestock producers than commercial producers. Possible reasons for this is the notion that smallholders do not implement appropriate animal health management practices and are not part of traditional livestock communication networks. These factors contribute to the effectiveness of passive disease surveillance systems. A cross-sectional study, using a postal survey (n = 1,140) and group interviews (28 participants in three groups), was conducted to understand the animal health management and communication practices of smallholders keeping sheep, cattle, pigs, dairy goats and alpacas in Australia. These practices are crucial for an effective passive surveillance system. Findings indicate that there is a need for improvement in animal health management practices, such as contact with veterinarians and attitudes toward reporting. Results also indicate that these practices differ depending on the livestock species kept, with sheep ownership being associated with lower engagement with surveillance activities and smallholders keeping dairy goats and alpacas having in general better practices. Other factors associated with surveillance practices among participant smallholders are gender and years of experience raising livestock. Despite the differences observed, over 80% of all smallholders actively seek information on the health of their livestock, with private veterinarians considered to be a trusted source. Emergency animal diseases are not a priority among smallholders, however they are concerned about the health of their animals. The finding that veterinarians were identified by producers to be the first point of contact in the event of unusual signs of disease, strengthens the argument that private veterinarians play a vital role in improving passive surveillance.

Other producers are also a point of contact for animal health advice, with government agencies less likely to be contacted. The effectiveness of on-farm passive surveillance could be enhanced by developing strategies involving both private veterinarians and producers as key stakeholders, which aim to improve awareness of disease and disease reporting responsibilities.

Keywords: smallholders, animal health management, passive surveillance, Australia, disease reporting

INTRODUCTION

Biosecurity and animal health management practices of smallholder livestock producers are often perceived as posing an increased risk for disease introduction and spread (1, 2). Key components and drivers of these practices are awareness and knowledge of diseases and attitudes toward monitoring disease and reporting to private veterinarians or relevant authorities. The effectiveness of passive surveillance systems for early detection of disease introductions rely on these practices. Previous studies investigating the effectiveness of surveillance systems in Australia for early detection of foot-and-mouth disease (FMD), reported that improving producer recognition of the presence of unusual signs of disease and reporting these signs would be the most effective strategy for reducing the time from incursion to detection and as such minimizing the potential impact of an FMD outbreak (3, 4).

Whilst increasing producer knowledge and understanding of disease is an essential component of passive surveillance, it is not the only factor that needs to be considered. The actions of an individual are influenced by a number of factors including knowledge, beliefs, attitudes and intentions (5). Studies conducted in the United Kingdom have reflected a disparity between what producers consider to be the "usefulness" of biosecurity practices and the actual implementation of such practices at a farm level, suggesting that the relationship between attitudes and actions in this area requires further investigation (6). Similarly, a study of Danish dairy cattle farmers, reports that despite Danish legislation that farmers with herds larger than 330 per year must develop a farm-specific biosecurity plan, the mandatory plan had not been developed by any of the farmers participating in the study. The researchers speculated that factors that influenced this lack of implementation were a lack of trust in other farmers' ability to maintain adequate biosecurity, uncertainty as to whether other farmers would contribute to the common good, a perception that the risk of disease introduction was low and, the expectation that there would be no social consequences associated with non-compliance (7).

The notion of responsibility is also important to consider. In a study investigating the limitations and incentives in reporting suspicion of Classical Swine Fever amongst pig farmers in the Netherlands, a gap was identified between the expectation of authorities and what pig farmers and veterinary practitioners considered to be their responsibility (8). Strengthening partnerships between government, producers, veterinarians and industry are the main tenets of a biosecurity system based on shared responsibility (9), with such relationships vital to safeguarding livestock industries. Perceptions of risk, consequences, intrinsic and extrinsic benefit or cost, responsibility and social elements, therefore must be at the forefront of discussions on drivers of producer-led passive surveillance.

Whilst the drivers for the on-farm practices of commercial producers are likely to be closely aligned with financial factors, the smallholder sector of livestock producers is arguably more complex. The smallholder sector encompasses a broad range of livestock keepers, in relation to species and number of animals kept, land size, and motivations for keeping livestock (10, 11).

Recent studies focusing on pig producers in Australia have found that herd size and the severity of perceived impact of the disease influence attitudes toward disease reporting. In addition, it was found that recent contact with veterinarians and the keeping of animal health records was less likely in small scale producers (12–14). Studies have also shown that the communication networks that exist between smallholders and industry and government stakeholders are often inadequate (10). It has been suggested that the risk of disease introduction and spread could potentially be reduced through improvements in extension and communication networks, given that this would increase producers' active engagement and participation within their industry (15).

In summary, a limited number of studies have investigated smallholder animal health management and communication practices which define producers' abilities to recognize and report diseases and therefore the effectiveness of passive surveillance systems in the country. This study aims to understand these practices and their influences among smallholder livestock producers in Australia.

MATERIALS AND METHODS

The study was conducted in two different phases over a 3 year period from 2013. The first phase involved a cross-sectional survey of Australian smallholder livestock producers (Phase 1) and the second phase involved group interviews with a cohort of these producers (Phase 2). Phase 1 of this study also involved consultation with stakeholders to conduct a stakeholder analysis as reported by Hayes et al. (11). Research proposals for both phases of the study were approved by the Human Research Ethics Committee at Charles Sturt University (20th May 2013) (protocol number 416/2013/05); 9th December 2014 (protocol number 400/2014/52) and 10th November 2015 (protocol number 400/2015/38). For the purpose of this study,

smallholders included were those keeping ≤50 head of: (1) cattle and sheep, (2) pigs, (3) dairy goats, and (4) alpacas, noting that participants could keep mixed herds provided that no individual species exceeded 50 head. Smallholders in the first category were those keeping cattle only, sheep only or cattle and sheep.

Phase 1: Cross-Sectional Study on Smallholder Producers in Australia

Development of the Questionnaire

A questionnaire was developed to gather information on demographics and general husbandry (9 questions), biosecurity (17 questions), animal health management (8 questions) and communication networks (3 questions) of smallholders. The questionnaire comprised short-closed, semi-closed and open questions and was prepared for both paper and electronic completion. The questionnaire was piloted with three representatives from state government departments of agriculture and three smallholders, with suggestions incorporated where appropriate. The electronic version of the questionnaire was delivered using the online survey tool, SurveyMonkey®.

Questionnaire Distribution

Stakeholders, as identified in the stakeholder analysis conducted as part of Phase 1 of the study (11), who agreed to assist with the cross-sectional study, were contacted to discuss best approaches to the questionnaire distribution. These stakeholders were broadly categorized as state based government departments/authorities, Natural Resource Management (NRM) groups, Catchment Management Authorities (CMA), Landcare Networks (LC), industry associations and community groups. A national level standard approach to distribution of the questionnaire was not possible due to differences between states of Australia in relation to available registers for smallholders and the information available on these smallholders, such the species kept. As such, different approaches of distribution were used for each state and livestock species.

For smallholder producers of cattle and sheep, available sampling frames were state government registers with postal addresses (Qld, SA, Tas and Vic) and with email addresses (WA and NSW). The availability of project funds limited the number of questionnaires which could be delivered by post so a sample of smallholder producers on the government register in Qld, SA, Tas and Vic was selected. The sample size calculation was performed using Epitools epidemiological calculators (epitools.ausvet.com.au), and assumed a population of smallholders >1,000, a 95% confidence level and 5% precision, with ∼20–30% of smallholders conducting a specific animal health management practice. The required sample size was between 245 and 322.

The questionnaire was mailed to a randomly selected sample of smallholders from Queensland ($n = 700$), South Australia ($n = 700$), Tasmania ($n = 696$), and Victoria ($n = 699$). For smallholders keeping cattle and sheep in Western Australia and New South Wales the questionnaire was emailed to all government registered smallholders with email addresses ($n = 780$ and $n = 1{,}239$, respectively). Although availability of resources was a driver for the postal distribution of the survey, a total of 4,814 (2,795 postal, 2,018 electronic distribution) smallholders keeping cattle and sheep were surveyed, which was considered adequate for obtaining a representative sample among this type of smallholder.

For smallholders keeping pigs, the sampling frame was all smallholders identified by Australian Pork Limited (the pork industry representative body) with an available email address ($n = 897$). The questionnaire was distributed by email through Australian Pork Limited. For smallholders keeping alpaca, the survey was distributed through the Australian Alpaca Association, among all members with an available email ($n = 1{,}370$). For dairy goat smallholders, all producers listed in the publicly available Dairy Goat Society of Australia herd book were sent the questionnaire via post ($n = 476$). Overall, a total of 3,271 questionnaires were distributed by post and 4,286 by email.

The postal questionnaire was sent to the selected smallholders and included the participant information sheet and an addressed, reply paid return envelope. Invitations to complete the questionnaire on-line were sent directly from the assisting organizations in an email containing an introduction to the project and a link to the full information statement and online questionnaire.

A repeat mail-out/electronic contact was used for the distribution of the questionnaire to increase response rate. To reduce potential for non-response bias and encourage participation, an incentive of entry into a lucky draw for five gift vouchers (each of AU $50) for each livestock species group was offered.

Data Analysis

Data from the returned questionnaires were entered into Microsoft Excel (2007) and checked for data entry errors. Descriptive and statistical analysis were conducted using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Associations were investigated between explanatory variables and animal health management practices (dependent variables), using logistic regression analyses. Animal health management practices included in the analysis were: (1) the frequency of livestock inspection; (2) keeping records of animal health events; (3) contact with veterinarians; (4) actions in response to recognizing unusual signs of disease; and (5) sources of animal health information. All of these dependent variables were binary. Association of animal health practices with the explanatory variable "species" was initially investigated using univariable logistic regression analysis. This step was conducted to identify differences in animal health practices between smallholders keeping different livestock species. The next step in the analysis of the data was to investigate those factors (explanatory variables) associated with animal health practices within smallholders keeping the same livestock species. For these analyses, univariable logistic regression was initially conducted to investigate preliminary associations of the animal health practices with a group of explanatory variables. These explanatory variables were: Age, gender, state, property size

(hectares), years owning livestock, species kept, number of animals kept and biosecurity knowledge. Variables with $P < 0.2$ in the univariable analysis were investigated further in a multivariable logistic regression model. Prior to building the multivariable model, correlation between these explanatory variables was tested by a chi-square test and only one of a pair of highly correlated variables was considered for inclusion in the multivariable model. Correlations were found between species kept and number of animals kept; property size and number of cattle or sheep kept; and, state and property size. Age and gender were included in the multivariable models as potential confounders. A backward selection method was used to build the multivariable logistic regression model for each animal health practice, with only those explanatory variables with a P-value < 0.05 being retained in the final model. Further, first order interaction terms were included to the final models and retained if significant at $P < 0.05$. The model fit was assessed using the Nagelkerke R^2.

To investigate the biosecurity knowledge of smallholders, participants were asked with an open-ended question, to provide a definition of the term, biosecurity. This information was qualitatively analyzed using content analysis and classification of answers into four categories: (0) No knowledge (I don't know/incorrect reference to introduction and spread of diseases); (1) Low level of understanding of biosecurity (general mention of disease prevention but no reference to introduction and/or spread); (2) Moderate level of understanding of biosecurity (correct reference to practices preventing the introduction or the spread); and (3) High level of understanding of biosecurity (correct reference to practices preventing the introduction and spread of disease).

The geographic location of participants in the study (respondents to the survey) according to species kept was mapped by postcode using ArcGISTM 9.3 (ESRI Inc., Redland, CA, USA).

Phase 2: Group Interviews

Recruitment of Participants

Group interviews were undertaken to gain a broader and deeper level of understanding of the attitudes, behaviors and communication networks of smallholders in relation to biosecurity and the management of animal health. This activity provided a follow up to the questionnaire, focussing on areas considered by the research team to be of high interest. The planned structure for the group followed that described by Morgan (16), with a highly moderated structured interview, 8–10 participants per group and three groups. From identified regions of smallholder population in South-Eastern Australia (17–19), three representative areas were selected for inclusion—Riverina region (NSW), South Coast region (NSW), and Euroa/Benalla region (Victoria). In the South Coast and Euroa/Benalla regions, a randomly selected group of 60 smallholders fitting the study criteria were invited to participate in the group interviews, through invitations sent by the NSW Small Farm Networks and Agriculture Victoria, respectively. In the Riverina region, invitations were distributed via community groups, university internal communications, letterbox drops and media. As a result of this recruitment process, eight to ten smallholder producers in each region volunteered to participate in the study. Each participant was offered an AU$50 gift voucher, the provision of lunch and an information package on biosecurity and animal health management.

Data Collection and Analysis

Smallholders who made contact with the researchers were emailed a participant information statement and consent form and asked to confirm their willingness to participate via telephone or email. Smallholders confirming their intent to participate were contacted 7 days prior to the group interview, serving as a reminder. Each group interview, which had 2–3 h duration, was facilitated by two researchers, alternating as moderator and scribe. The group interviews comprised structured activities and open discussions in relation to diseases of importance and communication networks. All discussions were recorded via a tape-recorder for subsequent transcription. Descriptive and categorical data from the structured activities was recorded and analyzed in Microsoft® Excel (Windows XP, 2006) and qualitative data was analyzed using applied thematic content analysis (20–22). This approach of analyzing qualitative data allows for the identification and examination of themes using a transparent method. To ensure integrity of the constructs resulting from the thematic analysis two researchers (MH-J and LH) conducted this analysis independently. Qualitative data was read by each researcher and the information coded. A second read was used to validate the initial coding, and from the codes, themes were identified based on topics and frequency of occurrence of these topics.

RESULTS

Demographic and Husbandry Characteristics of Smallholders

A total of 1,140 usable questionnaires were received, including 746 from cattle and sheep smallholders, 198 from pig smallholders, 103 from dairy goat smallholders and 93 from alpaca smallholders. Respondents who did not provide information on number of animals kept, kept no livestock, indicated higher than 50 animals in any of the livestock categories or were otherwise not in the target population have been excluded from the analysis. The response rate, considering only the usable responses, was 14.7, 23.9, 21.6, and 6.8%, for cattle/sheep, pig, dairy goat, and alpaca smallholders, respectively. The distribution of smallholders responding to the survey by species was mapped by postcode and is shown in **Figure 1**.

Table 1 provides a description of the main demographic and husbandry characteristics of smallholders. Overall, most smallholders were over 45 years of age, the majority of cattle and sheep and pig smallholders being males and for alpaca and dairy goat producers, the majority being female. The distribution of the number of years smallholders kept livestock was different between smallholder types ($P < 0.05$), with cattle and sheep smallholders having kept livestock for longer than other type of smallholders. The majority of participants kept livestock for

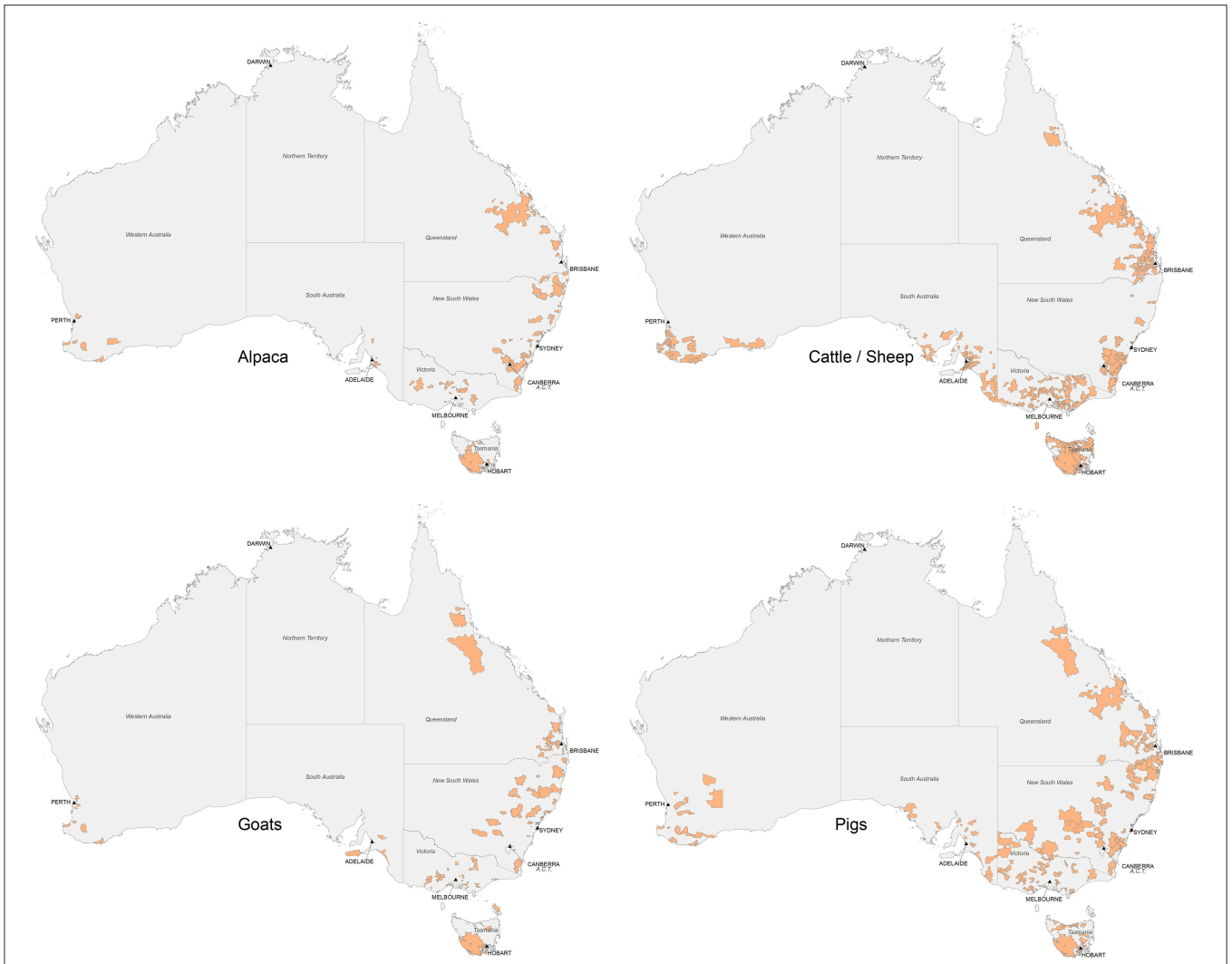

FIGURE 1 | Location by postcode of smallholders participating in a cross-sectional study in Australia in 2013–2015, according to species kept.

reasons other than primary income; mainly for extra income, home consumption and as a hobby.

In relation to the second phase of this study, a total of 28 smallholder producers participated across three group interviews. The demographic characteristics of group interview smallholders were similar to those of survey participants. Participants kept different livestock species, with most keeping cattle ($n = 19$) and approximately half keeping sheep on their property ($n = 13$). In addition, some kept goats ($n = 7$), horses ($n = 6$), poultry ($n = 4$), and pigs ($n = 2$). Twelve participants only kept one livestock species (cattle, $n = 6$; sheep, $n = 6$), whilst the rest of smallholders kept more than one livestock species. Over 60% of participants were over 45 years of age and the median years of experience raising livestock was 17 years, with a range from 2 to 60 years. Their properties ranged from 5 to 200 ha with a median size of 70 ha. Secondary income ($n = 15$) and family tradition ($n = 14$) were the most common reasons for keeping livestock, followed by home consumption ($n = 6$).

Animal Health Management Practices

Participant animal health management practices, related to producers' engagement with disease surveillance activities, are shown in **Table 2**. Most of these practices differed depending on the livestock species kept, with those smallholders keeping dairy goats and alpacas having in general better practices than other smallholders. Smallholders keeping dairy goats and alpacas are more likely ($P < 0.05$) to monitor their animals daily, keep animal health records and have more regular contact with veterinarians than smallholders keeping other livestock species. Furthermore, in relation to contact with veterinarians, results from this study suggest that a proportion of cattle (16.9%) and sheep (27.9%) smallholders had never contacted a veterinarian.

Despite the differences identified among smallholders and the low veterinary contact reported by some groups of smallholders, most respondents indicated that they would contact a private veterinarian if they saw unusual

TABLE 1 | Demographic and husbandry characteristics of 1,140 smallholder livestock producers participating in a cross-sectional study in Australia in 2013–2015.

Characteristic	Species							
	Cattle/sheep	%	Pigs	%	Dairy goats	%	Alpaca	%
AGE								
18–24 y	1	0.1	2	1.0	1	1.0	2	2.3
25–34 y	17	2.3	18	9.2	7	7.2	2	2.3
35–44 y	110	15.0	51	26.0	16	16.5	4	4.6
45–54 y	199	27.1	69	35.2	20	20.6	26	30.2
55–64 y	238	32.5	39	19.9	35	36.1	35	40.7
+65 y	168	22.9	17	8.7	18	18.6	17	19.8
SEX								
Male	486	67.5[a]	130	68.1[a]	21	21.9[b]	23	26.7[b]
Female	234	32.5	61	31.9	75	78.1	63	73.3
YEARS KEEPING THE LIVESTOCK SPECIES								
1–5 y	118	16.3[a]	97	50.0[b]	26	25.2[c]	25	27.2[c]
6–15 y	189	26.1[a]	47	24.2[a]	31	30.1[a]	51	55.4[b]
16–29 y	144	19.9[a]	15	7.7[b]	21	20.4[a]	16	17.4[a]
>30	274	37.8[a]	35	18.0[b]	25	24.3[b]	–	–
PROPERTY SIZE (HA)								
<10	223	30.6[a]	30	15.2[b]	49	48.0[c]	30	32.2[a]
10–29	226	31.0[a]	38	19.3[b]	26	25.5[ab]	28	30.1[a]
30–79	224	30.7[a]	35	17.8[b]	15	14.7[b]	22	23.7[ab]
≥80	56	7.7[a]	94	47.7[b]	12	11.8[a]	13	14.0[a]
OTHER AGRICULTURAL ACTIVITIES ON PROPERTY	324	43.4[a]	132	66.7[b]	52	50.5[a]	41	44.1[a]
STATE								
New South Wales/ACT	125	16.8	65	32.8	28	27.2	38	41.3
Victoria	148	19.8	47	23.7	25	24.3	18	19.6
Tasmania	126	16.9	18	9.1	8	7.8	9	9.8
South Australia	151	20.2	16	8.1	5	4.9	8	8.7
Western Australia	96	12.9	14	7.1	14	13.6	10	10.9
Queensland	100	13.4	37	18.7	23	22.3	9	9.8
REASONS FOR KEEPING THE LIVESTOCK SPECIES *(multiple responses)*								
Primary income	34	3.3	18	4.9	9	6.5	2	1.7
Extra income	461	44.8[a]	91	24.8[b]	26	18.7[b]	49	41.2[a]
Hobby/family tradition	106	10.3[a]	95	25.9[b]	69	49.6[c]	57	47.9[c]
Home consumption	356	34.6[a]	102	27.8[a]	22	15.8[b]	–	
Pet	106	10.3	15	4.1	13	9.4	11	9.2
Rare breeds	–	–	46	12.5	–	–	–	–

[a,b,c] *For each row, different superscripts differ $P < 0.05$.*

signs of illness or disease. However, most smallholders also reported that they would treat the animals themselves when faced with unusual signs of disease, and a quarter of smallholders keeping cattle, sheep and pigs reported that they would do nothing in such an event. Other frequent actions reported when faced with unusual signs of disease, were contacting other producers followed by contacting government agencies.

Given the differences in animal health management practices identified between smallholders keeping different livestock species, investigation of other factors influencing these practices was carried out for each type of smallholder, and results are presented in the following sections Animal Health Management Practices of Smallholders Keeping Cattle and Sheep, Animal Health Management Practices of Smallholders Keeping Pigs, and Animal Health Management Practices of Smallholders Keeping Dairy Goats and Alpacas.

The group interviews further investigated animal health management practices, diseases of concern, veterinary contact and actions when faced with unusual signs of disease in their animals, among participant smallholders. A clear animal health management theme emerging from the group interviews was the engagement of smallholders in preventative health measures, including specific preventative treatments, such as vaccination and internal parasite control, and close and frequent monitoring of their animals, as the following quote suggests.

TABLE 2 | Animal health management practices of 1,140 smallholder livestock producers participating in a cross-sectional study in Australia in 2013–2015.

Practice	Species							
	Cattle/sheep	%	Pigs	%	Dairy goats	%	Alpacas	%
FREQUENCY OF LIVESTOCK INSPECTION								
Daily	Cattle 375	62.3[a]	190	97.4[b]	96	97.0[b]	72	81.8[b]
	Sheep 184	66.9[a]						
Weekly	Cattle 200	33.2[a]	4	2.1[b]	3	3.0[b]	12	13.6[b]
	Sheep 76	27.6[a]						
Fortnightly	Cattle 21	3.5	1	0.5	0	–	2	2.3
	Sheep 6	2.2						
Monthly	Cattle 6	1.0	0	–	0	–	2	2.3
	Sheep 9	3.3						
KEEP RECORDS OF								
Animals with disease	351	54.8[a]	75	45.2[a]	83	89.2[b]	76	88.4[b]
Animals that died or euthanased	416	63.5[a]	91	54.8[a]	93	96.9[b]	78	90.7[b]
Treatment routine	425	66.3[a]	114	68.7[a]	76	77.6[a]	80	93.0[b]
CONTACTED A VETERINARIAN IN THE LAST 12 MONTHS	257	37.4[a]	64	35.8[a]	72	72.7[b]	70	79.5[b]
If no, last time veterinarian contacted								
Never	116	16.9[ab]	50	27.9[a]	4	4.0[b]	6	6.8[ab]
1–2 y ago	119	17.3	34	19.0	12	12.	11	12.5
3–5 y ago	108	15.7[a]	16	8.9[a]	10	10.1[a]	1	1.1[b]
>5 y ago	87	12.7[a]	15	8.4[a]	1	1.0[b]	0	–
ACTION AFTER IDENTIFYING SYMPTOMS OF DISEASE OR UNUSUAL BEHAVIOR IN YOUR LIVESTOCK *(always and sometimes)*								
Do nothing	184	24.7[a]	49	24.7[a]	4	6.3[b]	1	1.7[b]
Treat myself	551	73.8[a]	153	77.3[a]	75	91.5[b]	65	84.4[b]
Call other producer	496	66.5[a]	105	53.0[b]	46	67.6[a]	53	80.3[c]
Call veterinarian	658	88.2	179	90.4	90	94.7	80	97.6
Call livestock agent/saleyard	157	21.0[a]	24	12.1[b]	2	3.4[c]	3	5.6[c]
Call Government agency	269	36.1[a]	87	43.9[a]	22	34.4[a]	14	24.6[b]
Call the Emergency Animal Disease Watch Hotline	158	21.2[a]	61	30.8[a]	0	–	1	1.1[b]
SEEK INFORMATION ON MANAGEMENT AND HEALTH OF LIVESTOCK	597	82.9	163	82.3	89	92.7	82	95.3
MOST USEFUL SOURCES OF INFORMATION								
Government	311	52.1[a]	98	60.1[a]	36	37.5[b]	31	36.0[b]
Veterinarian	402	67.3[a]	109	66.9[a]	76	79.2[b]	70	46.5[c]
Rural supplier	227	38.0[a]	44	27.0[b]	28	29.2[b]	21	24.4[b]
Other producers	149	25.0[a]	32	19.6[a]	62	64.6[b]	67	77.9[b]
Industry breed groups	72	12.1[a]	34	20.9[a]	41	42.7[b]	34	39.5[b]

[a,b,c] *For each row, different superscripts differ $P < 0.05$.*

> "*I keep a close eye on my animals… I'm constantly around my cattle, so it is unlikely that I will miss any disease*" (Riverina region NSW, smallholder 5)

Participants were asked to list the three diseases considered being of most importance to themselves or their livestock operations and results indicate that producers are mainly concerned about endemic diseases, with internal parasites and clostridial diseases being the most frequently listed diseases. Some emergency and exotic animal diseases, such as foot-and-mouth disease, were listed by some producers; however, these were not considered a priority for smallholders. The thematic analysis of the open discussions identified two major reasons for a disease being considered of concern, these being animal welfare and loss of income, with approximately a third of smallholders identifying each of these as the main reason. Other reasons were the impact on livestock industries and the Australian economy, with 16.1% of smallholders identifying this as a reason, and impact on neighbors (7.7%) and personal/family health (4.8%).

Approximately half of group interview participants ($n = 13$) indicated that they had contacted a veterinarian in the past year. Thematic analysis of the data identified that the two most common reasons for using a veterinarian among participants were for pregnancy testing and animal health problems that producers could not deal with themselves. Other less common reasons for using a veterinarian were vaccinations of companion

animals kept on farm and general animal health advice. The quote below provides an example of when smallholders would use a veterinarian.

> "(I would use a veterinarian for])… *anything I can't handle myself*" (South Coast region NSW, smallholder 1)

The group interviews also identified the main barriers or challenges for a more frequent use of veterinarians, with the cost involved with the veterinary services being the main barrier. In agreement with the survey results, although the use of veterinarians could be improved, when participants were asked about the action they would take if faced with unusual signs of disease, most would contact a private veterinarian. However, over half of participants indicated that first they were likely to contact a neighbor, due to the perceived expertise and level of trust, as the following quote indicates.

> "*Their experience* (neighbors) *is valuable and can be contacted at any time for opinion and advice*" (Riverina region NSW, smallholder 3)

Only some smallholders would also contact the government veterinarian, with a smallholder showing a lack of trust of some government veterinarians, as seen in the quote below, and another participant perceiving contacting the government veterinarian for unusual signs of disease an overreaction. Most smallholders ($n = 22$) have not heard about the Emergency Animal Disease Watch Hotline.

> "*I know that they* (government veterinarians) *don't know what I want to know*" (South Coast region NSW, smallholder 2)

Animal Health Management Practices of Smallholders Keeping Cattle and Sheep

Tables 3 and **4** present results of logistic regression analyses investigating associations between demographic and husbandry characteristics and biosecurity knowledge (explanatory variables) and animal health management practices of smallholders keeping cattle and sheep.

Species kept ($P = 0.008$) and biosecurity knowledge ($P = 0.006$) were significantly associated with smallholder contact with veterinarians. A higher proportion of producers keeping cattle and sheep (46.2%) had contacted a veterinarian in the last year than smallholders keeping sheep only (29.8%) and those keeping cattle, sheep and pigs (18.8%). In addition, smallholders keeping horses were more likely to have contacted a veterinarian in the past year than those without horses (OR, 2.45; 1.6–3.7; $P < 0.001$). Producers who had a moderate to high understanding of biosecurity were more likely to have contacted a veterinarian (OR, 1.72; 1.2–2.5: $P = 0.006$).

Keeping records of animal health practices was associated with participant gender, property size and species kept ($P < 0.05$; **Table 3**). In general, keeping these records was more likely among female participants and those with larger properties, and less likely among those smallholders keeping sheep only.

Frequency of animal monitoring and inspection was associated with participant gender, years of experience raising cattle and property size (**Table 4**). Female respondents and those with more years of experience, reported to inspect their animals more frequently than male and less experienced respondents. In addition, the bigger the property the less frequent the inspection of animals was ($P < 0.001$).

Animal Health Management Practices of Smallholders Keeping Pigs

Table 5 presents the results of the logistic regression analyses investigating associations between demographic and husbandry characteristics and biosecurity knowledge (explanatory variables) with animal health management practices among smallholder livestock producers keeping pigs. The main characteristics associated with animal health management practices among smallholders keeping pigs were participant gender, species of livestock kept and the years of experience raising pigs.

Veterinary contact in the past 12 months was more likely among female pig smallholders ($P = 0.037$), smallholders keeping more than 10 sows ($P = 0.002$) and those not keeping sheep in their property ($P = 0.002$). Keeping animal health records was more likely ($P < 0.05$) among younger and less experienced smallholders and those with no sheep on their property.

Animal Health Management Practices of Smallholders Keeping Dairy Goats and Alpacas

Limited significant associations were found during the logistic regression analyses for smallholders keeping dairy goats. In agreement with results reported for the previous groups of smallholders, univariable analysis showed that dairy goat smallholders keeping sheep were less likely to have contact with veterinarians than those not keeping sheep ($P = 0.04$); however, when age and gender were included in the multivariable analysis as confounders, there was no association between keeping sheep and contact with veterinarians. The only practice where significant associations were identified by the multivariable logistic regression analysis was contacting a government veterinarian in the event of unusual signs of disease or behavior in goats (data not shown). Smallholders with more years of experience raising dairy goats were more likely ($P = 0.04$) to contact a government veterinarian than less experienced smallholders. No significant associations were observed between alpaca smallholder demographics and their animal health management practices.

Information Sources on Livestock Management and Health

The vast majority of smallholders indicated that they sought information on the management and health of their livestock (**Table 2**). Veterinarians were considered to be the most useful source of information by all smallholders (>65%), with the exception of alpaca smallholders, of which less than half of respondents considered veterinarians as a useful source of information. When comparing other sources of information among the different groups of smallholders, dairy goat and alpaca smallholders were more likely ($P < 0.05$) to consider information

TABLE 3 | Results of a multivariable logistic regression analysis investigating animal health management practices (contact with veterinarians and record keeping as dependent variables) of 746 smallholders keeping cattle and sheep participating in a cross-sectional study in Australia in 2013–2015 (Only significant associations are shown).

Practice	Producers	%*	*B*	SE	Odds ratio	95% CI	*P*
CONTACTED A VETERINARIAN IN THE LAST 12 MONTHS							
Species kept							0.008
Sheep	37	29.8	−0.62	0.25	0.54	0.3–0.9	
Cattle	154	34.6	−0.35	0.33	0.70	0.4–1.4	
Cattle and sheep	60	46.2	0		1.00		
Cattle, sheep, and pigs	6	18.8	−1.84	0.61	0.16	0.1–0.5	
Horses in the property							<0.001
No	144	28.3	0		1.00		
Yes	113	49.1	0.90	0.21	2.45	1.6–3.7	
Biosecurity knowledge							0.006
No-poor	90	31.5	0		1.00		
Mod-High	117	45.2	0.54	0.20	1.72	1.2–2.5	
Property hectares							0.012
<10	70	31.8	−0.39	0.26	0.67	0.4–1.1	
10–29	68	30.8	−0.71	0.24	0.49	0.3–0.8	
30–79	93	41.9	0		1.00		
≥80	20	38.5	0.28	0.41	1.32	0.6–3.0	
KEEP RECORDS OF ANIMALS WITH DISEASE							
Gender							0.001
Male	219	51.0	0		1.00		
Female	123	63.1	0.63	0.19	1.87	1.3–2.7	
Species kept							0.002
Sheep	39	35.5	0		1.00		
Cattle	239	60.7	0.89	0.24	2.43	1.5–3.9	
Cattle and sheep	59	53.6	0.63	0.29	1.89	1.1–3.3	
Cattle, sheep, and pigs	14	51.9	0.42	0.48	1.53	0.6–3.9.	
State							<0.001
NSW	84	76.4	0		1.00		
QLD	50	60.2	−0.82	0.33	0.44	0.2–0.8	
SA	52	42.3	−1.43	0.31	0.24	0.1–0.4	
TAS	57	49.1	−1.18	0.31	0.31	0.2–0.6	
VIC	65	51.2	−1.20	0.30	0.30	0.2–0.5	
WA	43	52.4	−1.00	0.33	0.37	0.2–0.7	
KEEP RECORDS OF ANIMALS THAT DIED OR EUTHANASED?							
Gender							0.002
Male	261	60.0	0		1.00		
Female	144	71.3	0.62	0.20	1.86	1.3–2.8	
Species kept							0.027
Sheep	48	41.7	0		1.00		
Cattle	270	68.7	0.71	0.24	2.03	1.3–3.3	
Cattle and sheep	80	68.4	0.73	0.31	2.08	1.1–3.8	
Cattle, sheep, and pigs	18	60.0	0.53	0.47	1.70	0.7–4.3	
Property hectares							<0.001
<10	86	45.3	0		1.00		
10–29	127	62.9	0.56	0.22	1.75	1.1–2.7	
30–79	156	77.6	1.20	0.24	3.33	2.1–5.4	
≥80	41	82.0	1.71	0.43	5.54	2.4–12.7	
KEEP RECORDS OF TREATMENT ROUTINE							
Biosecurity knowledge							0.016
No-poor	157	63.1	0		1.00		
Mod-High	176	75.5	0.51	0.21	1.67	1.1–2.5	
Property hectares							<0.001
<10	104	55.6	0		1.00		
10–29	133	65.8	0.48	0.26	1.62	1.0–2.7	
30–79	145	75.1	1.01	0.27	2.74	1.6–4.6	
≥80	37	80.4	1.50	0.56	4.49	1.5–13.6	

**Proportion of producers within each row conducting the specific practice investigated in the model (denominators not provided).*

TABLE 4 | Results of a multivariable logistic regression analysis investigating animal health management practices (monitoring and attitudes toward disease as dependent variables) of 746 smallholders keeping cattle and sheep participating in a cross-sectional study in Australia in 2013–2015 (Only significant associations are shown).

Practice	Producers	%*	*B*	SE	Odds ratio	95% CI	*P*
HOW OFTEN DO YOU INSPECT YOUR LIVESTOCK? CATTLE (DAILY INSPECTION vs. OTHERS)							
Gender							0.035
Male	235	59.2	0		1.00		
Female	130	70.3	0.45	0.22	1.57	1.1–2.4	
Property hectares							<0.001
<10	104	72.2	0		1.00		
10–29	128	66.7	−0.23	0.26	0.80	0.5–1.3	
30–79	115	56.1	−0.65	0.25	0.52	0.3–0.8	
≥80	20	40.8	−1.45	0.36	0.23	0.1–0.5	
Horses in the property							0.038
No	401	78.9	0		1.00		
Yes	143	60.3	0.44	0.21	1.55	1.0–2.4	
Years owning livestock							0.011
1–5	41	48.8	0		1.00		
6–15	95	61.7	0.62	0.30	1.85	1.0–3.3	
16–29	69	59.5	0.54	0.32	1.71	0.9–3.2	
≥30	158	68.1	1.00	0.30	2.72	1.5–4.9	
ACTION AFTER IDENTIFYING UNUSUAL SIGNS OF DISEASE (NEVER CONTACT-GOVERNMENT AGENCY)							
Property hectares							0.023
<10	89	58.6	0		1.00		
10–29	82	48.8	0.35	0.23	1.42	0.9–2.2	
30–79	67	41.4	0.74	0.24	2.09	1.3–3.4	
≥80	15	40.5	0.52	0.39	1.69	0.8–3.6	
Years keeping livestock							0.016
1–5	40	47.1	0.67	0.29	1.95	1.1–3.5	
6–15	85	59.9	0		1.00		
16–29	45	43.7	0.62	0.27	1.85	1.1–3.2	
≥30	80	43.2	0.73	0.25	2.08	1.3–3.4	

**Proportion of producers within each row conducting the specific practice investigated in the model (denominators not provided).*

from other producers and industry breed groups useful, and cattle and sheep and pig producers were more likely ($P < 0.05$) to consider government a useful source of information.

Within smallholders keeping cattle and sheep, and those keeping pigs, some significant associations were observed between the reported information seeking behavior and some explanatory variables. For smallholders keeping cattle and sheep, female and younger producers were more likely to seek information on livestock management and health than male ($P = 0.028$) and older producers ($P < 0.001$). Regarding usefulness of different sources of information, producers from NSW (54.9%) and Western Australia (59.5%) were more likely ($P < 0.001$) to consider government agencies as useful sources than producers from other states. Results also suggest that those smallholders keeping only sheep are less likely to find veterinarians a useful source of information than other smallholders within this group ($P = 0.006$), with aligns with the frequency of veterinary used previously reported.

Within smallholders keeping pigs, those with less experience raising pigs and with <10 sows, were more likely ($P < 0.001$) to seek information on pig management and health. Over 90% of producers with <5 years of experience reported seeking information compared to 68.6% among producers with more than 30 years of experience. In addition, less experienced smallholders were more likely ($P < 0.05$) to rely on other producers as useful sources of information.

DISCUSSION

The aim of this study was to develop an understanding of the demographic and husbandry characteristics that may influence smallholder livestock producer's engagement with animal health management and disease reporting, key practices of the on-farm component of a passive surveillance system. Over a thousand smallholder producers participated in the study; being one of the first studies among this sector of livestock producers in a developed country, with this number of participants. However, one of the limitations of the study was the low response rate, which could be due to the distribution methods and the topic of the study, which are known factors influencing response rate (23). Given no specific registers for smallholder producers exist and distribution was done through other organizations due to

TABLE 5 | Results of a multivariable logistic regression analysis investigating animal health management related practices of 198 smallholders keeping pigs participating in a cross-sectional study in Australia in 2013–2015 (Only significant associations are shown).

	Producers	%*	*B*	SE	Odds ratio	95% CI	*P*
CONTACTED A VETERINARIAN IN THE LAST 12 MONTHS							
Gender							0.037
Male	34	26.4	0		1.00		
Female	27	45.0	0.75	0.36	2.12	1.1–4.3	
Sows							0.002
0–10	35	26.1	0		1.00		
>10	29	46.8	1.15	0.37	3.15	1.5–6.5	
Sheep							0.002
No	30	42.3	0		1.00		
Yes	34	27.2	−1.08	0.36	0.34	1.2–0.7	
KEEP RECORDS OF ANIMALS WITH DISEASE							
Years keeping livestock							0.024
1–5	54	65.9	0		1.00		
6–15	18	43.9	−1.07	0.43	0.34	0.1–0.8	
16–29	6	50.0	−0.97	0.69	0.38	0.1–1.5	
>30	11	37.9	−1.23	0.53	0.29	0.1–0.8	
KEEP RECORDS OF ANIMALS THAT DIED OR EUTHANASED?							
Age							0.02
18–34	13	72.2	1.27	0.62	3.56	1.1–12.0	
35–44	21	43.8	0		1.00		
45–54	38	63.3	1.03	0.43	2.81	1.2–6.5	
55–64	24	72.7	1.47	0.52	4.35	1.6–12.1	
>65	4	40.0	0.06	0.75	1.06	0.2–4.6	
Sheep							0.01
No	47	72.3	0		1.00		
Yes	55	51.9	−0.93	0.36	0.39	0.2–0.8	
KEEP RECORDS OF TREATMENT ROUTINE							
Sheep							0.033
No	49	77.8	0		1.00		
Yes	65	63.1	−0.82	0.39	0.44	0.2–0.9	
ACTION AFTER IDENTIFYING UNUSUAL SIGNS OF DISEASE (NEVER CONTACT-NEIGHBOR/FRIEND/OTHER PRODUCER)							
Years							0.017
1–5	62	83.8	0		1.00		
6–15	24	64.9	−1.00	0.50	0.37	0.1–1.0	
16–29	41	36.4	−2.07	0.73	0.13	0.0–0.5	
>30	4	56.0	−1.01	0.56	0.36	0.1–1.1	

**Proportion of producers within each row conducting the specific practice investigated in the model (denominators not provided).*

confidentially reasons, follow-up with non-respondents was not possible and as such, selection bias could not be assessed. A selection bias is acknowledged in the group interviews with the possibility that those who agree to take part in research of this nature, may already possess an interest in the subject matter and as such, may not be representative of the smallholder population. Although participants represented a diversity of smallholders in relation to location and key demographic and farm characteristics results need to be interpreted with caution. Another potential limitation of the study is in relation to the data analysis approach, which could be associated with the multiple testing problem. This problem refers to the probability of false positives or Type I errors, which increases when more tests are conducted to investigate the association of a specific explanatory variable with several dependent variables. In this study, a total of 12 dependent variables (animal health management practices) were investigated for each smallholder type, but associations with the explanatory variables were only found for a low number of animal health management practices (up to six). As such, although possible, we believe the multiple testing problem is unlikely to have caused major impacts on the results. When interpreting results from the multivariable logistic regression models is important to consider that these models aim to identify potential factors influencing animal health

management practices, and should not be used as a predictive model. Findings from this study, generally indicate that sheep ownership was a factor associated with lower levels of inspection and engagement with veterinarians. To maximize the likelihood of the early detection of disease, it is essential to regularly observe livestock, with delays having potential economic and eradication implications (24). With over 95% of participants in the current study reporting that they inspected their livestock at a minimum weekly interval, it can be argued that smallholders are effectively engaging in passive surveillance. However, whilst this would appear to indicate that early signs of disease could potentially be identified by producers, for diseases that can be spread during the incubation period, such as foot-and-mouth disease, a weekly inspection interval, as reported by a third of smallholders keeping cattle and sheep, would not be adequate. The effectiveness of inspection is also dependent on the level of knowledge of clinical signs of disease and the actions taken once such signs are observed (24, 25). It is also important that accurate animal health records are maintained given that these can help to identify patterns of disease or deaths. With the exception of alpaca producers, improvement in record keeping is clearly needed across all species.

The time between when a problem is recognized and a veterinarian consulted has been shown to be a major influence on time to disease detection (3). In the current study it was found that the majority of smallholders, when observing symptoms of disease, would attempt to treat it themselves. This is likely to be influenced by the degree to which a disease is considered by the individual farmer to be of concern to their operation. The group interviews provided an opportunity for further exploration of some of the questionnaires key areas of interest. Results indicate that endemic diseases are the main diseases considered to be of importance. As reported in similar studies (26), despite smallholders being concerned about the health of their animals, they do not consider EADs to be a priority and as such, the effectiveness of animal inspection for early detection of diseases comes into question. Whilst EADs are understandably a high priority to those involved in protecting the integrity of the Australian livestock industries, for the individual producer it can be a case of competing priorities and motivations. Whilst for cattle and sheep producers in this study the motivation for keeping livestock was primarily associated with obtaining additional income and home consumption, for those keeping other species the motivation was less clear. This highlights the difficulty in making generalizations about smallholders as they are clearly not a homogenous group (11). Studies have shown that animal welfare is a motivator for disease prevention, across all species, particularly for those with non-intensive systems (27). The current study supports these findings, with group interview participants identifying animal welfare the main reason of concern in relation to animal health. For many smallholders, livestock are considered pets, regardless of whether they ultimately end up slaughtered for home consumption (18). These findings are interesting to consider in relation to the previously identified selection bias. If producers who agree to participate in studies of this nature are assumed to be more engaged in the topic of interest, the fact that they do not prioritize EAD's, suggests that more work needs to be done across the whole smallholder sector in terms of biosecurity engagement.

The relationship between producers and veterinarians has been explored in previous studies (11, 15, 28). For producer-led passive surveillance to be effective, producers must trust both, those from whom they receive information and those to whom they provide information (4). Veterinarians are considered to be a trusted stakeholder, thereby placing them in a strong position to influence the behavior of smallholders (11, 15, 29). In the current study, the frequency of contact with the veterinarian was different between species, with a low contact identified among cattle and sheep and pig smallholders. A relationship with a veterinarian is an important aspect of animal ownership and the finding that a proportion of cattle and sheep and pig smallholders had never contacted a veterinarian requires consideration. Whilst the reasons for this were not explored in this study, the level of experience of such producers as compared to alpaca and dairy goats may, in part, explain this finding. Horse ownership was positively associated with veterinary contact, possibly indicating that for those keeping higher value animals, their relationship with a veterinarian is established and as such may cross over to their livestock operation.

Producers with a moderate to high understanding of biosecurity were also more likely to have contacted a veterinarian, suggesting that an understanding of biosecurity may be a positive influence on behavior and attitudes toward surveillance (27). This supports the suggestion that producers who discuss the application of biosecurity measures with veterinarians are more likely to engage in stronger biosecurity behavior (30).

Results in relation to information sources should be interpreted with caution, as the questionnaire distribution method had the potential to impact the outcomes in this component of the survey. The involvement of industry bodies in the distribution of the questionnaire to alpaca and dairy goat producers, meant that the questionnaire was only distributed to those already aligned with an industry organization. Previous studies have reported a considerable level of mistrust of government sources (11, 15, 29, 31), a finding supported by the current study, with almost half of producers indicating that they would never call the State Department of Primary Industries or Agriculture in the event of unusual symptoms. In addition, there were state differences observed with producers from NSW and WA being more likely to contact a government source. At the time that this study was conducted, these states were the only Australian states or territories that had government services tasked solely with supporting smallholders, leading to the suggestion that services such as these, are an effective method of engaging smallholders.

It could be argued that for more experienced producers, in this study those keeping cattle and sheep, past exposure to a higher level of service from government agencies may have resulted in an ongoing positive relationship with such services. In recent decades there has been a reduction in government extension services (3, 15, 32–34) which for those in "newer" industries such as the alpaca industry, may mean that they have had no past experience with government support and as such, may not consider them to be a useful source of information as a

result of this. The relationship between producers must not be underestimated when it comes to animal health management, particularly as "other producers" were shown to be one of the primary contacts in the event of the observation of unusual symptoms of disease.

In summary, this study provides an insight into the animal health management practices of smallholder livestock producers in Australia and identifies some influencing characteristics that should be considered when developing strategies for improving their engagement with the surveillance system in the country. Species kept, the level of experience, the location as well as the local networks used by the smallholders are important factors to consider. It is important that the correct health related information is shared between producers, leading the authors to suggest that well-informed "champion" producers could be included as part an overall producer-led passive surveillance strategy. The need for the flow of information from government sources both to and subsequently between experienced and less experienced producers, highlights the importance of understanding and building upon these relationships.

DATA AVAILABILITY

The datasets for this study will not be made publicly available because All data was collected under confidentiality and following Human Ethics Committee requirements, data was not to be shared beyond the research team.

ETHICS STATEMENT

Research proposals for both phases of the study were approved by the Human Research Ethics Committee at Charles Sturt University (20th May 2013 (protocol number 416/2013/05); 9th December 2014 (protocol number 400/2014/52) and 10th November 2015 (protocol number 400/2015/38).

AUTHOR CONTRIBUTIONS

MH-J, LH, and J-AT designed the study and developed the questionnaire and the focus group activities. LH led the distribution of the questionnaire and the recruitment of focus group participants. MH-J and LH analyzed questionnaire data. MH-J, LH, RW, and LR organized and facilitated focus group discussions. MH-J and LH analyzed the focus group data. MH-J and LH prepared the manuscript. J-AT, RW, and LR provided input to the manuscript.

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Individual or Common Good? Voluntary Data Sharing to Inform Disease Surveillance Systems in Food Animals

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Livestock producers have traditionally been reluctant to share information related to their business, including data on health status of their animals, which, sometimes, has impaired the ability to implement surveillance programs. However, during the last decade, swine producers in the United States (US) and other countries have voluntarily begun to share data for the control and elimination of specific infectious diseases, such as the porcine reproductive and respiratory syndrome virus (PRRSv). Those surveillance programs have played a pivotal role in bringing producers and veterinarians together for the benefit of the industry. Examples of situations in which producers have decided to voluntarily share data for extended periods of time to support applied research and, ultimately, disease control in the absence of a regulatory framework have rarely been documented in the peer-reviewed literature. Here, we provide evidence of a national program for voluntary sharing of disease status data that has helped the implementation of surveillance activities that, ultimately, allowed the generation of critically important scientific information to better support disease control activities. Altogether, this effort has supported, and is supporting, the design and implementation of prevention and control approaches for the most economically devastating swine disease affecting the US. The program, which has been voluntarily sustained and supported over an extended period of time by the swine industry in the absence of any regulatory framework and that includes data on approximately 50% of the sow population in the US, represents a unique example of a livestock industry self-organized surveillance program to generate scientific-driven solutions for emerging swine health issues in North America.

Keywords: porcine reproductive and respiratory syndrome, epidemiology, surveillance, data sharing, US

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INTRODUCTION

Although porcine reproductive and respiratory syndrome (PRRS) is one of the most important economic constraints to pork production in the US (1, 2), reporting of PRRS outbreaks is not mandatory in the country. In the absence of a regulatory framework, PRRS control and elimination actions are voluntary. At the field level, producers and veterinarians make decisions that seek to

maximize profit while keeping the necessary standards for animal health and welfare. However, individual-level decisions may lead to complex and diverse epidemiological scenarios at a regional level. Because of the epidemiological features of PRRS virus (PRRSv) transmission, such as high levels of disease incidence, high variability and rapid mutation of the virus, intensiveness of production, and, in some cases, vertical integration of the industry, and limitations of current preventive and control methods, there is not much hope for disease control if programs are not simultaneously implemented at local (i.e., production system level) and regional levels (i.e., state or county levels) (3–6).

The perception that regional approaches are required to control the disease has led to the implementation of at least 30 voluntary regional PRRSv control or elimination projects in the US and Canada (7). None have succeeded in eliminating PRRSv regionally, and, arguably, most seem to have had limited success on significantly controlling the disease. Part of the limited progress on those regional projects may be attributed to inconsistencies in regional biosecurity compliance and suboptimal biosecurity of swine operations, incomplete regional producer participation, poor standardization, and availability of information on pathogen monitoring and surveillance systems, including lags in detection and communication about outbreaks (7). Lack of progress of regional control and eradication projects may also be at least in part associated with limited funding for disease and insufficient coordination of control activities. In contrast, one may argue that it is unknown what the epidemiological situation of the disease would be in the absence of those voluntary programs, and thus, the effectiveness of their implementation is debatable and subject to speculation.

Nevertheless, veterinarians and producers from individual farms and production companies, generically referred to as "production systems," still need to make decisions intended to maximize their results (8) using the information available to them, which may lead to scenarios that are not compatible with disease control at a regional level. The tension between "individual" and "common" good leads to a complex relation of behaviors and attitudes, resembling a "game." In this "game," certain "players" (production systems) make decisions that may be conditional to the decisions made by other "players," which, in turn, may result in a change of decisions taken by other "players." This "game," which in social economics corresponds to a concept referred to as "game theory" (9, 10), soon becomes dynamic, and the conditions required to control the disease at a regional level become, at minimum, difficult to reach. Thus, PRRSv control at the regional level becomes challenging, and often depends on regional leadership. If short- and long-term values of participation are not quantified and clear, it is unlikely that progress will be made.

Many stakeholders have perceived the social nature of PRRSv control at the regional level in the US. Spontaneous initiatives intended to voluntarily share knowledge have emerged in the country with the objective of promoting a greater good, i.e., control of a disease that affects the industry as a whole, even if such sharing may represent a potential risk or loss to their individual interest. Here, we review the largest voluntary initiative for data sharing among US swine producers, including a summary of its design and governance, and highlighting a number of epidemiological features of the disease that the project helped to elucidate over the last 10 years. The ultimate objective of this voluntary program is to build the capacity to respond in the event of an emerging disease, while supporting the prevention and control of endemic diseases of swine, such as PRRSv.

PRRS AND THE MORRISON'S SWINE HEALTH MONITORING PROJECT (MSHMP)

Since the late 1980's, PRRSv has been consistently generating losses in the US swine industry. Thanks to an effective collaboration between researchers, swine veterinarians, and swine producers, epidemiological characteristics of the disease were uncovered and preventive and control measures were implemented. Despite important improvements from a bioexclusion, biomanagement, and biocontainment standpoint, the virus continues to persist in US swine herds. Because there has historically been no documentation of disease occurrence metrics through space and time, the industry could not systematically assess whether the current situation was better or worse compared to previous years, generating uncertainty and speculation. Based on this knowledge gap and the need to further understand the epidemiology of the disease at larger spatial and temporal scales, a group of producers and practitioners decided to voluntarily share breeding herd PRRSv status for their respective production systems. Through weekly reporting, practitioners updated their respective PRRSv breeding herds' status, making the estimation of weekly cumulative incidence reports possible. The product of this effort was then shared back to participants in the form of weekly reports. Reports included information on disease prevalence, disease incidence, and proportion of herds in each PRRSv status. Reports also included benchmarking comparing numbers of participants to aggregated results from other participants in the database. The program's name changed through time and it is currently referred to as the Morrison's Swine Health Monitoring Project (MSHMP), in recognition of the late Dr. Robert Morrison, who was the driving force leading the inception and organization of the program. Compared to regional control projects, the MSHMP is larger, including information from reproduction farms (sow farms, multipliers, genetic nuclei) in a number of regions, whereas regional control projects are typically smaller, limited to a geographical area, and including also information nurseries, growers, and finishers.

MSHMP participants have agreed that PRRSv incidence graphs generated by their voluntary collaboration would be shared with the industry for benchmarking, disease monitoring, and promoting participation. In 2011, the project included 13 production systems that provided data related to PRRSv breeding herd status, location, and PRRSv interventions on a regular basis. Those systems represented approximately one million sows, which at the time, accounted for roughly 17% of the total US breeding herd based on USDA estimates (11). In 2013, porcine

epidemic diarrhea virus (PEDv) and porcine delta-coronavirus (PDCoV) emerged in the US swine population (12, 13). This dramatic and dynamic situation provided an opportunity for producers to continue working together, and therefore PEDv and PDCoV were added to the list of diseases being monitored and reported on a voluntary basis (14). Over the years, others were prompted to join the project, which ultimately increased the representativeness of the database and thus provided a more accurate benchmark for the industry. Senecavirus A and pathogens associated with central nervous system disease were later added to the list of pathogens reported to MSHMP. At the time when this manuscript was written in February 2019, 38 production systems, accounting for ∼50% of the US sow population, continue to provide their data for the benefit of the industry (**Figure 1**).

MSHMP PROJECT DESIGN

Briefly, after each participant has voluntarily agreed to participate, a participation form and a data privacy agreement are signed prior to data acquisition. Then, farm-level information, such as location, herd size, farm type (e.g., farrow-to-wean, farrow-to-feeder, farrow-to-finish), genetic level (e.g., multiplier or commercial herd) and air filtration status is provided by each participant and stored in a central database housed at the University of Minnesota. One person within each participating system serves as point of contact for the project, becoming responsible for data sharing in direct communication with the MSHMP's data coordinator. Each week, participants share the list of sow farms that have changed their status, either worsening (e.g., outbreaks) or improving (e.g., ceased shedding or completed elimination). Once the information is received by the MSHMP data coordinator, it is reviewed for quality control and entered into the main database. Data are then used to estimate measures of disease occurrence such as incidence and prevalence. For incidence, a graph featuring the weekly and yearly cumulative incidence is presented. Incidence data are also used to create an exponentially weighted moving average graph, in which the magnitude and duration of the outbreak is graphed through time. Additionally, that figure depicts a threshold level (upper confidence limit of the two lowest seasons in the previous years) that marks the start and end of epidemic periods. The prevalence graph for PRRSv is based on a classification from the American Association of Swine Veterinarians (AASV), in which each PRRSv sow herd status is defined (15). The MSHMP report is comprised of 6 pages including participant logos and supporting/funding sources (page 1), the aggregate incidence and prevalence graphs for PRRSv (page 2) and PEDv (page 3), the Seneca Valley Virus and Atypical central nervous system case counts (page 4), a space for sharing the latest developments in swine related research referred to as "science page" (page 5), and the names and affiliations of all the individuals that receive the public report (page 6). Additional pages are shared with the project participants referring only to their own systems, and including incidence and prevalence graphs for both PRRSv and PEDv.

MSHMP'S CONTRIBUTION TO SWINE HEALTH SCIENCE

Since its inception in 2011, the MSHMP has played a critical role in providing data that scientists translated into science-driven solutions to help the US swine industry mitigating PRRSv impact. Here, we summarize some important swine disease features that the MSHMP has helped to elucidate and that promoted engagement and participation among producers.

PRRSv Annual Occurrence Is Nationally Consistent but Regionally Different

For the last 10 years (2008–2018), PRRSv has maintained stable incidence levels on an annual basis, with an increase in the number of outbreaks, colloquially referred to as "PRRS season," consistently starting between mid-October to mid-November (16). The only period that showed a substantial different incidence, compared to other periods, was in 2013–2014, when PEDv was first detected in the US (17).

However, seasonal dynamics seemed to differ across different states, with Minnesota, North Carolina, and Nebraska having more consistent seasonality than Iowa, and Illinois. Furthermore, there seems to be a secular pattern in the southern and southeastern regions of the country, with large epidemics occurring every 2–4 years (18, 19).

PRRSv Impact May Be Mitigated by Implementing Certain Management Strategies

MSHMP investigators developed herd-level metrics of success of PRRSv control and elimination programs, such as time-to-stability (TTS), time to baseline productivity (TTBP), and total loss per thousand sows. Those metrics were used to compare the effect of multiple aspects or interventions on breeding herds affected with PRRSv. PRRSv-infected breeding herds achieved stability (i.e., producing PRRSv-negative piglets) significantly sooner, compared to other strategies, when they had reported a prior infection (i.e., existing partial herd immunity) and implemented herd closure (i.e., temporary interruption of replacement breeding stock introduction), and/or used wild type live virus inoculation as part of load-close-expose programs. Specifically, TTS was 7 weeks sooner for breeding herds adopting live-virus inoculation (LVI) as part of a load-close-expose program compared to those that used MLV vaccination protocols. Conversely, herds using MLV achieved TTBP 7 weeks sooner, and lost 1,443 piglets/1,000 sows less than herds using LVI. Altogether, economic analysis revealed an advantage for herds using MLV compared to LVI (20, 21).

More recently, MSHMP data have been used to estimate the breeding herd PRRS time-to-stability of a subset of breeding herds from six production systems that used similar testing criteria. A total of 82 breeding herds in the Midwestern US participated in this study, which accounted for ∼250,000 sows. These herds reported 161 PRRS outbreaks between 2011 and 2017. Breeding herds that had PRRSv outbreaks during the spring

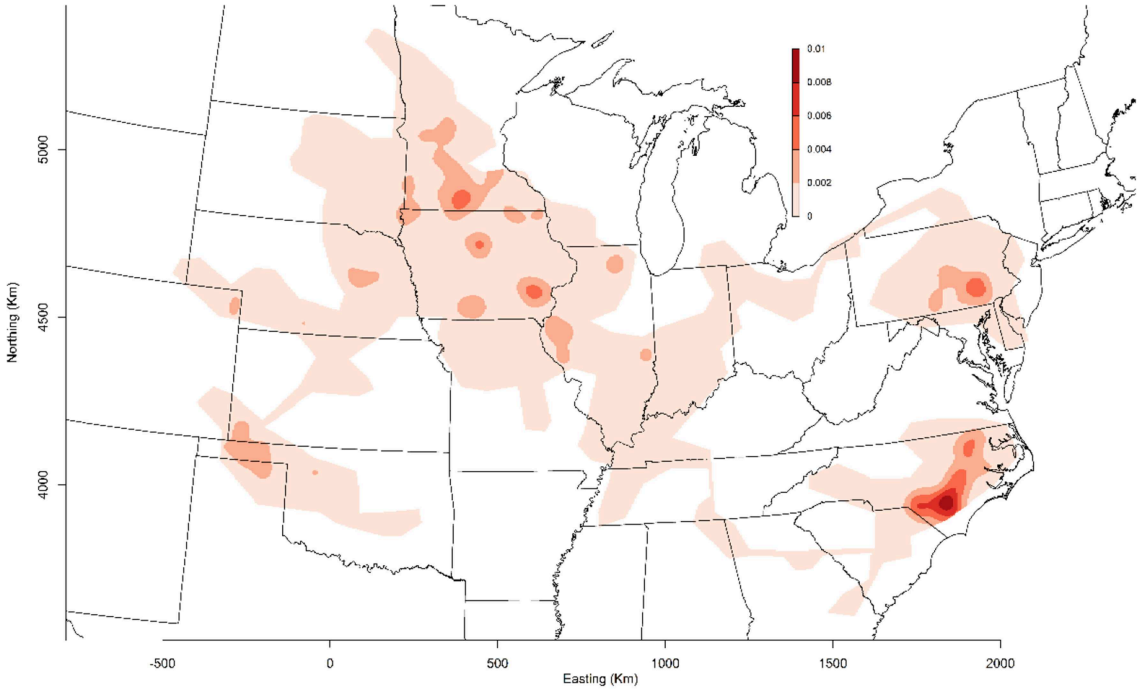

FIGURE 1 | Geographical distribution of farms enrolled in the Morrison Swine Health Monitoring Project (MSHMP) as of February 2019.

and summer had a significantly longer TTS than herds that had outbreaks during the fall and winter. In addition, there was a significant difference between TTS between production companies suggesting that there are particular factors within production systems that may drive viral persistence in breeding herds (22).

The Annual Cost of PRRSv to the Swine Industry has Decreased Since 2011

The MSHMP has been used to provide incidence data that helped to estimate the cost of PRRS, as part of various projects aimed at monitoring PRRS impact over time. Those data have helped in part to demonstrate that the annual cost of PRRS to the US swine industry has decreased $83.3 million from October 2010 ($663.91 million) to October 2016 ($580.62 million) (23), partially possibly due to increased number of breeding herds constantly immunizing sows with attenuated PRRS virus vaccines (**Figure 1**), which has been associated with reduced production losses (20).

Some have suggested that, coincidently with the dissemination of research results demonstrating the impact of control measures on TTS, the proportion of sow farms in the MSHMP that have implemented vaccine-based control strategies substantially increased, suggesting that producers may have seen some value in the adoption and implementation of those research findings in the field (**Figure 2**). Noteworthy, however, it is also possible that the shift may be explained, at least in part, by other factors that were not formally assessed such as, for example, PED emergence in the country. In any case, data provided by participants has allowed MSHMP to compile and visualize the rate at which different control strategies have been adopted by participants.

Environmental Factors Are Associated With the Odds of PRRS Outbreaks

Swine farm density has long been recognized as an important indicator for the risk of farms becoming infected with PRRSv and PEDv, even though the relative contribution of different routes of infection remain debated among swine practitioners and producers. Through the use of historical MSHMP data along with publicly available datasets, insights on environment-related factors that could affect the risk of PRRSv outbreaks were assessed. Swine sites located in regions with higher land slopes, and swine sites surrounded by trees and herbaceous coverage were protected from reporting a PRRSv outbreak compared to sites located in regions with lower land slopes and regions characterized by cultivated areas, respectively (24). However, the effects of slope may not be consistent across all regions, and may depend on the specific topography of the area (25). Precipitation, temperature, and land cover were all contributors for PRRSv spatial risk, with specific contribution amounts varying according to "subregion" in the US (26). For PEDv, temperature, wind speed, and vegetation have been identified as important modulators of risk, even in swine-dense areas (25). Taken together, these results demonstrate that, even for highly intensive pig production, environmental factors play an

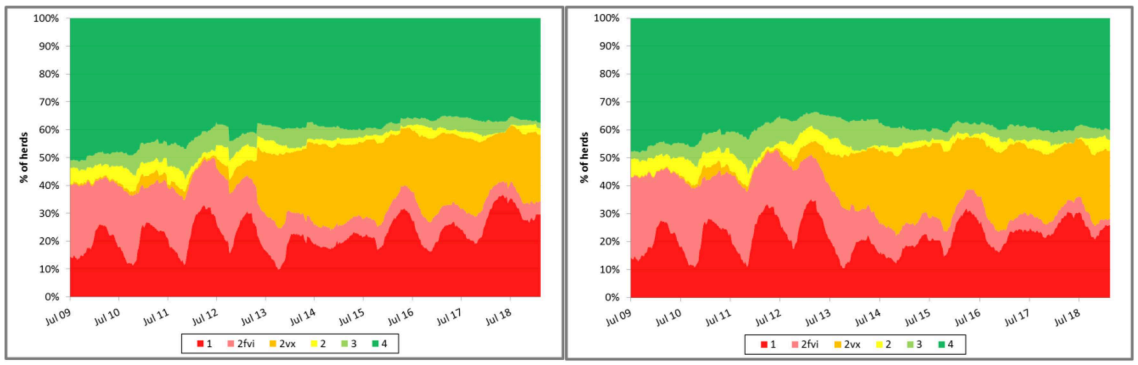

FIGURE 2 | Evolution of porcine reproductive and respiratory syndrome (PRRS) status recorded in the Morrison Swine Health Monitoring Project (MSHMP) between 2009 and 2017 (left) and in 12 systems that consistently reported the status during the same time period (right). Breeding herds have been classified using a slightly modified terminology to the one proposed by Holtkamp et al. (15) that incorporates breeding herd shedding and immune status to increase its relevance to industry practices. Category 1 (red): positive unstable breeding herds that are undergoing a PRRSv outbreak and are weaning positive piglets. Category 2fvi (pink): herds that continue to expose breeding replacements to a live field virus strain (e.g., live-virus inoculation). Category 2vx (orange): herds that continue to expose breeding replacements or sows to PRRSv through the use of a modified-live PRRSv vaccine. Category 2 (yellow): herds that have uncertain PRRSv shedding status and positive PRRSv exposure status (animals are seropositive) with no clinical signs, no evidence of weanling piglet viremia and have stopped gilt and sow exposure to live virus. Category 3 (light green): herds that have negative PRRSv shedding status and have introduced breeding replacements that maintain seronegative status for more than 60 days. Category 4 (dark green): PRRSv-naïve herds in which pigs are negative for both shedding and exposure status for at least a year after reaching Category 3.

important role in determining outbreak risk and drive further disease spread.

The Highly Infectious Nature of Swine Viral Infections, Along With the Strong Association With Epidemiological Factors May Facilitate Forecasting Disease Risk in Breeding Herds

For the past several decades, epidemiological modeling has been an important tool for understanding and predicting the spread of infectious diseases. The availability of farm-level outbreak data through MSHMP, combined with data on animal movements between farms (27), has enabled some of the first data-informed epidemiological models in the US swine industry. The availability of PRRSv genetic sequences from farms that became infected [see for example, (28)] allowed for the model to be fit to the observed spatiotemporal dynamics of the unfolding PED epidemic in 2013 (29). Data on which and when farms become infected also has opened the possibility of using this rich database to develop predictive models that could be used to forecast when a farm is expected to be at high risk. The potential for forecasting was initially explored using PED incidence data (25). Animal movement data available within the MSHMP was combined with environmental risk factors within a 10 km radius around breeding farms to identify major drivers of PED outbreaks, which included the total numbers of pig movements into neighboring farms, regional hog density, environmental, and weather factors such as vegetation, wind speed, temperature, and precipitation, and topographical features such as slope. Results suggest that PED occurrence may be predictable with an acceptable (i.e., >80%) level of accuracy, which eventually may lead to the design and implementation of a near real-time forecasting system for these diseases.

CONCLUSIONS AND PERSPECTIVE

The US swine industry continues to reach important production levels together with expanding their breeding and growing herds. Infectious diseases are one of the most important limiting factors as they deteriorate performance and increases production costs. Furthermore, foreign animal diseases are of concern for the industry as they could potentially affect the industry's export market. Therefore, the US swine industry continues to work closely with researchers to seek answers and implement procedures to mitigate the burden of endemic diseases, while building capacity to respond against emerging and foreign animal diseases. A program like MSHMP, which its foundation is trust and voluntary participation, has had an important impact on the surveillance of pathogens for the national industry. An academic institution-led program has motivated producer and veterinarian collaboration for the greater good of the industry. Reasons why producers and veterinarians have been willing to share their data are difficult t to assess. Initially, there was an intention to standardize data collection and sharing, to improve situational awareness and facilitate the decision-making process considering the epidemiological situation of the disease in near real time and in the entire region, rather than on their own farms and systems only. However, motivation declines with time, and it is important to demonstrate the added value of the effort. In that regards, even though shared data are private and managed only by the MSHMP team, producers and veterinarians still obtain a benefit because important outputs, that help inform their decisions, are routinely shared with them. PRRSv has been used as a way to bring the industry together and build the methodology to create a program that has the potential to be adaptable to other diseases. MSHMP has demonstrated over the years that voluntary organization of swine producers and practitioners toward a common goal resulted in

a powerful initiative that outweighed initial concerns about own data protection. Most importantly, such voluntary collaboration has also led to collaborative research involving a number of higher education institutions in the US that helped to elucidate some of the most important epidemiological features of endemic swine diseases in the US, and ultimately, provided a substantial support to the mitigation of disease impact in the country. The evolution of analytical capabilities for big data analysis is expected to result in novel and innovative tools that will allow for the routine analysis of big datasets, such as that collected in the MSHMP. The promotion of social initiatives, intended to promote data sharing and self-organization of producers, along with the application of those novel analytical techniques, may help to reshape in the near future the landscape of coordinated activities to promote the prevention and control of food animal diseases worldwide.

DATA AVAILABILITY

No datasets were generated or analyzed for this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Bayesian Assessment of the Accuracy of a PCR-Based Rapid Diagnostic Test for Bovine Tuberculosis in Swine

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Infection with the *Mycobacterium bovis (M. bovis)* causes a disease referred to as bovine tuberculosis (bTB), which affects a wide range of mammal hosts. Many countries have implemented control and eradication plans that have resulted in variable levels of efficacy and success. Although bTB is a notifiable disease in Argentina, and a control plan that targets cattle herds has been in place for decades, *M. bovis* is still prevalent in cattle, swine, and certain wild species. The aim of the paper here was to assess the sensitivity (Se), specificity (Sp), and positive and negative predictive values (PPV and NPV) of PCR from tissue, which is a test for rapid *M. bovis* detection in swine. Bacteriological culture was also performed for comparison purposes. A Bayesian approach was applied to estimate the accuracy of the diagnostic tests, PCR and bacteriological culture, in 266 swine samples with bTB-like lesions recovered during routine official inspections at slaughterhouses. A one-population model, assuming conditional dependence between test results, and incorporating prior information on the performance of the tests obtained from the literature, was used to estimate the tests Se and Sp. The accuracy of the combined (in parallel) application of both tests was also estimated. The Se of the PCR (82.9%) was higher than the Se of the bacteriological culture (79.9%), whereas the Sp of both tests was similar (88.5 and 89.0%, respectively). Furthermore, when both techniques were assessed in parallel, the Se of the diagnostic system increased substantially (Se = 96.6%) with a moderate Sp loss (Sp = 78.8%; PPV = 92.8%; NPV = 89%). Results suggest that the PCR, or the combined application of bacteriological culture and PCR, may serve as an accurate diagnostic tool to confirm bTB in swine samples. Results here will help the design and implementation of effective surveillance strategies for the disease in swine of Argentina and other settings in which the disease is prevalent.

Keywords: tuberculosis, swine, diagnosis, bacteriology culture, PCR, bayesian modeling

INTRODUCTION

Mycobacterium bovis (*M. bovis*) is a member of the *Mycobacterium tuberculosis* complex (MTC) (1). MTC members cause tuberculosis (TB) in a wide range of host species worldwide, and *M. bovis* is a major causative agent of bovine tuberculosis (bTB), holding a significant zoonotic potential (2). Globally, bTB prevalence is quite heterogeneous and somehow related to the social features of the setting, with the disease being endemic in most developing countries and eradicated from many developed regions (3). Factors suggested to have impaired the efficacy of bTB control programs include limited political willingness, resources scarcity, existence of wildlife reservoirs, and limitations of the available diagnostic tests (4–8).

There are many tests available and widely used to diagnose the disease (9, 10). Many bTB diagnostic tests have been applied for decades, and rely on a variety of biological principles, including the measurement of the cellular response in the host following the application of an intradermal test, the histopathology of postmortem specimens, or the bacteriological culture (BC) of the agent (11). Currently, there are many other testing strategies available, such as the interferon gamma essay, antibody-based assays, or the detection of bTB DNA by PCR, which presents new opportunities to improve or develop control plans, but for which there is still a need to gain understanding of their performance in field conditions (9, 12, 13). However, because those diagnostic techniques have values of sensitivity (Se) and specificity (Sp) that vary on each animal species and the specific epidemiological situation, estimation of the test accuracy is challenging (14–16).

In Argentina, bTB is endemic in both livestock and wildlife populations (17). The protein purified derivative (PPD) skin test and the meat inspection of carcasses at slaughterhouses are the actions approved and used in the bTB's Control and Eradication National plan (SENASA, Res.128/2012). However, PPD testing is compulsory only for dairy cattle, dairy goats, dairy sheep, and genetic nuclei and multipliers. Control activities are voluntary for other species, including swine.

Official records estimated that 0.3% of inspected pigs in Argentina showed TB-like lesions, as observed by the Argentine Animal Health Service (SENASA) inspectors at slaughterhouses. However, evidence suggests that the figure may have been underestimated (18–20).

The BC is considered the reference technique for bTB diagnosis, even though the Se of the test is only ∼80% (10, 12), impairing its systematic application on disease control programs. Moreover, it is a laborious technique, which requires high biosecurity facilities and relatively specialized workforce for implementation. Also, because the technique depends on the agent's viability, preservation, and quality of the collected sample drastically affects the results (10, 21). Another key limitation is the relatively long turnaround time of the techniques (on average, between 2 and 3 months), which jeopardizes the ability to inform decision-makers on a timely manner (22, 23). For that reason, the BC has important limitations as a confirmatory test for the macroscopic inspection at slaughterhouses (**Table 1**).

The direct PCR analysis from tissue samples has been developed as an alternative technique to obtain a relatively fast confirmation of the infection. Direct PCR is believed to allow for the rapid, specific detection of *Mycobacteria*, and it is independent of the agent's viability on the sample. Some studies have reported the performance of the direct PCR in samples from cattle, buffaloes, humans, and some wildlife species (10, 14, 16). However, to the best of the authors' knowledge, the accuracy of the test is yet-to-be-assessed in swine (12).

The evaluation of diagnostic test performance, traditionally, has been based on the comparison of test results against a gold standard, allowing the assessment and validation of new techniques in comparison to a reference test. The limitation of such analytical approach is that the assumption of perfect Se and Sp of the reference test is, typically, questionable for many diseases, including bTB (21). Alternatively, Bayesian methods have been proposed as an analytical option to assess the accuracy of diagnostic tests without the requirement of a reference test (24). Bayesian methods have previously been used to estimate the accuracy of TB diagnostic techniques in bovine populations (8, 10, 25).

The aim of the study here was to estimate the Se and Sp of the BC, and of a rapid diagnostic test (PCR from tissue) on swine TB-like lesions obtained at slaughterhouses, and thereafter to evaluate the combined performance of those tests. Results will inform current discussions regarding the evaluation and potential modifications to the bTB control strategies in the target population and in the context of the Argentine disease control plan. Results may

TABLE 1 | Key features of both diagnostic tests that influence the feasibility of implementation in the context of a control plan.

Test / Requirements	Bacteriological culture + identification by PCR	DNA extraction from tissue + identification by PCR
Laboratory biosafety	High: BSL-2. During all process. Special features: Viability and exponential amplification of the agent. Long process. High exposition risks.	High: BSL-2 For initial manipulation. Until extraction and inactivation of the remaining material.
Average turnaround time	2–3 months	3–4 days
Relative cost	1*	1.33#
Trained personnel	Highly dependent on the operator skills (technique sensitivity varies with the level of skills and experience of the operator)	Average training requirements. Allows broader automation. PCR tests are more robust and versatile tests for labs settings.

Relative cost: based on the comparison between average costs provided by 3 local labs.
**cost of Mycobacteriology culture includes: culture, Ziehl-Neelsen staining, and identification by PCR.*
#cost of direct PCR from tissue samples, includes: extraction kit and PCR.

also be useful for countries in which bTB is prevalent in swine populations.

MATERIALS AND METHODS

Sample Collection

Swine samples ($n = 266$) showing bTB-like lesions (TBL) were collected in multiple visits to three slaughterhouses located in the Province of Buenos Aires between 2015 and 2017. Those three slaughterhouses processed pigs from the main productive region of Argentina, which includes the provinces of Buenos Aires, Córdoba, Santa Fe, La Pampa, and Entre Ríos. Approximate 4×4 cm cuts of lymph nodes showing bTB-like lesions were collected. Additionally, tissue samples from swine shipped from bTB-free premises were also collected in order to validate the DNA extraction and PCR assay.

All samples were stored at −20°C. BC and PCR were carried out at the Infectious Disease Department's Mycobacterial diagnosis laboratory of the Veterinary School of the University of Buenos Aires. Because samples were collected from animals inspected post-mortem by the national authority and according to national regulations, no ethical or farmer's consent approval was required.

Diagnostic Procedures

Preparation of the Samples for PCR and Culture

Samples (4–7 g) of each individual lymph node were placed into a mortar and crushed with sterile sand and 10 mL of sterile bi-distilled water for homogenization. Two milliliters of this homogenate were transferred into a 15 mL tube and 4 mL of 4% NaOH were added to decontaminate the sample using the Petroff's modified method described elsewhere (26). A portion (∼400 μL) of the homogenate was separated and frozen at −20°C for further DNA extraction.

DNA Extraction

Invitrogen™ PureLink™ Genomic DNA Mini Kit (Invitrogen, California, USA) was used for DNA extraction directly from tissue, according to the manufacturer's protocol. The obtained DNA was stored at −20°C until use for the PCR assay.

Bacteriological Culture

Bacteriological culture was performed following a protocol established elsewhere (26). Stonebrink and Löwenstein Jensen media were inoculated and incubated up to 60 days at 37°C and examined every 2 weeks. When bacterial growth was observed, Ziehl Neelsen staining was performed to observe acid fast bacilli, and, if positive staining, a loop full of bacteria was suspended in 200 μL of bi-distilled water and thermal lysis was performed at 95°C for 45 min. Lysates obtained were stored at −20°C until PCR assay.

Polymerase Chain Reaction (PCR) Assay for *M. bovis* Detection

PCR was conducted in both DNA extracted from tissue and colonies lysates, for the detection of the insertion sequence *6110* (IS*6110*) characteristic of the MTC (27). Positive (bTB-confirmed sample) and negative controls (bi-distilled water) were also evaluated by the PCR. The primers used for the amplification, as well as PCR cycling conditions, have been described elsewhere (28). Colonies grown in Stonebrink media and IS*6110*-PCR positive were subject to spoligotyping to identify and to type the *M. bovis* isolates, following the protocol described by Kamerbeek et al. (29). Spoligotyping was carried out using the spoligotyping kit (Mapmygenome India). *M. tuberculosis* H37Rv (ATCC 27294) and *M. bovis* Bacillus Calmette-Guerin (BCG) (ATCC 27289) were included as reference strains for each assay.

Detection of *Mycobacteria* Other Than Tuberculosis

Ziehl Neelsen staining-positive isolates that were IS*6110*-PCR negative, were tested for its identification. The IS*1245*-PCR was used to detect the *Mycobacterium avium (M. avium)* complex (30). PCR controls were also conducted using a strain of *M. avium* obtained from a pure culture by thermal lysis as a positive control and bi-distilled water as a negative control.

Statistical Model

Latent Class Analysis

A Bayesian approach was used to estimate the Se and Sp of the BC and the PCR test (24) in samples showing bTB-like lesions ($n = 266$) and in the absence of a gold standard. Samples were considered to have originated from one single population, given that only samples showing bTB-like lesions were evaluated. For the analysis, results from both tests were assumed to be conditionally dependent because, although biological principles of both tests are different (the culture required that the pathogenic agent was viable, whereas the PCR only requires the presence of the genetic material in sufficient quantity), both tests are based on the detection of the mycobacteria. For that reason, we preferred to follow the conservative assumption that results were not independent.

Prior distributions for model parameters (including test Se and Sp and the disease prevalence, p) were initially approximated using information on the expected values and uncertainty around that expectation, from data reported in the peer-reviewed literature (**Table 2**). Beta distributions of the parameters were fitted using BetaBuster (https://betabuster.software.informer.com/), using a most likely value based on the median of estimates published in the literature, and a lower bound of the credibility interval that was approximately three standard deviations below the median, according to the published data (lower 99%CI Se = 0.58; Sp = 0.81). We preferred to use wide prior standard deviations to reflect the uncertainty around the true value of the parameters, considering that uncertainty related to the true value of the parameters is likely larger than simply the 95% CI of the results reported in the literature. Uniform distributions were used for the two co-variances (33). Field data were then used to modify the prior distributions and estimate posterior distributions in a Bayesian framework, using a one-population model and assuming conditional dependence between test results. Posterior distributions were reported as the posterior estimates of the median and posterior probability intervals (95% PPI). The code is provided as **Supplementary Table 2**. All analyses were implemented in the WinBUGS software, version 1.4, and results were computed for 10,000 iterations,

TABLE 2 | Parameters of the beta distribution and source of data used to estimate the accuracy of both bTB tests in swine samples from Argentina.

Parameter		Most likely value	Uncertainty	References	Parameters of the beta distribution	
					A	B
Bacteriology culture	SeBC	0.79	>0.6	(10, 31)	16.1034	5.0197
	SpBC	0.97	>0.8	(10, 31)	17.2976	1.5041
PCR	SePCR	0.81	>0.6	(10, 12, 14, 15)	13.7759	3.7573
	SpPCR	0.99	>0.8	(10, 12)	14.52192	1.13658
True prevalence	P	0.32	>0.2	(32)	5.025	7.0375

BC and MTC-PCR IS6110 (PCR). Uncertainty was measured as the 95% confidence level that the parameter was higher (>) than a certain value. Se, Sensitivity; Sp, Specificity.

after the first 1,000 were burnt-in. Autocorrelation was eliminated through thinning the chains by collecting one in 10 consecutive samples (https://www.mrc-bsu.cam.ac.uk/software/bugs/the-bugs-project-winbugs/). The influence of the selected priors on the posterior distributions was evaluated by comparing the initial models with a model fitted using non-informative uniform (0.1) distributions alternatively for the parameters of each test and the prevalence (**Supplementary Table 1**). The outputs including the MCMC trace-plots, posterior density distribution plots and convergence were visually assessed (**Supplementary Figure 1**).

Agreement between the results obtained from both test was measured using the kappa statistic. The Kappa coefficient, combined Se and Sp of the tests used in series and in parallel, and the positive and negative predictive values of the tests were calculated using the posterior estimates of the model and using the WinEpi software (34) as:

$Se\ series = Se_{PCR} \times Se_{BC}$

$Se\ parallel = 1–(1–Se_{PCR}) \times (1–Se_{BC})$

$Sp(series) = 1– (1–Sp_{PCR}) \times (1–Sp_{BC})$

$Sp(parallel) = Sp_{PCR} \times Sp_{BC}$

For presenting the results here, we followed the guidelines for reporting of diagnostic accuracy in studies that use Bayesian Latent class models (STARD-BLCM) described elsewhere (35).

RESULTS

Descriptive Results

Most (171/266, 64.8%) samples were culture-positive, and most of those samples (137/171, 80.1%) were also MTC-IS*6110*+ PCR-positive. Out of the PCR-positive samples (176/266, 66.2%), only some (39/176, 15%) were BC-negative. A few (13/56, 23.2%) of the remaining 21% culture and PCR-negative samples (i.e., 4.9% of all the samples) were *M. avium complex* (IS*1245*+)-positive (**Table 3**). All IS*6110*-positive samples showed spoligotypes that were characteristic of *M. bovis* species, due to the absence of the 3, 9, 16, and 39–43 spacers.

Estimation of Tests Se and Sp

The estimated (posterior) Se of the bacteriological culture and of the PCR were 79.9% (95% posterior probability intervals, PPI: 71.69–88.7%) and 82.9% (95% PPI: 74.35–92.3%), respectively. The estimated (posterior) Sp was similar for both tests, with a value of 88.5% (95% PPI: 67.2–99.5%) for culture, and of 89.05% (95% PPI: 69.8–99.1%) for PCR.

Bovine tuberculosis prevalence in TB-like samples was 74.39% (95% PPI: 63.3–83.5%). The agreement of both tests was moderate (Kappa coefficient = 0.395; 95% CI (confidence interval) = 0.304–0.486). The negative and positive posterior correlation estimated between the diagnostic tests was uncertain, −0.02 (95% PPI: −0.2–0.33) and 0.16 (95% PPI: −0.15–0.74), respectively. The low correlations between the two test Se and between the two test Sp for samples showing bTB-like lesions suggests that the results of both tests were independent from each other.

Combination of both tests was evaluated considering the estimated prevalence, obtaining a Se and Sp of 66.2 and 98.7% (positive predictive value, PPV = 92.8%; negative predictive value, NPV = 89%) when the test were combined in series, and a Se and Sp of 96.6 and 78.8% (PPV = 92.8%; NPV = 89%) when combined in parallel. Results for a broad range of hypothetical prevalence values are presented in **Figure 1** to illustrate the expected variation on the PPV and NPV for alternative scenarios of disease prevalence when those two techniques were combined in parallel.

Results were not sensitive to the selection of the prior distributions, as suggested by the relatively consistency (magnitudes of percent differences <9%) in the results when using non-informative priors (**Supplementary Table 1**), to the posterior distribution of Se and Sp for both tests except for the Sp of the tests in which a reduction of 26.6% for the culture and 29.1 for the PCR was estimated.

DISCUSSION

Although significant improvements have been made in the last 20 years, control and eradication of bTB continues to be a major challenge for Latin American countries (36, 37). Furthermore, the disease has re-emerged in humans in the region, reinforcing the need to understand the role of domestic and wild animals on the disease epidemiology, and, most importantly, the need for developing new strategies to effectively and efficiently prevent and control disease spread (37–40). Some of the challenges associated with bTB diagnosis in swine include presence of fewer bacilli in bTB lesions compared to other species (41, 42), relatively high susceptibility to avian TB infection, being those lesions undistinguishable from those produced by *M. bovis* (20, 32), and the lack, despite some promising advances (43), of simple, affordable, and sensitive diagnostic tools to identify infected animals at the farm level, like the PPD test use routinely

TABLE 3 | Distribution of the results for both bTB diagnostic tests applied.

	TB positive samples			TB negative samples	
	POS_{BC}/POS_{PCR}	POS_{BC}/NEG_{PCR}	NEG_{BC}/POS_{PCR}	NEG_{BC}/NEG_{PCR}	Total
TBL	137	34	39	56	266
	51%	13.8%	15%	21%	100

Samples of swine lymph nodes with TBL obtained from slaughterhouses were processed by BC and MTC-PCR IS6110 +PCR.

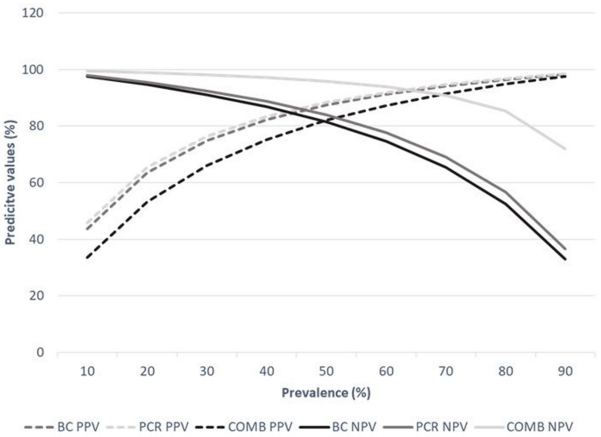

FIGURE 1 | Estimates of predictive values for a range of prevalence values based on the Se and Sp obtained in the analyses presented here. PPV, Positive predictive value; NPV, Negative predictive value; BC, Bacteriological culture; PCR, Polymerase Chain Reaction; COMB, Combined techniques in parallel.

in cattle (43–45). Here, we provided evidence suggesting that the PCR may be used as an effective tool for the rapid and effective detection of the infection in swine routinely inspected at slaughterhouses in Argentina. Ultimately, results presented here will help to inform decisions intended to update control strategies in endemic settings.

The high frequency of *M. bovis* infection in bTB-like lesions estimated in this assay (74.39%), suggests that the disease continues to be prevalent in swine populations of Argentina. Furthermore, the figure is substantially higher compared to those reported elsewhere (12, 32), who detected MTC in 32.7% of TBL samples from South West Iberian Peninsula and absence of MTC positive results from samples obtained in Sao Paulo region, respectively. Still, the high frequency (31%) of bTB reported in wild boar samples from the south of Brazil (46) would suggest the need for further studies, increasing the sample size, and targeting specific risk populations. The relative high bTB prevalence in Argentine swine is likely associated with different rearing systems. Such conclusion is also supported by the observation that the risk for bTB is relatively high in pigs reared in extensive, small (<50 sow) farms that are co-located with cattle (unpublished data). In this regard, unfortunately, not much evidence has been reported regarding the interspecies dynamic of transmission of bovine tuberculosis in farms where the cohabitation with other species, like cattle, exits. A report from the Association of Veterinarians of Buenos Aires province (47) provided preliminary evidence that supports this hypothesis, describing a different frequency of slaughterhouse findings of TBL based on rearing system being more frequent in extensive farms. Biosecurity in extensive farm is usually less strict than in intensive systems, increasing the risk for bTB (48).

The Se of the PCR test estimated here (83%) is similar to that reported for cattle (10), and higher than values previously reported for pig (12). The difference may be explained, at least in part, by differences in the study design, given that previous studies have used BC as the gold standard, whereas we have used a Bayesian approach that does not make use of such a questionable assumption. Another explanation may be that we used a larger sample size (4–7 g range of homogenized tissue for initial extraction) compared to previous studies (10, 12, 49). Increasing the volume of the samples may have increased the analytical Se (and thus the diagnostic test Se) of the test, without affecting the test Sp (13). One study (12) reported a detection rate of 77.3% of culture positive samples by RT-PCR and others (31) showed a Se of 66.1%, compared to 80.1% obtained in this work using direct PCR. Only one other study (49), reported a frequency of PCR-positive results similar to ours, working with bovine samples.

The bTB control program in Argentine swine is voluntary and its implementation is based on the use of PPD test at farm level and identification of bTB-like lesions in swine without the use of a confirmatory test. Here, we estimated that ∼25% of the bTB-like lesions observed at the time of slaughtering in swine are non-infected, suggesting a Sp of the inspection of ∼75% (95% CI: 63–85%). Furthermore, results suggest that the PCR may be used as a rapid, effective, confirmatory test for bTB-like lesions detected in pigs at the time of slaughtering, given that the test accuracy was similar (and on average, slightly higher) to that reported for the BC. Furthermore, the significant reduction of the turnaround time between the sample submission and the result would facilitate the follow up actions on positive cases by the sanitary authority. As expected, in-series combination of the tests impaired the combined Se value (66.6%), making it not suitable as a screening protocol. Conversely, in scenarios of relatively high bTB prevalence, such as those observed in Argentina, the in-parallel use of the BC and PCR (**Figure 1**) showed a good performance suggesting that the combined used of those techniques would be appropriate for the confirmation of the disease in bTB-like lesions. Consequently, it is recommended that bTB-like lesions were run by PCR and the positive samples be considered bTB infected, whereas negative samples would be run by BC, and considered as infected if positive, and non-infected

otherwise. Moreover, the NPV (probability that a sample negative to a screening test was non-infected) and PPV (probability that a sample positive to a screening test was infected) of the in parallel use of both tests (**Figure 1**) showed that this combination would be suitable for a wide range of prevalence. The combination of both tests showed much better NPV than the individual tests (% of increase, 8.5–35.3%) with prevalence values higher than 0.4; however, the combined used of the tests did not substantially impact the PPV, showing <1–8.5% reductions. Similar findings were also reported elsewhere (50–52). Results suggest that the combined use of those techniques would be appropriate for disease confirmation in bTB-like lesions and in the context of a TB control plan. The use of PCR as a routine confirmatory technique is commonly questioned taking into account only the associated direct costs (53). However, direct application of the PCR technique in tissues brings certain benefits such as the reduction of both the laboratory turnaround time and indirect costs associated with maintenance of personnel and facilities. Furthermore, bacteriological culture required significant investments in personnel training and the biosafety protocols and facilities, as it represents a much greater risk of exposure to the agent (**Table 1**).

In conclusion, these results suggest that bTB is still highly prevalent in swine populations of Argentina, and that the PCR may serve as an effective and rapid test for the confirmation of the agent in bTB-like lesions macroscopically detected at the time of slaughtering in the country. The results here may ultimately help to update current strategies used to prevent and control of the disease in settings in which the disease is yet-to-be eradicated.

AUTHOR CONTRIBUTIONS

SB, MP, and AP conceived and performed the statistical analysis. SB and MP drafted the manuscript. MJM, MM, NA, MZ, and SB participated in the generation, collection and curation of the data, and collaborated in interpretation of the results. SB and AP designed the study and coordinated the work. All authors read and approved the final manuscript.

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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.2019.00204/full#supplementary-material

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Comparison of Two Rift Valley Fever Serological Tests in Cameroonian Cattle Populations Using a Bayesian Latent Class Approach

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Rift Valley Fever is an important zoonotic viral disease of livestock occurring across much of Africa causing acute febrile illness, abortion, and neonatal death in livestock particularly sheep and cattle and a range of disease in humans from mild flu-like symptoms to more severe haemorrhagic fever and death. Understanding the epidemiology requires well-evaluated tools including antibody detection ELISAs. It is well-recognized that tests developed in one population do not necessarily perform as well when used in different populations and it is therefore important to assess tests in the populations in which they are to be used. Here we describe the performance of a commercial RVF ELISA (ID.Vet) and an in-house plaque reduction neutralization test ($PRNT_{80}$). A Bayesian no gold standard latent class model for two tests and ≥2 populations based on the Hui-Walter model was used to estimate the test parameters using a range of populations based on geographical separation and age to assess consistency of performance across different sub-populations. The ID.Vet ELISA had an estimated diagnostic sensitivity (Se) of 0.854 (0.655–0.991 95%BCI) and specificity (Sp) of 0.986 (0.971–0.998 95%BCI) using all the data and splitting the population by geographical region compared to 0.844 (0.660–0.973 95%BCI) and 0.981 (0.965–0.996 95%BCI) for the $PRNT_{80}$. There was slight variation in the mean Se and Sp in different sub-populations mainly in Se estimates due to small numbers of positives in the sub-populations but the 95% BCI generally overlapped suggesting a very consistent performance across the different geographical areas and ages of animals. This is one of few reports of serological evidence of RVF in Central Africa and strongly suggests the virus is actively circulating in this cattle population. This has important public health implications and RVF should be considered as a differential in both livestock disease cases as well as human febrile cases in West and Central Africa not just East Africa. We also demonstrate that the performance of the commercial

ELISA is comparable to the $PRNT_{80}$ but has the advantages of speed, lower cost and no containment needs making it a much more useful test for low and middle income settings (LMICs).

Keywords: Cameroon, no gold standard, sensitivity, specificity, Rift Valley fever (RVF)

INTRODUCTION

Rift Valley Fever (RVF) is a mosquito-borne zoonotic viral disease of ruminants, caused by a *Phlebovirus* in the *Bunyaviridae* family. It was first described in Kenya in 1931, and has since been reported in many African countries, as well as the Arabian Peninsula (1–3). It is considered one of the most important emerging zoonotic pathogens of public health significance affecting mainly African communities with low resilience to economic and environmental challenges (4). The epidemiology is characterized by explosive epidemics in both humans and livestock populations usually associated with flooding or dam construction and long inter-epidemic periods where there is little evidence of viral presence in those populations affected by epidemics. Where the virus persists in these inter-epidemic periods is still a major gap in our understanding of the epidemiology of RVF (4).

Aedes and *Culex* mosquitoes are the main vectors of the RVF virus (RVFV), and it can be transferred vertically from female mosquitoes to their eggs in some species of the *Aedes* genera (5–7). Sheep, goats, and cattle are the domestic species most affected but clinical signs are usually mild and inapparent in adult animals but can lead to major outbreaks of abortions and death in neonates during epidemic periods which result in direct significant economic losses (5, 8, 9). The disease can also affect other wild animals such as buffalo, as well as spill over into humans (10). RVF is transmitted between animals and to humans through the bite of an infected mosquito vector. The disease in humans can also result from direct contact with infected tissues, blood or body fluids (11). A rise in RVFV prevalence in domestic ruminants can sometimes precede epidemics in humans (1) and similarly a decline in herd immunity in the inter-epidemic periods coupled with extensive flooding appears to facilitate these explosive outbreaks. Symptoms of the disease in humans can vary, ranging from flu-like symptoms to more severe conditions such as meningoencephalitis, haemorrhagic fever, or death (5, 11). The case fatality rate for patients developing the haemorrhagic form of the disease can be as high as 50% (4).

Epidemiological studies have focused upon East Africa (12) where the virus was first isolated, with less known about its significance in Central-West African human or livestock populations although outbreaks in human populations in West Africa have been associated with dam projects (4). Within the Central African region, livestock and human cases of RVF have been reported in the savanna of northern Cameroon, Chad, and within forest areas in the Central African Republic. Livestock seroprevalences of 9–20% within goat herds of northern Cameroon (1, 13) and 4.4% in cattle, 10.7% in sheep and 8.6% in goats in Chad (14) have been reported. Most recently, in a large sample across Cameroon prevalence estimates of 13.5% (11.4–15.7) for cattle and 3.4% (2.3–4.7) for small ruminants were produced (15).

Cameroon is a significant cattle producer of the Central-African region with livestock contributing ∼$476 million to the national economy in 2010 (16) and being of cultural importance to rural communities. The Northwest Region (NWR) and the Vina Division (VD) of the Adamawa Region of Cameroon are major cattle keeping areas in the wider Adamawa Plateau of Central Africa. Cattle are kept for many reasons, including financial, draft power, dairy products, and trade. The area is mostly covered by sparse tree savannah, with a dry season between November and April, and the wet season from May until October (17). *Culex spp.* and *Aedes* spp. mosquitos are present in Cameroon and due to the close association between cattle and people in Cameroon, cattle may act as a reservoir for RVF although little is known about its epidemiology in this setting (15).

A number of tests have been developed to detect IgM and IgG antibodies in different species including a new commercial multi-species ELISA from ID.Vet (Montpellier, France) (15) and an in-house plaque reduction neutralization test by the Canadian (18). There are currently no validation studies in African cattle populations. It is well-recognized that diagnostic tests developed and validated in one population behave differently when used in different settings (19, 20). This is important for surveillance activities or risk factor evaluation in order for estimates at the population level to be correctly adjusted for the test imperfections and reliable estimates generated for evidence based decision making. Using population based serum banks from studies in Cameroon we are able to estimate test performance in naturally infected populations with a range of coinfections that may impact performance and thus get more reliable point estimates as well as capturing the variation and thus the uncertainty of these estimates.

Hui and Walter (21) developed a no gold standard model to estimate test sensitivity and specificity in the absence of a gold standard test under certain assumptions. These assumptions are that each test performs the same in each population, that the tests are conditionally independent (i.e., if a sample is positive in one test this does not influence the probability it will be positive on the other test conditional on its true status) and that the prevalences are different in each of the sampled populations (or the problem can become mathematically non-identifiable). Applying the model in a Bayesian framework and making some adjustment for the possible conditional dependence between tests (22) allows us to estimate the test parameters (sensitivity and specificity) as well as the population prevalence.

The aims of this study were to estimate the test performance of two RVF diagnostic tests in a naturally infected population and to assess the stability of these estimates in different geographical

regions and age groups. In particular we were interested in understanding how the ID.Vet test performed as it has wide potential use as a simpler screening serological test for low and middle income settings (LMICs). Secondly to describe the seroprevalence of RVF exposures in cattle in the NWR and VD of Cameroon in 2013.

MATERIALS AND METHODS

In reporting this analysis the authors have followed the STARD (23) and recent STARD-BLCM (24) guidelines for reporting diagnostic test accuracy.

Study Sites

The study was conducted in two sites in the NWR and VD of the Adamawa Region of Cameroon. Both are of similar geographical size of ∼17,000 km^2. The NWR is situated in the fertile mountainous highlands, 500–3,000 m above sea level. Bamenda, the capital, is Cameroon's third largest city. The Region is densely populated (1,804,695 people) and an estimated 506,548 cattle are grazed there (25). The VD is part of the fertile Adamawa Region's savannah plateau. The regional capital is Ngaoundere and the population of the VD (317,888 people) is much smaller than that of the NWR. The cattle population is also smaller with an estimated 176,257 head (26). Veterinary services are predominately provided by the government through the Ministry of Livestock, Fisheries, and Industrial Agriculture/Ministere de l'Elevage des Peches et Industries Animales (MINEPIA), with local veterinary technicians stationed at Zootechnical and Veterinary Sanitary Control Centers (ZVSCC) distributed across the country (17). Their responsibilities include registration of local livestock keepers, disease control mainly through annual vaccination campaigns, meat inspection, and regulation of livestock markets and animal movements.

Study Design

A cross sectional survey was conducted between January–May 2013 in the NWR and September–November 2013 in the VD. These were pastoralists whose herds were listed in the Ministry of Livestock, Fisheries, and Animal Industries vaccination records at 81 local veterinary centers in the NWR and 31 in the VD in 2012. A total of 5,053 pastoralist herds in the NWR and 1,927 in the VD, with a range of 1–215 cattle per herd were included in the sampling frame. The list of herds in each site was stratified by administrative area; seven Divisions in the NWR and eight sub-Divisions within the VD and a random sample of herds was taken from each site proportional to the total number of herds listed in each of the two sites. This survey was part of a larger study of bovine tuberculosis and liver fluke and the sample size was based on a clustered random sample of cattle assuming a cattle level prevalence of ∼10%, a within herd variance of 0.15 and between herd variance of 0.01, an average herd size of 70, a relative cost of 12:1 for herd:cattle and relative error of ±15% (Survey Toolbox; AusVet) (27). This gave a target sample size of 15 cattle per herd and 88 herds under the simplifying assumption of perfect test performance. To allow for potential losses or drop out and to have balanced samples from the two sites, we aimed for 50 herds in each of the two sites in the NWR and VD. Within each herd the 15 samples were stratified to each of three age classes; <2 years old (young), 2–5 years old (adult), and older than 5 years (old).

ID Screen® Rift Valley Fever Competition Multi-Species ELISA

The competitive ELISA was performed according to the instructions of the manufacturer and all the samples were run once. In brief, 50 µl of sample diluted 1:1 with the supplied kit buffer was added to each test well of the recombinant RVF nucleoprotein precoated plate and incubated for 1 h at 37°C. The plate was washed and 100 µl of the supplied anti-RVF-NP conjugate added and incubated for a further 30 min at 21°C and washed. 100 µl of supplied substrate solution supplied was added and incubated for a final 15 min at 21°C before adding the stop solution. The plate was read at 450 nm. To control the validity of each plate, the mean value of the two negative controls (ODNC) was calculated and the plate was considered valid when $OD_{NC} > 0.7$. For a valid plate, the mean value of the two positive controls divided by OD_{NC} should be <0.3. For each sample the competition percentage was calculated by dividing $(OD_{sample}/OD_{NC}) \times 100$. The manufacturers suggest if the value was ≤40% the sample was considered positive. A value >50% was a considered a negative result and values in between 40 and 50% indicated an inconclusive result.

RVF $PRNT_{80}$

RVFV strain ZH501 (28) was propagated in a mosquito cell line (C6/36, ATCC) as previously described (18). Briefly, C6/36 cells were infected at a multiplicity of infection (MOI) of 0.1 and maintained at 28°C in a 1:1 mixture of EMEM (Wisent) and ESF-921 (Expression Systems, Woodland, CA, USA) supplemented with 2.5% FBS, 25 mM HEPES and 1 mM sodium pyruvate. Vero E6 cells were used to determine the titers of RVFV as previously described (18).

Neutralizing antibody response to RVFV was determined by plaque reduction neutralization test ($PRNT_{80}$) modified from a previously described protocol (5). Serial 2-fold dilutions of serum in DMEM were made starting from 1 in 40 to obtain duplicates of 100 µl/well for each serum sample. One hundred microliter of DMEM containing 100 PFU of RVFV was added to each serum dilution, mixed, and incubated at 37°C, 5% CO_2, and 95% relative humidity for 1 h. Two hundred microliter of the virus/serum mixture was then transferred onto a 48-well plate containing confluent Vero E6 cell monolayer and incubated for another 1 h. An overlay of 1.75% carboxymethylcellulose in DMEM containing 0.3% BSA was then added to all wells and plates incubated at 37°C, 5% CO_2, and 95% relative humidity. Assay of negative and positive control sera as well as a back titration of the virus was performed at the same time as the test sera. After 4–5 days the cells were fixed with 10% formalin, stained with 0.5% crystal violet and plaques counted. The reciprocal of the highest serum dilution that prevented at least 80% CPE was taken as the $PRNT_{80}$ titer for that sample.

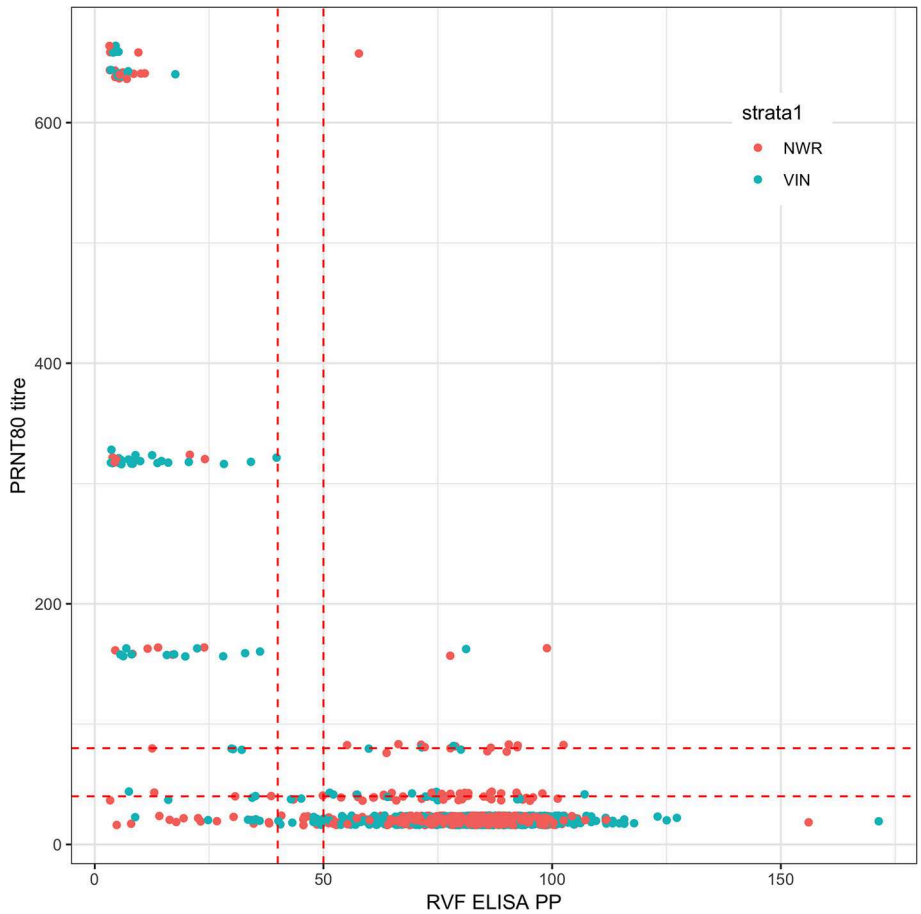

FIGURE 1 | Bivariate plot of the raw continuous values from the $PRNT_{80}$ and ID.Vet RVF tests. The points are colored by study site and jittered on the y axis. Horizontal and vertical lines in dashed red were added to mark the various cut-off values used. Note that for the $PRNT_{80}$ a positive result is greater than the cut-off while for the ID.Vet ELISA is lower than the cut-off.

Data Analysis

Hui and Walter (21) introduced a latent class approach to the evaluation of diagnostic tests in the absence of a "gold-standard." The Hui-Walter paradigm requires two (or more) tests evaluated in two (or more) populations. This model assumes that: (i) the prevalence of the disease is different within each population; (ii) the tests have the same properties across populations; (iii) and the tests must be conditionally independent given the disease status.

The Bayesian version of the Hui-Walter model (29) assumes that for the *i*th subpopulation the counts ($\mathbf{O}_i$) of the different combinations of test results, +/+, +/–, –/+, and –/– for the two tests, follow a multinomial distribution:

$$\mathbf{O}_i|\mathrm{Se}_j, \mathrm{Sp}_j, \mathrm{p}_i \sim \mathrm{Multinominal}(\mathbf{Pr}_i, \mathrm{n}_i) \quad \text{for} \quad \begin{matrix} i = 1, 2, \ldots, S \quad \text{and} \\ j = 1, 2, \ldots, T \end{matrix}$$

where S is the number of subpopulations, T is the number of tests and $\mathbf{Pr}_i$ is a vector of probabilities of observing the individual combinations of test results. Conditioning on the (latent) disease status, these probabilities can be specified using the sensitivity (Se) and specificity (Sp) of the tests and the prevalence (p) in subpopulations. As an example, for two tests the probability of observing both tests positive in the *i*th subpopulation is given as:

$$\Pr(\mathrm{T}_1+, \mathrm{T}_2+) = \mathrm{Se}_1\mathrm{Se}_2\mathrm{p}_i + (1 - \mathrm{Sp}_1)(1 - \mathrm{Sp}_2)(1 - \mathrm{p}_i)$$

The other three probabilities for the remaining three test scenarios may be similarly derived.

In a Bayesian analysis all parameters are given distributions. Hence, prior distributions for the test properties and the prevalences within the subpopulations must be specified. For prevalences where no information was available the distributions were modeled using a uniform distribution on the interval between 0 and 1 with a beta(1,1) distribution. The Se and Sp of the two tests were modeled using the priors $\mathrm{Se}_1 \sim$Beta(20,1) and $\mathrm{Sp}_1 \sim$Beta(5,1) for the ID.Vet ELISA based on published estimates of performance (30) and vaguer priors $\mathrm{Se}_2 \sim$Beta(5,2) and $\mathrm{Sp}_2 \sim$Beta(10,2) were used for the RVF $PRNT_{80}$. A sensitivity analysis was done to confirm that the priors were not overwhelming the posteriors and driving the estimates (see **Supplementary Material S3**).

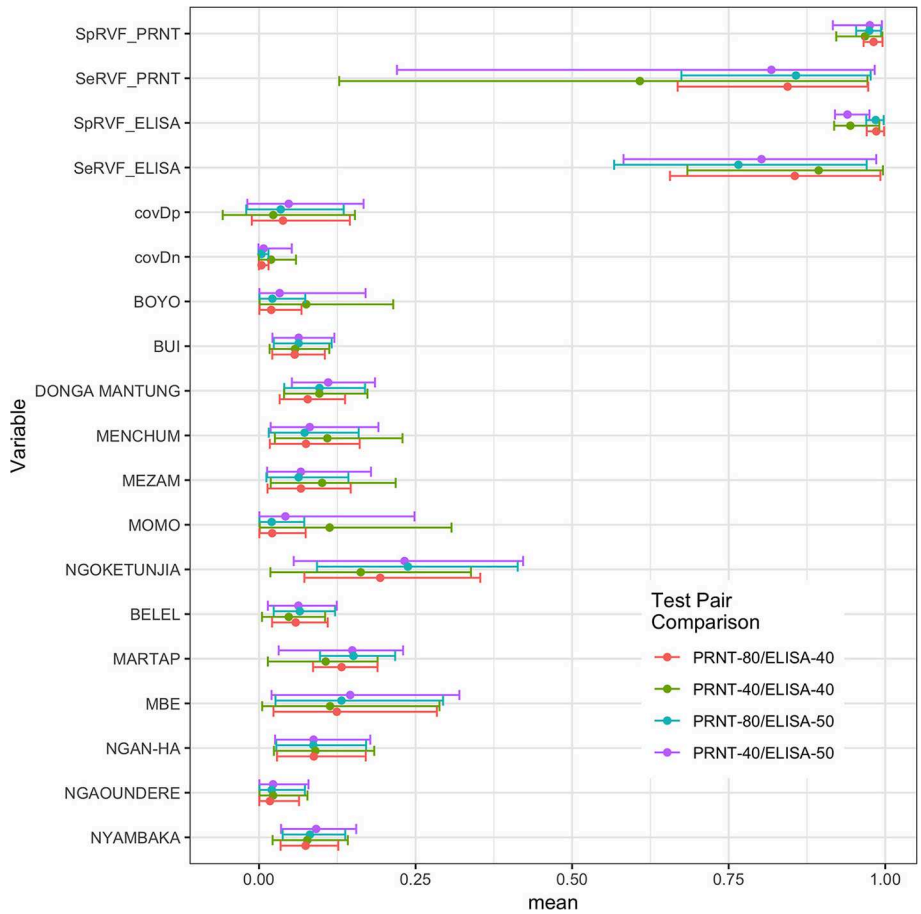

FIGURE 2 | Bayesian posterior means and 95% BCI for the two tests and their covariances (covDn, covDp) and the 13 prevalences for the subpopulations using the Hui-Walter NGS model allowing for conditional dependence between tests. The four test/cut-off combinations are presented together for comparison.

If the two tests cannot be reasonably assumed to be independent then the Hui-Walter model must be extended (19, 31) to account for the covariance structure between the two tests as below:

$$\Pr(T_1+,T_2+) = ((Se_1^*Se_2) + covDp)^*p_i + (((1-Sp_1)^*(1-Sp_2)) + covDn)^*(1-p_i)$$
$$\Pr(T_1+,T_2-) = (((Se_1)^*(1-Se_2)) - covDp)^*p_i + (((1-Sp_1)^*Sp_2) - covDn)^*(1-p_i)$$
$$\Pr(T_1-,T_2+) = ((1-Se_1)^*Se_2) - covDp)^*p_i + ((Sp_1{}^*(1-Sp_2)) - covDn)^*(1-p_i)$$
$$\Pr(T_1-,T_2-) = ((1-Se_1)^*(1-Se_2) + covDp)^*p_i + ((Sp_1^*Sp_2) + covDn)^*(1-p_i)$$

The covDp and the covDn are the covariances between the two tests when the animal is diseased and when it is not diseased, respectively. The covariance between the test outcomes for infected subpopulations satisfies $(Se_1\text{-}1)^*(1\text{-}Se_2) \leq covDp \leq (\min(Se_1,Se_2)\text{-}(Se_1Se_2))$ and for the non-infected subpopulation, $(Sp_1\text{-}1)^*(1\text{-}Sp_2) \leq covDp \leq (\min(Sp_1,Sp_2)\text{-}(Sp_1Sp_2))$. Therefore, for instance, a uniform $((Se_1\text{-}1)(1\text{-}Se_2), (\min(Se_1,Se_2)\text{-}(Se_1Se_2)))$ prior distribution can be used for covDp.

The model was implemented in JAGS using R (32) (code available in **Supplementary Material S1**). For this analysis the chains were used and the first 50,000 iterations were discarded as a burn-in. A further 250,000 iterations were run for each chain and then thinned by 100 to produce a set of 7,500 interactions kept for posterior inference. The parameter estimates are the mean of the posterior and the 0.025 and 0.975 quantiles were used to give the Bayesian credibility intervals (BCI). Convergence of the chain after the initial burn-in was assessed by visual inspection of the time-series plots for the parameters, as well as Gelman-Rubin statistic and diagnostic plots of model convergence based on the three sample chains with dispersed starting values (33).

Descriptive Statistics and Mapping

All statistical modeling and data visualization was conducted in R 3.5.2 (34).

RESULTS

A total of 1,498 cattle were sampled across the 2 study sites (January–May 2013 in the NWR and September-November 2013 in the VD). Eighteen samples failed the $PRNT_{80}$ test and a further seven failed the ID.Vet test due to excessive hemolysis leaving a final sample size of 1,473 for the remaining analyses. The raw continuous readings from the two tests are presented in **Figure 1** and there is generally good agreement.

An initial NGS analysis was conducted comparing the four different combinations of tests/cut-offs to identify the best combination for the rest of the analysis. The data was subset into 13 different populations based on the administrative Divisions (NWR) or sub-Divisions (VD) used in the sampling design. The posterior estimates for the test parameters and the different population prevalences are shown in **Figure 2**. From this the specificities are all very high with narrow 95% BCIs but as might be expected using the lower cut-off for the $PRNT_{80}$ in particular results in lowered specificities. The sensitivities are all lower than the specificities and have a much higher uncertainty around the estimates reflecting the relatively small number of positives in the sample (for all cut-offs used). Interestingly, all sets of four prevalence estimates for each population are very consistent across the test combinations. Overall the combination with the ELISA cut-off set at 40pp or lower and the $PRNT_{80}$ at 80 or greater were considered the optimal cut-offs as they produced on average the highest sensitivity and specificity estimates for both tests (**Table 1**).

The Hui-Walter model assumes that the test performs the same in each population. To explore this we repeated the analysis at a site level running independent models for the NWR and VD. The resulting estimates are given in **Figure 3** and show that although these is some variation between the

TABLE 1 | No gold standard estimates of the sensitivity (Se) and specificity (Sp) and Bayesian 95% credibility intervals (BCI) for the ID.Vet Rift Valley Fever ELISA at a cut-off of 40 and the in house $PRNT_{80}$ with a cut-off of 80.

Parameter	Mean	95% BCI
Se IDVet ELISA	0.854	0.655–0.991
Sp IDVet ELISA	0.986	0.971–0.998
Se $PRNT_{80}$	0.844	0.660–0.973
Sp $PRNT_{80}$	0.981	0.965–0.996

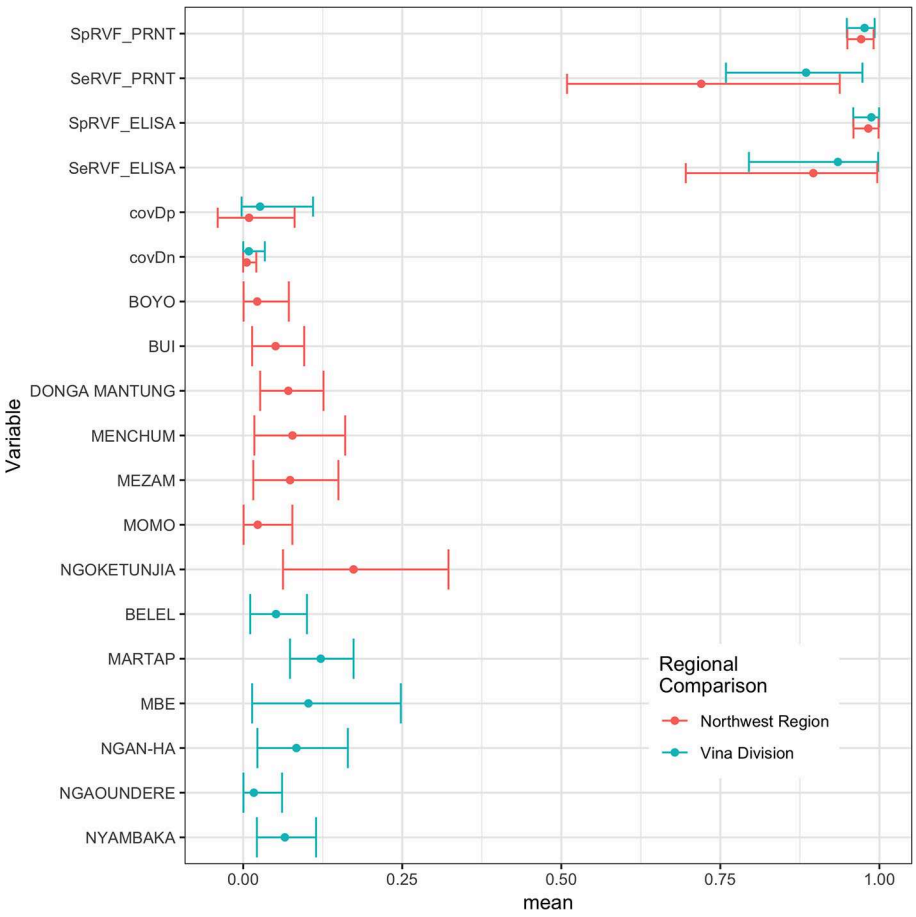

FIGURE 3 | Bayesian mean and 95% BCI for the ID.Vet ELISA (cut-off 40pp) and the $PRNT_{80}$ (cut-off 80) estimated independently in the two study sites in the Northwest Region and Vina Division.

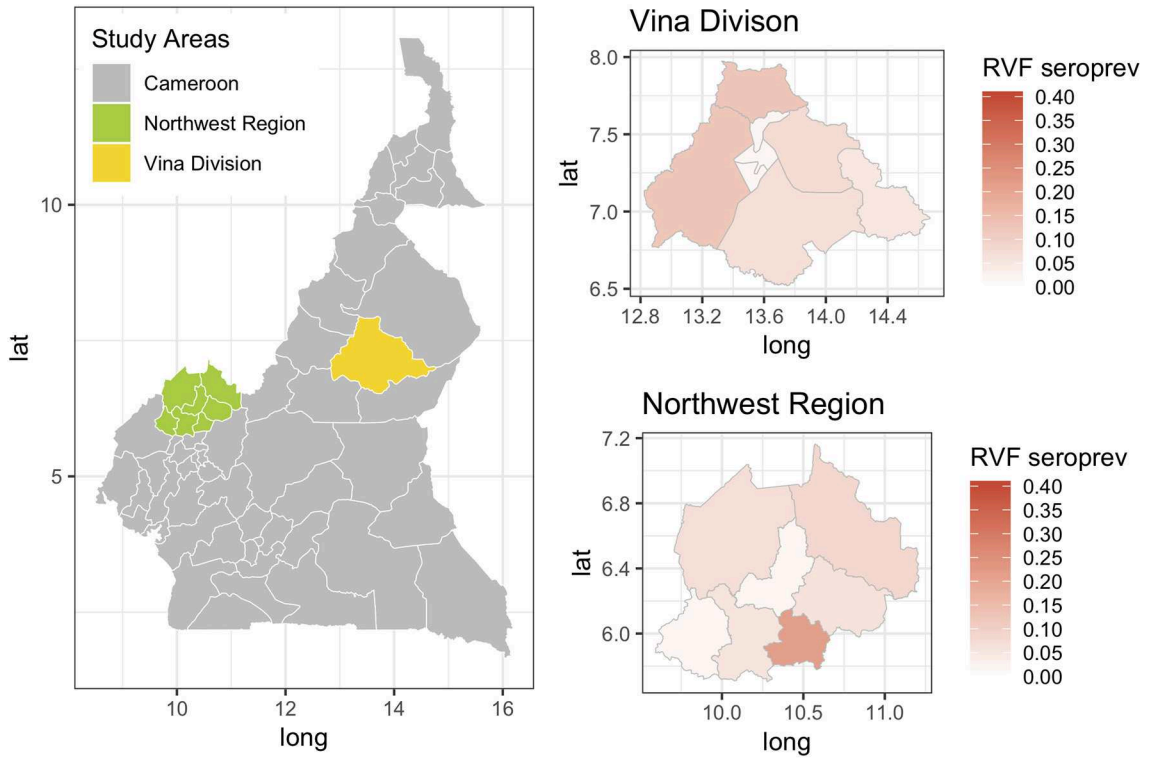

FIGURE 4 | Map showing the two study sites in the Northwest Region and the Vina Division of Cameroon. The smaller chloropeth maps show the mean estimated prevalence of rift valley fever seroprevalence for each site by Division (NWR) or sub-Division (VD).

sites the specificities remain very high and the sensitivities a little lower with considerable uncertainty as might be expected from the smaller sample sizes but overall a very similar estimate across the two study sites. The prevalence estimates are almost identical to those from the single model estates shown in **Figure 1**. These have also been plotted on a map in **Figure 4** to highlight the spatial variation in seroprevalence across the two sites.

Finally, we were interested to compare the performance of the tests in different age groups. The cattle were classified as young (<2 years old), adult (≥2 but <5 years old), and old (≥5 years old) based on the age and or dentition collected at the time of the sampling. The estimates based on the two tests and 13 sub-populations run as a single model are given in **Figure 5** with the test parameters further highlighted in **Table 2**. Again both tests appear to perform very consistently across the different age groups although the specificity seems to drop slightly in the old class for both tests for reasons that are not clear but which are reflected in higher levels of misclassification/lower agreement in this age class. It is also reassuring that seroprevalence generally increases with age group as one would expect for a vector borne infectious disease (**Figures 5** and **6**).

DISCUSSION

Rift Valley fever is an important emerging zoonotic disease in sub-Saharan Africa whose epidemiology is still poorly understood and there is a need to evaluate low cost surveillance tools for LMIC settings such as Cameroon. Here we have compared 2 RVF diagnostic tests, an in-house PRNT and a commercial ELISA (ID.Vet) using a latent class approach in a Bayesian framework to deal with the problem of having no gold standard test. The analysis first compared the tests using two different cut-offs and identified the optimal combination of cut-offs for the two tests that were then used for the remainder of the analyses (**Supplementary Table S2**). We also then compared their performances in geographically separated populations and also across age groups to assess consistency of performance and parameter estimates across these different groups. The results suggest that the tests perform consistently across the different groups with precise estimates of very high specificities and good sensitivities but with more uncertainty in these. There is some variation across the different subpopulation analyses, particularly in sensitivity estimates. This may be due to the relatively small numbers of positive animals per administrative area which results in stochastic noise when the sample is sub-setted by site and age.

The model assumes that the tests perform the same in the different populations and the results support this when the sample was split and analyzed separately for each site (**Figure 3**). We have included conditional dependence since both tests are serology based as suggested by Toft et al. (29). In addition, Toft et al. (29) warn about the potential inflation of the standard errors when the difference between the prevalences in the different subpopulations are small. In this study the range of

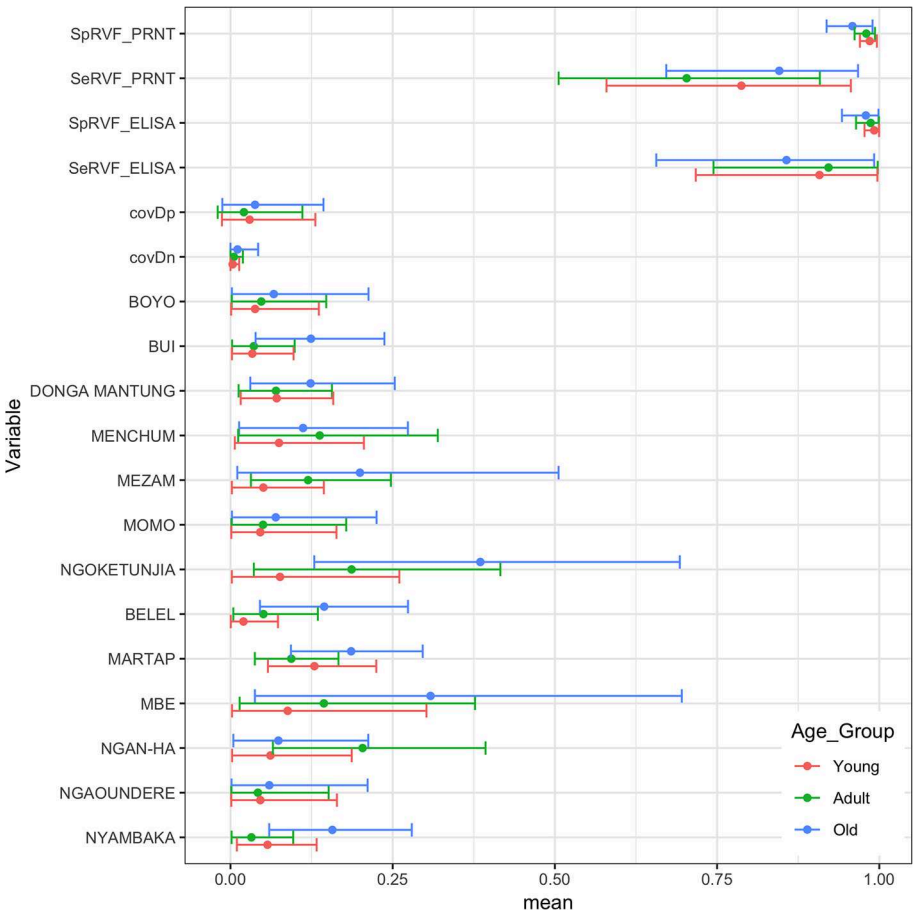

FIGURE 5 | Bayesian mean and 95% BCI parameter estimates for the ID.Vet RVF ELISA (cut-off 40pp) and the RVF PRNT80 (cut-off 80) estimated independently in the three age classes of cattle in Cameroon and the age class specific seroprevalence estimates across the 13 subpopulations.

TABLE 2 | No gold standard estimates of the sensitivity (Se) and specificity (Sp) with Bayesian 95% credibility intervals (BCI) for the ID.Vet Rift Valley Fever ELISA at a cut-off of 40 and the in house PRNT80 with a cut-off of 80 estimated separately for each age group.

Parameter	Age class	Mean	95% BCI
Se ID.Vet ELISA	Young	0.906	0.712–0.997
	Adult	0.941	0.817–0.998
	Old	0.857	0.655–0.992
Sp ID.Vet ELISA	Young	0.992	0.977–1.000
	Adult	0.990	0.973–1.000
	Old	0.979	0.944–0.999
Se $PRNT_{80}$	Young	0.786	0.577–0.956
	Adult	0.754	0.567–0.937
	Old	0.847	0.672–0.968
Sp $PRNT_{80}$	Young	0.985	0.970–0.996
	Adult	0.985	0.971–0.995
	Old	0.958	0.920–0.989

prevalences is fairly wide therefore this should not be an issue. Finally, the model assumes all individuals are independent and does not account for clustering by herd which may lead to underestimation of the standard error and narrower BCIs. The specificity estimates are similar to those previously published for both the ID.Vet RVF ELISA (0.997) (30) and $PRNT_{80}$ (0.960) (35). The estimates for the sensitivity of the ID.Vet RVF ELISA are lower than those published by Paweska et al. (30) who report a sensitivity of 0.963. Our results suggest that in this population it may perform less well but may be a better reflection of performance in a naturally infected population where we do not know the stage of infection and their is a wider range of infectious doses. Other ELISA tests are available including another competitive ELISA produced by Veterinary Medicine Research and Development (Washington State) which when compared to the $PRNT_{80}$ had similar sensitivities and specificities (36).

This represents the first report of estimating the ID.Vet RVF ELISA performance in an African cattle population and the ELISA shows great potential as a low cost easy to use surveillance tool with very good overall accuracy comparable to the more standardized $PRNT_{80}$. Although generally considered the reference test and widely used in reference laboratories the $PRNT_{80}$ is labor-intensive, time consuming, expensive, and requires virus appropriate biocontainment (36) and so it not

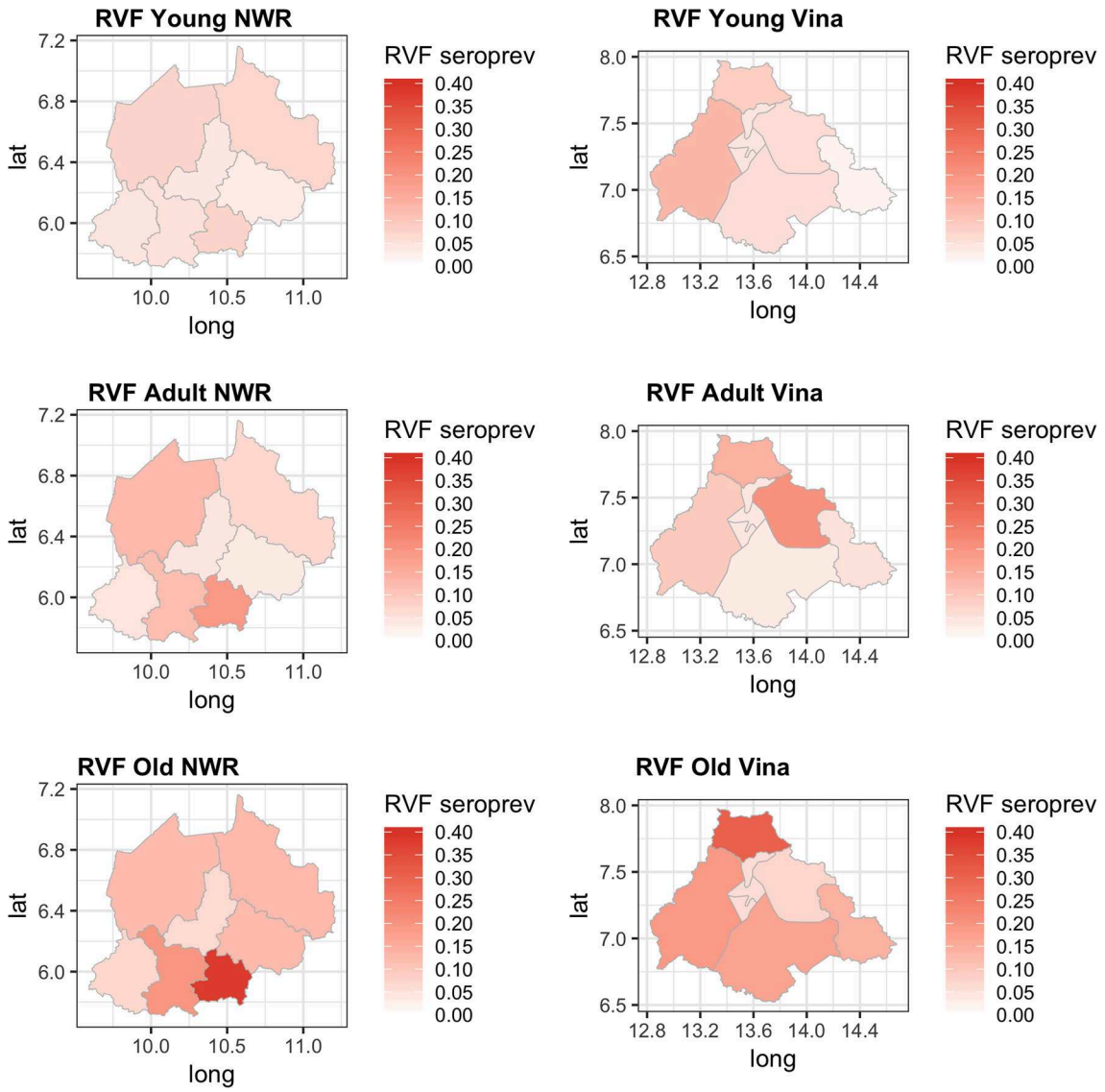

FIGURE 6 | Chloropeth maps showing the two study sites of the Northwest (Divisions) and Vina Division (sub-Divisions) of Cameroon and the Bayesian NGS mean seroprevalence estimates for the three age classes from young adult and old.

practical for most surveillance activities particularly in LMICs. Laboratories with only very basic equipment can use the kit making it very appropriate for use in Africa.

Previous studies have demonstrated that RVF virus is circulating in Cameroon and the Central African region more widely. Further, the results presented in this paper suggest that the seroprevalence of RVF in cattle varies across the study sites between 2 and 12% with the exception of Ngoketunjia in the NWR where it was particularly high at near 20%. This Division is to the south of the Region and includes a large dam and swamp areas where cattle are grazed and it would be consistent with much higher vector populations. There is also a clear pattern of increasing seroprevalence with age across the 13 sub-populations over the two sites consistent with viral circulation although no clinical disease has been reported. This may be because at the time of the study there was no animal surveillance for abortions. These estimates appear consistent with those recently reported from Cameroon for the Adamawa Region in 2018 (15) and 1995 (13). They do not report seroprevalences in cattle for the NWR. From discussions with local health personnel there is also very limited diagnostic exploration of human febrile illnesses which may lead into cases being missed. Given that there appears to be viral circulation, screening of high risk human groups such as slaughterhouse workers or veterinarians for evidence of exposure should be prioritized to provide further evidence for the need to include screening for RVF in febrile cases. Furthermore, RVF outbreaks have been associated with dam construction in West Africa and El Nino events in East Africa and changes in habitat

that favor mosquito populations are a potential risk for triggering epidemics (37). Interestingly the highest seroprevalence in this study was from Ngoketunjia which includes a dam. With new dams currently under construction in Cameroon the potential risks for RVF outbreaks should be considered.

In conclusion we have demonstrated that both the ID.Vet ELISA and $PRNT_{80}$ have comparable performances in cattle from Cameroon. This supports the use of the ELISA as a relatively low cost easy to use surveillance tool for the African context. The results also suggest that RVF virus is endemic and circulating in Cameroonian cattle and it is interesting that no clinical reports exist for cattle or humans. The results here and from other studies in Cameroon suggest that human screening of febriles illnesses should be considered by hospitals where malaria has been ruled out.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The study design and sampling methodology was reviewed and approved by the University of Edinburgh Ethics Committee, UK (ERC No: OS02-13) and by the Ministry of Scientific Research and Innovation (MINRESI), Cameroon. MINRESI gave permission to conduct the fieldwork and issued fieldwork permits. The research did not involve endangered of protected species and no further approvals were necessary to conduct fieldwork. All participants gave informed verbal consent to participate and were aware they could opt out at any stage. Verbal consent was deemed appropriate for the variety of dialects spoken, variable literacy amongst participants and due to the remote outdoor fieldwork environment. Information to be provided to participants, for informed verbal consent, was communicated to the interviewer in a written document. Additional training was provided to the interviewer regarding the consent procedure and interview process. Furthermore, the interviewer was experienced in conducting questionnaires in similar studies and spoke the various local dialects of study participants. Verbal consent was recorded on a cover sheet to the questionnaire by the interviewer and refusals were recorded in separate document along with reasons for refusal.

AUTHOR CONTRIBUTIONS

BB, VT, KM, and LN designed the original study in Cameroon. RK, SM, and VN collected the samples in the field. J-MB, RK, and CN carried out the diagnostic tests. IH and BB carried out the statistical analysis. BB, CN, and RK prepared the first draft of the paper and all authors commented.

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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.2019.00258/full#supplementary-material

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Conflict of Interest Statement: 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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frontiers in Veterinary Science

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Simulation Based Evaluation of Time Series for Syndromic Surveillance of Cattle in Switzerland

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Choosing the syndrome time series to monitor in a syndromic surveillance system is not a straight forward process. Defining which syndromes to monitor in order to maximize detection performance has been recently identified as one of the research priorities in Syndromic surveillance. Estimating the minimum size of an epidemic that could potentially be detected in a specific syndrome could be used as a criteria for comparing the performance of different syndrome time series, and could provide some guidance for syndrome selection. The aim of our study was to estimate the potential value of different time series for building a national syndromic surveillance system for cattle in Switzerland. Simulations were used to produce outbreaks of different size and shape and to estimate the ability of each time series and aberration detection algorithm to detect them with high sensitivity, specificity and timeliness. Two temporal aberration detection algorithms were also compared: Holt–Winters generalized exponential smoothing (HW) and Exponential Weighted Moving Average (EWMA). Our results indicated that a specific aberration detection algorithm should be used for each time series. In addition, time series with high counts per unit of time had good overall detection performance, but poor detection performance for small epidemics making them of limited use for an early detection system. Estimating the minimum size of simulated epidemics that could potentially be detected in syndrome TS-event detection pairs can help surveillance system designers choosing the most appropriate syndrome TS to include in their early epidemic surveillance system.

Keywords: syndromic surveillance, Holt-Winters, EWMA, syndrome selection, time series

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INTRODUCTION

Early warning systems are critically important for controlling emerging or reemerging diseases. Dealing with a disease epidemic in its early stages is easier and more economical than dealing with an epidemic that has become large and widespread (1, 2). Traditional passive early detection systems rely on reports submitted to veterinary public health authorities by various healthcare stakeholders when they observe suspect cases in the field. This surveillance activity covers a large part of the animal population and the costs associated with data collection and analysis are relatively low (3–5). However, the performance of these passive surveillance systems suffers from frequent under-reporting due to the lack of stakeholder awareness, especially regarding emerging diseases, and fear of the consequences of reporting a disease occurrence (4, 5). To enhance traditional passive

surveillance systems, real-time or near real-time surveillance systems have been developed. These systems, commonly called syndromic surveillance (SyS) systems (6), are based on pre-diagnostic often unspecific routinely collected data which is available prior to laboratory confirmation of the causative agent of an epidemic. A great variety of data can be used for syndromic surveillance (e.g., laboratory requests, milk production, Google queries, and many others). These data are converted to time series (TS) for monitoring and are referred to as syndromes (6).

Constant improvements in data science and computer technology have favored the development and implementation of SyS systems by facilitating data acquisition, and analysis. The number of operational SyS systems has constantly increased during the last decades in both human and veterinary medicine (7, 8). By simultaneously assessing information from different data sources related to different populations and/or symptoms, one can improve epidemic detection and in particular, the sensitivity and the specificity of epidemic detection (8). Choosing the syndrome TS to monitor in a SyS system is not an easy or straightforward process. Defining which syndromes to monitor in order to maximize detection performance is very challenging and has been recently identified as one of the research priorities in SyS (9). This is especially true when data can be subdivided into many syndrome classifications or definitions (9), or when the objectives of surveillance are unspecific. For these reasons selection of syndrome TS should be guided by data characteristics including representativeness and by the objectives of the surveillance system (10). However, when the objectives are broad, for example to detect not only known diseases of interest, but also new, emerging, exotic or unknown endemic diseases, they are of little help for selecting the most appropriate syndrome TS.

In operational SyS systems, syndrome TS are monitored with automated aberration detection algorithms in order to detect unexpected changes that could potentially be caused by an epidemic. A useful criterion for selecting a specific syndrome TS for a SyS system is an assessment of the nature of the change that can be detected. Any syndrome TS that is monitored with any aberration detection algorithm should be able to detect a sudden and very large variation in the number of cases reported. However, detecting a large change in a syndrome TS is of little interest for surveillance if the aim is early detection of disease epidemics. In this case detecting small changes in a time series which may represent the onset of an epidemic is of greater importance. Changes in syndrome TS should be detected with a high degree of certainty and as soon as possible after the epidemic has started. Estimating the minimum size of an epidemic that could potentially be detected in a syndrome TS may serve as a useful criteria for comparing syndrome TS performance and provide guidance for their selection.

In Switzerland, a cattle disease SyS system is currently being designed to meet the goals of the "Swiss Animal Health Strategy 2010+,"[1] which aims to maintain and improve the high standard of animal health in the country. The purpose of the SyS system is to detect abnormal health events such as disease epidemics occurring in the Swiss cattle population by monitoring syndrome TS extracted from a central database maintained by the Federal Food Safety and Veterinary Office (FSVO). The objective of our study was to evaluate different syndrome TS as candidates for inclusion in the system. Data quality and population coverage should be always carefully assessed before including a TS into a SyS system (10). However, evaluating these criteria was not the purpose of our study and these characteristics are only briefly presented and discussed in this paper. Since the goal of SyS is early detection of epidemics, our study focused on estimating the minimum size of simulated epidemic that can be detected in syndrome TS-event detection pairs, as a criterion for inclusion in a SyS system. To standardize the comparisons between syndrome TS-event detection pairs, we created a standard set of simulated epidemics of various shapes and sizes and used this standard set to compare the performance of all syndrome TS-event detection pairs. Our study objective differs from other studies that focus on evaluating the performance of event detection algorithms only. For practical purposes, the combined performance of an event detection algorithm operating on a specific syndrome TS should be more useful to surveillance system designers.

[1]See http://www.blv.admin.ch/gesundheittiere/03007/index.html?lang=en

MATERIALS AND METHODS

Data Sources and Associated Time Series

Three databases containing data from the Swiss national cattle population were used: (1) the *Swiss Animal Movement Database* (AMD), (2) the database owned by the *Association of Swiss Cattle Breeders* (ASR), and (3) the *Swiss Laboratory Information System* (ALIS). The AMD has been studied and reported to have potential value for SyS because of its relatively high quality in terms of population representativeness and reporting timeliness (11). The other two databases contain laboratory test orders (ALIS) and clinical data collected by farmers (ASR).These two databases haven't been investigated in Switzerland, but similar data have been reported to be of value for SyS in others countries (8).

The AMD contains data on cattle mortalities, including stillbirths, reported by farmers to the Swiss national system for the identification and registration of cattle. All reported on-farm deaths and stillbirths for the period from January 1st 2009 to September 28th 2016 were extracted from the AMD. Since the reporting of on-farm deaths was mandatory, we can assume a high population coverage from this source over this period excepting for stillbirths. Stillbirths were defined as non-living fetuses expelled before the end of gestation, or calves born dead within 24 h following birth since mid-2014. Before that date, no official definition of a stillbirth existed in Switzerland. It is not mandatory to report every stillbirth to the AMD and the population coverage of this syndrome TS is unknown at the time of writing. Four syndrome TS were created from the AMD database. One was based on stillbirths (*AMD_stillbirth*) and three were based on categories of on-farm deaths defined according to the age at death: up to 6 months old (*AMD_mortality_calves*), 6 months−2 years (*AMD_mortality_young*), and more than 2 years *(AMD_mortality_adults*).

The *ASR* (http://asr-ch.ch/en/asr/) is the private umbrella organization of the Swiss cattle breeding organizations. Beginning in 2013 the ASR developed and implemented a database containing cattle illness diagnoses reported by farmers and veterinarians. All cases were reported using a coding system with four levels ranging from least specific (i.e., organ affected) to most specific (e.g., infectious agent isolated). Data were available for the most common cattle breeds in Switzerland: Braunvieh, Fleckvieh, and Holstein, which represent the majority of the Swiss dairy cattle population. No data about beef cattle were available. The timeliness of reporting to this database is unknown. Data were available from January 1st, 2014 to December 31st, 2016. Three syndrome TS were created based on the age category of diseased animal: abortions (*ASR_abortion*), diseased calves (*ASR_calves*), and diseased adults (*ASR_adults*). In the ASR database calves are defined as cattle up to 6 months of age. Abortions are defined as calves born dead, or born alive but having died within the first 24 h of life. The syndrome TS *ASR_calves* and *ASR_adults* were each split into three syndrome TS based on the most frequent diagnostic classification found in the database: gastrointestinal symptoms (i.e., *ASR_GI_calves* and *ASR_GI_adults*), respiratory symptoms (i.e., ASR_*RESPI_calves* and *ASR_RESPI_adults*), and cattle having a classification of "other" in the ASR classification schema (i.e., *ASR_OTHER_calves* and *ASR_OTHER_adults*). The category "other" encompasses various unspecific symptoms such as fever, anorexia, changing behavior or reduced production. The precise coverage of the dairy cattle population by the ASR is unknown but it is expected to be high.

The ALIS database contains data from laboratory tests performed by the 25 accredited laboratories involved in the diagnosis of epizootics in Switzerland on behalf of the FSVO. All laboratory tests performed for the 70 notifiable epizootics of interest in Switzerland are collected in ALIS. The reporting timeliness (time between the sampling date and the date when the sample was received by a laboratory) was on average of 1 day. Data were analyzed from November 1st, 2013 to July 27th, 2016. All laboratory tests performed for mandatory reasons without any clinical suspicion were excluded (e.g., mandatory surveillance programs, importation, vaccination, research activities). One syndrome TS was created containing counts of stillbirth samples sent to the accredited laboratories *(ALIS_abortion)*. Two additional syndrome TS were created from samples sent to the accredited laboratories because of clinical suspicion of two diseases of interest in Switzerland: bovine viral diarrhea (*ALIS_BVD*) and infectious bovine rhinotracheitis (*ALIS_IBR*). Suspicious cases were individual cattle and they were always confirmed (or negated) with an FSVO approved laboratory test.

In total, data for 16 syndrome TS were extracted from the 3 databases and converted to weekly syndrome TS (see **Figure 1**).

Time Series Analysis and Preprocessing

To the best of our knowledge there were no epidemics reported in the target cattle population during the study period. However, there was considerable variation in the data that was known to be caused by non-epidemic events. Because the historical baselines in our study were very short, extreme outliers had a substantial effect the HW models, resulting in poor TS predictions. Extreme single time point aberrations were removed in order to obtain aberration-free historical baseline data that improved the prediction performance of our models and the performance of aberration detection algorithms (12–14). We chose a manual approach for outlier removal in order to preserve as much of the natural variation as possible in the data. We examined each syndrome TS visually and manually removed only the most extreme peaks. Extreme peaks were defined as weeks where the number of reported cases equaled at least two times the number of reported cases in the neighboring weeks. Once extreme peaks were identified, they were investigated in more detail to determine if they were associated with a specific health related event or not. Peaks that were associated with health related events were considered abnormal. They were removed from baseline syndrome TS and replaced by the weekly average of the 10 previous time points. The 10 week average was used as it has been reported to provide the best prediction performance for HW models. Extreme peaks that were not associated with health related events were considered part of the normal variation and left in the baseline syndrome TS. In total there were 7 abnormal values identified, 1 week in ALIS_abortion because of suspicions of Neosporosis, and 6 weeks in ALIS_IBR likely because IBR suspect cases were identified and this may have increased veterinarian awareness of the disease, causing them to increase IBR sample submission. The best HW models were evaluated using the autocorrelation and partial autocorrelation functions of the residuals (ACF and PACF, respectively) (15) and the root-mean-squared error (RMSE) (16). RMSE is a measure of the difference between the values predicted by a model and the values actually observed from the environment that is being modeled. We calculated RMSE for the differences between the observations and the predicted values within both the training period ($RMSE_t$) and the validation period (RMSEv). In both cases, the predictive performance of the HW model are better when the criterion is lower.

Regression models were fitted to the syndrome TS to estimate the linear trend and annual seasonality. Poisson and negative-binomial regression models were fit to the syndrome TS for the full time period available for each syndrome TS. Likelihood ratio tests were used to test for the significance of each predictor at a statistical significance level of 5%. Syndrome TS were then characterized using 4 parameters adapted from Choi (17):

1. Length of the historical baseline: long when >3 years, short when ≤ 3 years;
2. Linear trend: positive, negative, or none;
3. Annual seasonality: none when no monthly effect identified. When there was a monthly effect, the strength of the seasonality was assessed based on the value of F_s (18) calculated as equal to 1–Var (R)/Var(S + R). Var (R) is the variance of the remainder component of the syndrome TS and Var (S + R) is the variance of the detrended syndrome TS. Seasonality was considered to be weak when F_s was below 0.5, and strong when it was ≥ 0.5;

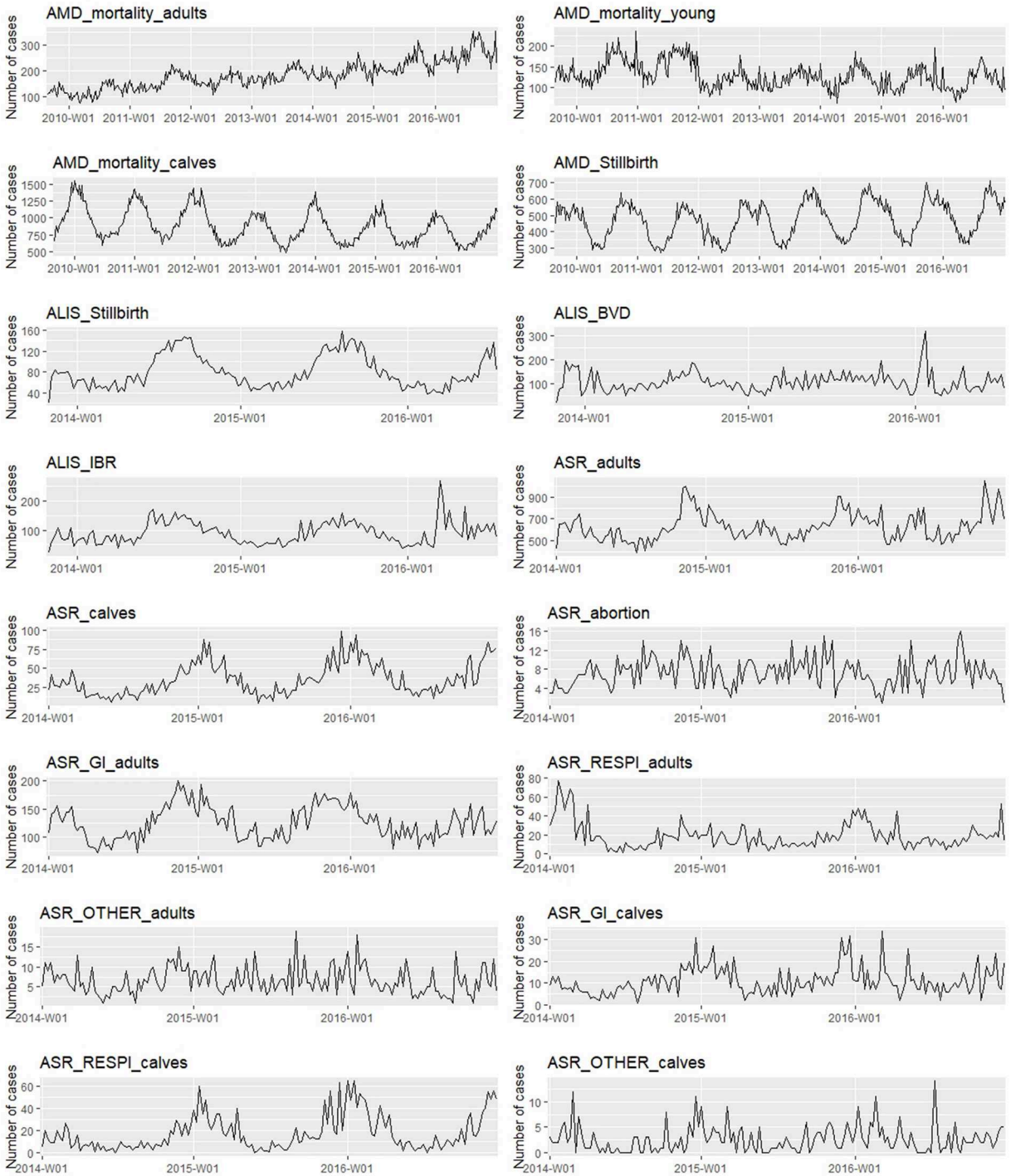

FIGURE 1 | Weekly syndrome time series extracted for the study (abnormal peaks have been removed).

4. Mean and standard deviation (SD) of the weekly counts, and corresponding coefficient of variation (CV).

Aberration Detection Algorithms

Two different aberration detection algorithms were compared: Holt–Winters generalized exponential smoothing (HW) (19, 20) and Exponential Weighted Moving Average (EWMA) (21, 22). Ten different detection limits, or alarm thresholds, were tested for both algorithms. To avoid contamination of the baseline with cases from gradually increasing epidemics, a guard-band of 2 weeks was used between the baseline and the current value being evaluated.

Holt-Winters

HW is a triple exponential smoothing method which involves exponentially decreasing the weights of observations over time, such that oldest observations have the smallest weight. The forecast is continuously revised according to the most recent observations. HW incorporates three components: a level term, a trend term, and a seasonality term, respectively, defined by the smoothing constants α, β, and γ. HW can be applied to raw time series containing trend and seasonality. All the data available before 31-12-2015 were used for model training. The data available after December 31st, 2015 were used for model validation and for the estimation of model prediction performance. The training data contained data for periods from 2 to 7 years and the validation data contained data for periods from 7 to 12 months, depending on the length of syndrome TS. Optimal HW parameters were determined through minimization of the squared prediction error (23). Model fit was evaluated using the autocorrelation and partial autocorrelation functions of the residuals (ACF and PACF, respectively), normality Q-Q plot, and the root-mean-squared error (RMSE). ACF is the linear dependence of a variable on itself at two points in time and PACF is the autocorrelation between two points in time after removing any linear dependence between them (15). ACF and PACF were used to find any remaining repeated patterns in the model residuals. RMSE is a measure of the difference between the values predicted by a model and the values actually observed in the real data (16). This criterion was calculated for the differences between the observed and the predicted values within both the training period ($RMSE_t$) and the validation period ($RMSE_v$). In both cases, the predictive performance of the model were better when the RMSE was lower. The alarm thresholds tested for evaluating event detection performance were based on constant values multiplied with the standard error of the predicted value for each week (21, 22). The following constant values were used: 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5.

EWMA

EWMA is the simplest form of exponential smoothing and it relies on cumulative differences between observed data in a time window and a threshold. It is based on the equation: $E_t = (1 - \lambda)^t E_0 + \Sigma_{ti=1} (1 - \lambda)^t \lambda I_t$ where λ is the smoothing parameter (>0) that determines the relative weight of current data in relation to past data, I_t is the observed value at time t, and E_0 is the starting value. EWMA is recommend only for stationary and normally distributed TS (21, 22). A 1 week differencing (i.e., computation of the difference between consecutive weekly time points) was used to remove the largest temporal effects present in the raw data. The differenced residuals were saved as a new TS. Autocorrelation and normality in the TS of residuals were assessed using ACF, PACF and normality Q-Q plot in order to evaluate whether pre-processing enabled transformation of the weekly auto-correlated TS into stationary and normally distributed TS. EWMA was then applied to the residual TS using a smoothing parameter λ of 0.2. The same constant values that were used for HW were also used for calculating the alarm thresholds for the EWMA algorithm.

TABLE 1 | Methods for epidemic simulation adapted from Dórea et al. (24) and Lotze et al. (25).

Epidemic shape	Method used for epidemic simulation
Single spike	Epidemic length was always 1 week and on that week, $extra_k$ equals the epidemic magnitude (25, 50, 150, 300, or 500).
Flat	$Extra_k$ always equals to the epidemic magnitude (25, 50, 150, 300, or 500) for the 12 weeks of the epidemic time period.
Linear	$Extra_k$ increases linearly until it reaches a maximum value equal to the epidemic magnitude on week 12 of the epidemic time period. Example for an epidemic magnitude of 25, starting on week *i*, $extra_{i+k}$ equals successively 2, 4, 6, 8, 10, 12, 15, 17, 19, 21, 23, 25 for *k* varying from 1 to 12.
Exponential	$Extra_k$ increases exponentially until it reaches a maximum value equal to the epidemic magnitude on week 12 of the epidemic time period. For the duration of 12 weeks, this was achieved by assigning the maximum number of extra cases (i.e., the epidemic magnitude) to the last week of the epidemic time period, and dividing each week by 1.3 to obtain the value for the preceding week. Example for an epidemic magnitude of 25, starting on week *i*: $extra_{i+k}$ is calculated as follow: $extra_{i+12} = 25$, $extra_{i+11} = extra_{i+12}/1.3$, $extra_{i+10} = extra_{i+11}/1.3$, etc. $extra_{i+k}$ equals thus successively 1, 2, 2, 3, 4, 5, 7, 9, 11, 15, 19, 25 for *k* varying from 1 to 12.
Lognormal	$Extra_k$ increases following a log normal curve until it reaches a maximum value equal to the epidemic magnitude on week 12 of the epidemic time period. The percentage of increase in week *k* of the epidemic time period equals the x^{ieme} percentile of a log normal distribution [lognormal (4, 0.3)]. Example for an epidemic magnitude of 25, starting on week *i*, $extra_{i+k}$ equals successively: 6, 10, 12, 14, 16, 17, 19, 20, 21, 22, 23, 25 for *k* varying from 1 to 12.

$count_i$, The number of cases in week i which equals $fit_i + extra_i$; fit_i, the mean fitted value of the Poisson distributions that characterized this week i of the baseline; $extra_k$, number of cases added due to the epidemic on week k.

Data Simulation

We simulated epidemic-free baseline TS for each syndrome using the model predictions obtained from the best fitting HW model that was developed using the data available before 2016. The mean fitted value for each week of the year was used as the mean of weekly Poisson distributions (one for each week of the year). We then randomly sampled from each weekly Poisson distribution to simulate 300 epidemic-free baseline TS for each syndrome.

Twenty five different epidemics types were simulated based on five different epidemic shapes and five epidemic magnitudes (see **Table 1** and **Appendix 1**). Five epidemic shapes representing different temporal progressions of an epidemic within a population were created, based on (24, 25): single spike, flat, linear, exponential, and log normal. The length of all simulated epidemics was fixed at 12 weeks except for the epidemic shape "single spike" which lasted only 1 week. We choose an epidemic length of 12 weeks (3 months) because we were interested in evaluating the syndrome time series for early epidemic detection. We were not interested in alarms after 12 weeks as in our opinion these would not qualify as early detection. Epidemic magnitude represents the severity of the epidemic and was defined as

the maximum number of additional cases added to the weekly epidemic-free baseline during the epidemic time period. In this context a case equals a diseased animal reported in the data. Five different epidemic magnitudes were tested: 25, 50, 150, 300, and 500 corresponding, respectively, to very small, small, medium, large, and very large epidemics. The magnitudes represented the maximum number of extra cases added per week to the epidemic-free baseline during the epidemic time period. As an example, for an epidemic with a magnitude of "150"; 150 cases were added to the epidemic-free baseline at the peak of the epidemic which was on week number 12 of the epidemic time period. Smaller numbers of epidemic cases were also inserted for each of the 11 weeks prior to the epidemic peak. The exact number of extra cases added to the epidemic-free baseline for the pre-peak weeks was calculated according to the different epidemic shapes (see **Table 1** for details). For the "single spike" epidemic, which lasted 1 week, the epidemic magnitude always represented the total number of cases in the epidemic.

Three hundred epidemics of each type were simulated and randomly inserted within the 300 simulated baselines. Only one

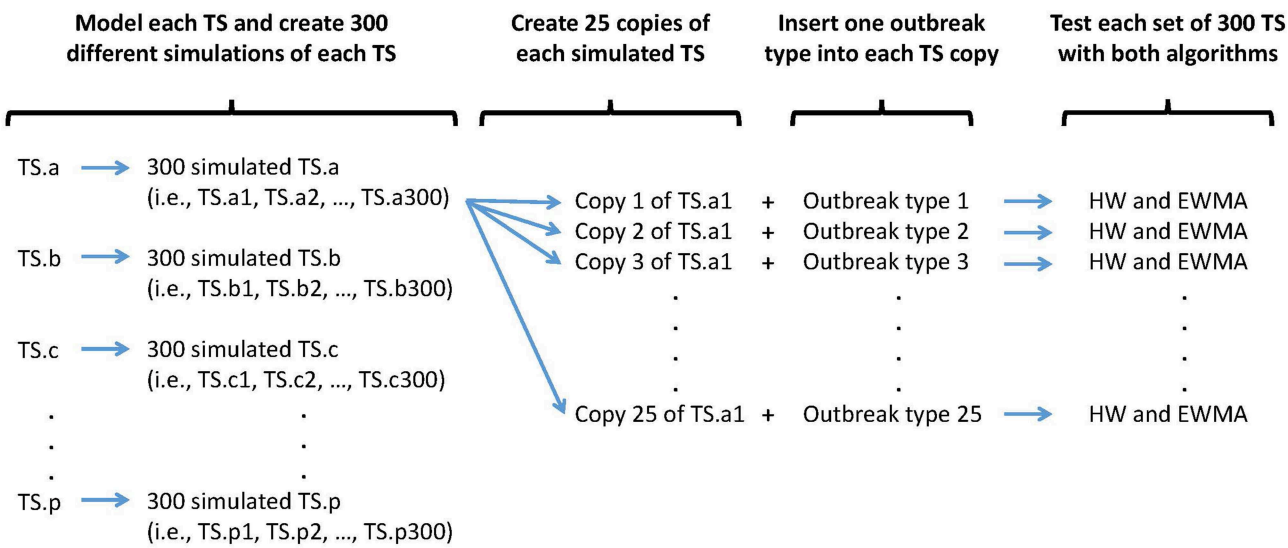

FIGURE 2 | Synthetic outbreak and baseline simulation process. An outbreak type is defined by a specific shape (i.e., single spike, flat, linear, exponential, or lognormal) and a specific size (i.e., very small, small, medium, large, very large).

TABLE 2 | Time series characteristics.

Time series	Length of the historical data	Trend	Seasonality (F_s)	Mean weekly count (SD)	Coefficient of variation (CV)
AMD_mortality_calves	2009–2016	–	0.91–strong	891.8 (241)	0.27
ASR_adults	2014–2016	0	0.74–strong	629 (129.2)	0.20
AMD_Stillbirth	2009–2016	+	0.92–strong	466 (108)	0.23
AMD_mortality_adults	2009–2016	+	0.54–strong	170 (42)	0.24
AMD_mortality_young	2009–2016	–	0.58–strong	130.4 (31)	0.23
ASR_GI_adults	2014–2016	0	0.74–strong	125 (29.3)	0.23
ALIS_BVD	2013–2016	0	0.38–weak	105.4 (41.9)	0.39
ALIS_IBR	2013–2016	0	0.54–strong	94.8 (39.5)	0.41
ALIS_abortion	2013–2016	0	0.89–strong	79.6 (31.7)	0.40
ASR_calves	2014–2016	+	0.75–strong	36 (20.9)	0.58
ASR_RESPI_adults	2014–2016	0	0.46–weak	19 (14)	0.73
ASR_RESPI_calves	2014–2016	+	0.69–strong	17.7 (15.8)	0.89
ASR_GI_calves	2014–2016	0	0.50–strong	11 (6.3)	0.57
ASR_Abortion	2014–2016	0	0.36–weak	7 (3.1)	0.44
ASR_OTHER_adults	2014–2016	0	0.36–weak	6 (3.5)	0.58
ASR_OTHER_calves	2014–2016	0	0.36–weak	2.6 (2.6)	1

Length of the historical baseline, trend (positive, negative, none), seasonality [none, weak (F_s < 0.5), strong ($F_s \geq 0.5$)]. TS were ordered according to the weekly average number of notifications from the largest (top row) to the smallest (bottom row).

epidemic was inserted per simulated baseline to avoid epidemic overlap. Each simulated baseline was used 25 times to detect 25 different epidemics types characterized by different epidemic shapes and magnitudes. In other words, to assess the algorithms and time series capacities to detect a certain epidemic shape of a certain magnitude, 300 different simulated baselines were used. The process and resulting syndrome TS are presented in **Figure 2**.

Detection Performance Estimation

We calculated sensitivity (*Se*) based on the number of epidemics detected out of all inserted epidemics. An epidemic was detected when it triggered at least one true alarm, defined as a week that produced an alarm within an epidemic period. *Se* was calculated as:

$$\text{Se} = \text{Epidemics detected}/\text{Total number of epidemics inserted} \quad (1)$$

We also calculated the specificity (*Sp*), the positive predictive value (*PPV*) and the negative predictive value (*NPV*) as:

$$\text{Sp} = \text{TN}/(\text{TN} + \text{FP}) \quad (2)$$

$$\text{PPV} = \text{TP}/(\text{TP} + \text{FP}) \quad (3)$$

$$\text{NPV} = \text{TN}/(\text{TN} + \text{FN}) \quad (4)$$

where TP is the number of true positive alarms (i.e., alarms raised on a week which is part of an epidemic period), TN the number of true negative alarms, FP the number of false-positive alarms (i.e., alarms raised on a week which is not part of an epidemic period), and FN the number of false negative alarms.

A receiver-operating characteristic (ROC) curve was generated and, assuming equal costs for false negative and false positive alarms, we graphically defined the optimal alarm threshold where *Se* and *Sp* were at a maximum. The timeliness of the first alarm raised during an epidemic time period was computed. Detection timeliness was the time lag (in weeks) between the start of the epidemic and the first alarm. A value of

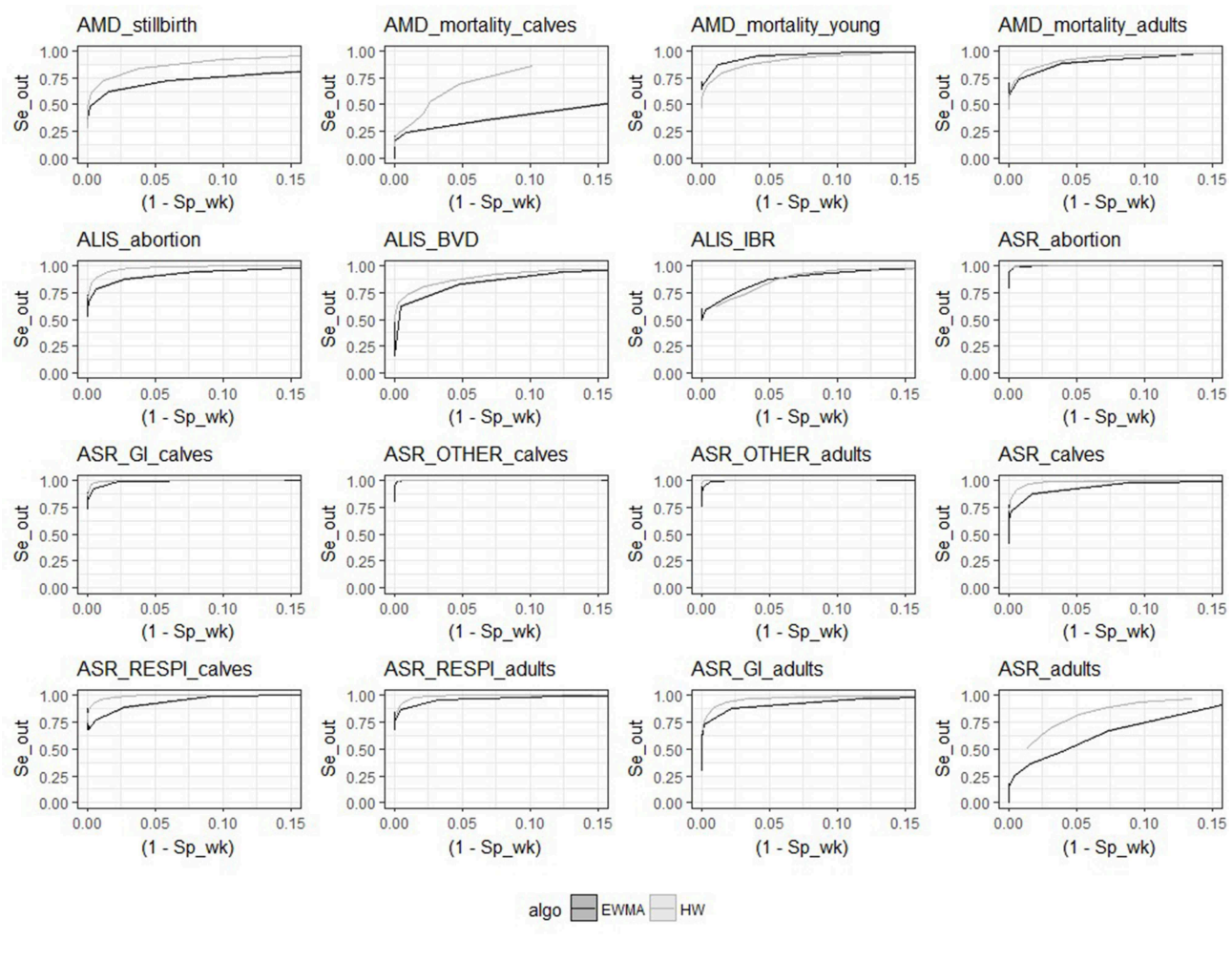

FIGURE 3 | Receiver-operating characteristic (ROC) curves for the 16 syndrome TS and the 2 aberration detection algorithms (all epidemic sizes and shapes are collated).

1 meant that the first alarm was raised during the second week of the epidemic. Single spikes were excluded from the computation of the detection timeliness as they always lead to detection on the first, and only week of the epidemic. The cumulative number of cases occurring because of the epidemic when the first alarm was raised (*cum_cases*) was also calculated. We calculated Spearman's nonparametric correlation coefficients (ρ) to test the association between the size of the syndrome TS (in terms of counts per week) and the detection performance of the syndrome TS.

Software Implementation

All statistical analyses were implemented in R x64 version 3.4.1 (26). Dynamic regression was performed with the functions glm (package "stats"), glm.nb [package "MASS" (27)], and stl [package "forecast" (28)]. The stl function was also used to estimate the detrended and remainder component of each syndrome TS and calculate the strength of the seasonality. The expected numbers of counts at time t for HW were estimated with the predict functions of the "forecast" packages. EWMA and HW aberration detection algorithms were executed using the package "Vetsyn" package (29).

RESULTS

Time Series Description

Seven of the syndrome TS in this study had a linear trend and all 16 syndrome TS had seasonality, however, the syndrome TS peaked in different seasons (see **Table 2** and **Figure 1**). The main differences between these syndrome TS were the length of historical data available, which ranged from slightly less than 3 years to more than 7 years; and the average number of reports per week that varied from a low of 2.6 for *ASR_OTHER_calves* to a high of 891 for *AMD_mortality_calves.* An interesting observation was that in general the coefficient of variation (CV)

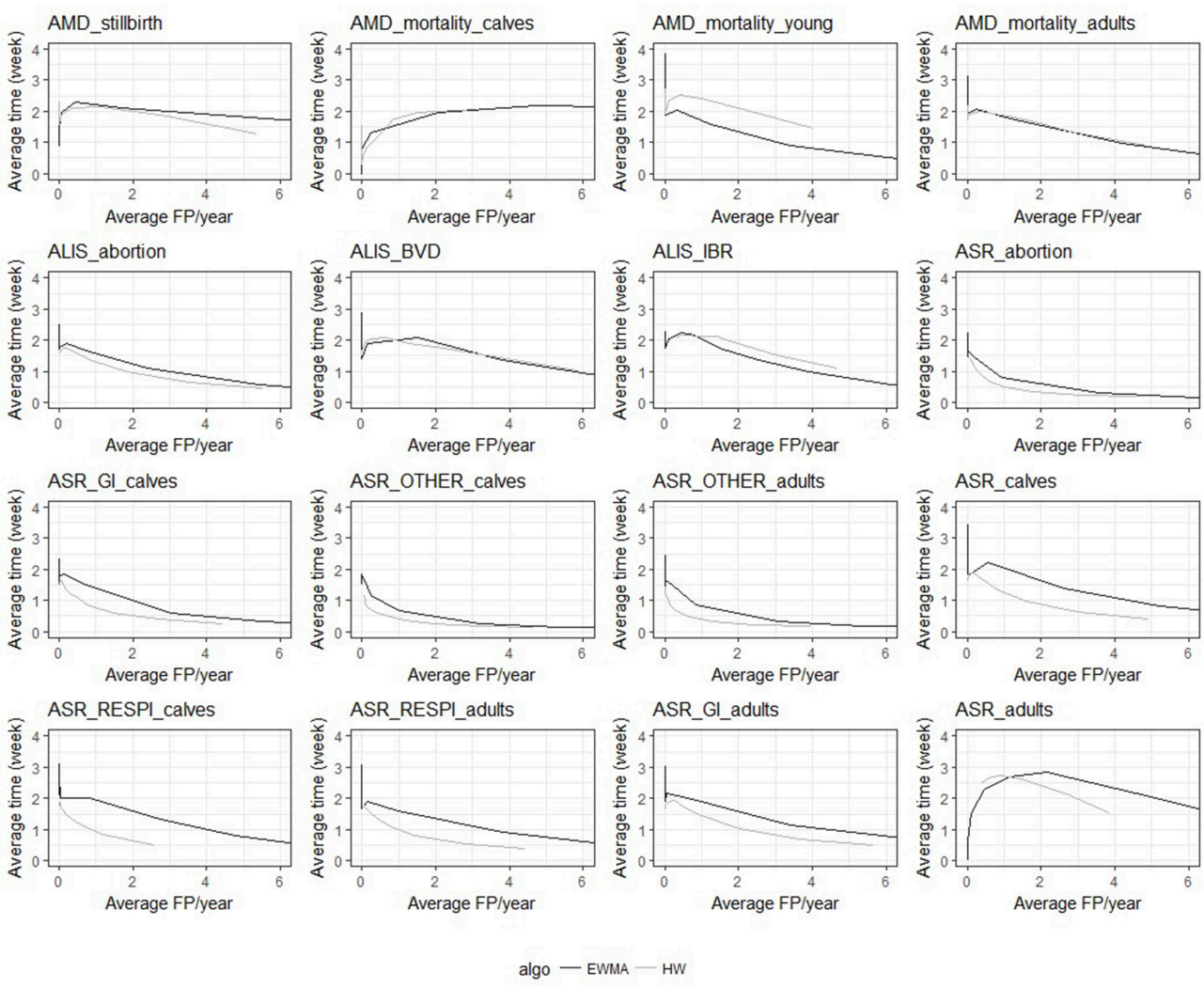

FIGURE 4 | Overall average detection timeliness (week) and corresponding average number of expected false positive alarms (FP) per year for the two algorithms and the 16 TS (all epidemic sizes and shapes are collated).

was greater for syndrome TS with smaller average weekly counts. All syndrome TS with counts >100 counts/week had on average a CV of <0.30. All syndrome TS with average weekly counts around or <100 had CV values of >0.39.

Time Series Modeling and Preprocessing

The fitting and prediction performance of HW for each syndrome TS is shown in **Appendix 2.A**. The HW method removed most of the autocorrelations present in the raw data but sporadic autocorrelations remained. The HW method correctly predicted the values of the validation dataset. However, the accuracy of the predictions varied a lot depending on the syndrome TS evaluated (see **Appendix 2.A**).

Autocorrelation function plots of the 1 week differencing for the 16 syndrome TS are shown in **Appendix 2.B**. All the syndrome TS had similar results and 1 week differencing removed most of the autocorrelations present in the raw syndrome TS (**Appendix 2.C**). Some autocorrelations remained, especially at lag 1. One-week differencing did not remove this residual autocorrelation and even produced some residual TS with a higher number of significant autocorrelations. The syndrome TS created with 1 week differencing were used to implement the aberration detection algorithm EWMA.

Detection Performance

Comparing Algorithms

As expected, both aberration detection algorithms performed better with large epidemics as compared to small epidemics (i.e., higher sensitivity, specificity, and detection timeliness). Flat epidemics were always detected with higher sensitivity, specificity, and timeliness than log normal and linear increases. Single spikes and exponential increases had the worst performance and were the epidemic shapes most difficult to detect for both algorithms. There was no difference in the performance of the two algorithms for different epidemic shapes (see **Appendix 3**, **Figures 1**, **2**).

Despite the similarities mentioned above, the two algorithms had different relative performance depending on the syndrome TS. The Holt-Winters algorithm outperformed EWMA for 12 syndrome TS: *AMD_stillbirth, AMD_mortality_calves, AMD_mortality_adults, ASR_OTHER_adults, ALIS_abortion, ALIS_BVD, ASR_GI_calves, ASR_calves, ASR_RESPI_calves, ASR_RESPI_adults, ASR_GI_adults*, and *ASR_adults*. The EWMA algorithm outperformed HW for only 2 syndrome TS: *AMD_mortality_young*, and *ALIS_IBR*. Both algorithms had equivalent sensitivity and specificity for 2 syndrome TS: *ASR_abortion,* and *ASR_OTHER_calves* (see **Figure 3**). The HW algorithm had equivalent or a better balance between detection timeliness and the average number of false positive alarms than EWMA in most syndrome TS (see **Figure 4**). However, EWMA had better timeliness for *ALIS_IBR* and *AMD_mortality_young*.

The HW algorithm performed better detection for the following time series: *AMD_stillbirth, AMD_mortality_calves, AMD_mortality_adults, ASR_OTHER_adults, ALIS_abortion, ASR_GI_calves, ASR_calves, ASR_RESPI_calves, ASR_RESPI_adults, ASR_GI_adults*, and *ASR_adults*. This algorithm was thus considered to be the optimal algorithm for these syndrome TS, and was used for all further analyses of these syndrome TS. Whereas, the EWMA algorithm demonstrated better detection performance with the syndrome TS: *AMD_mortality_young* and *ALIS_IBR*. The detection timeliness of *ALIS_BVD* were

TABLE 3 | Global Detection performance obtained with the optimal algorithm at the optimal alarm threshold.

Time series	Mean weekly count	Optimal algorithm	Optimal alarm threshold	Se	Sp	PPV	NPV	FP per year	T
AMD_mortality_calves	891.8	HW	0.05	85.9 (85.1–86.7)	88.8 (89.2–90.5)	44.9	84.8	4.0	2.0
ASR_adults	629	HW	0.5	93.4 (92.8–94)	90.4 (89.8–91.1)	56.5	88.3	3.8	2.1
AMD_Stillbirth	466	HW	0.5	92.4 (91.8–93.0)	90.2 (89.5–90.9)	53.1	87.1	3.9	1.8
AMD_mortality_adults	170	HW	0.75	94.4 (93.9–94.9)	93.7 (93.7–93.8)	65.4	88.1	2.5	1.6
AMD_mortality_young	130.4	EWMA	0.75	95.5 (95.0–95.9)	95.7 (95.7–95.8)	77.4	90.6	1.7	1.6
ASR_GI_adults	125	HW	1.5	97.1 (96.7–97.5)	96.5 (96.1–96.9)	77.6	88.7	1.4	1.4
ALIS_BVD	105.4	HW	1	92.3 (91.6–92.8)	92.5 (91.9–93.1)	59.6	87.4	3.0	1.7
ALIS_IBR	94.8	EWMA	0.75	92.4 (91.8–93.0)	91.6 (91.5–91.7)	62.2	89.6	3.3	1.4
ALIS_abortion	79.6	HW	1.5	97.6 (97.3–98.0)	96.9 (96.5–97.3)	81.3	89.8	1.2	1.3
ASR_calves	36	HW	2	96.2 (95.7–96.6)	98.0 (98.3–98.8)	85.9	88.0	0.6	1.3
ASR_RESPI_adults	19	HW	2	97.4 (97.1–97.8)	98.5 (98.2–98.7)	87.3	89.0	0.6	1.0
ASR_RESPI_calves	17.7	HW	1	98.4 (98.1–98.7)	98.0 (97.7–98.3)	88.7	91.7	0.3	1.1
ASR_GI_calves	11	HW	2.25	98.9 (98.7–99.2)	99.0 (99.0–99.1)	93.3	89.7	0.4	1.2
ASR_Abortion	7	HW	3	99.4 (99.3–99.6)	99.4 (99.3–99.6)	95.7	89.3	0.2	1.2
ASR_OTHER_adults	6	HW	3	99.9 (99.8–99.9)	99.8 (99.7–99.9)	98.3	90.1	0.1	1.0
ASR_OTHER_calves	2.6	HW	3.5	99.5 (99.3–99.7)	99.7 (99.5–99.8)	97.4	89.7	0.1	0.9

The optimal alarm threshold is defined as a multiple of the standard error. FP/yr, mean number of false positive alarms per year; T, timeliness in weeks; Se, Sensitivity; Sp, specificity; PPV, the positive predictive value; NPV, the negative predictive value. TS were ordered according to the weekly average number of cases from the largest (top row) to the smallest (bottom row).

TABLE 4 | Detection performances obtained with the optimal algorithm at the optimal alarm threshold.

Time series	Very small epidemics (25)				Small epidemics (50)				Medium epidemics (150)			
	Se	Sp	T	CC	Se	Sp	T	CC	Se	Sp	T	CC
AMD_mortality_calves	60.6	89.1	2.5	24	71.6	89.3	2.6	36	97.1	89.9	2.4	90
ASR_adults	78.8	86.7	3.6	33	88.1	87.6	3.0	40	100	90.0	2.1	80
AMD_Stillbirth	75.1	88.1	3.0	28	87.0	88.6	2.7	40	100	89.7	1.6	68
AMD_mortality_adults	78.3	92.0	2.9	19	93.8	92.4	2.7	38	100	93.5	1.4	63
AMD_mortality_young	80.2	94.4	3.1	20	97.4	94.8	2.5	34	100	95.7	1.3	67
ASR_GI_adults	87.0	94.1	3.0	21	98.0	95.2	2.3	29	100	97.6	1.0	60
ALIS_BVD	71.8	88.8	2.6	17	89.6	89.9	2.8	38	100	93.1	1.6	75
ALIS_IBR	71.7	89.7	2.1	14	90.4	90.3	2.0	33	100	91.7	1.5	71
ALIS_abortion	88.0	94.0	2.9	20	99.0	96	2.3	29	100	97.9	1.0	58
ASR_calves	83.1	96.9	3.5	23	98.0	98.2	3.0	40	100	99.2	1.3	66
ASR_RESPI_adults	87.8	96.8	2.9	18	99.4	98.2	2.5	31	100	99.1	1.0	58
ASR_RESPI_calves	91.9	95.5	2.8	18	100	97.9	3.0	35	100	98.8	1.4	67
ASR_GI_calves	99.9	92.8	3.2	21	100	94.9	2.0	27	100	95.7	0.8	62
ASR_Abortion	97.0	99.3	3.2	22	100	99.4	2.0	30	100	99.4	0.6	55
ASR_OTHER_adults	99.2	99.7	3.1	21	100	99.7	1.8	28	100	99.7	0.4	54
ASR_OTHER_calves	97.4	99.6	2.7	17	100	99.6	1.5	23	100	99.6	0.3	59

Se, Sensitivity; Sp, specificity; T, timeliness in weeks; CC, cum_cases, cumulative number of cases occurring because of the epidemic when the first alarm was raised. Results obtained for Large and very large epidemics are not shown as the detection performances were similar to those obtained for medium epidemics. TS were ordered according to the weekly average number of notifications from the largest (top row) to the smallest (bottom row).

equivalent for both HW and EWMA, but the overall sensitivity and specificity was slightly better with HW. HW was chosen as the most appropriate aberration detection algorithm for *ALIS_BVD*.

Comparing Syndrome Time Series

The optimal alarm threshold for the optimal algorithm previously selected for each syndrome TS was estimated as the alarm threshold where *Se* and *Sp* were at a maximum. This assumes equal costs for false negative and false positive alarms. The detection performances obtained at the optimal alarm thresholds are summarized in **Tables 3**, **4**, and in **Appendix 4**.

Syndrome TS with lower mean weekly counts (e.g., *ASR_RESPI_calves, ASR_GI_calves, ASR_abortion, ASR_OTHER_adults, ASR_OTHER_calves*) were better for detecting all epidemics, as there was a general increase in the overall detection performance for all metrics as the mean weekly count in the syndrome TS decreased (**Table 3**). However, the relationship varied between metrics. *Se* and *Sp* decreased in syndrome TS with the largest mean weekly counts (Spearman ρ coefficients equal to 0.878; $P < 0.0001$ for *Se*, and 0.941; $P < 0.0001$ for *Sp*). PPV had the strongest relationship with syndrome TS mean weekly count. The smaller the mean weekly count the larger the PPV ranging from 44.9 to 97.4 in syndrome TS with the largest to smallest mean weekly counts, respectively (Spearman ρ coefficient 0.95; $P < 0.0001$) (see **Figure 5**). The average number of false positive signals per year decreased for syndrome TS with the largest to the smallest mean weekly counts, respectively (Spearman ρ coefficient −0.945; $P < 0.0001$). Timeliness decreased when the weekly mean number of counts increased (Spearman ρ coefficient −0.943; $P < 0.0001$). NPV had the weakest relationship with syndrome TS mean weekly count (Spearman ρ coefficient 0.610; $P = 0.012$). The observation that increased detection performance was associated with decreased mean weekly counts was not related to decreasing relative variance in syndrome TS with smaller weekly mean counts. The CV increased as the mean weekly count of the syndrome TS decreased (Spearman ρ coefficient −0.863; $P < 0.0001$) (see **Figure 5**).

Small epidemics were detected earlier on average than very small epidemics but with a higher number of cumulative cases. This is consistent with the method used for simulating the epidemics. The three syndrome TS with the largest weekly baseline counts (i.e., *AMD_mortality_calves, ASR_adults, AMD_stillbirth*) tended to detect small and very small epidemics later and with a higher average cumulative number of cases at the time of detection than syndrome TS with smaller weekly baseline counts. Only half of the syndrome TS were able to detect very small epidemics (i.e., magnitude 25) with a sensitivity above 85%. However, all syndrome TS except *AMD_mortality_calves* detected more than 85% of the small epidemics (i.e., magnitude 50). Time-series with high weekly counts were very poor for detecting very small and small epidemics. Only syndrome TS with small weekly counts detected more than 90% of the very small epidemics. The syndrome TS with the highest average number of reports per week, *AMD_mortality_calves*, detected only 60.6% of these epidemics. Only the syndrome TS with <200 counts per week on average could detect more than 90% of the small epidemics

To test the theory that changing the alarm threshold may increase detection performance, we modified the alarm threshold for the 11 aberration detection algorithm-syndrome TS pairs

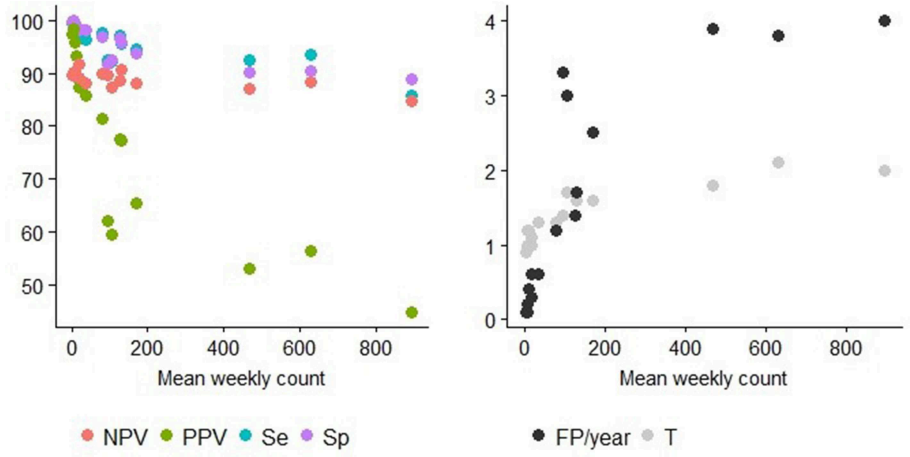

FIGURE 5 | Detection performance at the optimal alarm threshold and Mean weekly count. Y axis of the left graph: percentage of specificity (Sp), sensitivity (Se), positive predictive value (PPV), or the negative predictive value (NPV). Y axis of the right graph: number of false positive alarms per year (FP/year) or number of weeks before the first true positive alarm is raised (T = detection timeliness). The different TS can be distinguished using the information provided in **Table 2**.

TABLE 5 | Detection performance obtained with the optimal algorithm at the optimized alarm threshold.

Time series	Optimal algorithm	Optimized alarm threshold	Overall Se	Overall Sp	Very small epidemics (25)		Small epidemics (50)	
					Se	Sp	Se	Sp
AMD_mortality_calves	HW	0.05	85.9	89.8	60.6	89.1	71.6	89.3
ASR_adults	HW	0.05	96.3	86.4	87.2	82.5	94.5	83.6
AMD_Stillbirth	HW	0.05	96.3	82.1	87.1	79.3	94.6	80.1
AMD_mortality_adults	HW	0.05	98.0	83.8	91.8	80.9	98.3	81.6
AMD_mortality_young	EWMA	0.5	98.6	88.5	93.6	85.8	99.7	87.0
ASR_GI_adults	HW	1	98.4	93.0	93.0	89.4	99.2	90.7
ALIS_BVD	HW	0.5	96.7	87.4	86.5	82.4	97.0	83.6
ALIS_IBR	EWMA	0.25	98.7	79.2	94.0	74.9	99.6	76.3
ALIS_abortion	HW	1	99.2	93.8	96.1	89.4	100	92.1
ASR_calves	HW	1.5	98.4	97.1	92.5	94.6	99.8	96.3
ASR_RESPI_adults	HW	1.5	99.0	97.3	95.4	95.0	100	96.6

The optimized alarm threshold is define as a multiple of the standard error. Se, Sensitivity; Sp, specificity.

that did not detect more than 90% of the very small epidemics. The smallest alarm threshold able to provide a sensitivity for very small epidemics equal to or above 90 % was defined as the optimized alarm threshold. The new detection performances obtained with this optimized alarm threshold are presented in **Table 5**. Most of the syndrome TS were able to detect more than 90% of the very small epidemics by using the optimized alarm threshold. However, three time series *AMD_mortality_calves, ASR_adults,* and *AMD_stillbirth* were never able to reach this level of detection performance even when using a very low alarm threshold (i.e., 0.05 times the standard error of the prediction). In addition, increasing the sensitivity for very small epidemics reduced the overall specificity of the detection. For example the specificity of *AMD_Stillbirth* dropped from 90.2 to 82.1% when the alarm threshold changed from 0.5 to 0.05 times the standard error of the prediction, resulting in more than 7 false alarms per year.

DISCUSSION

In this study, different syndrome TS performed differently depending on the type and magnitude of simulated epidemic, suggesting that all syndrome TS are not equally suited for detecting all types and magnitudes of epidemics. Our study illustrates that the event detection performance is dependent on the characteristics of three components: the syndrome TS, the epidemic, and the aberration detection algorithm. Since these three components are interrelated, they should be evaluated together.

The two detection algorithms used in this study were selected because they are easy to automate and they can be implemented on short baseline TS and (30). We expected to see differences in detection performance between the two algorithms for the different epidemic shapes. The EWMA algorithm has been reported to perform well for detecting small but repeated

differences between observed and expected values, as seen in flat or linear epidemics (16, 21, 23). Holt-Winters method has been reported to be more effective for detecting large epidemics with a sudden increase in cases such as in single peak or exponential epidemics (22, 23). These performance differences were not supported by the results of our study. Somewhat unexpectedly, we identified optimal algorithms for each syndrome TS that performed equally well across all epidemic shapes. The HW algorithm outperformed the EWMA algorithm for most of the syndrome TS (i.e., 14 out of 16 syndrome TS) which confirms reports from previous studies (23, 27). However, the EWMA algorithm outperformed the HW algorithm on the *AMD_mortality_young,* and *ALIS_IBR* syndrome TS. This may partially be explained by the high mean weekly counts in these syndrome TS combined with the poor data forecasting performance of the HW algorithm. The latter may be at least partly due to the complex temporal patterns observed in these syndrome TS (see **Table 2** and **Figure 1**) which has been reported to make the HW algorithm less well-adapted to TS (31). There are many other methods available for aberration detection and some of these could also be used for TS selection [see for example (31)]. Adding information about the total cattle population under surveillance and working with proportions instead of count data could also be tested to take into account shifts in submissions and possibly improve detection performance. In addition, testing different values of the smoothing parameters for the EMWA algorithm could be explored for improving detection performance. However, longer historical syndrome TS would be needed to develop better models, especially for syndrome TS in the ALIS database which had <3 years of data.

In our study, overall detection performance (collated for all epidemic types) differed greatly from individual detection performance for epidemics of different magnitudes. It was not surprising that detection performance was lower for small and very small epidemics compared to larger epidemics. Small increases in cases per unit of time can easily remain unnoticed in the background noise of a TS, especially when the TS contains on average, a large numbers of cases per unit of time. Other studies have assessed TS detection performance using epidemics of different magnitude, but only reported overall detection performance by collating the results obtained for epidemics of different magnitude [see for example (17, 24)]. Our study demonstrated that overall detection performance may result in misleading interpretations of the sensitivity and specificity of the surveillance system. Overall detection performance may mask the fact that a specific detection algorithm applied to a specific TS may actually only detect large increases in the number of cases. For example, *AMD_mortality_calves* syndrome TS performed very well overall (Sensitivity = 85.9 and specificity = 89.9 for all epidemics combined) but performed poorly for detecting very small and small epidemics, as only 60.6% of the very small epidemics and 71.6% of the small epidemics were detected. We strongly recommend that in future studies researchers report the specific detection performance obtained for different epidemic sizes and shapes in order to avoid overestimating the overall detection performance of the surveillance system.

In our study, small epidemics remained largely unnoticed in certain syndrome TS, especially when the mean baseline count, and the background noise were high (e.g., syndrome TS extracted from AMD). Adjusting the alarm threshold is a strategy for increasing sensitivity, but it increases the number of false alarms. Increasing the number of false alarms is a problem when monitoring several syndrome TS at the same time, as surveillance systems monitoring multiple syndromic TS have been reported to intrinsically suffer from a lack of specificity (32). An alternative approach to improve detection performance is to split large syndrome TS into smaller sub-TS or, in other words, to change the level of TS clustering. If syndrome TS are sufficiently large, splitting them into sub-TS can reduce the background noise in the sub-TS. This will increase the ratio of "epidemic cases" to "baseline cases," and potentially improve detection performance. But only if the epidemic cases are not expected to be split among the sub-TS. For example, when geographical information is available, hierarchical time series approaches (33) or other spatiotemporal methods [see for example (34, 35)] could be used to improve detection performance as epidemics of transmissible diseases are supposed to start in a localized geographical area. When the epidemic is expected to be split among the sub-TS (e.g., when splitting a syndrome TS according to the production type and when all production types are susceptible to the disease), the benefit of splitting the data may be reduced. Splitting the data into different sub-TS should be carefully discussed as the benefit in terms of improved detection performance may not always offset the extra effort needed to properly monitor additional TS. Monitoring syndrome TS with low counts also has disadvantages. There is an increased risk of producing excessive numbers of false positive alarms (24), especially when the mean count per time unit is ≤5 (36, 37).

Syndrome TS that do not perform well for detecting small epidemics may have other uses in surveillance (8). SyS data can be used to define the normal behavior of disease and pathogens in animal populations in the absence of a specific epidemic. This information may have value for setting national benchmarks (38) or for supporting other surveillance programs (39). SyS may provide some evidence for the absence of certain diseases, or it may help to better understand farmers' production practices and veterinarians' clinical practices. Bovine Virus Diarrhea and IBR in Switzerland and the associated syndrome TS (*ALIS_BVD* and *ALIS_IBR)* are a good illustration of the potential alternative use of syndrome TS. Switzerland started an eradication program for BVD in 2008 which dramatically reduced the number of BVD cases (40) and the country has been officially free from IBR since 1990 (41). In our study, both syndrome TS had poor detection performance. Using other syndrome TS alone or a combination of syndrome TS, could potentially be more effective for early detection of a new epidemic of BVD or IBR in Switzerland. The syndrome TS *ALIS_BVD* and *ALIS_IBR* may have more value for monitoring long term trends in the epidemiological situation of the two diseases. This information could be especially relevant for BVD, as Switzerland is not free from the disease. The *ALIS_BVD* syndrome TS could be used to monitor long term trends in the number of suspect BVD cases, which may be useful

for monitoring the impact of control measures, or farmer and veterinarian responses to these control programs.

The epidemics used in our study were simulated as vectors containing a fixed number of extra epidemic cases, which were added to all epidemic free baseline syndrome TS. This method proposed by Lotze et al. (25) was chosen because it allows the creation of standardized simulated epidemics that are constant for all syndrome TS being evaluated. Using standardized epidemics allows for the direct comparison of the performance of different syndrome TS. For example, a "small" epidemic will have the same number of epidemic cases when it is inserted into either a small (having a small mean number of cases per unit of time) or large (having a large mean number of cases per unit time) baseline syndrome TS. The size of the inserted epidemic will also be constant for baseline syndrome TS, which have small or large variation in the number of cases per unit of time. The other commonly reported method for epidemic simulation defines the number of epidemic cases as a multiple of the standard deviation of the baseline syndrome TS [see for example (17, 24, 25, 42)]. Both approaches are perfectly suitable for epidemic simulation and the choice of one or the other depends on user preferences (25). However, the second method may not be as easy to use for the direct comparison of syndrome TS that have different standard deviations. Difficulty arises because the size of the simulated epidemics inserted into syndrome TS with different standard deviations will not be the same. For example, a simulated epidemic with a magnitude 2 times the standard deviation will produce 20 epidemic cases for a syndrome TS with a standard deviation of 10, and 200 epidemic cases for a syndrome TS with a standard deviation of 100. Computationally the two methods are comparable because transforming a multiple of the standard deviation into the corresponding number of extra cases and vise versa is quite straightforward. However, interpreting epidemics based on multiples of standard deviations is more difficult and may in some situations result in misleading interpretations of detection performance. For example, consider the case where an algorithm has been shown to detect more than 90% of small simulated epidemics and where the small simulated epidemic magnitude equals 2 times the standard deviation of the syndrome TS being evaluated. This detection performance may appear sufficient for detecting small epidemics, but if the standard deviation of the syndrome TS was quite large, it could mean that only large epidemics were being detected. When the objective of syndrome TS evaluation is to operationalize a SyS system for field use, we recommend the approach used in our study, where each type of simulated epidemic has a constant number of cases for all syndrome TS being evaluated. This approach more closely resembles field situations where we expect the size of an epidemic to have no relationship to the standard deviation of a syndrome TS. It also closely aligns with the way that surveillance practitioners characterize epidemics, which is by counting cases to map epidemic growth and geographic spread. They do not characterize epidemic growth in terms of increases in the number of standard deviations of the baseline case TS.

The objective of the SyS system currently being developed in Switzerland is to detect an epidemic of any disease in the Swiss cattle population. Based on our approach and results, most of the syndrome TS considered in this study may have value for this SyS system. Indeed, it was possible to accurately and timely detect small changes occurring in most of the syndrome TS considered. Our results also indicated that some syndrome TS should be excluded from an early detection SyS system because of their poor detection performance. This is the case for the syndrome TS *ALIS_IBR* and *ALIS_BVD*. However, they may have value for other surveillance purposes. The syndrome TS extracted from the AMD dataset also performed poorly and their usefulness for early epidemic detection is questionable. Except for the *AMD_stillbirth*, syndrome TS, all other syndrome TS from the AMD dataset consisted of counts of cattle mortalities. Cattle mortalities may not be the best indicator for early disease detection. To obtain a detectable signal in these syndrome TS, the excess mortality from an epidemic in the population would have to be high. High mortality is easily noticed by veterinarians or farmers and would likely be reported through traditional passive surveillance. Smaller epidemics caused by diseases with low mortality could remain unnoticed or signals may not be generated in these syndrome TS until late in the course of an epidemic. However, cattle mortality syndrome TS may be of interest for investigating the consequences of an epidemic. The objective of our study was to present a method that surveillance practitioners could use to help select syndrome TS-event detection pairs for inclusion in a surveillance system. The method estimates the minimum size of various types of simulated epidemics that could potentially be detected in syndrome TS-event detection pairs. We wish to point out that this is not the only evaluation criterion that should be used to select TS for inclusion in a SyS system. Before drawing any final conclusion regarding which syndrome TS to include in the Swiss SyS system, other selection criteria such as the representativeness and quality of the data should be carefully considered (10, 43–45). For example, the ASR data that we used did not contain data about the Swiss beef cattle population, which might reduce the benefit of this data sources for disease early detection. Assessing the population coverage of this data source would be essential before including this data source in a national surveillance system. The lack of consistency in the definition of stillbirth in the AMD data may also be an issue and might lead to inconsistent data reporting. We recommend a holistic approach that considers all TS characteristics. The criterion "the minimum size of event that could be detected in syndrome TS-event detection pairs" that we presented in this study should be only one of the criteria considered. In addition, in this study TS were evaluated individually but in future studies it would be interesting to evaluate TS together using multivariate aberration detection algorithms.

CONCLUSION

Our study results demonstrate that syndrome TS are not all of equal value for early epidemic detection. Event detection performance is dependent on the characteristics of the syndrome TS, the nature of the epidemic being targeted, and the event detection algorithm. Final selection of specific syndrome TS for

inclusion in an operational SyS system will be dependent on the performance characteristics of the syndrome TS and also on the goals of the surveillance initiative. It is not possible to set specific decision rules that can apply to all situations. However, the results of our study suggest that surveillance system designers should carefully assess each candidate syndrome TS before including it in their early epidemic surveillance system. The assessment should include fitting an optimal event detection algorithm to the syndrome TS and then evaluating the detection performance of the syndrome TS-algorithm pair on a variety of epidemic types. Only those syndrome TS which have acceptable performance for epidemics types that are similar to epidemics of the disease under surveillance should be included in the SyS system. Evaluating the ability of syndrome TS for early detection of epidemics is essential for selecting syndrome TS for a syndromic surveillance system, as early epidemic detection is the central task of syndromic surveillance.

DATA AVAILABILITY STATEMENT

The datasets for this study will not be made publicly available because the data contain confidential information on Swiss farms that cannot not be shared with a third party.

AUTHOR CONTRIBUTIONS

CF and JB designed the study. CF performed the analysis. CF, SS, and DH provided access to the data. All authors wrote and reviewed the paper.

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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.2019.00389/full#supplementary-material

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REVIEW
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Infectious Disease Risk Across the Growing Human-Non Human Primate Interface: A Review of the Evidence

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Most of the human pandemics reported to date can be classified as zoonoses. Among these, there is a long history of infectious diseases that have spread from non-human primates (NHP) to humans. For millennia, indigenous groups that depend on wildlife for their survival were exposed to the risk of NHP pathogens' transmission through animal hunting and wild meat consumption. Usually, exposure is of no consequence or is limited to mild infections. In rare situations, it can be more severe or even become a real public health concern. Since the emergence of acquired immune deficiency syndrome (AIDS), nobody can ignore that an emerging infectious diseases (EID) might spread from NHP into the human population. In large parts of Central Africa and Asia, wildlife remains the primary source of meat and income for millions of people living in rural areas. However, in the past few decades the risk of exposure to an NHP pathogen has taken on a new dimension. Unprecedented breaking down of natural barriers between NHP and humans has increased exposure to health risks for a much larger population, including people living in urban areas. There are several reasons for this: (i) due to road development and massive destruction of ecosystems for agricultural needs, wildlife and humans come into contact more frequently; (ii) due to ecological awareness, many long distance travelers are in search of wildlife discovery, with a particular fascination for African great apes; (iii) due to the attraction for ancient temples and mystical practices, others travelers visit Asian places colonized by NHP. In each case, there is a risk of pathogen transmission through a bite or another route of infection. Beside the individual risk of contracting a pathogen, there is also the possibility of starting a new pandemic. This article reviews the known cases of NHP pathogens' transmission to humans whether they are hunters, travelers, ecotourists, veterinarians, or scientists working on NHP. Although pathogen transmission is supposed to be a rare outcome, Rabies virus, Herpes B virus, Monkeypox virus, Ebola virus, or Yellow fever virus infections are of greater concern and require quick countermeasures from public health professionals.

Keywords: zoonoses, interspecies adaptation, monkey alarm calls, emerging disease, threat

DRIVERS OF NHP TO HUMAN CONTACT AND INTERSPECIES PATHOGENS' TRANSMISSION

The recognition that the AIDS pandemic originated as a simian retrovirus transmitted to humans has increased public health concerns about the risk that humans become infected by other pathogens prevalent in NHP. The human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), etiological agents of AIDS that cause about 1 to 2 million annual deaths, have been linked to cross-species transmission of simian immunodeficiency virus (SIV) from chimpanzees (*Pan troglodytes*) and sooty mangabeys (*Cercocebus atys*) (1). Humans might have been infected with SIV either by NHP hunting and wild meat consumption or by keeping infected NHP as pets (2). In the past decades, viruses as deadly as rabies, Herpes B virus, Marburg and Ebola viruses were transferred from NHP to humans. It is likely that during centuries and until recently, the main route of simian pathogen transmission to human was NHP hunting and wild meat consumption (3).

NHP MEAT CONSUMPTION

In Central Africa, Asia and Latin America, wildlife is the primary source of meat for low-income people living in rural areas (4–6). The practice of NHP hunting is part of the culture; it has been happening for centuries and the sale of wild meat is considered legal in many countries despite being illegal in some. Even in France, in the French Guiana two species of NHP, the howler monkey (*Alouatta maconnelli*) and the squirrel monkey (*Saimiri sciureus*), are allowed on the hunt but prohibited for sale (7). This results in regular close contacts between animal carcasses and hunters as well as between raw meat and women who prepare food. The meat is usually cooked before being shared with children (8). Most recently, illegal hunting for wild meat consumption or traditional medicine, also known as the bushmeat trade, as well as extermination of wild animals by troops foraging for food during wars have accelerated the NHP populations decline. The impact on NHP populations varied from lightly to heavily hunted. Human predation went hand in hand with an increase in contacts between NHP carcasses and humans. Fa and collaborators (9) calculated that more 150,000 carcasses of NHP per year are traded in Nigeria and Cameroun. NHP meat in Congo basin's local market is a cheap source of food (10) (**Figure 1**). Although wild meat consumption is associated with an increased risk of acquiring zoonotic diseases, people eating NHP ignore or express indifference to the risk of contracting simian pathogens, mainly because their own experience suggests that they can do it without incident. Even when governments imposed a ban on the hunting and consumption of wild meat after the 2013 to 2016 outbreak of Ebola virus in West Africa, the trade and consumption of NHP meat were not deeply affected (14). Over the past decade, the washing-based Bush Meat Crisis Task Force has regularly reported alarming information about wild animals being harvested for food in the Congo basin every year (15). A study in Liberia reported that 9,500 NHP are trade annually on the Liberia-Ivory Coast wild meat markets. According to journalists from the Guardian, there has been a massive chimpanzee decline in DRC due to hunting, with more than 400 chimpanzees being slaughtered each year. The hunting of gorillas and chimpanzees by poachers in Cameroun was also reported by the Ape Action Africa in Mefou. However, in some tribes, women refuse to eat or cook ape as it goes against their beliefs. The consumption of NHP meat is not limited to people living in poverty in Central Africa, Asia, or Latin America; wealthier households consumed only slightly less wild meat than others. Spider monkey dishes are popular in Southern Mexico. Although currently banned from dishes, NHP brain has long been viewed as a prized delicacy in Asia. The CITES/GRASP (16), reported that in Indonesia orangutans could be purchased for $100 and that some restaurants prepare dishes containing orangutan meat if specifically requested by customers.

ILLEGAL IMPORT OF NHP MEAT IN USA AND EUROPE

Illegal NHP meat importation into the United States was documented in a pilot study project intended to monitor the presence of pathogens in samples of wild meat confiscated in several US international airports that included specimens from chimpanzees (*Pan troglodytes*), mangabeys (*Cercocebus*), guenons (*Cercopithecus*), baboons (*Papio*), and green monkeys (*Chlorocebus*). This pilot study revealed the presence of simian foamy virus and herpesviruses (cytomegalovirus and lymphocryptovirus) in several NHP samples (17). In Europe, bushmeat was found in personal baggage at the Charles de Gaulle airport in Paris and Toulouse Blagnac airport, France. A metagenomic sequencing performed on African NHP bushmeat seized at Toulouse Blagnac airport demonstrated the presence of sequences related to several viruses related to the *Siphoviridae*, *Myoviridae,* and *Podoviridae* bacteriophage families; some of them infecting bacterial hosts that could be potentially pathogenic for humans (18). Journalists from the Independent reported that gorilla and chimpanzee meat is said to be on offer to African communities in Hackney and Brixton, UK, at hundreds of pounds per kilogram (19). The problem of smuggled wild meat in the French and English airports was not isolated but is relevant to most major airports in Europe and worldwide. The Tengwood organization also reported illegal bushmeat trade in Switzerland's airports (20). About 300 kg of bushmeat or wild meat found in luggage of passengers arriving in two international airports between 2008 and 2012 were confiscated by the Swiss customs, among which 12 kg were of NHP origin. The confiscated bushmeat probably represents only a very small percentage of what is smuggled into Europe annually. To date, it remains impossible to draw a global picture of the NHP meat international traffic.

LAND CONVERSION FOR HUMAN USE

Long-term deforestation has resulted in the fragmentation of about 60% of subtropical and 45% of tropical forests (21). Risk of NHP pathogens' transmission to humans has increased

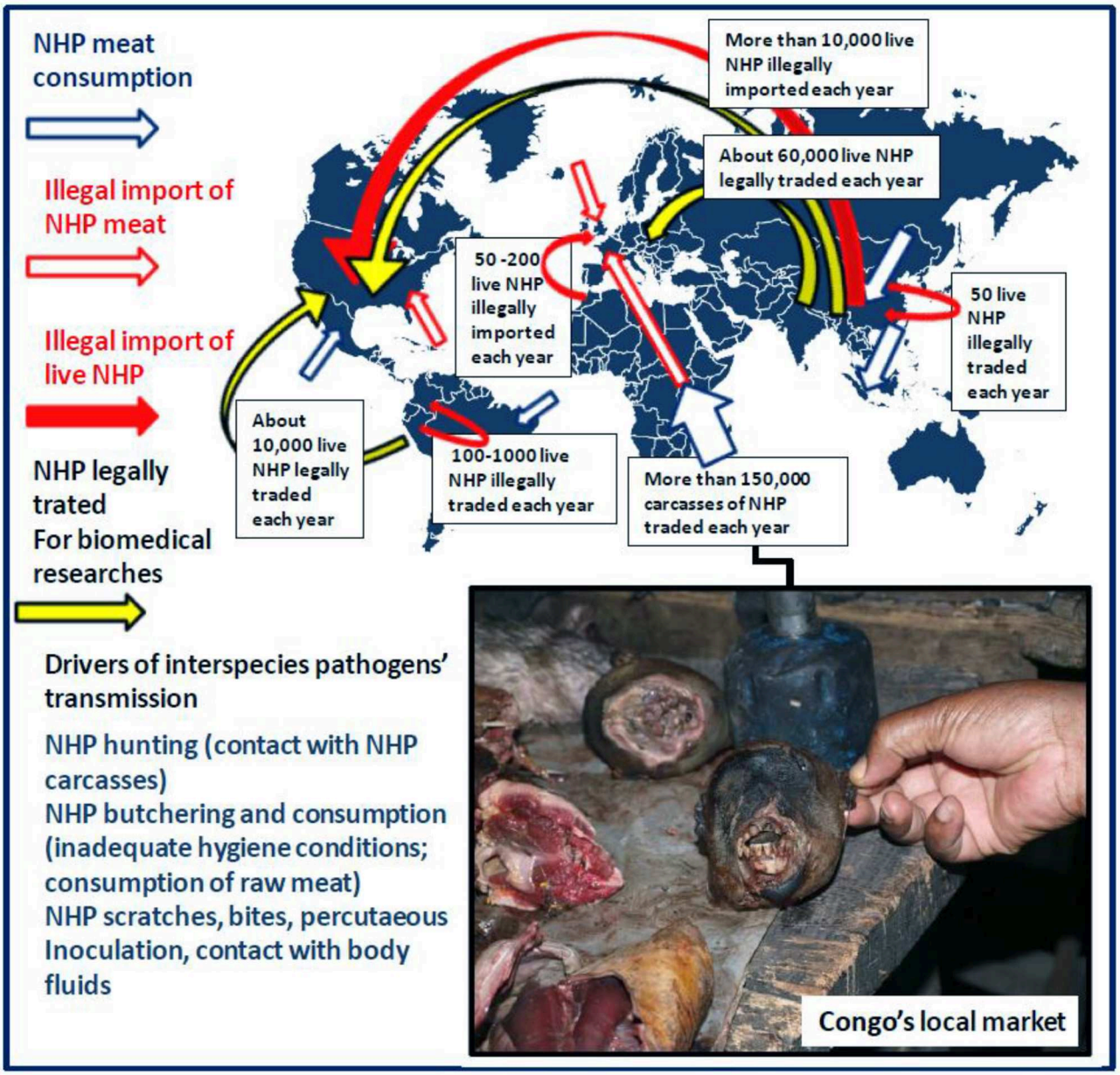

FIGURE 1 | Drivers of interspecies pathogen transmission from NHP to humans including incidents with NHP (in wildlife ecosystem or after illegal import of live NHP), contact with NHP carcasses, NHP consumption (local consumption and illegal import of NHP meat). According to the CITES database for 2005–2014, the global primate trade was estimated 450,000 NHP/year. The figure illustrates the dynamics of the annual legal trade and illegal traffic of live NHP at the international level as well as the local trade and consumption of NHP carcasses according to Fa et al. (9), Van Lavieren (11), Smith et al. (12), and Nijman et al. (13). Obviously, it is almost impossible to draw an exhaustive view of the global primate trade and particularly of the NHP illegal trade as the information available is only based on reports of animal confiscations by customs or health services. Information on NHP meat consumption in South America and Asia is not available. Box: NHP wild meat in Congo's local market is a cheap source of food (Picture credit: Oleg Mediannikov).

along with the growing human-NHP interface. Due to changing ecosystems, a consequence of road development (22) and intensified agriculture that reduce wildlife habitat in tropical countries (23), humans living in these geographic areas are more frequently exposed to closer contacts with wildlife. In addition, for economic reasons the immigration of workers and jobseekers results in permanent urbanization of frontier forests (24). For example, in West Africa the NHP habitat fragmentation by agriculture, road infrastructures and human settlements, rather than continuous intact forest, strongly affected the geographic distribution of NHP groups (25). What is true for Africa is also true for NHP living in South America and Asia (26, 27).

NHP ILLEGAL TRADE

Beside the traditional hunting and wild meat consumption, live NHP illegal trade has increased over time, along with the escalating demand for wild animal as pets. As in many other countries, in the USA and European countries NHP import is rigorously regulated by laws and the underground import and trading of NHP is prohibited. It is obviously extremely difficult to draw an exhaustive overview of the NHP illegal trade as the information available is only based on reports of animal confiscations by customs or health services. When documentation exists it usually only concerns a site at a given time and annual data is extrapolated. China and Southeast Asia was considered a primary region of origin for US wildlife imports with ∼150,000 live macaques during the 2000–2013 period (12). A few years ago, the "Libération" French newspaper reported the illegal importation in France of young Barbary macaques (*Macaca sylvanus*) from Morocco which were sold as pets in the suburbs and became aggressive during adulthood (28). French police reported in 2007 that they seize approximately 50 live macaques each year (11). As it is generally the case for illegal trade, a substantial part remains hidden. For example, from 1997 to 2008 about 2000 NHP (mainly *Macaca fascicularis*, *Nycticebus coucang*, and *Macaca nemestrina*) were sold openly in North Sumatra markets despite being totally protected in Indonesia (29). The CITES reported that about illegally sold 1,000 orangutans (*Pongo*) were rescued by the judicial services in Kalimanatan, Indonesia (16). In South and Central America, about 100 live night monkeys (*Aotus nancymaae*, *A. vociferans*, and *A zonalis*) are traded each year. Despite night monkey trade being regulated by the CITES convention, there is evidence for illegal trade at the Columbia, Peru and Brazil cross-border (30). In Peru, between 100 and 1,000 NHP are illegally traded each year (5). Dues to intensive management on wildlife domestic markets in China since the outbreak of severe acute respiratory syndrome (SARS) in 2003, more than 730 NHP (mainly *Macaca mulatta* and *Nycticebus spp*.) were rescued and confiscated in Chinese markets from 2000 to 2017 (31). In a recent survey conducted by a New York Time reporter it was claimed that there is a specific annual underground market for thousands of baby apes sold alive to local traders for $10 only whereas their cost can go up to as much as $250,000 when shipped abroad for international illegal business (32). Obviously, when NHP are kept as pets, they might be a direct source of wildlife pathogens.

NHP AS MODEL ANIMALS

NHP are traded both domestically and internationally to supply biomedical industry and pharmaceutical markets. In the early 1970s, India legally exported about 20,000 to 50,000 NHP per year and Peru exported about 30,000 NHP per year to supply the demand for biomedical studies. In the past 15 years, a linear increase in the export of live NHP has been observed (each year 3,500 more NHP are exported), with China being the largest exporter (13). The CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Trade Database (Cambridge, UK) from 2005 to 2014 reported a global NHP trade of 450,000 live individuals (430,000 individuals in trade were Asian species), plus an additional 11,000 body parts. About 70,000 NHP are legally exported each year for the biomedical industry. With the high demand for NHP in biomedical research there is also a risk of infection for persons working in primate centers. Regarding the NHP legally exported or imported, they are routinely subjected to a careful health check. The Federation for Laboratory Animal Science Associations (FELASA) provide an approach to monitor and control both endemic and incoming pathogens that may cause zoonotic and anthroponotic diseases or interfere with research outcomes. For example: monitoring for tuberculosis or rabies is required upon arrival of the NHP and renewed every year (33).

TOURISM AND ECOTOURISM

The development of international tourism and changes in the behavior of travelers brought about new risks, especially as some destinations might combine immersion into wildlife and the worst health requirements. NHP are present in 90 countries, however two-thirds of all species occur in just 4 countries: Brazil, Madagascar, Indonesia, and Democratic Republic of Congo (6). Several countries in Maghreb, Mashreq, Middle East, Central and West Africa, and Southeast Asia display a high prevalence of free-roaming NHP in urban settlements (e.g., Queen's Gate in Gibraltar; Taif city, Saudi Arabia, Lopburi Khmer temple, Thailand; Ubud monkey Forest, Bali; Katmandou monkey temples, Nepal; or Dehli city, India). Hindu and Buddhist temples have become sanctuaries for NHP who tolerate the presence of humans, and a high prevalence of Herpes B virus was reported in these NHP (mainly rhesus macaques) (34). In Bali, more than 700,000 tourists visit the monkey temples each year. A case of a tourist infected by a simian foamy virus after an incident with a *Macaca fascicularis* at monkey temple in Bali was reported (35). A recent survey reported evidence that chimpanzees and gorillas have transmitted pathogens to 33 ecotourists who visited wild great apes in Africa (36). In addition, tourists should also take care of NHP kept as pets by owners in several countries (e.g., 6,000 gibbons -*Hylobates moloch*- are kept as pets in Borneo, Java and Sumatra). For example, despite no quotas being allocated for trading of NHP as pets in Indonesia, during the last decade in North Sumatra NHP such as long-tail macaques (*Macaca fascicularis*) and pig-tailed macaques (*Macaca nemestrina*) were illegally sold at markets in Medan (29). In addition, between 1993 and 2016, at least 440 orangutans (*Pongo abelii* and *Pongo pygmaeus*), protected by the Indonesian law since 1931, were formally confiscated at the receivers in Indonesia (37), indicating that this illegal trade continues to exist despite prison sentences handed down to offenders.

ARTHROPOD BITE

Another source of concern for public health resides in pathogens which can be transmitted during the blood meal of arthropods. The outbreaks of yellow fever (YF) in South American NHP are a recent example. A first YF outbreak in 2008 left more than 2,500

dead NHP in Brazil. The most recent outbreak from 2016 to 2018 killed at least 732 monkeys in Southeast Brazil. The black and brown howler monkeys (*Alouatta* spp.) were the most affected (mortality rate of 90%), but other species such as the endangered golden-headed lion tamarin (*Leontopithecus chrysomelas*) and wooly spider monkeys (*Brachyteles arachnoides*) became sick, indicating that they are vulnerable to YF (38). It has been hypothesized that this NHP outbreak was of human origin. Humans infected in an urban cycle through *Aedes aegypti* mosquito bites can travel long distances over short periods of time, shuttling the disease from urban areas to forests where the sylvatic cycle (NHP to NHP transmission through *Haemagogus* mosquitoes bite) occurs. Conversely, non-infected humans can be infected when they visit wild sites were infected NHP and mosquito vectors are present. Such situation has forced the Brazilian public health authorities to urgently launch a national anti-YF vaccination campaign.

"ONE HEALTH, ONE EARTH": IMPACT OF EVOLUTIONARY PROCESSES

The "one health" concept defines a fundamental principle of biology: it recognizes that the health of people is connected to the health of animals and the environment (39). The inter-species transmission of pathogens is primarily a matter of the number of encounters between two species over time. To understand this process, it is necessary to keep in mind the long co-evolution of microorganisms and their hosts, the history of species evolution, the adaptation of pathogens to hosts in which they persist without seriously affecting their health, eventually if the transmission of the pathogen requires its transfer via an intermediate vector insect, the nature of the mutations that can allow the infectious pathogens to change host when they meet a new host, and the dynamics of encounters between different ecosystems. When entering more deeply in the dynamics of infectious diseases, the first challenge is to identify what are the microorganisms that are present in humans and those that colonize the wildlife and could cross the species barriers. Although many microorganisms (almost 15,000 bacteria and 2,000 viral species) have been identified so far (40, 41), the classification of microbes is still a source of debate (42) and many more unknown microorganisms could be at the origin of pathologies of NHP and human. According to molecular clock analyses, viruses and bacteria already populated the planet a few billion years ago (43), long before mammals appear on Earth. *Cyanobacteria* are considered as the source of oxygen found in Earth's atmosphere and several microorganisms have then contributed to the evolution of life until becoming essential to biological functions in humans (44, 45). It was estimated that the divergence time between Archeabacteria and Eubacteria (prokaryotic group comprising all bacteria excluding Archebacteria) was 3 to 4 billion years ago (46). The divergence of Old World monkeys (OWM) and hominoid primates (apes, humans) was an estimated 23 million years (My) ago (**Figure 2**). Heritable individual differences contributing to change for survival are likely to have played a crucial role in human differentiation from ape-like ancestors (53). *Homo sapiens* emerged 0.15 My ago and have spread and evolved through the entire planet while being subject to natural selection. Thus, viruses and bacteria had thrived for billions of years before *Homo sapiens* emerged in this ecosystem and they are exquisitely adapted to host parasitization. Over time, pathogens might select host traits that reduce their impact on the host's life span (54).

Understanding the evolution of species and pathogens may be useful to better identify the current threat. An ancient biological fight between microbial pathogens and human is likely to have shaped human evolution over the millennia through selection of alleles that were advantageous in the new ecosystem (55). Natural selection includes positive selection (selection of advantageous alleles), purifying selection (removal of disadvantageous alleles), and balanced selection (maintenance of polymorphism via heterozygote statute). Remarkably, ancient trans-species polymorphisms have been described for the major histocompatibility complex (HLA in humans) believed to result from its role in the recognition of pathogens. Consequently, HLA antigens from different species share identical epitopes (56, 57). Conversely, as humans dispersed throughout the world, populations encountered new pathogens providing strong selective pressures. The transcriptional responses of macrophages to *Listeria* spp. or *Salmonella* spp., indicated that the immune response varied between African and European individuals living in America, suggesting ancestry differences in immune response to pathogens (58). The extinction of entire tribes of Native Americans was partly linked to the importation of smallpox by Europeans in the Western Hemisphere (59). After European contact, the Native American population showed a marked decrease in HLA-DQA1 alleles, likely due to gene selection (60). The distribution of ABO alleles across human and NHP reflects the persistence of an ancestral polymorphism that originated at least 20 My ago (61). These antigens are associated with an immune response produced in the gut after contact with bacteria and viruses carrying A-like and B-like antigens and are known to act as cellular receptors for pathogens (62). In countries highly exposed to *Plasmodium falciparum* (the agent of malaria), adaptation selected defense mechanisms preventing the most serious consequences of the disease. Persons who expressed sickle hemoglobin or those who present glucose-6-phosphate dehydrogenase deficiency, evaded the worst complications of malaria (63, 64). The chemokine receptor mutant CCR5-delta32, expected to have been selected by bubonic plague or smallpox (65), confers resistance to HIV by preventing the virus co-receptor's expression at the cells surface (66).Yet, what is true for host-pathogen interaction after a long co-evolution is generally not for EID insofar as the new human host is not supposed to have undergone genetic selection driven by this emerging pathogen.

It is likely that only some of the microorganisms that are potentially pathogenic for human have been identified to date. In the early 2000s, it was estimated that infectious diseases were responsible for 15 million of 57 million annual deaths of humans. Among the causative pathogens, the deadliest infectious disease in humans was caused by HIV, a retrovirus which found its origin in wildlife NHP (67). Each year, about 2 million

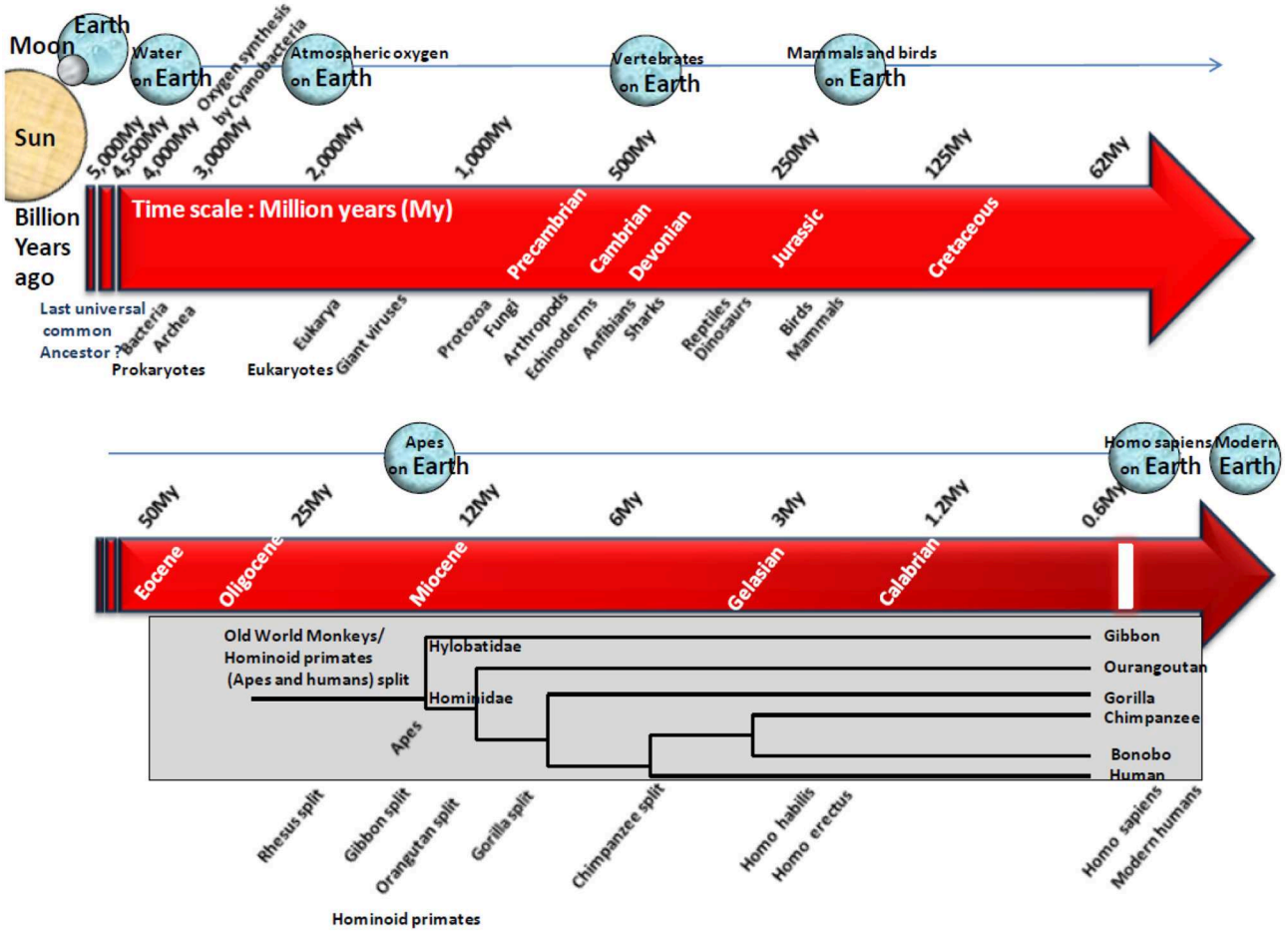

FIGURE 2 | Schematic representation of the origin of life on Earth and simplified phylogenetic tree illustrating the evolution of hominoid primates. Earth is expected to have formed roughly 4.5 billion years ago according to radiometric dating. The most common hypothesis suggests that life arose gradually from inorganic molecules to more complex structures able to self-replicate. On the time scale of life evolution on Earth, microorganisms and NHP are at the other end of the spectrum. Microorganisms were the earliest self-replicating structure present billion years ago whereas hominoid primates are the latest, with an estimated appearance 23 My ago. Cyanobacteria are expected to have first contributed to the presence of atmospheric oxygen on Earth. Then, microorganisms have shaped the world of life during million of years controlling processes essential to life of complex organisms. Almost 15,000 bacteria species have been characterized to date (40), yet they represent a small part of the estimated 10 million bacteria species that are expected to colonize the Earth (47). More than 2,000 viral species have also been characterized (41). The recent proposal of a fourth branch named "TRUC" (for Things Resisting Uncompleted Classifications) a new branch in species evolution that includes giant viruses, has challenged the previous evolution tree that discriminated among Archea, Bacteria, and Eukarya (42). The divergence between Eubacteria and Eukaryotes was >3.5 billion years ago (48). Indeed earliest eukaryotic cells are expected to have got their cytoplasm from Eubacteria, their nucleus from Archebacteria, and their mitochondries from an aerobic prokaryote (49). Old World monkeys (OWM, referring to Africa and Asia) and hominoid primates (apes, humans), share a common ancestor. The divergence of OWM and hominoid primates was estimated 23 million years (My) ago at the Oligocene-Miocene boundary, although estimating divergence remains a difficult task since the molecular clock in OWM (rhesus monkeys, pig-tailed macaques, bonnet macaques, cynomolgus monkeys, colobus monkeys, proboscis monkeys, African green monkeys, baboons, and langurs), apes (gibbons, orangutans, gorillas, chimpanzees, bonobos), and humans, seems to run at a lower rate than for other mammals (50). It was recently claimed that human had diverged 12 My ago from chimpanzees and 15 My ago from gorillas (51), whereas previous reports estimated that human had diverged from chimpanzees (*Pan troglodytes* and *Pan paniscus*) 6 My ago, from gorillas (*Gorilla gorilla* and *Gorilla beringei*) 6.5 My ago, from orangutans (*Pongo pygmaeus* and *Pongo abelii*) 12 My ago from gibbons 15 My ago and from rhesus monkeys (*Macaca mulatta*) 21 My ago, respectively (50). Our first ancestor, *Sahelanthropus tchadensis*, lived in Africa 6 My ago, whereas *Homo sapiens* emerged much later, 0.15 My ago. Subsequently, *Homo sapiens* has spread and evolved through the entire planet while being subject to natural selection. A study of short tandem-repeat *Alu* polymorphism indicated that non-African populations have high frequencies of *Alu*(+) allele, whereas African populations have low frequencies of the *Alu*(+) allele. In chimpanzees and gorillas, only the *Alu*(–) allele was observed, supporting the hypothesis that *Alu*-insertion occurred after the divergence of human and great apes (52). At most the hosts are genetically close at most the risk of pathogen transmission is high.

people died from AIDS, but fortunately this number has more recently dropped to about 1 million and HIV is no longer the deadliest pathogen for humans (68). Currently, *Mycobacterium tuberculosis,* the causative agent of tuberculosis, kills 1.7 million people annually from tuberculosis, making it the leading cause of death from infectious disease (69). This pathogen is sometimes found in NHP. In addition, more than 1.6 million people die from diarrheal disease caused by infectious pathogens, and 800,000

from malaria. Rare outbreak of malaria in human found their origin in NHP (70). Contacts between species that do not meet naturally put both species at risk for infectious diseases and the risk is magnified when they are genetically close (71). As NHP and particularly great apes are our closest relatives, protein sequence homologies are very high between great apes and humans and it seems reasonable to hypothesize that their pathogens are more likely to jump and easily adapt to humans (e.g., find an appropriate cellular receptor). Therefore, it is not surprising that NHP share many diseases with humans (72). Although the risk of accidental transmission is likely very low, the NHP pathogens which have not yet crossed the species barrier represent a possible threat for humans. The fact that almost 7.5 billion people populate Earth to date and that the human population is growing steadily is probably a factor contributing to increase this risk.

Humans are nowadays very often in contact with pets or farm animals such as cattle, pigs, poultry, and horses. Although there are many infectious diseases typically found in people working with livestock, according to the microorganisms-driven genetic selection theory humans should have less to fear from livestock than from wild animals regarding the risk of deadly zoonotic diseases since they shared the same ecosystem with livestock for millennia. This does not mean humans should not remain vigilant (this is the reason why milk pasteurization exists and why meat must be cooked before consumption) and not worry about pathogens abnormally present in their livestock because of the interconnectedness of different ecosystems. Despite veterinary

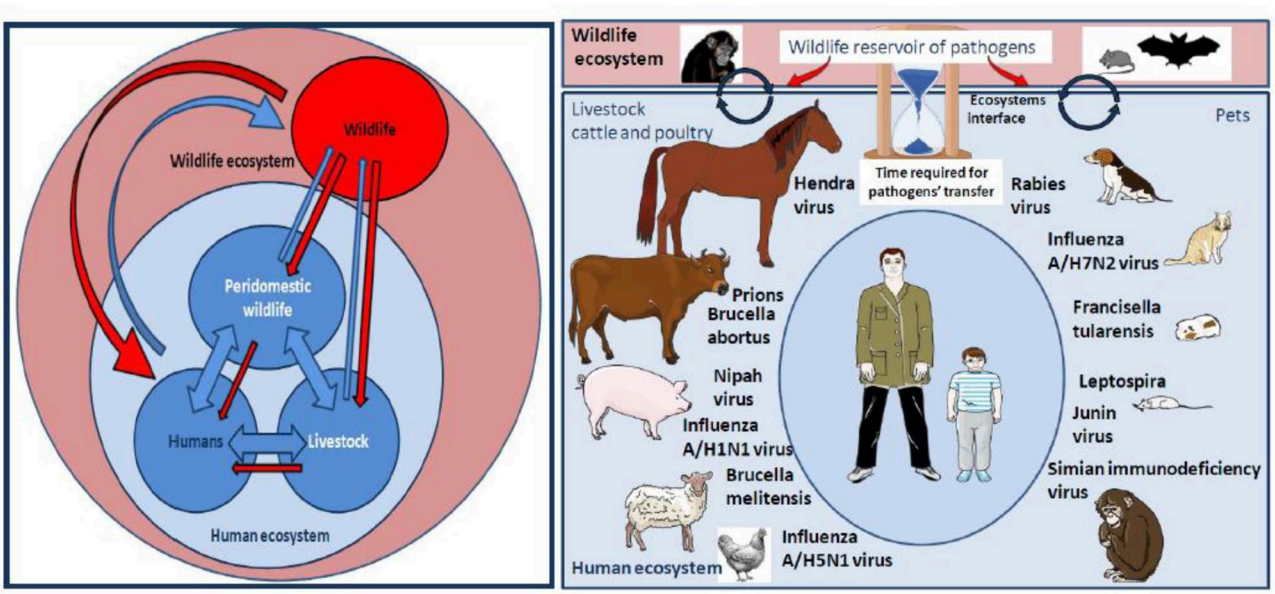

FIGURE 3 | Left panel: Pathogens flow at the human-livestock-wildlife interface. Many factors associated to this interaction network contribute to a trend toward the globalization of the distribution of pathogens: Ecosystems evolution, extensive agriculture, and water control projects, anarchic urbanization, migration of humans and animals, agroecology and livestock-wildlife interactions, spread of vectors, climate change, human behaviors, development of regional, and international transports. **Right** panel: NHP can be sometimes wildlife ecosystem actors and sometimes actors of the human ecosystem, especially when they are kept as pets. At the level of wild ecosystems, NHP are not the only source of pathogens, they are important players alongside other actors such as bats, rodents, or birds. When NHP come in close contact with humans as pets they can either directly transmit pathogens to humans or infect actors of the human ecosystem such as farm animals (livestock, cattle, poultry) or pets that can become intermediates in the transmission of pathogen to humans. Of course, most pathogens transmitted to humans by farm animals or pets do not originate from NHP. The figure summarizes examples of pathogens' transmitted from cattle, poultry, or pets to humans. The SARS-like bat CoV was transmitted to humans after evolution in the Himalayan palm-civet. The MERS-like bat CoV, originated in vespertilionid bats and evolved in dromedary prior to its transmission to human. The Hendra virus was passed from the *Pteropus* bat to horses, followed by transmission from horses to humans. *Pteropus* bats were also responsible for Nipah virus transmission to pigs which infected humans. In fact, bats are among the major reservoirs of viruses (including lyssavirus, filoviruses, coronaviruses, and paramyxoviruses), and a threat because they are adapted to flight on long distances, thus dispatching pathogens to a larger area. For the same reasons of mobility over long distance, birds are also exquisitely adapted to carry pathogens (such as influenza viruses) to farm animals before being passed on to humans. Pigs play a role in the transmission of influenza A/H1N1 to humans. Transmission of A/H7N2 to humans by cats was reported. Poultry was involved in the transmission of A/H5N1 to humans. Viruses can also be transmitted in unconventional manners such as the West Nile virus, a vector-borne pathogen maintained in a bird-mosquito cycle that infects horses and contaminated a veterinarian during the brain autopsy of a horse, or a pig found carrying a rabies virus. Investigation of wounds of humans bitten by farm animals has often shown the presence of *Actinobacillus lignieresii, A. suis*, *Staphylococcus aureus*, *Prevotella melaninogenica*, *Escherichia coli*, and *Pasteurella multocida* among others. In the USA, dogs cause about 1,000,000 bite cases/year (Bic/y) and cats 400,000 Bic/y for 90 million pets. Infection rates was about 15% following dog bites and 40% following cat bites and almost half of the wounds were polymicrobial with aerobic and anaerobic organism. There is a specific concern for rabies transmission. Most deaths from rabies occur in India, Africa, Latin America and Southeast Asia with a hotspot in Thailand were 10% of stray dog in Bangkok, are infected with rabies (compared to 1% in the US). Children have developed tularemia after contact with hamsters carrying *Francisella tularensis.* Guinea pig and rat pets could possibly spread Junin virus through their urine, feces and saliva. Cases of children bitten by rats infected by *Leptospira* were reported. The bites of monkeys kept as pet are also a source of concern.

cares, these animals may serve as intermediate for transmission of wildlife-borne pathogens (including NHP-borne pathogens on rare occasions) to the livestock owners and thereby represent the main source of human infectious disease and pandemics (**Figure 3**). According to WHO (73), a zoonosis is any disease or infection that is naturally transmissible from vertebrate animals to humans. Today, as was the case centuries ago, most EID in humans originate from zoonoses after hazardous events, the occurrence of which is impossible to predict (23). EID can refer to different epidemiological situations: (i) the disease is caused by a newly identified pathogen and did not exist previously in humans (e.g., AIDS or SARS); (ii) the disease existed before but a new etiological agent was discovered (e.g., hepatitis C); or, (iii) the disease existed before and the causative agent was identified, but it appeared for the first time in a geographic area where no case had been diagnosed previously (e.g., West Nile Virus epidemic in the USA) (74). An EID is obviously unusual; it is surrounded by uncertainty and anxiety. Epidemics of Ebola Filovirus in 1977, AIDS/HIV in 1983, Hantavirus in 1993, Influenza A/H5N1 in 1997, Nipahvirus in 1998, Severe Acute Respiratory Syndrome/SARS-Coronavirus in 2003, and MERS-Coronavirus in 2012, were of zoonotic origin (23).

DOCUMENTED EVIDENCE OF TRANSMISSION OF PATHOGENS FROM HUMAN TO NHP

Due to the growing human-NHP interface, all great ape species are considered to be endangered (75). In particular, gorillas (**Figure 4**) that spend most of their lives at ground level rather than in the trees like most other NHP are critically endangered. This ecological disaster has had surprising consequences by attracting more curious nature lovers into places where apes still live (**Figure 5**). Hundreds of tourists flock each year to wildlife national parks to view the last apes in their natural forest environment (92, 93). In the national parks of Rwanda, Democratic Republic of Congo (DRC) and Uganda, almost 500 gorillas acclimated to the presence of humans. NHP from some groups of mountain gorillas (*Gorilla gorilla beringei*) can be exposed up to 2,000 h of human visits/year (94). The influx of tourist brings in "eco-dollars" which found preservation of wildlife and help the local economy, but these practices can also introduce human pathogens in the ecosystem increasing the risk of accelerating the disappearance of great apes (36, 95).

The threat is a direct function of the pathogens' mode of transmission and their ability to survive in aerosols, soil, water, food, or feces. Several diseases affecting NHP have been considered to be of human origin; they include: (i) respiratory viral pathogens such as measles virus, influenza virus, respiratory syncytial virus, rhinovirus, adenovirus, cytomegalovirus, and bacteria such as *Streptococcus pneumoniae* or *Mycobacterium tuberculosis*; (ii) enteric pathogen virus such as poliovirus, coxsackie virus, herpes simplex virus, Hepatitis virus, or bacteria such as *Salmonella* spp., *Shigella* spp., *Campylobacter* spp.; and, (iii) parasites such as, *Giardia lamblia* or *Schistosoma* spp. The diversity of human pathogens found in NHP leaves no doubt as to the susceptibility of great apes to pathogens widely present in humans. In recent years, primatologists pointed out the misdeeds and risks of ecotourism and have advocated: (i) to limit the frequency and duration of visits; (ii) to reduce the number or visitors; (iii) to prohibit access to people with known diseases; (iv) to banish the ecotourists' consumption of food on wildlife site; (v) to define a minimum distance of observation or physically separate NHP and visitors in wildlife parks; and, (vi) to wear a facemask (93).

In 1965, an outbreak of polio was observed among NHP at the Yerkes Primate Center, USA (96). In 1971, a deadly outbreak

FIGURE 4 | Great apes in their ecosystem. **Upper** panel: bonobo in RDC. Bonobos inhabit mature, mixed-species lowland forests located primarily on terra firma, but they can be found in secondary forests, occasionally in seasonally inundated areas, and in the forest of savannah mosaics. They appear to be more arboreal—adapted for living in trees—than other African great apes; **lower** panel: gorilla in Republic of the Congo. Gorillas spend most of their live at ground level rather than in the trees like most other primates. There are at least five different gorilla ecosystems, depending on geographical location (Picture credit: Oleg Mediannikov). In wild gorillas, infection with human metapneumovirus, human respiratory syncytial virus, human adenovirus, human measles virus, human gut *Salmonella* and *Campylobacter* were reported Knowledge about great apes microbial flora (Retroviruses, Adenoviruses, *Mycobacterium tuberculosis, Microsporidia*, *Cryptosporidium*, or *Giardia*), remains to be further documented (76, 77). Apes in African parks showed a high prevalence of parasitic gastroenteritis which could pose a severe threat to tourism; furthermore, visiting tourists showed high prevalence of malaria (78). Among apes, chimpanzees are particularly threatened by infectious disease as a result of their gregarious organization (79, 80).

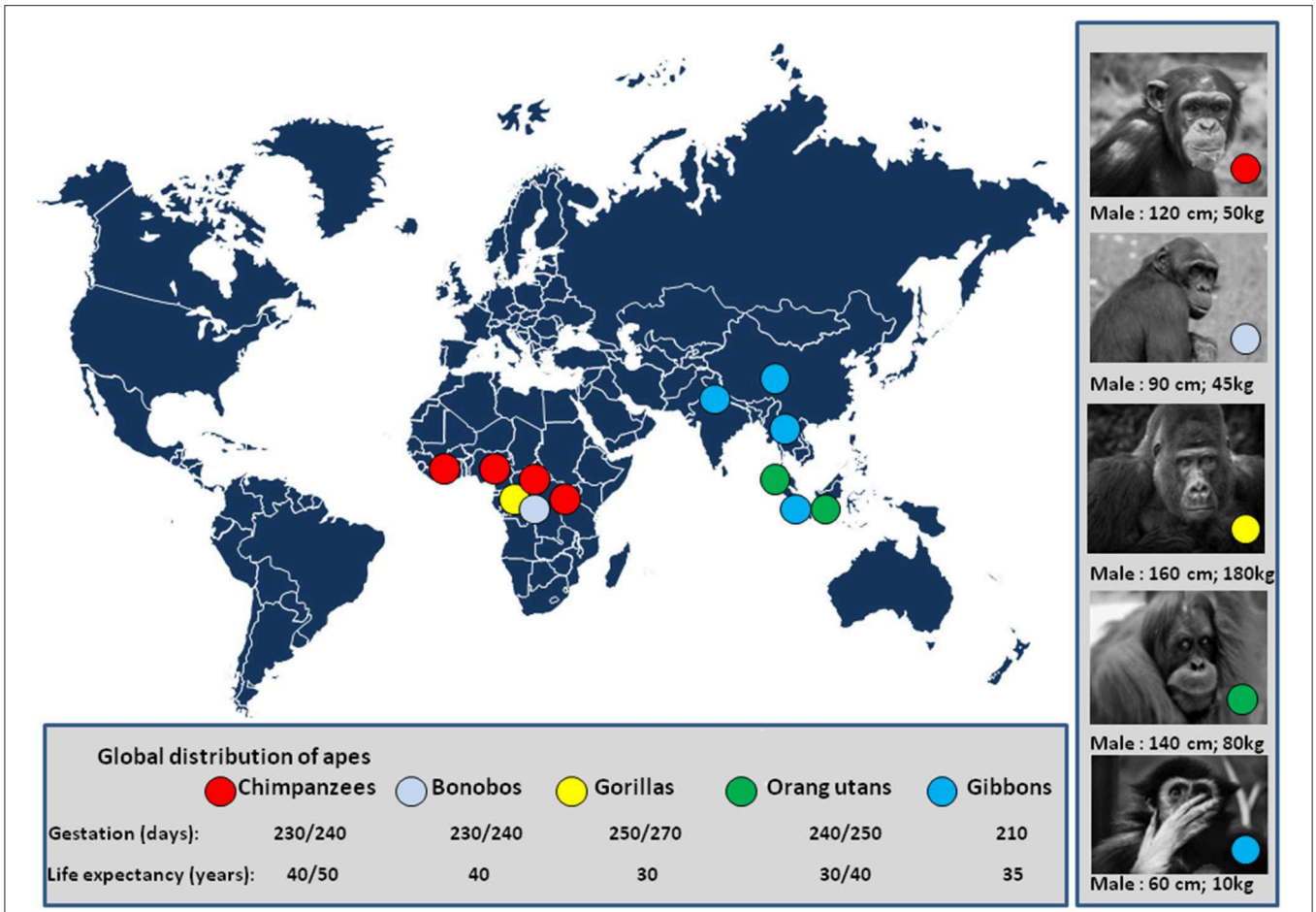

FIGURE 5 | Global distribution of apes. The number of chimpanzees was estimated one million 50 years ago. A decade ago, the estimated number of chimpanzees was 250,000 individuals in a wild area extending from Western Africa (Gabon) to Central Africa (81), and it is likely about 200,000 individuals to date. The population of bonobos is estimated to be 20,000 individual living in DRC (82); The mountain gorilla population comprises about 1,000 individuals in two populations; the first lives in the Uganda's Bwindi national park (Napa) (83) and the second in the Virunga mountains a wildlife area shared by the Uganda's Mgahinga Napa, the Rwanda's volcanoes Napa, and the Virunga Napa of DRC (84). A population of about 4,000 Grauer's gorillas (eastern lowland gorillas) is found in DRC, mostly in the Kahuzi-Biega and Maiko Napa (85). About 250 Cross River gorilla live in Cameroun and Nigeria (86). The population of orangutans in Borneo is about 100,000 individuals, 6% live in captivity (87), and 15,000 individuals live in Sumatra (88). The global demand for natural resources eliminated more than 100,000 Bornean orangutans in the past decades with projection below 50,000 individuals in 2025 (89).The population of gibbon is about 47,000 in Thailand; 4,500 agile gibbons in the Bukit Barisan Selatan NP of Indonesia; 5,000 sylver gibbons in Java; and, 2,000 individuals in China (90). There are other places where apes have been acclimated in Napa from the US and Europe (e.g., among 9 zoos/parks presenting bonobos in Europe, "Vallée des singes"/monkey valley park, France, maintains 20 captive/semi-free bonobos originating from DRC or born in captivity). Pictures of apes are from the sponsored shutterstock free website https://pixabay.com/fr/. Illegal trade with some zoos is also a threat for apes. The international trafficking of apes was well-documented in a report from UNEP and UNESCO (91).

of influenza virus was reported in gibbons (97), followed by another outbreak in NHP in 1978. In 1996, a new influenza virus outbreak, probably transmitted by veterinarians, killed 11 chimpanzees in Tanzania. In 1988, a measles virus outbreak, probably of human origin, killed 6 gorillas and sickened 27 more in Rwanda (98). During the next few years, other outbreaks of measles, polio, and scabies in great apes were reported (99). A case of hepatitis in a captive group of great apes was found after contact with an HAV-infected staff member (94). In addition, HBV was found to be highly prevalent (60%) in a colony of 143 ourangutans (*Pongo pygmaeus*) (100). Human herpesvirus 1 (HHV1) can kill New World monkey (NWM), as was suggested after a man was bitten by his pet, a marmoset monkey (genus *Callithrix*), which had acute stomatitis. For exclusion of possible pathogen transmission, a sample from the marmoset's oral mucosa was tested and found positive for HHV1 and despite treatment, this NWM died 2 days later (101). Human metapneumovirus infection was reported in wild mountain gorillas in Rwanda (102). Simultaneous detection of a human respiratory syncytial virus (HRSV) infection in western lowland gorillas and in the local human population was reported (103). Evidence of high prevalence (22%) of human coronaviruses (HCoV) in baboons (*Papio hamadryas*) of the kingdom of Saudi Arabia, was reported (104). It was recently reported that human adenoviruses (HAdV-B and HAdV-E) are frequently found in wild gorillas (55%) and chimpanzees (25%)

(105). However, using a Bayesian ancestral host reconstruction method these authors found that the human HAdV-B circulating in humans are of zoonotic origin (multiple cross-species transmission events between gorillas and chimpanzees) and were transmitted to humans more than 100,000 years ago. Transmission of human pathogens to NHP is not limited to viruses. During the mid 80s, a tuberculosis outbreak (caused by meat contaminated with bovine tuberculosis that was found by the NHP in an open garbage of a tourist lodge) afflicted a troop of savana baboons (*Papio anubis*) living in the Maasai Mara Reserve of Kenya and half of the males died (106). *Mycobacterium tuberculosis* and *M. bovis* can be acquired from infected humans or ruminants (107). In 1988, an outbreak of respiratory illness affected more than 20 gorillas in the Volcanoes National park in Rwanda. Five animals succumbed to the disease and, beside measles, the NHP had seroconverted to *Mycoplasma pneumoniae* a pathogen of probable human origin (108). Attempts to investigate NHP microbiome suggested that bacterial diversity is shaped by ecology, life history and physiology rather than phylogenetic relationships (109). In Uganda, it was observed that the number of gorillas carrying human gut *Salmonella* or *Campylobacter* had doubled in 4 years, and *Shigella* was isolated for the first time in this group of apes, probably because of ecotourism (110). What concerns wild NHP also applies to animal in captivity; outbreaks of human metapneumovirus, human respiratory syncytial virus and *Streptococcus pneumoniae* have caused the death of captive chimpanzees (111, 112) (**Figure 6**).

Regarding pathogens that pass from humans to NHP (named reverse zoonotic disease transmission) and are capable to infect a variable diversity of hosts (also named wide host plasticity) (113–115), it can be hypothesized that bidirectional transmission (pathogen passed back from NHP to humans) (116), is likely to occur. For example, this could be the case for fecal bacteria repeatedly ingested by different hosts or pathogens transmitted by a blood-feeding insect.

BACTERIAL AND/OR PARASITE RISKS FOR HUMANS SHARING THE NHP'S ECOSYSTEMS

Pathogen transmission from NHP to humans can occur by air droplets, fecal-oral contamination, cutaneous contact, bite or by an arthropod vector (**Figure 7**). There are serious risks that humans can be bitten by monkeys when they keep them as pets, when scientists maintain monkeys for medical research, when staff members handle NHP in zoos or national parks, when travelers visit sites with high prevalence of free-roaming monkeys, and when ecotourists escape from conventional sightseeing to meet great apes in Africa. NHP bites remain poorly documented to date. Incidence and type of NHP bites will depend on geographic location, industrialized vs. developing country, ecosystems, and cultural factors (117). According to WHO, monkey bites accounts for 2 to 21% of animal bites injuries worldwide. Among 332 patients who sought medical attention for bite wounds in 1975 in USA, five (1.7%) claimed to be injured by monkeys (118). A retrospective analysis of incidents caused by NHP in the UK on primatologists indicated that 85 *Cynomolgus* monkey bites had occurred over a 6 year period (119) [Tribe and Norenux2c 1983]). In 1 year, 55 patients presented to St Bernard's Hospital, Gibraltar, with a primate bite (120).

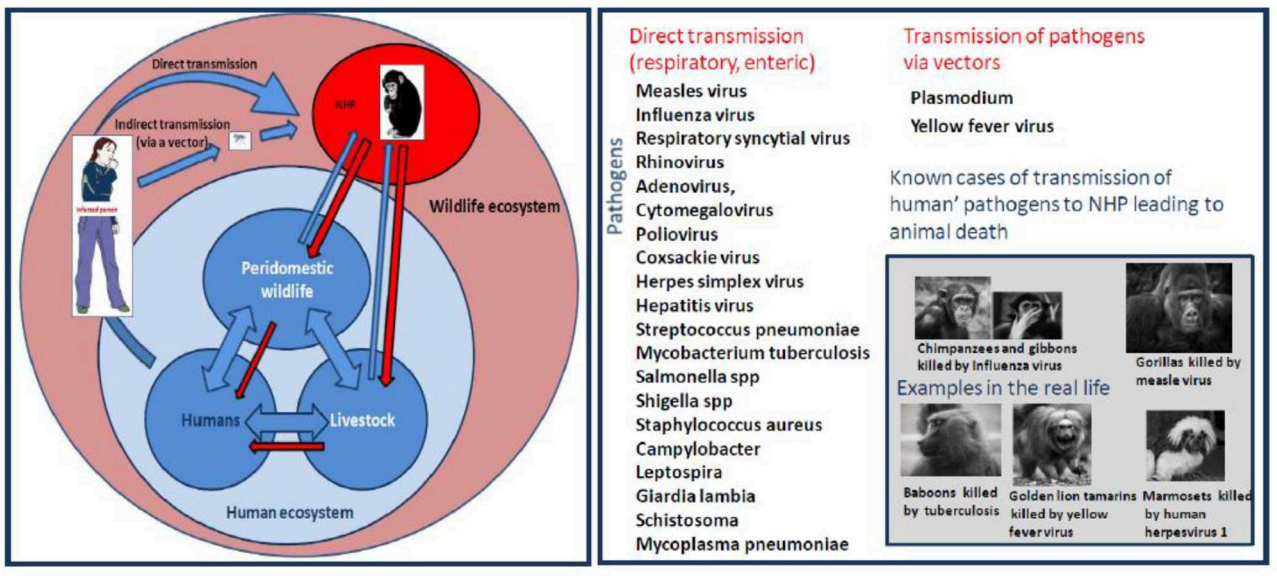

FIGURE 6 | Left schematic representation of interspecies transmission of pathogens from humans to NHP. **Right** Human diseases threatening monkeys/apes. This schematic representation summarizes the known infectious pathogens (virus, bacteria, and parasites) reported to have been transmitted to NHP after contact with infected humans. The risk of infection is a direct consequence of the pathogen's mode of transmission and its ability to survive in aerosols, soil, water, food, or feces.

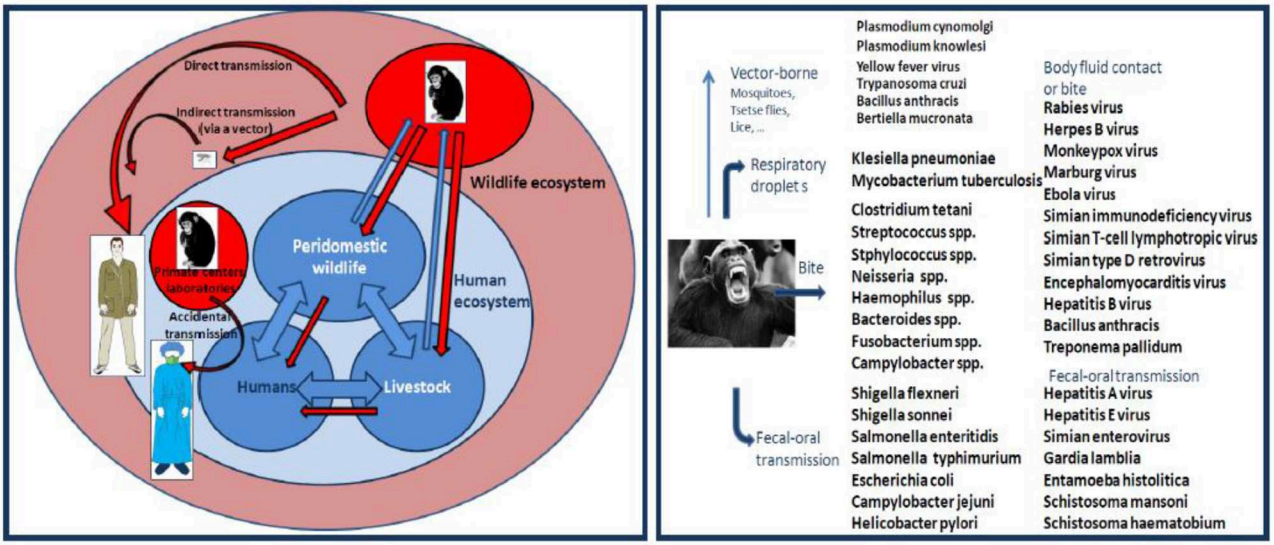

FIGURE 7 | Left panel: schematic representation of interspecies transmission of pathogens from NHP to humans. **Right** panel: Monkey and ape transmission of pathogens to humans. This illustration summarizes some of the known cases of pathogen interspecies transmission.

Most frequently (75%), bites are located on the hands, arms and legs (121). Depending on the force of the animal bite it might cause a crush injury with a variable amount of tissue damage. The severity of the injury ranges from superficial abrasion to crush wounds and major tissue loss. The risk of infection increases with the size of tissue destruction. Bacteria isolated from humans bitten by monkeys cover a large spectrum of species including *Streptococcus* spp., *Enterococcus* spp., *Staphylococcus* spp., *Neisseria* spp., *Haemophilus* spp., *Bacteroides* spp., and *Fusobacterium* spp. (122). A bacteriological analysis of 17 rhesus monkeys indicated that *Neisseria* spp., *Streptococcus* spp. and *Haemophilus parainfluenzae,* were the species most frequently isolated from the tongue microbiota of these animals (123). The sub-gingival microbiota of macaques (*M. mulatta*) was found to include *Haemophilus* spp., *Fusobacterium cleatum*, *Peptostreptococcus micros*, *Streptococcus* spp., *Actinobacillus actinomycetemcomitans*, *Wolinella* spp., *Campylobacter* spp., *Eikenella corrodens*, and spirochetes (124). Regarding respiratory pathogens, *Klebsiella pneumoniae* was found in the nose and throat of NHP (125). Tuberculosis is rare in wild NHP, yet animals carrying *Mycobacterium tuberculosis* could infect back humans (**Table 1**). Natural infections with *Mycobacterium leprae* was reported in chimpanzees and sooty mangabeys (*Cercocebus atys*) (126). Recently, different strains of *Mycobacterium leprae* were isolated from NHP, including chimpanzees, sooty mangabeys and cynomolgus macaques (127). *Mycobacterium orygis* was found in captured rhesus monkeys (128). As far the fecal-oral transmission, *Shigella flexneri* and *S. sonnei* infections are common in NHP, as well as enteropathogenic *Escherichia coli*, *Salmonella enteritidis*, *S. typhimurium*, *Campylobacter fetus*, *C. jejuni*, *Helicobacter pylori*, and many others (129). Special attention should be drawn to endemic *Treponema pallidum* infection with genital stigmata in NHP from Guinea, Senegal, and Tanzania. Many NHP in Africa, including *Papio papio, P. anubis, P. cynocephalus, Chlorocebus pygerythrus,* and *Cercopithecus mitis,* were found to suffer from treponematoses (130). An isolate called Fribourg-Blanc obtained from a baboon lymph node was found to be genetically linked to *Treponema pallidum pertenue,* the causative agent of human yaws and, could be transmitted to humans by contact (131, 132). Vigilance is required regarding *Bacillus anthracis,* since anthrax killed chimpanzees and gorillas in West and Central Africa (133).

Regarding parasites, many NHP have been found infected with *Trypanosoma cruzi*, a pathogen isolated in 1924 from South American squirrel monkeys (*Chrysothrix sciureus*), that causes anorexia, weight loss, dehydration in monkeys and Chagas disease in humans. Natural *T. cruzi* infection has been reported for several NHP such as marmosets, spider, cebus, rhesus monkeys, and gibbons (134) and could be transmitted to humans via triatomine bugs after feeding on infected animals. *Giardia lamblia*, an enteric flagellate, induces diarrhea in monkeys and children (135). The parasite *Entamoeba histolitica,* common in OWM, has been reported in most NHP including the great apes (gibbons, orangutans, and chimpanzees) as a cause of severe enteric disease. It can infect humans as well, leading to dysentery. OWM such as mangabeys and great apes such as chimpanzees can carry either *Schistosoma mansoni* or *S. haematobium* (136). *Leishmania major* has been identified in wild gorillas' feces (137).

Several other parasites such as *Amoeba*, *Toxoplasma*, *Babesia*, *Cryptosporidia*, Coccidia, nematodes, and cestodes can be found in NHP possibly presenting a risk for humans (138).

The *Plasmodia* that infect great apes are usually of a different group than those found in OWM, and they are related to parasites inducing malaria in humans. Indeed, characterization of

Table 1 | The table illustrates some examples of bacteria that infect NHP and can possibly be transmitted by NHP to humans as well as the expected health consequences for the infected people.

Pathogens	Symptoms in monkey/ape	Transmission to human	Symptoms in human	What to do?
Campylobacter jejuni; Enterotoxigenic *Escherichia coli*	May be asymptomatic. Diarrhea, blood-tinged feces, nausea, rapid dehydration, prostration Fatal cases	Fecal-oral transmission contaminated water	Incubation: 12 h to 7 days Fever, diarrhea, nausea vomiting, rapid dehydration	Maintain normal fluid and electrolyte balance Antibiotics (ciprofloxacin, azithromycin, rifaximin)
Klebsiella pneumoniae	Coughing, sneezing, dyspnea, pyrexia, lose weight	Coughing, respiratory droplets, body fluids	Incubation: 1 day to 4 weeks Pneumonia; fever, cough, shortness of breath	Severe: meropenem. Non-severe: ceftriaxone, cefotaxine, ciprofloxacin (and secondarily adapted to antibiotic-resistance profile) Hydration. Oxygen
Streptococcus pneumoniae	Coughing, sneezing, dyspnea, pyrexia, lose weight	Coughing, respiratory droplets, body fluids	Incubation: 1 day to 4 weeks Pneumonia: fever, cough, shortness of breath Meningitis	Amoxicillin. Hydration. Oxygen
Streptococcus pyogenes; Staphylococcus aureus	Oral microbiota May be asymptomatic	Bite	May cause necrotizing cellulitis	Post-exposure prophylaxis Broad antibiotic coverage (amoxicillin clavulanic acid)
Mycobacterium tuberculosis	May be asymptomatic Cough, lose appetite or weight and abdominal symptoms short before death Fatal cases	Coughing, respiratory droplets	Incubation: 2 to 12 weeks Tuberculosis may infect any part of the body but most commonly occurs in the lungs	Preventive TB vaccine (low efficiency). Antibiotics (isoniazid, rifampicin, pyrazinamide, ethambutol)
Treponema pallidum pertenue	May be asymptomatic Skin lesions	Skin-to-skin contact	Incubation: 1 to 24 weeks Yaws primarily infects children Lesions: bumps on the skin of the face, hands, feet, and genital area	Antibiotics (benzathine-benzylpenicillin)

The medication mentioned in the right column is indicative but must obviously be adapted according to the characteristics of the pathogen and the patient's symptoms. For more detail see MSD Manual. Bacterial diseases of non-human primates: https://www.msdvetmanual.com/exotic-and-laboratory-animals/nonhuman-primates/bacterial-diseases-of-nonhuman-primates. About the parasites that can be passed from NHP to humans (not shown), the majority of them are transmitted by the fecal-oral route and cause gastrointestinal symptoms in humans. The treatment will depend on the nature of the parasite and the symptoms. Usually, mebendazole, albendazole, and ivermectin are used for antihelmenthic medications, praziquantel is used for treatment of cestodes and trematodes, metronidazole, benzimidazole, artemisin, mefloquine are used in the treatment of intestinal protozoa. It is worth noting that malaria can be transmitted to human by infected NHP in areas where the mosquito vectors are present. Artemisin-based combination therapies are usually the first line treatment for malaria. However, chloroquine is the preferred treatment when the parasite is sensitive to the drug. For more detail see WHO, International travel and health -chapter 7-Malaria: https://www.who.int/ith/2017-ith-chapter7.pdf; MSD Manual. Parasitic diseases of non-human primates: https://www.msdvetmanual.com/exotic-and-laboratory-animals/nonhuman-primates/parasitic-diseases-of-nonhuman-primates.

Laverania spp. found in various apes identified lineages in eastern chimpanzees as well as western lowland gorillas that were nearly identical to *P. falciparum* and *P. vivax* (139, 140). Among others, *P. knowlesi* that circulates in cynomolgus, leaf monkey and pig-tailed macaques in Southeast Asia inducing moderate symptoms in these natural hosts, can be fatal for rhesus monkeys. In contrast, *P. cynomolgi*, found in cynomolgus, toque monkeys, pig-tailed macaques, Formosan rock macaque and leaf monkeys, induces moderate symptoms in rhesus monkeys (141). To note, *P. cynomolgi, P. siminovale* and *P. inui* are related to *P. vivax*, *P. ovale* and *P. malariae* in humans, respectively. Cross infection of *P. knowlesi* has been documented in humans (70, 142, 143). Other plasmodia are found in great apes such as *P. pitheci* in orangutans in Borneo, and *P. rodhaini* in chimpanzees and gorillas. African apes can be considered as the source of parasites responsible for the human malaria.

Other blood sucking insects such as flies, ticks, fleas, sandflies, lice could also transmit pathogens from NHP to human; tsetse flies might transfer trypanosomiasis and lice can transfer *Bertiella mucronata* tapeworm (144).

VIRAL RISKS FOR HUMANS WHO SHARE ECOSYSTEMS WITH NHP

It is impossible to describe herein all the viruses of NHP considered at risk of transmission to humans. We can, however, draw attention to enteroviruses that have a wide distribution in monkeys and can be transmitted to humans. Enteroviruses A and B have been isolated from OWM, macaques (*M. mulatta* and *M. nemestrina*), sooty mangabeys (*C. atys*) and baboons (*Papio doguera*) with diarrheal disease (145). Members of human enteroviruses (HEV-A) presented VP1 sequences that were more similar to those of some simian enteroviruses than to those of the others HEV (146). More recently, new simian enteroviruses have been isolated from chimpanzees, including a D type enterovirus (EV111) that was found phylogenetically related to a human isolate from the DRC (147). It was also recently reported that Mountain gorillas (*Gorilla beringei*) are widely infected (43%) with lymphocryptoviruses (148).

The risk for retroviruses transmission from NHP to humans has been deeply studied. These viruses include simian

immunodeficiency virus (SIV), simian T-cell lymphotropic virus (STLV), simian type D retrovirus (SRV), and simian foamy virus (SFV). The emergence of HIV-1 and HIV-2 in humans, has been linked to cross-species transmission of SIVcpz from chimpanzees (*Pan troglodytes*) and SIVsm from sooty mangabeys (*Cercocebus atys*) (1). The seroprevalence of SIV in monkey is high, about 35%. African green monkeys (AGM) vervet (*Chlorocebus pygerythrus*), grivet (*C. aethiops*), sabaeus (*C. sabaeus*), and tantalum (*C. tantalus*) are the natural hosts of SIVagm, and do not show symptoms of immunodeficiency. SIVmac infection of macaques, is often accompanied by lymphadenopathy and immunodeficiency (149). Accidental transmission of a SIVmac to a laboratory worker after a dirty needle prick, has been reported. NHP are also natural hosts for STLV-I (150). STLV-I is frequently found (5–45%) in OWM, gorillas (*Gorilla gorilla*), chimpanzees (*Pan troglodytes*), and baboons, whereas the related STLV-II infects bonobos (*Pan paniscus*) and cause lymphomas in baboons (151). Cross-species transmission to humans might result from bushmeat hunting or animal bite (152). It is worth noting that 5% of humans infected with HTLV-I, the human counterpart of STLV-I, suffer from adult-T leukemia or tropical spastic paraparesis (153). Actually, four subtypes of STLV, which have their HTLV counterpart, have been identified. The SRV was found in pigtailed macaques, crab-cating macaques, rhesus macaques, celebes macaques and cynomolgus monkeys (154), and it induced deadly hemorrhagic disease in captive rhesus macaques (*M. mulatta*) and Japanese macaques (*M. fuscata*) colonies (155). SRV-2 was reported to cross species barriers since it has been found in healthy persons occupationally exposed to infected NHP (156). Another simian retrovirus, SFV, present in the saliva of infected animals, is widespread (up to 70% prevalence) in NWM, OWM and apes (157). The human foamy virus isolated from a Kenyan patient in 1971 and considered non-pathogenic, was phylogenetically linked to a chimpanzee-like SFV. Transmission of SFV to humans during monkey bites was documented in hunters living in Cameroun and a person who has had contact with macaques (*M. fascicularis*) in Indonesia (158). SFV infection has been reported in 1–4% of persons who worked with NHP in zoos, primate centers, and laboratories and up to 24% in workers after bite or scratch by gorillas or chimpanzees (159–161).

It is necessary to keep in mind that the encephalomyocarditis virus (EMCV) a picornavirus, induces outbreaks of fatal myocarditis in NHP. It was responsible for heart failure, renal failure and cerebral infarction causing the death of bonobos (*Pan paniscus*); gibbons (*Hylobates lar*) (162), *Macaca sylvanus* (163), and *Papio hamadryas* (164). EMCV was described as an encephalitis-type illness in humans rarely resulting in severe clinical symptoms (165). EMCV was reported as being responsible of a deadly disease causing up to 100% mortality in apes (162) In Peru, human febrile illness caused by EMCV infection in two patients has been reported, and the reservoir of the virus has not been identified (166). This virus did not spread in the human population.

Rabies is a zoonotic disease characterized by severe neurologic signs caused by rabies virus (genus Lyssavirus). This disease is responsible for almost 55,000 human deaths every year, mostly in Asia and Africa. Natural rabies was described a long time ago in laboratory monkeys (167). In countries of endemic canine rabies, cases of rabies attributed to NHP are often underreported (168, 169). Survey studies in Brazil indicated that the white-tufted marmoset (*Callithrix jacchus*) was a source of human rabies in the state of Ceara (170). Between 1990 and 2016, at least 19 human cases of rabies following the incident with *C. jacchus* were registered (171). Another report described that 11.1% of free-ranging capuchin monkeys (*Cebus apella*) had antibodies against rabies virus. Rabies was also reported in great apes such as chimpanzees. In India, rabies was reported in macaques (*M. mulatta*) (169). In Thailand, an Asian country known to be at high risk for rabies, a study of 2,622 Thai children consulting for possible rabies virus exposure revealed that stray dogs were involved in 86.3%, cats in 9.7% and NHP in 1% of cases and, a meta-analysis of data in the city's urban population confirmed these percentages, the mean animal bites being 992 Bic/y, with 657 bites from dogs, 324 bites from cats, and 11 bites (1.1%) from monkeys (172). This observation contrasts with another meta-analysis indicating that among 2,000 travelers in Southeast Asia seeking care for rabies post-exposure prophylaxis, 31% consulted after they had been injured by NHP (173). The difference between indigenous populations and travelers might be explained by frequenting old Khmer temples where long-tail macaques (*M. fascicularis*) are used to being fed by tourists, and sometime bite visitors. In India, rabies is not a notifiable disease, yet there are a few reports of human rabies following exposure to NHP (174). For example, a young boy from Australia developed rabies after he returned to his country following a trip to Northern India during which he was bitten on his finger by a wild monkey (175).

The Cercopithecine herpesvirus 8/B virus commonly infects rhesus, cynomolgus, stump-tailed and other macaques. It is highly prevalent (90%) in adult macaques (*M. mulatta* and *M. fascicularis*). Related viruses named SA8 and Herpes papio 2 were isolated from vervet (*Cercopithecus aethiops*) and baboon (*Papio*), respectively (176). Asymptomatic macaques can shed virus in oral and genital secretions. This herpesvirus provokes conjunctivitis, flu-like symptoms and might cause ascending paralysis and a potentially fatal meningoencephalitis in humans (117). There are documented cases of Cercopithecine herpesvirus transmission to humans by NHP bites, scratches and contacts (urine, feces, and brain). Davenport et al. reported the cases of three workers from the same animal research facility in Michigan, USA, who were infected with Cercopithecine herpesvirus by macaques; clinical symptoms varied from self-limited aseptic meningitis to fulminant encephalomyelitis and death; two patients survived after treatment with ganciclovir (177). Transmission of Cercopithecine herpesvirus by scratches and percutaneous inoculation with contaminated materials was also documented (178). A woman working in a primate center was exposed to biological material from a rhesus macaque which splashed into her eye, and she died from Cercopithecine herpesvirus infection (179). About 50 human cases have been reported among which 29 were fatal. Acyclovir treatment reversed the neurological symptoms and was life-saving in a few cases. Specific public health measures are required considering that seroprevalence in macaques of temples in Nepal and Bali

was 65 and 80%, respectively (35). It was claimed that monkey temple workers had developed an immune response against Cercopithecine herpesvirus without disease, suggesting these workers might present a natural resistance to Cercopithecine herpesvirus. Although tourists are immunologically naive, there is a lack of evidence of Cercopithecine herpesvirus infections among travelers, all reported cases being laboratory workers (**Table 2**). Recently, this virus was found in 14% of free-ranging rhesus macaques of Silver Springs State Park, a popular public park in Florida USA (182). This population of wild NHP, breeding very rapidly, is currently considered as a public health threat.

The monkeypox virus (MPXV), isolated in 1958 from cynomolgus monkeys (*M. fascicularis*) is a zoonotic virus endemic in Western and Central Africa. MPXV was then found in monkeys caged in zoos and primate research facilities (183). Yet, the main host of MPXV appear to be wild squirrels. A serosurvey conducted on almost 2,000 NHP in West Africa, revealed that 8% of vervet (*C. aethiops*) and 6% of colobus monkeys had antibodies against MPXV (184). A similar seroprevalence was found in *Cercopithecus ascanius* from DRC. In captive animals, outbreaks of MPXV have been recorded in rhesus monkeys, pig-tailed macaques, squirrel monkeys, owl-faced monkeys, African green monkeys, baboons, orangutans, gorillas, chimpanzees, and gibbons (185). MPXV was also isolated from a sooty mangabey (*Cercocebus atys*) found dead with pox-like lesions in Tai National Park in Ivory Coast. In 2014, a monkeypox outbreak among chimpanzees was reported in a sanctuary in Cameroon with 6 out of 72 monkeys infected by MPXV; the sick animals lacked appetite and showed gradual appearance of vesicles and nodules on the forelimbs and the face and one died from the disease (186). Humans can be infected with monkeypox and the disease, characterized by a flu syndrome, rash, pustules, occurs sporadically in villages within the tropical rain forest of West and Central Africa. Between 1970 and 1983, 155 human cases have been reported (80% expected to be of animal origin) (187). Person-to-person transmission of MPXV through respiratory droplets or body fluids, has contributed to a larger outbreak in human populations with a case fatality rate up to 10% (188). Since 2016, human monkeypox cases were reported in DRC (>1,000 cases reported per year; incidence rate: 5.53 cases per 10,000 people), Republic of the Congo (88 cases/y), Nigeria (89 cases/y), Central African Republic (19 cases/y), Liberia (2 cases/y), and Sierra Leone (1 case/y) (189). Smallpox vaccine confers partial cross-protection to monkeypox, reducing the case fatality rate. In Nigeria, an outbreak of human MPXV was reported by the end of 2017 with 146 suspected cases and 42 laboratory confirmed cases (190). Two human cases of MPXV infection were reported in the United Kindom (191). The first case was a Nigerian who traveled to England and upon arrival to UK presented fever, lymphadenopathy and a rash in the groin area that had developed

Table 2 | Pathogenic NHP' viruses represent a major threat to humans, due to the severity of symptoms in infected persons.

Pathogens	Symptoms in monkey/ape	Transmission to human	Symptoms in human	What to do?
Rabies virus	Neurological symptoms (aggression, fear, salivation, paralysis)	Bite (saliva), scratches, licking. Animals can be contagious 2 weeks before the onset of symptoms	Incubation: weeks to months Neurological symptoms Nearly 100% fatal cases	Preventive rabies vaccine Post-exposure prophylaxis (anti-rabies immunoglobulins) Broad antibiotics coverage (to avoid bacterial infections related to bite)
Herpes B virus	Asymptomatic	Contact (mucosal, urine, feces) Bite	Incubation: 3 days to 5 weeks Flu-like symptoms. Fatal meningoencephalitis cases (up to 70%)	Post-exposure prophylaxis acyclovir therapy
Monkeypox virus	Fever, facial edema, Pox-like lesions Fatal cases	Bite, scratches, scraping, cough, respiratory droplets, insufficiently cooked meat consumption	Incubation: 14 days Pox-like lesions (rash with vesicles and pustules) Polyadenopathy, diarrhea Fatal cases (up to 10%)	Smallpox vaccine confer partial cross-immunity Symptoms treatment Antiviral therapy under evaluation (cidofovir)
Marburg virus; Ebola virus	Severe hemorrhagic fever Fatal cases	Contact (blood, body fluids) Bite	Incubation: 2 days to 3 weeks. Severe hemorrhagic fever. Nausea, vomiting, diarrhea. Fatal hemorrhagic cases (around 50%)	Vaccine under evaluation. Symptoms treatment (fever, pain, dehydration) Antiviral therapy under evaluation
Yellow fever virus	Hemorrhagic fever Fatal cases	Mosquito vector	Incubation: 3 days to 2 weeks. Hemorrhagic disease Nausea, vomiting, diarrhea, jaundice Symptoms disappear in 3–4 days Toxic phase in a small % of cases. Fatal cases (around 50%)	Preventive YFV vaccine (99% immunity). Symptoms treatment (fever, pain, dehydration)

The table summarizes which viruses known to infect NHP, can be transmitted to humans and their deleterious effects for the infected people. The medications mentioned in the right column (pre-exposure prophylaxis, post-exposure prophylaxis, and/or post-infection medication) are indicative but protocols should be adapted to each clinical situation. For more detail see (172, 180, 181). Also see: WHO guide for rabies pre and post exposure prophylaxis in humans: https://www.who.int/rabies/PEP_Prophylaxis_guideline_15_12_2014.pdf; CDC B virus: https://www.cdc.gov/herpesbvirus/prevention.html; CDC Monkeypox: https://www.cdc.gov/poxvirus/monkeypox/clinicians/treatment.html; WHO Ebola: https://www.who.int/csr/resources/publications/ebola/patient-care-CCUs/en/; CDC Marburg: https://www.cdc.gov/vhf/marburg/treatment/index.html; CDC Yellow fever: https://www.cdc.gov/yellowfever/index.html.

the day before leaving Nigeria and the second was a UK resident who returned from a 3 week holiday in Nigeria. He presented fever, lymphadenopathy, a scrotal lump, and rash on the face and hands that had become pustular. The origin of infection was likely a contact with a person carrying infected lesions.

The transmission of the Marburg virus (MARV), from NHP to laboratory workers in 1967, is quite interesting to analyze regarding the spread of the pathogen via inter- and intra-species events. The source of the human outbreak was traced back to African green monkeys (*Chlorocebus aethiops*); indeed, these MARV-infected animals were imported in Europe from the Lake Kyoga region of Uganda for further experimentation in animal facilities in Marburg, Germany, aimed at obtaining kidney cells required to culture the poliomyelitis vaccine. Thirty-one persons (25 staff members and 6 secondarily infected humans) developed severe hemorrhagic fever, among which 6 died from the disease (192). Eight years after the isolation of this first filovirus, MARV was found to cause the death of a young Australian who had traveled throughout Zimbabwe. In the following years, only sporadic outbreaks affected small numbers of individuals in Africa (193). Yet, there were two large MARV outbreaks reported, first in the DRC from 1998–2000, associated with persons who engaged in illegal mining activities, and second in Angola from 2004 to 2005. In 2014, a single fatal case of MARV was identified in a healthcare worker in Kampala, Uganda (194). Another filovirus, the Ebola virus (EBOV), emerged in Africa in 1976. This virus is expected to be transmitted to humans by bats (23) and NHP are considered as intermediate host (195). However, each human outbreak of the Zaire EBOV strain (ZEBOV) was linked to reports of gorilla and chimpanzee carcasses in neighboring areas of Gabon and Congo, the sick persons having had contact with the NHP carcasses (196). It has been estimated that the ZEBOV outbreak killed 5,000 gorillas in West Africa in 2002–2003 (197). Moreover, a member of the Ebola virus family, the Reston ebolavirus (RESTV), was discovered in 1989 after an outbreak of hemorrhagic fever in cynomolgus macaques shipped from the Philippines to Reston, Virginia, USA (198). Seropositivity to the RESTV was estimated to be of 10% in rhesus, African green and cynomolgus monkeys. Several outbreaks of the death of monkeys were reported in cynomologus macaques caged in primate facilities in Sienna (Italy, 1992), in Alice (Texas, 1993), and in Manila (Philippines, 1996 and 2015) (199). Humans cases of RESTV infection remained asymptomatic, but surveillance is required to limit the risk of transmission of a mutant virus to humans.

DISCUSSION

Since the dawn of time, humans knew that the changing ecosystem exposes them to becoming sick. Today, traveling to regions of the world where hygienic conditions remain inadequate (lack of drinkable water, lack of sewerage), and touching wildlife with a special attraction for NHP, may still have undesirable consequences, especially that of being contaminated by a foreign pathogen infecting the NHP (the five main routes of pathogen' transmission being aerosol, direct contact, fomite, oral and vector). Conversely, there is also a risk of introduction of new infectious pathogens in the visited ecosystem. The "One Health" concept recognizes that human and animal health are intimately connected (39). Implementing this concept requires tracking the spread of pathogens from wildlife to humans. Insofar as part of the threat is unknown, it remains important to identify which behaviors increase exposure, how to quickly identify the type of pathogen which has passed the species barrier, how important is the risk to the health of the infected individual and that of the people he/she frequents, and what measures need to be taken. To this end, a public health approach to the problem is required. The risks will be very different depending on whether it involves bushmeat, contacts with NHP in laboratory, or NHP living in their natural ecosystem. The risk will also vary depending on the frequency of contact, the time spent in close proximity with NHP, the prevalence of the microorganism in the NHP population, the route of transmission (direct or indirect), the ability of hosts to transmit the pathogen, the time of incubation, the number of secondary infections produced in a completely susceptible population by an infected individual—known as R_0 (basic reproduction ratio: for a pathogen to invade and spread, R_0 must be >1)—(200, 201). Unfortunately, emergence of a new pathogen in the human ecosystem is impossible to predict (202, 203), and there is no guarantee of quick identification (e.g., HIV was discovered decades after its introduction and spread in the human population). Over the past decade, EID have increased, prompting the need for faster outbreak detection, monitoring, early warning, reports and intervention (74).

As for hunting, butchering, and consumption of NHP, serious health crises are very rare even if there are examples of major EID such as HIV or Ebola virus (2, 196). There is still no vaccine against HIV while the results for Ebola vaccine trials are encouraging (204, 205). An Ebola vaccine should help to prevent the spread of disease in countries where the epidemic is rife (206). Due to inadequate hygiene conditions (lack of drinkable water, lack of sewerage), bacterial, viral and parasitic intestinal infections are common, but they are rarely serious and most of them can be treated fairly easily. However, this can become a serious medical problem if the infected individual is sick in a rural area far away from any hospital. Regarding NHP caged in zoos, primate centers, and laboratories, the pathogens can be transmitted by scratches, bites, percutaneous inoculation, or contact with body fluids. In these working environments, (i) professionals have a good knowledge of the risks; (ii) the risk is limited because animals are subject to pre-import surveillance and post-import quarantine (e.g., in Europe Council Directive 92/65/EEC of 13 July 1992 laying down animal health requirements governing trade in and imports) (207); (iii) the workers adopt preventives measures (e.g., vaccine), and laboratory biosafety equipment with protective masks, glasses, gloves (208); and, (iv) prophylaxis actions are rapidly set up after an incident. In these workplaces, the pathogen is easy to identify because: (i) NHP are caged; (ii) the natural history of the animal involved in the incident is known; (iii) all NHP have regular veterinary and serological monitoring; (iv) the animal can be placed in quarantine and be subject to enhanced biological and veterinary surveillance. However, cases

of accidental transmission of Marburg virus and Cercopithecine herpesvirus to laboratory staff should not be forgotten (179, 192). These accidents should serve as examples to strictly apply the precautionary principle in laboratories. Another source of worry comes from *in situ* NHP recovery centers, such as the Pan African sanctuary Alliance (209) in Africa or Wildlife Alliance in Asia (210), where there exist primate nurseries attended daily by workers and volunteers who come into very close contact with the animals to save them but also share microorganisms. It could become a potential public health problem and a conservation problem when trying to reintroduce these animals in a wild ecosystem. What remains the most difficult biohazard threat to assess is associated with the illegal detention of NHP as pets and tourists contact with NHP during trips (211). When an incident involves a wild NHP, it is frequently difficult to know the species and natural history of the NHP and the pathogens borne by this wild animal.

Whatever their destination, travelers are frequently victims of health problems because they are foreigners to the visited ecosystems. The ill rate of travelers varies from 15 to 70% according to the destinations, the conditions of stay and the epidemiological survey carried out. Diarrhea—mainly associated with bacteria or virus infections with a preponderance of bacterial infections—is still the most common undesirable incident encountered when traveling abroad (212, 213). It is followed by upper respiratory diseases, dermatitis and fever. Beside these common disorders, the threat might change in nature as more travelers end up moving into area where wildlife is present. Coming into contact with wildlife increases the risks of meeting pathogens whose presence was limited to weakly anthropized ecosystems. On some tourist sites in Thailand, Indonesia, India or Bali, it is not rare (incidence about 1/1,000) to be bitten by an NHP during feeding of the animals or when tourists refuse to give them food (214). As described in this review, when humans got into contact with NHP they could also come into contact with known pathogens such as *C. tetani*, rabies, Herpes B, monkeypox, Marburg, or Ebola viruses, and other pathogens—known or so far unknown—which could pass the species barriers. Rabies is a small part of the problem since high-risk travelers are usually vaccinated (215). On the other hand, there is no vaccine for most pathogens present in the NHP to which these tourists could be exposed. If we take the example of the Cercopithecine herpesvirus which can cause a potentially fatal meningoencephalitis in humans (case fatality rate above 50%), the review of the scientific literature indicates that the virus is widespread in wild NHP groups and those living in freedom on tourist sites (prevalence of 60 to 90% in adult macaques depending the NHP group studied). Although hundreds of thousands of tourists come annually into contact with these infected NHP, there is so far a lack of evidence of Cercopithecine herpesvirus infections among travelers. Yet, several serious cases have been reported in primate center research workers. A single case of human-to-human transmission of Cercopithecine herpesvirus was reported in a woman who became infected after applying hydrocortisone cream to her husband's Cercopithecine herpesvirus skin lesions (216). Recently, genomic sequence variations between Cercopithecine herpesvirus isolated from different macaque species have been reported confirming the existence of different genotypes of Cercopithecine herpesvirus (217). This might suggest that some genotypes of this Herpesvirus might be more suitable than others to cross the species barrier. There has also been no report of serious case in the population of people living in close proximity with NHP and it was claimed that monkey temple Thai workers had developed a protective immune response (not scientifically demonstrated) against the Cercopithecine herpesvirus. What this example tells us is that, despite knowing the threat, no current model can predict the probability of transferring Cercopithecine herpesvirus infection to tourists after an incident involving a NHP. The situation is totally different in Central Africa with the monkeypox virus threat. The seroprevalence of MPXV ranges between 5 and 10% in several NHP groups. Humans can be infected by MPXV and develop a Flu syndrome with a case fatality rate up to 10%. Once transmitted to humans, the virus is very contagious and person-to-person transmission of MPXV occurs through respiratory droplets or body fluids leading to larger outbreaks in human populations. However, there is evidence suggesting that without repeated zoonotic introductions of the virus, human infections would eventually cease to occur (218). In both cases discussed above, the threat is known and it is possible to take preventive measures or to promptly set up therapy after infection of an individual. Of course, it's even worse if we do not know the nature of the threat (unknown pathogen) and if a human undergoes a long incubation period during which the infectious agent is present, but it is not yet causing clinical signs. Travelers should endorse responsibility for taking protective measures aimed at reducing exposure to pathogens. They should follow strict hygiene protocols, including the appropriate vaccination, maintenance of distance with NHP, and not feeding wild NHP (219). It can't be ascertained that travelers are always aware of the biohazard risks. There is therefore a need for more information to travelers via public health professionals, national authorities, and media. In addition, proactive approaches to surveillance, health assessment and monitoring of NHP populations, should be encouraged.

Professionals in charge of travel medicine know perfectly that they should recommend standard vaccination (including tetanus, rabies) according to National Advisory Committees, and the greatest caution to those who wish to meet NHP in their natural environment (220). Pre- and post-travel clinical surveillance is strongly recommended. Even in the absence of animal scratches or bites, travelers/ecotourists should be encouraged to self-screen clinical signs following any meeting with NHP. Tetanus is a preventable disease that is declining worldwide due to vaccination, but surveillance is still required. Before a stay in an area known as high-risk for rabies, preventive vaccine (pre-exposure) may be recommended. In all cases of scratches and bites by NHP, medical consultation is needed. If it is assumed that it is not possible to predict which pathogen could be transmitted to humans during an incident involving a NHP, emergency physicians and medical professionals not familiar with the field of primatology must adopt an attitude based on the precautionary principle (221). In cases of suspected or proven exposures, post-exposure prophylaxis (PEP) with anti-rabies

immunoglobulins (not always available on site) should be started. Pre-exposure rabies vaccine exempts of PEP. In case of superficial NHP scratches, patients often underestimate the seriousness of injuries. Wounds should be cleaned immediately by a 15 min deep irrigation with soapy water, and when possible by saline or antiseptic solution (e.g., chlorhexidine gluconate or povidone-iodine/betadine) to remove foreign bodies and pathogens. The injury may affect different layers of skin. Ischemic lesions promote microbial proliferation. Patients can be divided into low- and high-risk groups depending on the location and importance (superficial or severe) of the injury and the medical state (if known) of the animal that caused the injury. After adequate cleansing, evaluation of the risk of pathogen transmission (the patient's vaccine statute against tetanus and rabies should be questioned), examination, assessment of health status and investigation of any unusual symptom of the offending animal is required (when possible). Blood samples from the NHP and the victim should be collected and immediately sent for serological testing (a rapid transport time of the samples is critical; adequate information should be given to the laboratory for the research of unusual pathogens). In addition, buccal and conjunctival swabs from NHP should be used for culture and rapid PCR-identification of pathogens. The culture of pathogens classified BSL-3 or BSL-4 (for biosafety level), requires specialized facilities (e.g., herpes B virus that is of major concern with NHP bite, is classified BSL-4) (222). The victim should be directed to an emergency medical service where he/she should be considered for immunoprophylaxis and broad coverage antibiotic treatment against NHP's bacteria (such as Amoxicillin clavulanate and moxifloxicin or fluoroquinolone and metronidazole) (172). To prevent viral infections, initiating PEP with an antiviral drug such as valacyclovir (1g by mouth every 8 h for 14 days), or acyclovir (800 mg by mouth five times daily for 14 days or 5 mg/kg/8 h intraveinously for 3 days) and anti-rabies prophylaxis (20 IU/kg infiltrate around the wound and any remaining amount intramuscularly), may be needed (180, 181). Parenteral ganciclovir (5 mg/kg intravenously every 12 h for 2 days) is reserved for treatment of infection with central nervous system symptoms.

Post-exposure clinical survey of the patient is necessary to identify possible signs of illness (such as fever, pain, or shock). If there is evidence for a new pathogen, warning signal are needed for early detection and control of new infectious disease, and biosurveillance of humans and NHP in the area of emergence should be established to determine its evolutionary potential, its impact on health and the ability of leaders and stakeholders to control the phenomenon. The most serious risk for public health is a deadly pathogen able to spread through human-to-human transmission with high $R_{0,}$ or a deadly pathogen transmitted from NHP to humans via a flying blood-sucking vector insect.

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CD, OM, HM, and DR conceived the paper. CD wrote the paper.

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Assessing the Adoption of Recommended Standards, Novel Approaches, and Best Practices for Animal Health Surveillance by Decision Makers in Europe

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Animal health surveillance is an important tool for disease mitigation and helps to promote animal health and welfare, protect human health, support efficient animal production, and enable trade. This study aimed to assess adoption of recommended standards and best practice for surveillance (including risk-based approaches) in Europe. It included scoping interviews with surveillance experts in Denmark, the Netherlands, Norway, and Switzerland to gather information on knowledge acquisition, decisions and implementation of surveillance, and perceptions. This was followed by an online survey among animal health and food safety surveillance users in EU, EEA, and Schengen countries. A total of 166 responses were collected from 27 countries; 111 were eligible for analysis. A strong preference for legislation and established standards was observed, with peer-reviewed publications, conferences, symposia, and workshops to be major sources of information. The majority of respondents indicated a need for international evaluation for surveillance and implied that considerations of cost-effectiveness were essential when making a decision to adopt new surveillance standards. However, most of the respondents did not use a formal evaluation to inform the adoption of new standards or only conducted a descriptive assessment before their implementation or adaptation. Only a few respondents reported a quantitative economic evaluation despite economic efficiency being considered as a highly relevant criterion for surveillance implementation. Constraints mentioned in the adoption of new surveillance standards included insufficient time, financial and human resources, and lack of competency. Researchers aiming to achieve impact by their surveillance work are advised to consider ways of influencing binding standards and to disseminate their work pro-actively using varied channels of engagement tailored to relevant target audiences and their needs. Generally, a more

formal linkage between surveillance information and disease mitigation decisions—for example, by using systematic evaluation—could help increase the economic value of surveillance efforts. Finally, a collaborative, international platform for exchange and learning on surveillance as well as co-design and dissemination of surveillance standards is recommended.

Keywords: animal health, surveillance, standards, evaluation, disease control

INTRODUCTION

The current European Union (EU) Animal Health Law provides enhanced opportunities to apply alternative surveillance approaches achieving comparable levels of evidence. This allows increasing economic efficiency and effectiveness of surveillance while taking into account local practices and farming conditions. It requires a shift in the design of surveillance systems toward output-based (what has to be achieved) rather than input-based approaches (which activities must be undertaken) (1). According to Article 27 of the EU Animal Health Law (2), prevention and control measures for transmissible animal diseases should be disease-specific, taking into account disease epidemiology and associated risks, as well as characteristics of the target population. The Animal Health Law also encourages the application of risk-based approaches for surveillance. Risk-based surveillance was defined by Hoinville et al. (3) as: "making use of information about the probability of occurrence and the magnitude of the biological or economical consequence of health hazards to plan, design, and/or interpret the results obtained from surveillance systems." It seems, however, that the benefits of risk-based surveillance are not (yet) fully exploited by all beneficiaries of surveillance. A study carried out by the European Union's Seventh Framework Programme funded project Risk-Based Animal Health Surveillance Systems (RISKSUR)[1] showed that within the 11 EU Member States and Switzerland surveyed in 2011, slightly more than half of the surveillance components used risk-based sampling (4).

Surveillance systems are usually designed following recommended standards, i.e., guidelines issued by an authority (e.g., World Organization for Animal Health, OIE; Food and Agriculture Organization of the United Nations, FAO; World Health Organization, WHO; Codex Alimentarius Commission) or by general consent ensuring that processes and/or outputs are consistent and fit for purpose. Standards issued by international bodies like the Tripartite institutions (OIE, FAO, and WHO) are often used as international references. In principle they are not legally binding, unless they have been included in a country's national legislation (5). Also, if a country is member of the World Trade Organization, these standards are referenced in the agreement on sanitary and phytosanitary measures and can become relevant in a trade dispute. International guidelines or standards are being developed and regularly updated by the issuing organizations, such as the Tripartite institutions, in a transparent and responsive procedure. For the OIE, for example, all 182 OIE Member Countries are encouraged to contribute (6). It then takes two years for the adoption of new texts in the OIE codes, during which time the texts are submitted and then circulated to the Member Countries several times. However, in case of emergencies, standards may be developed in a shorter period and once adopted by the Assembly, they are then being circulated to the Member Countries. Some standards recommended by international authorities and/or elaborated by the EU Commission are translated into directly binding legislation relevant to a country.

Communication and knowledge transfer between the different actors involved (such as academia, livestock industry, and policy makers) in the design and implementation of surveillance systems are key and thus need to be enhanced by ensuring that the latest up-to-date standards are being applied (7). In an optimal situation, a standard should be cost-effective, feasible to implement, and robust (8), as well as politically, economically, and socially acceptable to decision makers and stakeholders. In reality, this is not always achieved because of political reasoning.

Standards set by public authorities are usually referred to as technical regulations and they are mandatory in many cases. Private standards are generally voluntary (9, 10) and developed by a non-government entity (9). In practice, private standards become de facto mandatory where compliance is required for entry into certain markets. The number of private standards and their influence on trade has risen steadily, a trend which is foreseen to continue. In the field of veterinary public health, the requirements of different stakeholders in the food chain for safe food and high welfare standards have been at the forefront of this development with retailers and other private institutions increasingly determining decisions regarding public health risks and other impacts (10–12).

To set up cost-effective risk-based surveillance systems, best practices should be applied (8). These have been defined by the RISKSUR consortium as: "working practices that are good examples using state-of-the-art methods and approaches under real-life conditions." RISKSUR developed approaches and tools for the design and evaluation of surveillance and promoted them using publicly available educational materials as well as a best practice document. The evaluation tool (13) provided guidance on how to evaluate functional, performance, and value attributes in relation to surveillance, including least-cost analysis, cost-effectiveness analysis, and cost-benefit analysis.

While it is important to use an agreed and common terminology, standards should also be flexible so that they can be adapted to accommodate local (e.g., national) needs as well as new hazards. If an international standard cannot be implemented

[1]https://www.fp7-risksur.eu/

at national level this might constitute a barrier to effective surveillance (14). Inconsistent implementation of international guidelines in countries can weaken international disease reporting and response to health risks. Moreover, comparisons of health status between countries may be hampered by large variations in implementation of surveillance and monitoring, as for example shown with regards to the monitoring of antimicrobial residues in meat in the Netherlands, Denmark, and Switzerland (15). Another barrier to implementation of standards is that data are not always available, accessible or easy to obtain. In aquaculture, for example, it is difficult to progress in the design of risk-based surveillance, due to lack, non-availability, or paucity of data (16).

Being aware of these challenges and barriers, the follow-up project to RISKSUR called risk-based surveillance for animal health in Europe (SANTERO)[2], aimed to promote the enhancement of risk-based surveillance methods suitable for implementation across industries and countries in Europe as well as their dissemination and integration into existing surveillance routines. However, the development of surveillance methodologies is of limited value if not adopted by the target users. Hence, the aim of this study was to gather information regarding the adoption and use of recommended surveillance standards, novel approaches and best practices across EU, European Economic Area (EEA), and Schengen countries from decision-makers for surveillance and/or their technical advisors, and/or technically competent users or data analysts who design, implement, or assess surveillance. Specific objectives were to identify drivers and constraints to uptake of surveillance standards and to identify preconditions required to achieve changes in surveillance policy in EU, EEA, and Schengen countries.

METHODS

A two-step approach was used: key informant interviews (Step 1) were conducted in a scoping study to inform the design of a questionnaire-based, online survey (Step 2). Standards were defined as "something considered by an authority or by general consent as an approved model or quality that can serve as a basis of comparison across countries" (17). It could include OIE standards (e.g., OIE surveillance guide), private standards and industry guidelines (e.g., private surveillance and monitoring in the pig industry to conform with an international private standard), best practice recommendations (e.g., RISKSUR document), EU regulation (e.g., disease notification rules), and national regulation (e.g., enhanced passive surveillance rules for the UK).

Key Informant Interviews

SANTERO collaborators conducted 12 key informant interviews in their countries, namely Switzerland ($n = 3$), Denmark ($n = 5$), the Netherlands ($n = 2$), and Norway ($n = 2$) with target interviewees being aquatic, livestock, and food safety decision-makers for surveillance and/or their technical advisors and/or technically competent users who design, implement, or evaluate surveillance. The aim of these key informant interviews was to gain an overview on the surveillance information context in those countries, the use of existing standards/guidelines, what influences the participants' actions and decisions and potential reasons for use or non-use. The interview guide included questions on three thematic areas: (1) how and where people acquire surveillance information and knowledge; (2) factors that influence the decisions to adopt surveillance standards; and (3) perceptions of implementation of standards in their institution. The full question guide can be found in **Supplementary File 1**. Each interviewer conducted the interview in the language of their choice and provided the answers to each question in the form of written notes in English for each interview conducted. Once all key informant interviews were complete, an interpretive summary was generated, i.e., an active interpretation of the answers given with the aim to inform the development of the survey.

Online Survey

Next, the resulting information was used to design an online survey in English (**Supplementary File 2**) that was directed at decision makers for surveillance and/or their technical advisors and/or technically competent users or data analysts who design, implement, or assess surveillance across EU, EEA, or Schengen countries. The survey included questions on respondents' characteristics (in particular their role in surveillance), the use and relevance of existing standards for animal health surveillance, procedures for data, information sharing and learning (both formal and informal), drivers and hindering factors for the adoption of new surveillance standards and evaluation of surveillance. The survey was pilot tested among collaborators in the consortium and then circulated widely online and by email using the SANTERO website, the RISKSUR newsletter and professional networks of all collaborators. The survey was open from May to July 2017. As an incentive for participation, respondents were given the opportunity to enter their names for a draw of three Amazon gift vouchers. Survey responses were monitored by the authors and where low participation was observed, the collaborators engaged their professional networks by direct contact thereby encouraging survey uptake and participation in a targeted manner. Responses were considered if a respondent answered about half of the questionnaire, i.e., all questions up to (and including) the use of existing tools (apart from the tools for aquaculture which were deemed to be more specialized). Descriptive statistics were conducted using IBM SPSS statistics 24 and Microsoft Excel. Open-ended questions (e.g., further explanations given or suggestions made) were coded manually by theme or topic and summarized in an interpretative way. Direct quotes were included where deemed appropriate from respondents that had given permission to use quotes.

Ethics

Ethical approval for the scoping interviews and the survey was requested and granted from the Royal Veterinary College, United Kingdom; approval number "URN SR2017-1049."

[2]https://santero.fp7-risksur.eu/

RESULTS

Scoping Interviews With Surveillance Stakeholders

Twelve interviews were conducted in the Netherlands, Denmark, Switzerland, and Norway with government representatives for food safety (3), government and private sector representatives for animal health including fish (4), consultants to government in animal health or food safety (2), veterinary advisors to government (3). They were all involved in the planning, design, implementation, and/or evaluation of terrestrial, aquatic or food safety surveillance in their countries.

Information and Knowledge Acquisition

Interviewees reported keeping up to date on surveillance standards and acquiring information about surveillance developments in several ways. All respondents reported using sources of literature, either peer-reviewed or official material about standards and guidelines produced by OIE, EU, EFSA, or national bodies to different extents. Requesting information from national disease experts was also described. The RISKSUR best practice guide (8) was known by three interviewees; two of them knew it because they had been involved in RISKSUR and one discovered it through course attendance. All respondents explained that they gained knowledge in their professional networks through: colleagues in formal international and national meetings or working groups; informal exchange during interactions with people at work, in projects, or at conferences; (formal) professional advice from experts on disease, in academia and industry; or as attendants at courses, workshops, or conferences.

Respondents in Denmark appeared to rely mainly on their institutions for data, information, and guidance, and explained that for current surveillance of food safety, their system and data produced (based on a combination of surveillance and disease experts) allowed them to be ahead of the classic sources of surveillance information such as EU and OIE guidance and Codex Alimentarius.

Opinions about the amount and ideal data differed among respondents. Some respondents were content with the quality, suitability and amount of data, while one person observed that "*more data is always desirable*." One informant criticized the information flows and availability, observing that final outputs and results are often not circulated despite being involved in projects and discussions. One interviewee observed that the most informative activity was the development of a new approach, while another one explained that surveillance tools are often not user-friendly and cannot be applied in official places with strict firewall protection. One interviewee emphasized that a platform for information exchange (and learning purposes) should be created, because only successful stories are widely spread, while failures, problems, or negative experiences are only known by small networks.

Decisions and Implementation

The use of surveillance standards and best practice guidance varied widely across countries and species. Interviewees from Denmark explained that surveillance was so entrenched in the system that the use of standards was not something noticeable or to highlight. Aquatic experts declared using various standards, in particular EU legislation that establishes requirements for surveillance and diagnostics (Council Directive 2006/88), OIE guidelines for diseases not described in EU legislation or for trade with non-EU countries, and national programme standards for notifiable diseases. For surveillance to document freedom from disease in aquatic animals, an informant from Norway had consulted peer-reviewed literature to design a programme with implementation of actions based on Council Directive 2006/88.

In terrestrial animal health, export requirements by third countries were an important driver to go beyond EU legislation and OIE requirements. Technical practicality such as data availability, added value and financial means were further criteria—sometimes with the power to overrule best practice—that influenced the inclusion of new surveillance components in the system. Legislation and cost-effectiveness were regarded as the most common drivers when setting new standards or implementing new surveillance. Reluctance to change appeared to be a strong hindering factor for the adoption of new approaches or implementation of current programmes in two countries (Denmark and the Netherlands), whereas fear of remaining in a programme forever and the associated unpopularity was a strong consideration in Switzerland. Other hindering factors to implementation of best practice and standards were financial and human resources, requirements from third countries, markets, lack of knowledge and uncertainty about the effect of the change.

Decision-making processes for surveillance differed among respondents, but there was a common theme, namely that decisions are often taken in groups, and are of multidisciplinary character bringing together public, private and academic stakeholders. Differences were found in the level of formality spanning the whole spectrum from informal to mixed to formal processes and decision making.

Perceptions

Almost all informants stated to be satisfied with the work of their institution in the use of surveillance standards. However, several criticisms were made, and suggestions put forward for improvement. They included the need for the development and implementation of standardized approaches to promote harmonization across countries and avoid making decisions based solely on factors like political pressure, gut feeling and media influence; implementation of risk-based approaches and improvement of EU regulations in support of the approach; wider uptake of evaluation of surveillance to demonstrate effectiveness, efficiency and best practice across countries; re-consideration of passive surveillance as a valuable approach; exploring and/or enhancing models for private-public-academic partnerships; more efficient use of and access to data (in real-time if feasible); and enhancement of community-based surveillance and engagement for more pro-active information sharing. It was pointed out by several interviewees that standards for the evaluation of surveillance were missing and would be necessary.

Informants attributed differing importance to existing standards with some rating design, implementation, and evaluation as critical, whereas others attributed most importance to prioritization.

Finally, most interviewees perceived a high-quality level of outputs produced by the international surveillance community but listed critical needs. These included a need for an umbrella organization to support the international surveillance community; formal evaluation standards; surveillance standardization across countries; involvement of industry partners; and management of influencing factors (e.g., political, consumer concerns, perceptions, and emotions). Moreover, informants perceived a lack of international agreements on what high quality, fit-for-purpose surveillance constitutes. They acknowledged the existence of a wide range of contexts with differing infrastructure, capacity, political factors, consumer demands, among others, and explained that there would be many different opinions on what good quality would constitute. It was suggested that context-specific barriers to implementation of new standards should be investigated at the country level to pave the way for effective implementation.

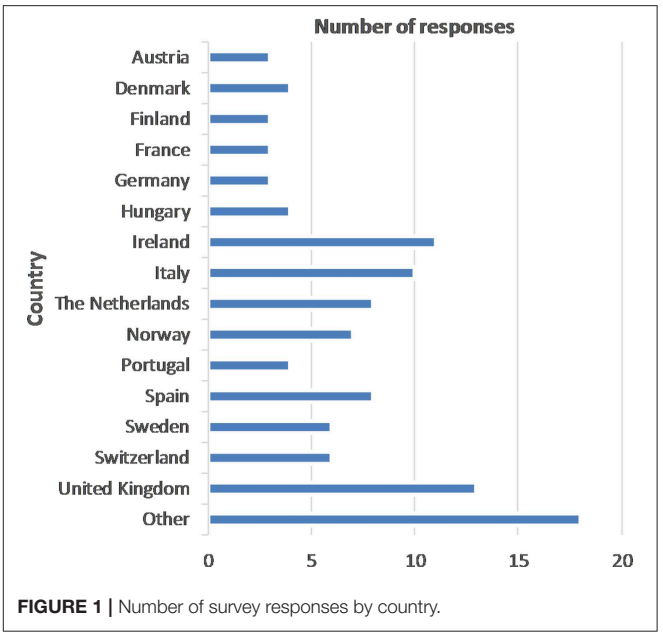

FIGURE 1 | Number of survey responses by country.

Survey

Response Rate and Respondent Characteristics

A total of 166 people started the survey, of which four did not give consent, 30 gave consent without providing any answers, 36 completed the survey partially and 96 completed the survey in full. After consideration of the inclusion threshold, 111 responses were analyzed.

The number of responses by country are presented in **Figure 1**. The "other" in **Figure 1** includes one or two responses each received from Belgium, Bulgaria, Croatia, Czech Republic, Greece, Lithuania, Luxembourg, Malta, Poland, Romania, Slovakia. A total of 58% of respondents were from the public sector, 17% from academia, 11% from research institutes, 6% from the private sector, 4% from non-government organizations, 3% from other organizations, and 2% from small or medium sized enterprises. A total of 28% of respondents were senior researchers, 23% each from middle and upper management, 9% trained professionals, 5% each administrative staff and junior researchers, 3% junior management, and the remaining respondents (<2% each) were self-employed/partner, student, temporary employee, policy advisor, and technical officer. Surveillance responsibilities of respondents included (with a few exceptions) multiple activities with analysis of surveillance data mentioned 80 times out of 483, development of surveillance design 33/483, communication of surveillance information to decision-makers 68/483, implementation of surveillance 53/483, assessment of surveillance system performance 48/483, development of new methods for surveillance designs 45/483, decisions on whether to run a surveillance component or programme and development of new methods for surveillance evaluation 33/483 each, assessment of surveillance system value/economic efficiency 32/483, decision on resource allocation for surveillance 17/483, and other (e.g., policy advisor, methods for fraud detection, diagnostics) 9/483. With regards to species focus, 42% of respondents stated that their roles were general and not species focused. For the other respondents, multiple species were often covered. Most frequently listed were terrestrial livestock (51/134 responses), wildlife (22/134), and fish and molluscs (14/134). Bees, camelids and deer, companion animals, equidae, insect vectors, and other (e.g., humans, food safety) were listed between 6 and 11 times.

With regards to the purpose of surveillance people were in charge of, multiple answers were given by respondents. The most frequently mentioned purposes were to demonstrate absence of disease or infection (73/445), confirm disease status (69/445), identify changes in disease status to facilitate early response (67/445), provide information for assessing and managing risks (64/445), and to assess if intervention measures are efficient (monitor progress, verify success) (46/445). Other surveillance purposes were mentioned 30 times or less. When asked what hazards people were responsible for, 32% of respondents said that their role was general and did not have a specific hazard focus. Among the other respondents, multiple hazards were commonly cited with the most frequent being emerging/re-emerging infectious diseases (54/258), zoonoses (52/258), endemic infectious disease (48/258), exotic infectious disease (40/258), and antimicrobial resistance (28/258). Chemical hazards, antimicrobial use, physical hazards and other (e.g., nanoparticles) were mentioned less frequently.

Use of Existing Surveillance Standards

When asked about the quality and adoption of surveillance standards (**Figure 2**), the majority of respondents predominantly agreed or fully agreed with the statements provided apart from the statement "in our institutions we are aware of surveillance standards, but adoption is limited," where 41 people fully agreed or agreed and 34 totally disagreed or disagreed. A total of 52% of respondents affirmed that national government or industry standards went beyond regional (e.g., EU) or international (e.g.,

OIE) standards; 29% stated that they did not think so and 19% did not know.

Existing standards used by the large majority of respondents were EU legislation (89%), peer-reviewed publications (87%), national legislation (84%), and the OIE codes for terrestrial and/or aquatic species (75%) (**Table 1**). The other standards were used by less than half of the respondents with standards for aquatic species being used by the smallest number of people. The most frequently mentioned purposes for using most standards were planning of surveillance activities and surveillance design. For peer-reviewed literature, the most frequently mentioned purposes were surveillance data analysis and surveillance data interpretation. The OIE codes for terrestrial and aquatic species were also used frequently for diagnostic procedures for surveillance. Private standards and the FAO technical paper "surveillance and zoning for aquatic animal diseases" were frequently mentioned for the purpose of surveillance implementation. Standards were used in the majority of responses "several times a year" (median 52.3%, min 33.3%, max 85.7%). Other standards and resources that were used by respondents were ISO standards; OIE training manuals on surveillance and international reporting of diseases in wild animals; statistical, surveillance and veterinary epidemiology textbooks; "own" standards, i.e., standards adapted for application to One Health for use by the institution; standards from breeders' associations; FSA, WHO, USDA, AECOSAN, and MAPAMA standards; surveillance standards and gray literature from other countries including non-EU countries (e.g., strategic reviews or programme reports of surveillance systems in specific countries).

The relevance of the standards for respondents' surveillance work was mostly deemed very relevant, relevant or moderately relevant; only a minority of respondents stated a slight or no relevance (**Figure 3**).

Information and Data Exchange

When enquiring about procedures for data and information sharing as well as learning (formal and informal) (**Table 2**), the most regularly used international sources were the exchange with international colleagues, international scientific publications, and international conferences or symposia. Sources used less often were international online courses and official communications by private standard setting bodies. For the national sources, most frequently used were exchange with colleagues at the workplace, exchange with national colleagues outside the workplace, and collaboration in national surveillance research or projects. The least frequently used national source were national online courses.

When asked whether they felt sufficiently informed about existing surveillance standards and best practice, 59/107 of respondents affirmed, 30/107 said no, and 18/107 did not know. Among those who said no and gave an explanation of what was missing, reasons cited included issues related to time (*"never enough time to learn about everything that is out there"*), difficulties to have an overview of everything (*"difficult to have an overview, learnt about new ones through this questionnaire"*) and too many sources to use (*"it is difficult to get an overview because of the amount of sources"*). Also, respondents pointed out topics that did not receive enough attention including guidelines for passive surveillance, non-statutory surveillance, common coding and parametric language, and design prevalence to prove freedom of certain diseases. Several suggestions were made on how people could be better informed including the coordination of sources, i.e., creation of a network, mailing list, website, forum, or regular gathering with information about surveillance standards, face-to-face or online training, or using national reference laboratories or an international bulletin for dissemination of information.

When asked whether they received information on new surveillance standards and best practice in a timely manner, 49/107 respondents said yes, 35/107 said no, and 23/107 did not know. Barriers mentioned included a lack of coordination; absence of a central (single) platform, group, association or mechanism with a (formal or coordinated) procedure for critical review and regular dissemination (e.g., with newsletters or other forms of notification), and a lack of open source data or

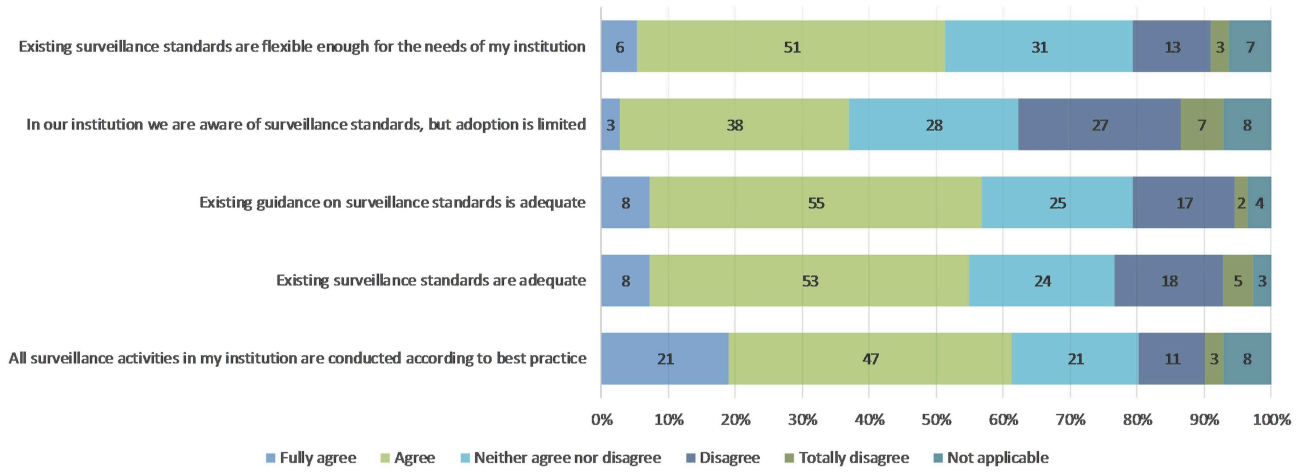

FIGURE 2 | Respondents' answers to statements on quality and adoption of surveillance standards, $n = 111$.

TABLE 1 | Surveillance standards used by respondents and surveillance purpose the standards were used for.

	Standards used			Surveillance purpose standard was used for (multiple answers possible)											
	Yes	**No**	**No answer**	**Prioritization of hazards for surv.**	**Planning of surv. activities**	**Surv. design**	**Surv. implementation**	**Diagnostic procedures for surv.**	**Surv. data analysis**	**Surv. data interpretation**	**Communication /reporting of surveillance findings**	**Evaluation of surveillance**	**Requirements for certification or accreditation**	**Other**	**Total**
EU legislation	99 (89%)	12 (11%)	0	34 (8.8%)	59 (15.2%)	54 (14%)	39 (10.1%)	41 (10.6%)	23 (5.9%)	27 (7%)	41 (10.6%)	28 (7.2%)	35 (9%)	6 (1.6%)	387
Peer-reviewed publications	97 (87%)	14 (13%)	0	33 (7.6%)	53 (12.2%)	64 (14.7%)	39 (8.9%)	49 (11.2%)	59 (13.5%)	62 (14.2%)	33 (7.6%)	40 (9.2%)	4 (0.9%)	0 (0%)	436
National legislation	93 (84%)	18 (16%)	0	26 (7.1%)	50 (13.7 %)	46 (12.6%)	40 (11%)	37 (10.1%)	28 (7.7%)	33 (9%)	43 (11.8%)	27 (7.4%)	29 (7.9%)	6 (1.6%)	365
OIE codes for terrestrial and/or aquatic species	83 (75%)	28 (25%)	0	19 (6.6%)	34 (11.8%)	48 (16.6%)	21 (7.3%)	46 (15.9%)	21 (7.3%)	23 (8%)	26 (9%)	24 (8.3%)	25 (8.7%)	2 (0.7%)	289
OIE Guide to Terrestrial Animal Health Surveillance	47 (42%)	64 (58%)	0	12 (8%)	18 (12%)	23 (15.3%)	14 (9.3%)	17 (11.3%)	15 (10%)	12 (8%)	15 (10%)	11 (7.3%)	12 (8%)	1 (0.7%)	150
Private standards	31 (28%)	80 (72%)	0	10 (8.8%)	15 (13.2%)	13 (11.4%)	14 (12.3%)	11 (9.6%)	8 (7%)	14 (12.3%)	11 (9.6%)	7 (6.1%)	8 (7%)	3 (2.6%)	114
Codex Alimentarius	31 (28%)	80 (72%)	0	12 (12%)	15 (15%)	9 (9%)	6 (6%)	7 (7%)	10 (10%)	13 (13%)	10 (10%)	9 (9%)	6 (6%)	3 (3%)	100
FAO risk-based disease surveillance manual	30 (27%)	81 (73%)	0	10 (9.5%)	17 (16.2%)	24 (22.9%)	9 (8.6%)	7 (6.7%)	9 (8.6%)	7 (6.7%)	10 (9.5%)	8 (7.6%)	4 (3.8%)	0 (0%)	105
RISKSUR best practice document	21 (19%)	90 (81%)	0	7 (8.4%)	14 (16.9%)	17 (20.5%)	8 (9.6%)	4 (4.8%)	10 (12%)	7 (8.4%)	5 (6%)	11 (13.3%)	0 (0%)	0 (0%)	83
Book "Epidemiological surveillance in animal health"	14 (13%)	97 (87%)	0	4 (9.1%)	5 (11.4%)	10 (22.7%)	4 (9.1%)	4 (9.1%)	5 (11.4%)	4 (9.1%)	3 (6.8%)	4 (9.1%)	0 (0%)	1 (2.3%)	44
OIE Guide for Aquatic Animal Health surveillance	7 (6%)	90 (81%)	14 (12%)	2 (6.1%)	6 (18.2%)	6 (18.2%)	3 (9.1%)	3 (9.1%)	2 (6.1%)	1 (3%)	3 (9.1%)	4 (12.1%)	3 (9.1%)	0 (0%)	33
Survey toolbox for aquatic animal diseases: a practical manual and software package	5 (5%)	92 (83%)	14 (12%)	0 (0%)	1 (5.6%)	5 (27.8%)	2 (11.1%)	2 (11.1%)	3 (16.7%)	3 (16.7%)	1 (5.6%)	1 (5.6%)	0 (0%)	0 (0%)	18
FAO technical paper "surveillance and zoning for aquatic animal diseases"	4 (4%)	93 (84%)	14 (12%)	2 (13.3%)	3 (20%)	2 (13.3%)	3 (20%)	2 (13.3%)	2 (13.3%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	15

Surv, surveillance.

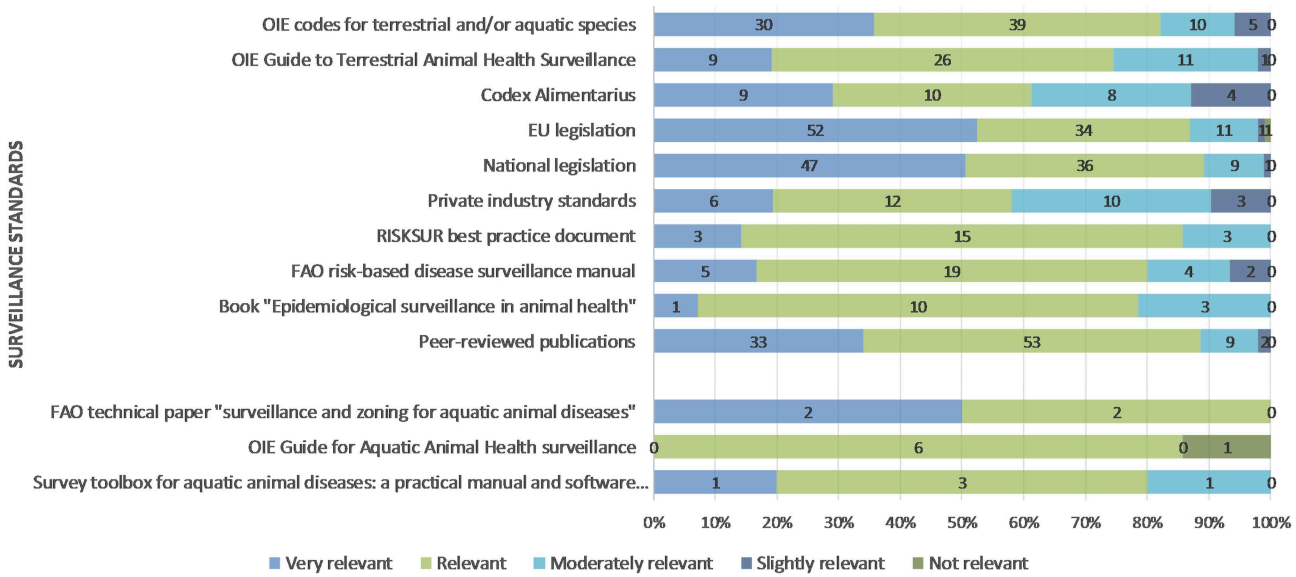

FIGURE 3 | Relevance of standards for the respondents' surveillance work.

information. Several respondents described difficulties related to having to search actively for information, the number of sources to consult, the time required to do so, and the challenge to decide what information to consider. For example: *"There are too many different web pages for standards and lot of standards. It is time consuming to study every single document and to decide to follow it or not."* A suggestion made by several respondents to tackle these challenges included the creation of a central repository or platform (termed by one respondent as "*knowledge bank*") including information on the quality and applicability of the different standards and an information dissemination mechanism with the possibility to subscribe for regular updates (e.g., email list, social media groupings). Other suggestions included the provision of practical training sessions, more regular exchange between different stakeholders, generation of applied examples/case studies, and elaboration of coding standards for surveillance. A few respondents stated that existing dissemination channels (e.g., EUR-Lex) were effective and that they did not have a need for improvement.

Evaluation of Surveillance, Drivers, and Hindering Factors

A total of 75/99 of respondents said yes to the question whether there was a need for international evaluation standards for surveillance; 7/99 said no and 17/99 did not know. When asked to explain why they had given this answer, the most frequent answer (given by 41 people) related to the need for standardization, harmonization, and comparison of evaluation outcomes across countries (e.g., *"To enable comparison and help understand surveillance results from other countries"* or *"To be able to compare surveillance performance and efficiency across countries"*). Five people stated that the evaluation standards in place and evaluations conducted were already good enough (e.g., *"there is already enough guidance for evaluation of surveillance"*). Six people described the importance for risk mitigation and achievement of health outcomes such as animal and public health and food safety (e.g., *"These standards are necessary to control animal health and food safety world wide"*). Four people pointed out the importance of learning from other countries' or people's experiences (e.g., *"I think it would be helpful as a way to learn from others; if we have the same task, how do we solve it in our own context?"*). Evidence for trade partners and the ability to enable trade was mentioned three times. Other (single) responses included benchmarking, evaluation of national standards, and improvement of capacity and expertize. One person stated that standards were too general for varied contexts: *"Most of the time standards are to (sic) general to be useful in specific situations. What is needed is deep teoretical (sic) knowledge and experience to approaches."*

When asked about who should be in charge of developing evaluation standards for surveillance, OIE (64/237), the scientific community (55/237), and the EU (53/237) were selected most often, followed by the FAO (24/237). Several respondents also suggested other possibilities including a combination of multiple institutions and people (e.g., *"all the main stakeholders"*), national authorities, and the WHO. For the question "In your opinion, what are the three principal subject matters that such surveillance evaluation standards should cover?," a wide range of answers was provided. The single most frequently listed item related to economic efficiency (e.g., cost-effectiveness, cost-efficiency, economics, costs, cost-benefits, economic implications). Also frequently mentioned were the types of hazards and topics to focus on (e.g., zoonoses, antimicrobial resistance, food safety, food fraud, epizootic disease, endemic disease) and surveillance attributes. Among the latter, most often mentioned were effectiveness, sensitivity, timeliness, and representativeness. Several persons suggested standards on methods for different surveillance activities (e.g., sampling or testing procedures) and

TABLE 2 | Respondents' frequency of using national and international sources to learn about new surveillance standards or best practice for surveillance; $n = 107$.

	Very often	Often	Sometimes	Rarely	Never
NATIONAL					
National conference or symposium	9 (8.4%)	31 (29%)	43 (40.2%)	15 (14%)	3 (2.8%)
Scientific national publications	9 (8.4%)	30 (28%)	37 (34.6%)	22 (20.6%)	4 (3.7%)
Lay national publications	9 (8.4%)	17 (15.9%)	27 (25.2%)	26 (24.3%)	20 (18.7%)
Official communications by private standard setting bodies	3 (2.8%)	11 (10.3%)	29 (27.1%)	30 (28%)	24 (22.4%)
Official communications by national public bodies	19 (17.8%)	34 (31.8%)	27 (25.2%)	15 (14%)	7 (6.5%)
National, non-institutional training event	0 (0%)	12 (11.2%)	29 (27.1%)	35 (32.7%)	26 (24.3%)
Institutional training event	4 (3.7%)	21 (19.6%)	39 (36.4%)	20 (18.7%)	18 (16.8%)
Exchange with colleagues at my workplace	38 (35.5%)	44 (41.1%)	16 (15%)	6 (5.6%)	1 (0.9%)
Exchange with national colleagues outside my workplace	20 (18.7%)	41 (38.3%)	30 (28%)	8 (7.5%)	3 (2.8%)
Collaboration in national surveillance research or projects	11 (10.3%)	40 (37.4%)	32 (29.9%)	14 (13.1%)	5 (4.7%)
National online courses	0 (0%)	7 (6.5%)	18 (16.8%)	28 (26.2%)	41 (38.3%)
INTERNATIONAL					
International conference or symposium	7 (6.5%)	29 (27.1%)	48 (44.9%)	20 (18.7%)	2 (1.9%)
International scientific publications	22 (20.6%)	44 (41.1%)	34 (31.8%)	5 (4.7%)	0 (0%)
International lay publications	3 (2.8%)	22 (20.6%)	37 (34.6%)	28 (26.2%)	10 (9.3%)
Official communications by OIE or FAO	5 (4.7%)	23 (21.5%)	41 (38.3%)	23 (21.5%)	9 (8.4%)
Official communications by private standard setting bodies	3 (2.8%)	8 (7.5%)	25 (23.4%)	39 (36.4%)	25 (23.4%)
EU bulletin	7 (6.5%)	17 (15.9%)	29 (27.1%)	30 (28%)	20 (18.7%)
International training event	2 (1.9%)	15 (14%)	40 (37.4%)	29 (27.1%)	18 (16.8%)
Exchange with international colleagues outside my workplace	7 (6.5%)	38 (35.5%)	41 (38.3%)	15 (14%)	3 (2.8%)
Collaboration in international surveillance research or projects	9 (8.4%)	20 (18.7%)	34 (31.8%)	29 (27.1%)	10 (9.3%)
International online courses	0 (0%)	6 (5.6%)	27 (25.2%)	42 (39.3%)	28 (26.2%)

design of surveillance, as well as an agreed conceptualization of the surveillance aim and purpose such as possibility for action, early warning and prevention. Answers focusing more specifically on the evaluation process suggested having guidance and/or an evaluation framework to help focus and design an evaluation (4/80 respondents) including guidance on suitable approaches (1/80), agreed metrics (1/80), minimum requirements (1/80), interpretation of evaluation results (2/80), documentation and reporting (2/80) including evaluation visualization (1/80), and communication (3/80). One person suggested the use of coding language to capture attributes and tiered data engines for analysis.

Asked about how relevant considerations of cost-effectiveness were when making a decision to adopt new surveillance standards, 79/99 deemed them to be absolutely essential or very important, whereas 18/99 said that they were of average importance and 2/99 of little importance. A total of 39/99 respondents stated that they did not conduct a formal evaluation to assess the economic efficiency when a new surveillance standard becomes available. Among those who conducted a formal evaluation, quantitative assessment of costs of the change in surveillance, quantitative assessment of the effectiveness of the change in surveillance (e.g., timeliness, sensitivity, acceptability), quantitative cost-benefit analysis, descriptive assessment of consequences, and descriptive assessment of costs were all used with similar frequency (between 21/141 and 31/141). Only quantitative cost-effectiveness analysis was used less (8/141).

When asked about the availability of resources for the adoption of new surveillance standards in their institutions, respondents indicated that there were largely sufficient or somewhat sufficient resources in terms of learning processes, information exchange, formal guidance on approaoches and methods, epidemiological skills and evaluation knowledge (**Figure 4**). Resources that were largely considered to be insufficient or somewhat insufficient were time, financial, human resource (i.e., labor) and economics skills. The question about rating the availability of resources for the adoption of evaluation standards for surveillance yielded very similar results.

DISCUSSION

The value of enhanced surveillance approaches and setting up risk-based surveillance systems can be realized when adopting best new practices and standards. This study showed that there is a substantial heterogeneity in the use and adoption of recommended surveillance standards, novel approaches and best practices among users from EU, EEA, and Schengen countries. Further, advancing on the findings from the RISKSUR project, it provides insights on the acquisition and use of information and

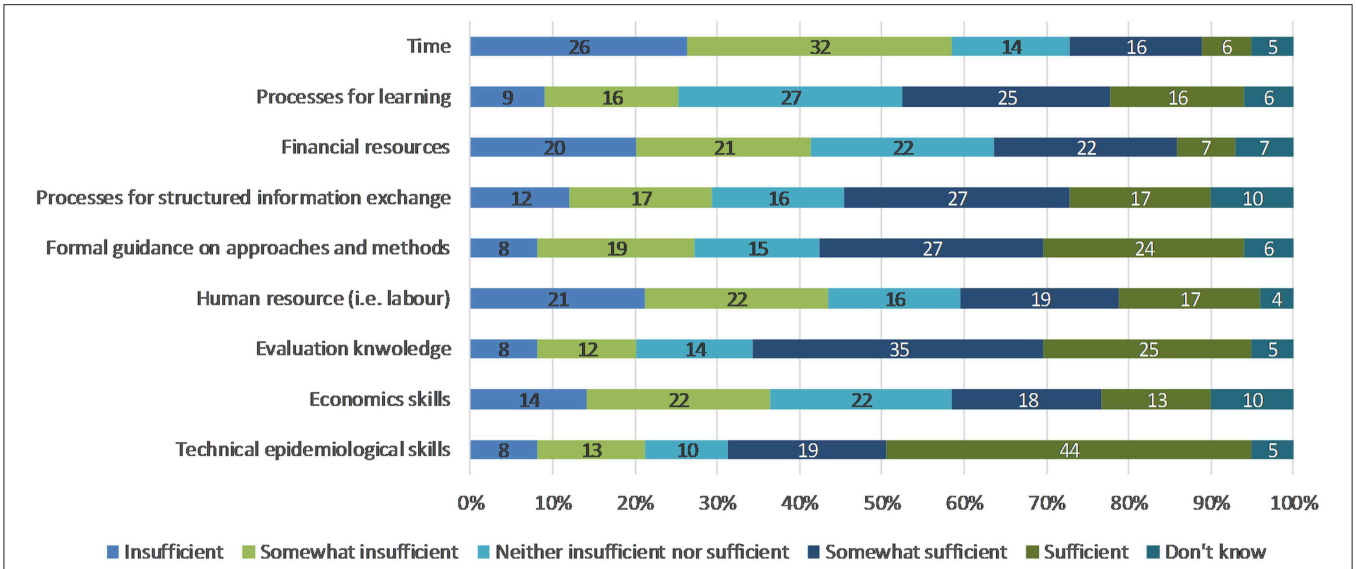

FIGURE 4 | Respondents' rating of the availability of resources for the adoption of new surveillance standards in their institution.

available tools for decision-making, on drivers and barriers for their implementation and considerations for the development of new standards (in particular for evaluation of surveillance).

This study provided a clear picture of the standards most commonly used by respondents with EU and national legislations, peer reviewed publications, and OIE standards found to be core sources of information for most users. The dominant use of EU legislation was to be expected given that the target respondents were from EU, EEA, and Schengen countries. Other available tools including the RISKSUR best practice document were used only by a minority of respondents. An explanation of this may be that the reported information overload among users might be driving the high reported use of certain standards. Information overload can occur when the requirements needed to process (information needed to complete a task) are larger than the capacity available to process the information (18–20) which may lead to arbitrary information analysis (e.g., omission of information and being highly selective) and sub-optimal decision making (20). One possible strategy of being highly selective may be to rely more heavily on standards that are legally binding or of international importance. A wide range of countermeasures for information overload have been described in the literature; e.g., intelligent information management, decision support system, measurement system of information quality, intelligent interfaces, and coordination through inter-linked units (20). The implementation of such measures could be addressed in a coordinated, central platform—as requested by many respondents—with a mechanism for quality control and timely dissemination of information. Such a mechanism would also allow reaching target groups more effectively and thereby stimulate the uptake of new knowledge and innovation. For such a platform to be effective, clear leadership, maintenance as well as continual monitoring and recommendation are needed (21). The development of the platform would start ideally with a wide-reaching consultation to describe in depth the needs and preferences of potential users (21, 22) who may be different in the way they assimilate information or their professional needs (e.g., technical vs. decision-making requirements); this would allow to design a platform that is user-centered (21, 23). Moreover, to increase utility, it should also be open access with easily accessible and interpretable content (23). Because the development, adoption and use of standards is a cyclical process, the platform should also have a function to assimilate information from its users to produce a system where information flows back and forth (21)—essentially a process of co-design and co-production of knowledge with collaboration between stakeholders.

While the external validity of the findings results is limited by the geographical boundaries of the study, some findings may also apply to non-European countries. The findings in this study are generally in line with the outcome of a survey conducted by the OIE prior to its 86th General Session in 2018, as part of a technical item addressing the implementation of its standards. The study, directed at OIE members, showed a high level of support for implementing OIE standards, but identified key challenges including a lack of technical expertise among Member States and pointed toward the need of training to further facilitate their uptake (24). One of the more overarching outcomes of that discussion was the launch of the OIE Observatory project, an implementation monitoring function that will assist the OIE in ensuring that its standards are relevant and fit for purpose, and to develop a more strategic focus to its capacity building activities (25). This is also in line with other developments: The challenge of the "know-do-gap," or bridging the gap between research and implementation, is gaining increased recognition; the WHO has even identified it as one of the most influential contemporary challenges (26). Consequently knowledge utilization or implementation science is gaining momentum to avoid the costs associated with underutilized knowledge. Multiple activities are suggested by

the WHO to promote knowledge translation including various ways to exchange, share, and promote knowledge supported by dynamic learning networks (26).

To avoid duplication of efforts and increase efficiency by using data (and communication channels) that already exist (27), a dissemination, development, learning, and exchange platform as suggested above could be generated in a joint effort by international organizations (e.g., the Tripartite), legislators (e.g., EU commission), implementers (e.g., national government institutions), and researchers. Bringing partners together in this way would promote cumulative knowledge and enhance capacity building (27). Regular coordination meetings, active dissemination of new information including training could be linked to relevant international conferences such as the International Conference on Animal Health Surveillance (ICAHS), the International Symposium of Veterinary Epidemiology and Economics (ISVEE), or the International Society for Economics and Social Sciences of Animal Health (ISESSAH).

While such mechanisms to create more capacity are being designed and/or implemented, people developing new approaches, tools or guidelines may want to reflect on best ways to disseminate knowledge effectively. For effective dissemination of research findings, it has been recommended to consider impact pathways, elaborate a clear description of the target audience, select a range of dissemination channels in line with the target audience, and consider viability and funding issues (21, 27, 28). Respondents in this study acquired information on surveillance standards occasionally to very regularly from scientific and lay publications, conferences, workshops or courses, exchange with colleagues, collaboration, and official communications, i.e., they used a multitude of different channels. Because target users have different preferences for communication channels, it is recommended that researchers elaborate dissemination plans and identify pathways to reach stakeholders (e.g., practitioners, policy makers) via the different media in the short, medium, and long term (21). Importantly, social media in particular, but also other channels, should not just be advocated because they appear to be popular, but be used in a targeted way with careful audience selection, message formatting, and delivery to achieve the desired effects (22). For example, Kapp et al. (29) showed how twitter could be effective in dissemination of information to policy makers following an analysis of target users and their twitter use.

When elaborating such dissemination plans, pathways on how to get standards into legislation or OIE standards (including evaluation standards) may be considered which would necessitate effective collaborations and networking. Also, continued research on the topic can have an impact, as implied by one respondent "*This survey, was actually good to increase the awareness of different sources to search for information. However, the links to the different sources and specific matters might be merged together in one internet page which needs to be regularly updated*." The idea of peer-learning could also be extended to the country level by making descriptions of animal health surveillance activities and their evaluations publicly available. In order to promote a more consistent approach to communication of animal health surveillance activities and their outputs, for the benefit of stakeholders, trade partners, decision makers, and risk assessors, a set of Animal Health SUrveillance guiDelines (AHSURED) have been developed, partly within SANTERO, and partly in an EFSA funded project (HOTLINE). The full AHSURED checklist with detailed item descriptions can be accessed on https://github.com/SVA-SE/AHSURED/wiki.

The survey underlined the importance of using economic evaluation criteria when planning the adoption of new standards, but only a bit more than a third of respondents indicated to conduct a formal financial or economic evaluation. Common constraints mentioned were a lack of human, time and financial resources as well as a shortage of economics skills. Consequently, new evaluation standards for surveillance that include economic evaluation guidance would need to be accompanied by knowledge transfer and capacity building. There is a role for governments to support the implementation of this process by enabling training opportunities, promoting innovation and making resources available within their institutions for capacity building as well as evaluation of surveillance. Moreover, tertiary education systems may want to consider integrating more economics into basic animal health and surveillance training. With enhanced (economic) evaluation capacity and skills, surveillance planners, implementers, and evaluators will have new tools at their disposal to create inventive evaluation and surveillance designs with limited resources.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request from the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Social Science Research Ethical Review Board, Royal Veterinary College. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualization of the study, designed the interview guide, and survey and actively disseminated the survey. LA, ST, ALé, and GS performed the interviews with decision makers. MG and BH analyzed the interviews qualitatively and results from the surveys. BH and BB wrote the first draft of the manuscript. All the authors contributed to reviewing and revising the full manuscript.

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SUPPLEMENTARY MATERIAL

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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.

The reviewer JF declared a past co-authorship with one of the authors KS to the handling editor.

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Application of Mixed Methods to Identify Small Ruminant Disease Priorities in Ethiopia

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Animal health interventions tend to focus on transboundary or zoonotic animal diseases and little attention is given to diseases that mainly affect livestock production and productivity which are of concern for smallholder farmers. To understand disease priorities of men and women livestock keepers and how these impact households, this study used participatory methods to elucidate priorities, reasons for prioritization, knowledge on small ruminant diseases and their transmission pathways. The study was conducted in 23 sites distributed across 14 districts in four regional states of Ethiopia. Ninety-two focus group discussions (FGD) were conducted with men or women only groups. Various tools, such as semi-structured interviews, simple scoring and proportional piling were used to facilitate the process. A follow-up household survey involving 432 households/interviewees collected in-depth data on key small ruminant diseases. Each focus group identified and scored their top five diseases. During analysis, the diseases were grouped in to seven major categories based on local names and clinical signs reported. Highest scores in proportional piling (out of 100 counters) were obtained for respiratory diseases and gastrointestinal parasites in highland areas (mixed crop-livestock systems) with strong agreement among respondent groups using Kendall's coefficient of concordance (W) ($W = 0.395$, $p < 0.01$); whereas in lowland areas (pastoral and agro-pastoral systems), the priorities were respiratory and neurological diseases, also with very strong agreement ($W = 0.995$, $P < 0.01$). There was no significant difference between men and women in prioritizing disease constraints. The reasons for prioritization were also used to define categories of impact of disease. The household survey confirmed disease priorities and highlighted the role of mortality for respiratory diseases. Despite differences in household roles, both men and women unvaryingly described the clinical signs in live animals the same way and reported similar observations of disease in carcasses of slaughtered animals. Overall, both men and women farmers had low awareness of zoonotic diseases. In conclusion, the priorities of national disease control programs do not fully match priorities of farmers. Such participatory tools should therefore, play a pivotal role when designing sustainable livestock health interventions.

Keywords: participatory epidemiology, small ruminant, diseases, gender, smallholders

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INTRODUCTION

In Ethiopia, small ruminants serve multiple livelihood functions by providing food and nutrition, income and raw material for industries. They also serve various important cultural purposes such as wedding gift, charity to poorer relatives and inheritance. Women have a lot of control over small ruminants compared to other livestock species and are closely involved in small ruminant health management. The animals often serve as emergency sources of funds for household obligations and personal use. The increase in demand for small ruminant meat products both locally and internationally presents an opportunity for small ruminant keepers to access better markets. However, this opportunity has not been used because of underperformance of the value chain in part attributed to the inability of producers to supply safe products in required quantities.

Infectious diseases have a huge impact on productivity of smallholder livestock systems and repeatedly come up as major constraints in household surveys in Ethiopia (1). The impact of animal diseases is seen through direct losses due to mortality, cost of treatment and indirect effects through slow growth and low fertility linked to morbidity. Annual mortality ranges from 12 to 14% for sheep and 11 to 13% for goats in Ethiopia (2). Animal health research and development projects and government interventions tend to deal with animal diseases which affect trade, are transboundary in nature, or are zoonotic. Comparably, little work has been done on endemic diseases and their contribution to loss of productivity is poorly documented even though these diseases potentially play an important role in adversely affecting food security and the livelihood of smallholder farmers.

To ensure that views of farmers are understood, veterinarians began using participatory methods in the 1980s, particularly in community-based livestock projects in Africa and Asia. By the late 1990s, there was increasing use of these methods and the term "participatory epidemiology" was commonly used to describe veterinary applications of participatory rural appraisal (PRA)-type approaches and methods. While PRA is a multidisciplinary approach to various development problems in rural communities, participatory epidemiology has increasingly been used by veterinarians with a focus on livestock diseases (3).

Besides participatory qualitative research approaches, use of mixed methods, which involves collecting and analyzing qualitative and quantitative data in a single study, have been also successfully applied in animal health (4–7). These approaches can provide a better understanding of the research problem than either approach alone (8).

This gender sensitive study on disease constraints was conducted using a mixed methods design with the aim of generating evidence to design interventions to address key animal health constraints. The study objectives were to identify the main small ruminant disease constraints as perceived by men and women, the impact of the diseases on households, knowledge of men and women on small ruminant diseases, their respective involvement in disease management and understanding disease transmission pathways. The findings will help to influence the national policy on livestock disease surveillance systems and disease control to ensure that smallholders' problems are addressed.

MATERIALS AND METHODS

Study Areas

The study was conducted in 23 villages across 14 districts in Amhara, Oromia, Tigray and Southern Nations Nationalities People's (SNNP) regions of Ethiopia. Five districts in the Amhara region (Basona Worena, Menz Gera, Menz mama, Abergelle and Ziquala), three districts in the Oromia region (Sinana, Yaballo, and Horro), three districts from SNNP (Lemo, Doyogena and Menjiwo/Adiyo), and three districts in the Tigray region (Endemehoni, Atsbi wonberta, and Tanqua Abergelle) participated.

The agroecology and production system characteristics of the study sites are shown in **Table 1**. Livestock production in Ethiopia is broadly classified into pastoral, agro-pastoral and mixed crop-livestock, peri-urban and urban production systems (9).

The highland agroecology with mixed crop-livestock system is typical for areas above 2,200 m above sea level (masl) and is characterized as a system in which livestock husbandry and rain fed cropping are closely interlinked. Livestock provide inputs (draft power, transport, manure) to other parts of the farm system and generate consumable or saleable outputs (milk, meat, eggs, hides and skins, wool, hair and manure). Crop residues are used as livestock feed; animals can be sold and revenues reinvested in agriculture or sold when the crop is failing because of weather or pests; cereals and most staple foods are produced in quantities that cover the needs of the family and excess is sold. The principal objective of farmers engaged in mixed farming is to gain complementary benefit from an optimum mixture of crop and livestock and spreading income and risks over both crop and livestock production (10).

The lowland agroecology with mixed crop-livestock system denotes elevation $\leq$1,500 masl where farmers herd livestock in rangelands and produce crops on fertile land. The system is understood in dual sense: firstly, it refers to farming systems entirely based on livestock but practiced in proximity to and perhaps functional association with cropping farming systems; secondly, it refers to the livestock subsystem of crop-livestock farming.

The lowland agroecology is typical for the pastoral production system characterized by sparsely populated pastoral rangelands, where subsistence of pastoralists is mainly based on livestock and livestock products. Livestock husbandry in this system is dominated by goats, cattle, sheep, and camels. Since the main source of food is milk, pastoralists tend to keep large herds to ensure sufficient milk supply and generate income by selling dairy products or live animals. The pastoral production system in some areas has been evolving into an agro-pastoral system (9). The agro-pastoral form of livestock production dominates in mid agroecological zones where a tendency for crop production over livestock production has been observed. Agro-pastoralists are sedentary farmers who grow crops and raise livestock. Livestock are used for draft, savings, and milk production. The production system is subsistence type of milk and or meat production (11).

TABLE 1 | Study sites, agroecology, and production system characteristics.

Region	Districts	Sites/villages	Agroecology	Production systems	Altitude (m)	Rainfall (mm)	Temperature (°C)
Amhara	Basona Worena	Goshe bado	Moist highland	Mixed crop-livestock	2,419	948	16
	Basona Worena	Gudo beret	Moist highland	Mixed crop-livestock	3,142	1,118	12
	Menz Gera	07 (Yedilfere)	Moist highland	Mixed crop-livestock	3,097	1,261	12
	Menz Mama	06 (Delfanna)	Moist highland	Mixed crop-livestock	3,097	1,261	12
	Abergelle	Sazaba	Dry lowland	Mixed crop-livestock			
	Ziquala	Bilewaqu	Dry lowland	Mixed crop-livestock	1,486	732	22
Oromia	Sinana	Selka Bakaye	Moist highland	Mixed crop-livestock	2,486	1,017	14
	Sinana	Ilu sambitu	Moist highland	Mixed crop-livestock	2,372	1,039	15
	Yabello	Elewaya	Dry lowland	Pastoral/agro-Pastoral	1,181	493	22
	Yabello	Derito	Dry midland	Pastoral/agro-Pastoral	1,588	625	20
	Horro	Lakku iggu	Wet highland	Mixed crop-livestock	2,678	1,621	13
	Horro	Gitilo Dole	Wet highland	Mixed crop-livestock	2,640	1,604	14
SNNPR	Lemo	Jawe	Moist mid-land	Mixed crop-livestock	2,152	1,136	17
	Lemo	Upper Gana	Moist mid-land	Mixed crop-livestock	2,151	1,086	17
	Doyogena	Ancha Sadicho	Moist highland	Mixed crop-livestock	2,616	1,314	14
	Doyogena	Hawara Arara	Moist highland	Mixed crop-livestock	2,499	1,275	15
	Adiyo	Boka	Wet highland	Mixed crop-livestock	2,464	1,910	15
	Adiyo	Shuta	Wet highland	Mixed crop-livestock	2,316	1,871	15
Tigray	Endemehoni	Embahasti	Dry highland	Mixed crop-livestock	2,884	746	14
	Endemehoni	Tsebet	Dry highland	Mixed crop-livestock	3,184	796	13
	Atsbi wonberta	Golgol Naele	Dry highland	Mixed crop-livestock	2,691	608	15
	Atsbi wonberta	Habes	Dry highland	Mixed crop-livestock	2,559	588	16
	Tanqua Abergelle	Hadnet/Hibiret	Dry lowland	Mixed crop-livestock	1,442	653	22

Cattle and small stock play a critical role in the agro-pastoralist household economy.

Ethics Approval Statement

This study was carried out in accordance with the recommendations of the ILRI Institutional Research Ethics Committee (ILRI IREC). ILRI IREC is accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya. The protocol was approved by the ILRI IREC (Certificate Ref. No: ILRI-IREC2015-17). The respondents provided consent to participate in the study by completing the questionnaires.

Methodology

We used an exploratory sequential type of mixed methods design, using both the qualitative and quantitative approaches equally (QUAL→ QUAN) (8, 12, 13). The qualitative data collection preceded the quantitative data collection. The intent was to first explore perceptions of smallholder men and women farmers on priority small ruminant diseases and then follow up on this exploration with quantitative data collection that allows studying a larger sample so to be able to infer results to the targeted population.

Qualitative data were first gathered through participatory focus group discussions. Quantitative data were then collected through a cross sectional study based on structured interviews for further detail exploration and in order to confirm initial findings. The qualitative part helped to identify emerging questions to be tested, allowed us to define disease categories and impact categories, and also ensured that the quantitative instruments were relevant and adequate. The conceptual overview of the mixed methods and analytical approach used are outlined in **Figure 1**.

Focused Group Discussion

Gender relevant framework

For the design of this study, it was hypothesized that men, women, boys, and girls have different knowledge and skills on livestock health depending on their roles and responsibilities in animal husbandry. However, very few studies have explored gender roles and views for small ruminant production in Ethiopia. Following the "gender relevant framework," which proposed to speaking separately to men and women (14), we paid great attention to capture the views of men and women by conducting separate FGDs and by ensuring women-headed households were included in the household survey. This methodologically relevant frameworks assumes that if interviewed separately, men, and especially women, are more likely to respond openly. Data collected in this way, can be analyzed to understand how to design, deliver and monitor livestock interventions to meet the needs and realities of both men and women (15, 16).

A workshop was held for veterinarians and researchers from national agricultural research institutes to train them in participatory epidemiology and gender. Participants learnt about the use of participatory epidemiology tools for studies on animal

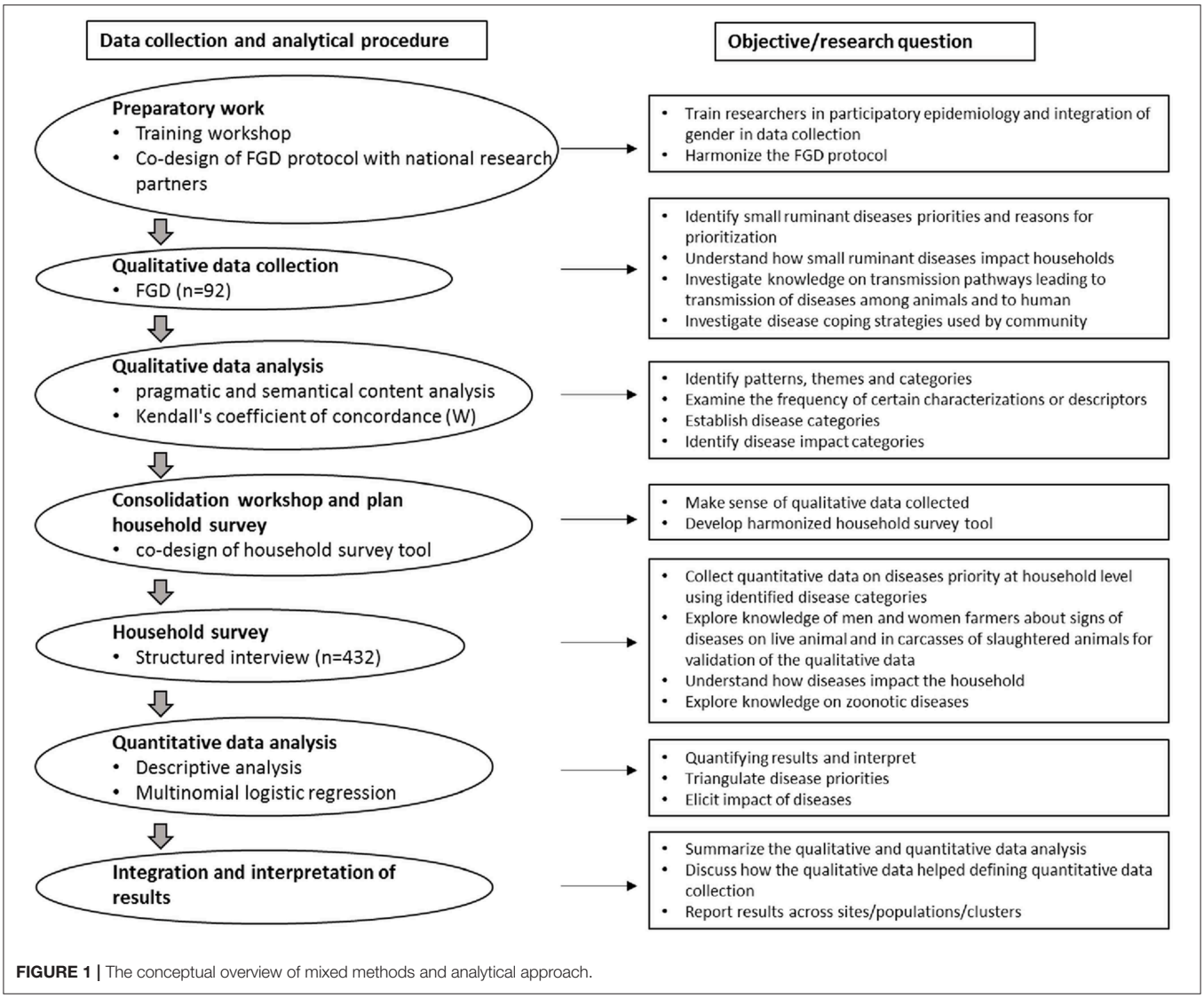

FIGURE 1 | The conceptual overview of mixed methods and analytical approach.

diseases problems, and the training had a strong focus on gender, its implication on study design and implementation of research. As part of the workshop, the study protocol was developed and tested in role play, and details of the field work were planned.

Four research teams, each consisting of 4–5 researchers, conducted the surveys.

Before each FGD, a meeting was held with local administration officials and site coordinators to introduce and explain the objectives of the study emphasizing the relevance of disease in small ruminants and their impact on household members. Site coordinators with local knowledge facilitated contact with development agents and farmers. Each team comprised a facilitator and note taker responsible for recording information. As part of the preparation, suitable locations to conduct the FGDs were identified. In each village, four FGDs took place with separate FGDs for men, women, male youth and female youth and a total of 92 FGD were conducted across the study villages. Each FGD had 6–8 participants who were actively involved in small ruminant production and had their own small ruminant herd. Attempts were made to ensure that the men FGDs comprised at least 1–2 local elders and a traditional healer.

The FGDs for men and women were held in parallel and findings of each were briefly presented in a joint session at the end of the FGDs. Similarly, the FGD for young male and female small ruminant keepers were held in parallel and their findings shared at the end during feedback sessions. Key informants (para-veterinarians and community animal health workers) were interviewed for triangulation purposes and to collect additional information or to clarify issues that may have come up during FGDs with farmers.

Various participatory tools were used to facilitate discussions, including simple ranking and proportional piling. First, participants listed five important small ruminant diseases that affect their herds and described the clinical signs of these diseases (local disease names were noted). For the ranking of diseases, 100 beans/counters were distributed among the diseases to

indicate their relative importance. In the process, participants were asked to explain the reasons for assigning the scores. The impact of these diseases on households were also identified. For the top two diseases, participants were asked to explain how the disease is spreading between animals, if it can transmit between animals and people, and who of the household is involved in activities that may lead to disease transmission. Ways of transmission or transmission situations were listed. As a last question, participants were asked to explain what the community does in response to small ruminant diseases to understand their disease coping strategies.

Household Survey

The findings of the FGDs informed the design of a household survey involving 432 households in the same communities. The FGD participants were excluded from the household survey. This survey aimed to gather more quantitative data to assess disease priorities, knowledge of men and women farmers about small ruminant diseases, the impact of diseases and investigate perceptions of different household members on their involvement in small ruminant health management.

Stratified multistage sampling (17), with four hierarchical stages, was used as sampling strategy. The regions, districts and villages were sampled purposively to include different agro-ecological zones and production systems. The first level of selection was the regions, within each of the selected regions, specific districts and villages were also selected. Households within each study villages were then selected by a stratified sampling approach targeting men-headed households and women-headed households.

To determine the sample size required for the cross-sectional household survey, the sample size and power calculation tool of Epi InfoTM 7 (CDC, Atlanta, GA) was used. The required sample size of 423 was calculated allowing us to be 95% confident of detecting a certain household characteristic or activity if it was practiced by 50% of the households, assuming allowable error of 5% and account for a design effect of 1.1 for clustering at village level. A sampling frame of all households having small ruminants from each of the selected villages was obtained from the respective administration office. In each of the 23 study site/villages, 15 men-headed households and 4 women-headed households were selected using systematic random sampling and then a few extra were added to account for eventual drop-outs, and finally the survey was conducted in 432 households.

Similar to the planning of the participatory survey, the study protocol was developed during training with researchers involved in data collection to ensure harmonization of data collection. For disease priorities, respondents were asked to indicate the top three small ruminant diseases. Impact categories of diseases were derived from answers received in the participatory survey. Knowledge of clinical signs of small ruminant disease in live animals and observation of diseases in carcasses of slaughtered animals was elicited. Information on zoonotic disease were also recorded by asking participants about the type of diseases they know that are transmitted from animals to people and their clinical signs.

Data Analysis

Epi info software version 7 was used for data collection and entry and exported into a Microsoft Excel 2013 spreadsheet. Data was analyzed using SPSS version 23.

Although the clinical signs described for most of the diseases mentioned by farmers are consistent with clinical signs and indicators described in veterinary literature and textbooks (18, 19), and were crosschecked with key informants, a conclusive diagnosis based on clinical signs and without laboratory confirmation is difficult, unscientific and unreliable. Thus, instead of using specific disease names, the diseases were grouped into seven disease categories.

For the FGD data, pragmatic and semantical content analysis was employed (20). For the pragmatic content analysis, diseases were grouped into categories according to their probable signs and attributed effects as described by the focus groups. Semantical content analysis used attribution analysis to examine the frequency with which certain characterizations or descriptors were mentioned. Descriptive statistics were computed for frequency and the percentage of FGD groups who described different disease priorities, reasons for prioritizing them and disease coping strategies.

The level of agreement among the scores of informant groups (men and women) for priority diseases in different agroecologies and production systems was assessed using Kendall's coefficient of concordance (W) (21). Consequently, evidence of agreement between informant groups was categorized as "weak," "moderate," and "strong" according to published guidelines on the interpretation of W and P-values assigned. Agreement was termed "weak" for $W < 0.26$ and $P > 0.05$; "moderate" for $W = 0.26$–0.38 and $P < 0.05$; and "strong" for $W > 0.38$ and $P < 0.01$.

Descriptive statistics were computed with frequency and percentage on the household data to understand knowledge of farmers on diseases.

For both the household data and data from FGDs on disease categories, multinomial logistic regression analysis was performed (17, 22, 23). The seven disease categories were considered as nominal dependent variables in the model. Each of the listed prioritized diseases with their corresponding explanatory variables were treated as one observation, leading to an inclusion of multiple observations per FG/household in the regression. The potential clustering effect was, however, not adjusted because a random effect multinomial regression model cannot be implemented in SPSS. We believe the effect of clustering was minimal because of the small number of observations per FG/household. The perception and preference of farmers were measured by proportional piling, resp. the number of beans allocated to each of the categories of the dependent variable. Reasons behind the scores (impacts of diseases), agroecology and production system, and gender were considered as independent variables. Multicollinearity was checked with simple correlations among the independent variables. Pairwise correlation coefficient > 0.8 considered as evidence of collinearity. Variables with a $p < 0.2$ in the univariable analysis were included in the multivariable analysis. A backward stepwise model building process was employed. The significance

of predictors was assessed using a likelihood ratio test and some variables were excluded from the final model because these did not improve the model prediction based on the likelihood ratio test with a *p*-value for variable removal of 0.157 as suggested (17).

The likelihood ratio chi-square test parameter was used to assess if the model predicts significantly better, or more accurately, than the null model. A *p*-value of <0.05 was considered to suggest model fit in the likelihood ratio test. The goodness-of-fit was also assessed through the Pearson chi-square tests, with $p > 0.05$ signifying better fit (24).

In the model (17), for an outcome variable that has *J* categories, the probability of membership in each of the outcome categories was computed by simultaneously fitting *J*-1 separate logistic model (with one category serving as the baseline or reference category). Consequently, for the dependent variable with 7 levels (leaving the first level as the baseline category), we estimated 6 sets of coefficients [$\beta^{(2)}$, $\beta^{(3)}$, $\beta^{(4)}$, $\beta^{(5)}$, $\beta^{(6)}$] corresponding to the remaining outcome categories. Because $\beta^{(1)} = 0$, the predicted probability that an observation is in category 1 was:

$$P\ (y = 1) = 1/1 + exp(x\beta^{(2)}) + exp(x\beta^{(3)}) + \ldots\ldots\ldots + exp\ (x\beta^{(7)}) \quad (1)$$

while the probability of being in category 2 was:

$$P\ (y = 2) = exp(x\beta^{(2)})/1 + exp(x\beta^{(2)}) + exp(x\beta^{(3)}) + \ldots\ldots + exp\ (x\beta^{(7)}) \quad (2)$$

and similar for categories 3, 4, 5, 6, and 7.

To identify any difference in the disease priorities of the men and women groups, the multinomial regression was applied separately for the female and male FGD data. The top three diseases categorized in to the seven disease categories were considered as a nominal dependent variable. The farmers' preferences and perceptions measured as the number of beans/scores allocated to each of the categories of the dependent variables was an independent continuous variable (covariate) in the model.

RESULTS

Priority Diseases and Reasons for Ranking

Farmers identified several diseases and syndromes that affect small ruminants with local names (vernacular names) and described their clinical signs. Based on this information and in consultation with key informants, the diseases described by participants of FGDs were grouped into seven major disease categories according to their clinical manifestation. These manifestations were respiratory, neurological, skin, gastrointestinal tract (GIT), external parasites, and systemic diseases. Diseases that did not fit into any of these categories were grouped as "others." Some of the reported diseases were difficult to name scientifically, therefore the descriptions of clinical signs and postmortem lesions mentioned were used to classify them into the respective category (**Table 2**). The probable corresponding scientific name of these diseases was added based on the clinical manifestations described by the informants. The vernacular names of diseases varied across study regions depending on the local language, even with small variations between districts.

Ten specific diseases or disease syndromes were mentioned by at least 15% of the FGD groups among their top five most important diseases (**Table 2**). These were pasteurellosis, pneumonia, coenurusis, sheep and goat pox, orf, fasciolosis, diarrhea, mange mite infestation, anthrax, and foot rot.

Some diseases were reported across agroecologies and production systems, while others were particular to an agroecology and production system in one or two districts. For example, pasteurellosis and coenurosis were repeatedly mentioned across production systems and agroecologies with different local names by 68 and 57% of focus groups, respectively. Peste des petits ruminants; however, was mentioned only in lowland mixed crop-livestock production systems (13% of focus groups).

There were important differences when disaggregating data based on the agroecology and production systems of the study districts. Respiratory diseases ranked first in the highland agroecology and mixed crop-livestock production system, followed by GIT diseases and neurological diseases with strong agreements among the informant groups ($W = 0.395$, $P = 0.000$). The most frequently mentioned diseases within the respiratory disease category were pasteurellosis, pneumonia, and contagious caprine pleuropneumonia (CCPP) among 54, 17, and 11% of focus groups, respectively. Diarrhea and fasciolosis were the priority health problems mentioned in the GIT disease category (**Table 2**).

In the lowland mixed crop-livestock system, the ranking was different with systemic diseases followed by GIT and skin diseases considered priorities with strong agreement among the focus groups ($W = 0.442$, $P = 0.000$) (**Table 3**). Anthrax and PPR were the major diseases mentioned in the systemic disease category among 40 and 21% of focus groups, respectively. For skin diseases, sheep and goat pox was mentioned by 60% of the focus groups, followed by Orf with 36%.

In pastoral/agro-pastoral production systems in the lowlands and midlands, respiratory diseases and neurological diseases were most important, followed by systemic diseases, again with very strong agreements among the informant groups ($W = 0.995$, $P = 0.001$). Coenurosis (95%) was the major disease stated in the neurological disease category (**Table 3**).

The main reasons for allocating the scores during the FGDs are presented in **Table 4**. For example, 56.8% of FGDs mentioned high mortality as the reason for scoring respiratory disease category highest. The main reason described by the farmers for prioritizing neurological diseases was the fact that there is no treatment available.

The reasons mentioned by the farmers implied the impact of the disease. Interesting to note is the fact that none of the focus groups mentioned impact on human health as a reason for prioritizing diseases. Farmers described economic impact as the loss of income from sale of animals and hides. Fifty focus groups stated that they cannot sell hides from infected animals if the animals were affected by skin disease like sheep and goat pox. Most of the time, they also cannot slaughter infected animals for consumption or sale in market. Diseases like PPR, anthrax

TABLE 2 | Disease categories, descriptions or diseases mentioned in the FGDs.

Major disease categories	Description	Local names of diseases mentioned	Probable scientific name	Count of FGDs	% of FGD	% within the category
Respiratory disease	Diseases of the respiratory tract: common clinical signs like coughing, sneezing, nasal discharge, dyspnea, abnormal respiratory sounds and different lung lesions at slaughter	Sombeessa[b]/Bubbutaa caccabsaa[b]/Argansoo[b]	Contagious Caprine Pleuropneumonia	13	14.13	11.21
		Sale[a]	Coughing	11	11.96	9.48
		Fuun Duuda[b]	–	1	1.09	0.86
		Engib[a]/Wotewut[a]/Furroo[b]/ Surridoo[b]/Halkafean[c] /Tegta[c]/Mieta[c] /Ganshu[d]/Gunfan[d]/Oshiyo[d]	Pasteurellosis	62	68.48	54.31
		Hudhaa[b]/Kokkee[b]	–	1	1.09	0.86
		Qedefera[d]	–	2	2.17	1.72
		Qeli Nefo[d]	–	4	4.35	3.45
		Samba mich[a]/Qufaa[b]/	Pneumonia	20	21.74	17.24
		Sillisaa[b]	–	1	1.09	0.86
Neurological disease	Diseases of the central nervous system: signs of circling, convulsion, staggering, abnormal behavior, abnormal gait and ataxia	Baria wez[a]/Azurit[a]/Tinan[a]/Sirgoo[b]/Lafan Martoo[b]/Jaanjjoo[b]/ Zarti[c]/Hsake Resi[c]/kenin[c]/Azar[c]/Aqnine[c]/Boko hucha[d]/Qele Gudo[d]	Coenurosis	53	57.61	94.64
		Gurgurit[c]/Haseka Riesi[c]	Oestrus ovis	1	1.09	1.79
		Riqannoota[b]	–	2	2.17	3.57
Skin disease	Skin diseases: different skin lesions and signs like hair loss, crusts, scabs, irritation, itching	Fentata[a]/Finnoo[b]/Darrabo[b]/ Shihure[c]/Bededo[c]/ Shilimat[c]/Enfrir[c]	Sheep and goat pox	35	38.04	60.34
		Afemended[a]/Abdarraa[b]/ Dorrobboo[b]/Umburura[b] Af'tetem[c]/Afe'mear[c]/Kurkursa[d]	Orf	21	22.83	36.21
		Gogimos[d]/Qodi Mosu[d]	–	2	2.17	3.45
Gasto-intestinal tract (GIT) disease	Diseases of the gastrointestinal tract: diarrhea, emaciation, erected hair, ascites, bottle jaw, and presence of different stages of parasite in feces and in GIT at slaughter	Albaatii[b]	–	2	2.17	2.08
		Mawule[a]/Dodo'o[b]/Malullaa[b]/Jiitoo[b] /Efeel[c]/Haseka Kebdi[c]	Fasciolosis	29	31.52	30.21
		Tsihtsah[c]/Gurgurit[c]/ Hamasu[d]/Temu[d]	GIT Parasites	8	8.70	8.33
		Himam sunba[c]	Lung warm	6	6.52	6.25
		Macho achi cheno[d]	Ascites	6	6.52	6.25
		Malula[d]/Lomme'eta[d]/wochiwocha[d]	Bottle jaw	10	10.87	10.42
		Mototo[d]/Qete nafo[d]	–	1	1.09	1.04
		Teqmate[a]/Albaasaa[b]/Zeaso[d]/Adora[d]	Diarrhea	34	36.96	35.42
External parasite	External parasites resulting in alopecia, itching, irritation, disturbance while grazing, visible to naked eye, externally on the skin of animals (tick, lice, sheep ked)	Ekek[a]/Cittoo[b]/Abeq[c]	Mange mite	19	20.65	55.88
		Aliqaru[d]/Bacharuta[d]	Lice infestation	3	3.26	8.82
		Mezger[a]/Benqera[d]	Tick infestation	11	11.96	32.35
		Qurdid[c]	Sheep ked	1	1.09	2.94
Systemic disease	Multi-systemic diseases with a range of clinical signs, incl. acute severe syndromes	Dhukkuba Tiruu[b]	Necrotic hepatitis	8	8.70	14.55
		Entit[a]/Hamot kebdi[c]/Minkae[c]/Tafia[c]/Megerem[c]/ kenkento[d]/	Anthrax	21	23.91	40.00
		Marra-Reeba[b]	–	3	3.26	5.45
		Qandhoo[b]	General septicemia	6	6.52	10.91
		Sheleme[a]/Tsehtsah[c]/weqie[c]	PPR	12	13.04	21.82
		Dira[a]/Werchi[c]	Black leg	4	4.35	7.27

(Continued)

TABLE 2 | Continued

Major disease categories	Description	Local names of diseases mentioned	Probable scientific name	Count of FGDs	% of FGD	% within the category
Others	Diseases with unknown cause, non-infectious, metabolic in nature and difficult to be classified in the above listed categories	Yehode menefat[d]	Bloating	1	1.09	2.38
		Dengetegna Besheta[a]	Sudden disease	1	1.09	2.38
		Dhukkuba hinbeekkamne[b]/Maqaan hin beekamu[b]	Unknown Disease	6	6.52	14.29
		Ayin Beshita[a]/Elemoosu[d]/Elitiso[d]	Eye disease	6	6.52	14.29
		Ye egir til[a]/Maasaa[b]/Barga'oo[b]/Ho'ichoo[b]/ Nutero[d]/ Loka Hucha[d]/ loka shokota[d]/ Moyale[d]/Naqarisa[d]	Foot rot	18	19.57	42.86
		Himam tub[c]	Mastitis	1	1.09	2.38
		Koyooo[d]/wugati[d]	–	1	1.09	2.38
		Mbray[c]	Abortion	1	1.09	2.38
		Mecho[d]/mechi[d]	–	5	5.43	11.90
		Raammoo miilaa[b]	–	1	1.09	2.38
		Senbecha/chinyako[d]	–	1	1.09	2.38

[a] *Amhara.*
[b] *Oromia.*
[c] *Tigray.*
[d] *SNNP.*

TABLE 3 | Summary of ranking of priority disease categories in different agroecology and production systems (top ranked disease in each FGD got a score of 7).

	Highland mixed crop-livestock		Lowland mixed crop-livestock		Midland mixed crop-livestock		Lowland pastoral and agro-pastoral		Midland pastoral and agro-pastoral	
Disease category	**Mean rank score**	**Test statistics**	**Mean rank score**	**Test statistics**	**Mean rank score**	**Test statistics**	**Mean rank score**	**Test statistics**	**Mean rank score**	**Test statistics**
Respiratory	6.07	$N = 64$	3.63	$N = 12$	4.75	$N = 8$	6.50	$N = 4$	6.00	$N = 4$
Neurological	4.27	Kendall's	2.92	Kendall's	1.81	Kendall's	6.50	Kendall's	7.00	Kendall's
Skin	4.03	$W = 0.395$	4.46	$W = 0.442$	4.38	$W = 0.389$	2.38	$W = 0.955$	2.75	$W = 0.847$
GIT	5.18	$\chi^2 = 151.7$	4.46	$\chi^2 = 31.83$	5.50	$\chi^2 = 18.68$	2.38	$\chi^2 = 22.93$	2.25	$\chi^2 = 20.33$
Ectoparasites	2.70	Df = 6	3.67	Df = 6	4.44	Df = 6	2.88	Df = 6	2.25	Df = 6
Systemic	2.58	Sig. = 0.000	6.58	Sig. = 0.000	2.56	Sig. = 0.005	5.00	Sig. = 0.001	4.00	Sig. = 0.002
Other	3.17		2.29		4.56		2.38		3.75	

and pasteurellosis can cause high loss because of high mortality rates. This aggravates poverty for those whose livelihoods depend on these animals. Important to note is also that 73.3% of the focus groups which prioritized GIT diseases mentioned frequent occurrence or endemicity as a reason for scoring them high.

The odds of allocating higher scores to respiratory and GIT disease categories than the reference "other" category were 1.10 (10%) and 1.08 (8%) times for men and 1.14 (14%) and 1.07 (7%) for women focus group participants, respectively. Women and men also gave similar scores to neurological diseases OR = 1.04 (4%) than to the reference "other" category, but the result was significant for women ($p < 0.05$) unlike the men (**Table 5**). The scoring of diseases provided by the women FGD groups was similar to the scoring by the men groups, this showed the perceptions and priorities of the men and women groups were very similar.

Impact of Small Ruminant Diseases on Households

The FGDs helped to define categories of how small ruminant diseases impact farmers in terms of financial loss that arises from mortality, lower productivity, loss of marketing value, and treatment costs. It also affects human health including through malnutrition, migration in search of other jobs, children drop out of school because of the extra time needed to take care of sick animals and other related financial constraints; all these causing severe social and psychological impact.

Farmers described financial loss as the loss of income from sale of animals and hides. They also can't slaughter infected animals for consumption or sale in market or sell hides from infected animals. Participant farmers said that it is not even possible to use hides as rugs at home if the animals were affected by diseases like sheep and goat pox.

TABLE 4 | Percentage of farmers providing specific reasons for ranking diseases from 1st to 3rd.

Reasons for ranking	Disease category	1st rank		2nd rank		3rd rank	
		%of total FGD	% within the disease category	% of total FGD	% within the disease category	% of total FGD	% within the disease category
High mortality	Respiratory diseases	22.8	56.8	14.1	39.4	5.4	27.8
	Neurological diseases	2.2	11.8	4.3	36.4	0.0	0.0
	Skin disease	3.3	37.5	3.3	27.3	0.0	0.0
	GIT diseases	10.9	66.7	4.3	22.2	9.8	39.1
	External parasites	0.0	0.0	0.0	0.0	0.0	0.0
	Systemic diseases	6.5	75	3.3	37.5	6.5	37.5
	Others	2.2	50	1.1	11.1	1.1	11.1
Acute and fatal	Respiratory diseases	7.6	18.9	3.3	9.1	1.1	5.6
	Neurological diseases	1.1	5.9	0.0	0.0	0.0	0.0
	Skin disease	0.0	0.0	0.0	0.0	0.0	0.0
	GIT diseases	1.1	6.7	0.0	0.0	0.0	0.0
	External parasites	0.0	0.0	0.0	0.0	0.0	0.0
	Systemic diseases	6.5	75	0.0	0.0	2.2	12.5
	Others	1.1	25	4.3	44.4	1.1	11.1
Unmarketable skin	Respiratory diseases	0.0	0.0	0.0	0.0	1.1	5.6
	Neurological diseases	0.0	0.0	0.0	0.0	0.0	0.0
	Skin disease	4.3	50	4.3	36.4	0.0	0.0
	GIT diseases	0.0	0.0	1.1	5.6	0.0	0.0
	External parasites	1.1	33.3	0.0	0.0	2.2	33.3
	Systemic diseases	0.0	0.0	0.0	0.0	0.0	0.0
	Others	1.1	1.1	0.0	0.0	0.0	0.0
Render the meat inedible	Respiratory diseases	4.3	10.8	0.0	0.0	1.1	5.6
	Neurological diseases	0.0	0.0	0.0	0.0	0.0	0.0
	Skin disease	4.3	50	3.3	27.3	0.0	0.0
	GIT diseases	1.1	6.7	2.2	11.1	0.0	0.0
	External parasites	0.0	0.0	0.0	0.0	1.1	16.7
	Systemic diseases	1.1	12.5	0.0	0.0	0.0	0.0
	Others	0.0	0.0	0.0	0.0	0.0	0.0
High transmission rate/affect most of the flock	Respiratory diseases	21.7	54.1	14.1	39.4	6.5	33.3
	Neurological diseases	1.1	5.9	2.2	18.2	0.0	0.0
	Skin disease	6.5	75	7.6	63.6	6.5	50
	GIT diseases	4.3	26.7	3.3	16.7	2.2	8.7
	External parasites	1.1	33.3	0.0	0.0	3.3	50
	Systemic diseases	3.3	37.5	3.3	37.5	0.0	0.0
	Others	1.1	25	4.3	44.4	1.1	11.1
Frequent occurrence/seen throughout the whole season/endemicity	Respiratory diseases	6.5	16.2	7.6	21.2	3.3	16.7
	Neurological diseases	5.4	29.4	0.0	0.0	0.0	0.0
	Skin disease	1.1	12.5	2.2	18.2	3.3	25
	GIT diseases	12	73.3	7.6	38.9	4.3	17.4
	External parasites	2.2	66.7	0.0	0.0	0.0	0.0
	Systemic diseases	1.1	12.5	2.2	25	0.0	0.0
	Others	1.1	25	2.2	22.2	0.0	0.0
No treatment and recovery	Respiratory diseases	4.3	10.8	2.2	6.1	0.0	0.0
	Neurological diseases	13	70.6	5.4	45.5	8.7	100
	Skin disease	0.0	0.0	0.0	0.0	0.0	0.0

(Continued)

TABLE 4 | Continued

Reasons for ranking	Disease category	1st rank		2nd rank		3rd rank	
		%of total FGD	% within the disease category	% of total FGD	% within the disease category	% of total FGD	% within the disease category
	GIT diseases	2.2	13.3	0.0	0.0	0.0	0.0
	External parasites	0.0	0.0	0.0	10.0	0.0	0.0
	Systemic diseases	0.0	0.0	1.1	12.5	1.1	6.3
	Others	1.1	25	2.2	22.2	0.0	0.0
Market price devaluation/affect market	Respiratory diseases	0.0	0.0	4.3	12.1	0.0	0.0
	Neurological diseases	2.2	11.8	1.1	9.1	0.0	0.0
	Skin disease	0.0	0.0	0.0	0.0	0.0	0.0
	GIT diseases	1.1	6.7	0.0	0.0	0.0	0.0
	External parasites	0.0	0.0	0.0	0.0	0.0	0.0
	Systemic diseases	0.0	0.0	0.0	0.0	0.0	0.0
	Others	0.0	0.0	0.0	0.0	0.0	0.0
High morbidity/affect productivity/reduce body weight/ lead to abortion	Respiratory diseases	0.0	0.0	3.3	9.1	3.3	16.7
	Neurological diseases	1.1	5.9	1.1	9.1	0.0	0.0
	Skin disease	1.1	12.5	3.3	27.3	2.2	16.7
	GIT diseases	1.1	6.7	5.4	27.8	6.5	26.1
	External parasites	0.0	0.0	1.1	50	1.1	16.7
	Systemic diseases	1,1	12.5	1.1	12.5	0.0	0.0
	Others	0.0	0.0	2.2	22.2	3.3	33.3
Not common in the locality	Respiratory diseases	0.0	0.0	2.2	6.1	2.2	11.1
	Neurological diseases	0.0	0.0	0.0	0.0	0.0	0.0
	Skin disease	0.0	0.0	1.1	9.1	5.4	41.7
	GIT diseases	0.0	0.0	3.3	16.7	5.4	21.7
	External parasites	0.0	0.0	0.0	0.0	0.0	0.0
	Systemic diseases	0.0	0.0	0.0	0.0	10.9	62.5
	Others	0.0	0.0	0.0	0.0	4.3	44.4

TABLE 5 | The odds of farmers allocating different scores to disease categories by male and female focus groups.

DC[a]		Male focus groups				Female focus groups			
	B	Std. error	Sig. (P)	Odds ratio	B	Std. error	Sig. (P)	Odds ratio	
Respiratory	Intercept	−1.350	0.489	0.006		−2.040	0.429	0.000	
	Score	0.098	0.028	0.000	1.103	0.127	0.021	0.000	1.135
Neurological	Intercept	−0.466	0.397	0.240		−0.419	0.282	0.137	
	Score	0.048	0.027	0.080	1.049	0.043	0.019	0.027	1.044
Skin	Intercept	−0.275	0.381	0.471		−0.306	0.275	0.265	
	Score	0.031	0.028	0.256	1.032	0.033	0.019	0.087	1.034
GIT	Intercept	−0.859	0.433	0.048		−0.796	0.308	0.010	
	Score	0.073	0.027	0.006	1.076	0.068	0.019	0.000	1.071
Ectoparasites	Intercept	0.028	0.360	0.938		0.241	0.248	0.330	
	Score	−0.004	0.030	0.892	0.996	−0.044	0.025	0.076	0.957
Systemic	Intercept	−0.072	0.366	0.843		0.011	0.258	0.966	
	Score	0.010	0.029	0.739	1.010	−0.002	0.021	0.942	0.998

[a] *The reference category is "others"; NE, non estimable.*

Some of the diseases are treatable but farmers explained that expenses incurred because of treatment affects the economy of the household. Diseases can also cause loss because of high mortality rates. This aggravates poverty for those whose livelihood is dependent on these animals. Some respondents reported that they were not able to send

TABLE 6 | Percentage of farmers providing 1st and 2nd priority diseases in different agroecology and production systems.

	Highland mixed crop-livestock		Lowland mixed crop-livestock		Mid-land mixed crop-livestock		Lowland pastoral and agro-pastoral		Mid-land pastoral and agro-pastoral	
Diseases	**1st priority (%)**	**2nd priority (%)**	**1st priority (%)**	**2nd priority (%)**	**1st priority (%)**	**2nd priority (%)**	**1st priority (%)**	**2nd priority (%)**	**1st priority (%)**	**2nd priority (%)**
Pasteurellosis	32.4	14.5	12.5	16.4	27.8	25				
Coughing	18.1	14.5			11.1					
Coenurosis	20.6	21.2					12.5	41.7	8.3	25
Sheep and goat pox	3.9	4.5		31.1						
Liver fluke	4.3	9.3			8.3					
Anthrax			47.2	21.3						
PPR			16.7	11.5						
Diarrhea		12.6	16.7	18		16.7				
CCPP							70.8		75	16.7
GIT disease							16.7	12.5		
Orf					19.4					
Foot rot					16.7	11.1				
Necrotic hepatitis								16.7	16.7	50
Tick infestation						19.4				

children to school anymore. It also predisposes household members to migrate to towns or other areas in search of other work.

Farmers also explained that lambs and kids cannot get enough milk from diseased dams, which may lead to death and thus reduce the flock size and greatly affect the overall farm productivity. In some areas where goat milk is consumed, respondents mentioned that there is a drop in milk supply from diseased animals affecting the nutrition of their children.

Also mentioned was the psychological impact that results from losing animals to diseases because in most of the surveyed rural areas ownership of animals is an indicator of wealth determining one's social status. Once a disease enters their sheep and goat flock it makes them give up on rearing other livestock as well.

Adverse social impact was described as being unable to pay taxes, buy fertilizer or pay membership fees to their "Idir" (traditional financial association). In addition, a household which encounters a disease in its flock first will not be allowed by neighbors to mix its herd on grazing fields and watering areas. This sometimes leads to social conflicts.

Household Survey: Disease Priorities and Impact

The household survey asked for the top three diseases at household level and how these diseases impact the household. The priority diseases mentioned were not different from those in the FGDs (**Table 6**). Based on results from the FGDs, categories of how of small ruminant diseases impact farmers were defined—economic/income loss, mortality, loss of productivity, loss of value, treatment costs, migration for other jobs, wastage of time treating the animals, children drop out of school, malnutrition, social and psychological impact. In addition, human health was added as a possible impact category.

The multinomial logistic regression results shown in **Table 7** revealed the odds of mentioning the given impact for a given disease category compared to the reference category. Respiratory diseases like CCPP and pasteurellosis had significantly higher odds for high scores due to associated high mortality compared to all other disease categories except systemic diseases (PPR, anthrax etc.). For example, neurological diseases (Odds Ratio, OR = 0.23), skin diseases (OR = 0.47), gastrointestinal diseases (OR = 0.3), and ectoparasites (OR = 0.04) have got significantly lower scores than respiratory diseases due to the high mortality impact associated with respiratory diseases. Systemic diseases (OR = 6.08) compared to respiratory diseases were more likely to affect the lowland mixed crop-livestock production system than midland pastoral and agro-pastoral production system. Other important significant findings were that the amount of time it took to treat diseases was an important reason for scoring "other diseases" high. Diseases mentioned there were foot rot, mastitis, abortion, eye disease, and bloating.

Disease Knowledge of Farmers

The FGDs found that, in general, farmers have a fair amount of knowledge about disease transmission pathways between animals. Identified common transmission pathways or situations leading to transmission of diseases among animals were feeding and watering troughs, common barns, sharing grazing areas, communal markets, slaughter and skinning places, and suckling (**Figure 2**).

Farmers said transmission of respiratory diseases was mainly related to inadequate ventilation in barns, poor sanitation, common grazing areas, market places and during use of

TABLE 7 | Multinomial logistic regression coefficients and odds ratios of disease impacts for the top two priority disease categories at household level.

DC	Financial/income loss					Mortality					School					Productivity				
	β (ref)	Std. error	e^{β} (OR)	95% Confidence interval for e^{β}		β (ref)	Std. error	e^{β} (OR)	95% Confidence interval for e^{β}		β (ref)	Std. error	e^{β} (OR)	95% Confidence interval for e^{β}		β (ref)	Std. error	e^{β} (OR)	95% Confidence interval for e^{β}	
R	1.22 (O)	0.537	3.39*	1.184	9.727															
N						−1.46 (R)	0.381	0.23***	0.111	0.492	1.25 (R)	0.628	3.49	1.022	11.976					
S	1.71 (O)	0.639	5.55**	1.585	19.432	−0.75 (R)	0.374	0.47*	0.227	0.984						1.00 (N)	0.470	2.72*	1.084	6.841
G	1.109 (O)	0.557	3.03*	1.018	9.030	−1.19 (R)	0.286	0.30***	0.172	0.528						0.89 (N)	0.390	2.45*	1.140	5.266
E	−1.65 (S)	0.707	0.19*	0.048	0.767	−2.91 (O)	1.000	0.05**	0.008	0.386										
						−3.36 (R)	0.873	0.04***	0.006	0.192										
						−1.91 (N)	0.915	0.15*	0.025	0.895										
						−2.61 (S)	0.903	0.07**	0.013	0.430										
						−2.16 (G)	0.870	0.12*	0.021	0.634										
Y	1.82 (O)	0.741	6.17*	1.443	26.380	1.05 (N)	0.502	2.85*	1.066	7.630						−2.00 (O)	0.705	0.14**	0.034	0.537
	1.76 (E)	0.791	5.79*	1.228	27.322	0.791 (G)	0.384	2.21*	1.040	4.682						−1.44 (R)	0.450	0.24***	0.098	0.571
						2.95 (E)	0.917	19.17***	3.177	115.638						−1.76 (S)	0.504	0.17***	0.062	0.446
																−1.69 (G)	0.452	0.19***	0.076	0.448
																−1.66 (E)	0.799	0.19*	0.040	0.915

R, respiratory diseases; N, neurological diseases; S, skin diseases; G, gastrointestinal diseases; E, ectoparasites; Y, systemic diseases; O others.

****The parameters β were significant at 0.001 level; **at 0.01; *at 0.05.*

The disease impacts were compared to each of the seven disease categories (each considered as a reference category in turn) in a series of analyses to determine the statistical significance of the differences.

TABLE 7 | Continued

DC	Time it took to treat animals					Cost					Value					Malnutrition				
	β (ref)	Std. Error	e^{β} (OR)	95% Confidence interval for e^{β}		β (ref)	Std. Error	e^{β} (OR)	95% Confidence interval for e^{β}		β (ref)	Std. Error	e^{β} (OR)	95% Confidence interval for e^{β}		β (ref)	Std. Error	e^{β} (OR)	95% Confidence interval for e^{β}	
R	−1.75 (O)	0.573	0.17**	0.056	0.534															
N	−3.16 (O)	0.753	0.04***	0.010	0.185	−1.44 (O)	0.718	0.24***	0.058	0.963										
	−1.41 (R)	0.552	0.244*	0.083	0.719	−1.62 (R)	0.468	0.19***	0.079	0.493										
S	−1.50 (O)	0.681	0.22*	0.059	0.845	1.58 (N)	0.599	4.84**	1.496	15.647										
	1.659 (N)	0.683	5.26*	1.378	20.050															
G	−1.72 (O)	0.599	0.18**	0.056	0.583	1.34 (N)	0.505	3.82**	1.423	10.281						1.60 (N)	0.613	4.97**	1.496	16.529
	1.45 (N)	0.586	4.25*	1.35	13.403															
E	−2.29 (O)	0.961	0.10*	0.015	0.660											2.16 (N)	1.022	8.63*	1.164	63.922
Y	−2.54 (O)	0.831	0.08**	0.015	0.402						−1.29 (R)	0.553	0.28*	0.093	0.816					
											−1.32 (S)	0.617	0.27*	0.080	0.896					

R, respiratory diseases; N, neurological diseases; S, skin diseases; G, gastrointestinal diseases; E, ectoparasites; Y, systemic diseases; O, others.

****The parameters β were significant at 0.001 level; **at 0.01; *at 0.05.*

The disease impacts were compared to each of the seven disease categories (each considered as a reference category in turn) in a series of analyses to determine the statistical significance.

TABLE 7 | Continued

DC	Psychological				Human health				Agroecology and production system			
	β (ref)	Std. Error	e^{β} (OR)	95% Confidence interval for e^{β}	β (ref)	Std. Error	e^{β} (OR)	95% Confidence interval for e^{β}	β (ref)	Std. Error	e^{β} (OR)	95% Confidence interval for e^{β}
N					−2.29 (R)	1.067	0.10*	0.012	0.814			
S									LMC 4.43 (R)	1.255	83.84***	7.17 980.43
									MMC 3.87 (N)	1.576	47.84*	2.18 1049.62
									MPA^			
G	−1.24 (R)	0.579	0.29*	0.09 0.90	−1.32 (R)	0.604	0.27*	0.082 0.870				
	−1.67 (N)	0.656	0.19*	0.05 0.68								
E												
Y	1.91(S)	0.932	6.78*	1.09 42.13	2.93 (N)	1.145	18.64*	1.97 175.73	HMC −2.06 (R)	0.70	0.13**	0.03 0.50
	1.84 (G)	0.709	6.31**	1.57 25.30	2.34 (S)	1.075		1.26	LMC 1.805 (R)	0.867	6.08*	1.11 33.24
					1.95 (G)	0.663	10.3*	1.92 85.36	HMC −2.93 (S)	1.243	0.05*	0.01 0.61
							7.03**	25.76	LMC −2.62 (S)	1.318	0.07*	0.01 0.96
									MMC −4.16 (S)	1.580	0.02**	0.001 0.35
									MPA^			

N, neurological diseases; S skin diseases; G; gastrointestinal diseases; E; ectoparasites; Y; systemic diseases; O; others; HMC highland mixed-crop; LMC, Lowland mixed-crop; MMC, midland mixed-crop; MPA; midland pastoral and agro-pastoral.

^Reference for agroecology and production system.

****The parameters β were significant at 0.001 level; **at 0.01; *at 0.05.*

The disease impacts were compared to each of the seven disease categories (each considered as a reference category in turn) in a series of analyses to determine the statistical significance.

communal feeding and watering troughs. Farmers were also knowledgeable about predisposing stress factors associated with housing, handling and marketing that can cause clinical presentation of pasteurellosis (25, 26). Farmers clearly stated that GIT parasites are mostly transmitted during grazing.

The household survey explicitly prompted households to acquire information on zoonotic diseases. Forty-six percent of the respondents said they knew about zoonotic diseases, but only 36% were actually able to name and describe a zoonotic disease. Anthrax, rabies, bovine tuberculosis and taeniasis were mentioned by 19.9, 7.6, 1.9, and 2.5% of respondents, respectively, while 1.4% of respondents named both anthrax and rabies. Anthrax was mostly mentioned in households in lowland agroecologies. There was no significant difference on knowledge about zoonoses between gender and age groups. Overall, both men and women farmers had low awareness of zoonotic diseases.

Regarding the knowledge of men and women farmers about small ruminant diseases, the household survey revealed that despite the differences in household roles, both men and women unvaryingly described the clinical signs in live animals similarly (**Figure 3**) and reported similar observations of disease in carcasses of slaughtered animals (**Figure 4**).

Disease Coping Mechanism or Strategy

The FGDs investigated disease coping strategies. These included mechanisms to prevent and control diseases, combined with strategies to reduce economic loss and ensure survival at difficult times. The use of modern veterinary services included vaccination and treatment with modern drugs. Traditional treatment and beliefs relied on traditional remedies and treatment practices. Health management activities covered isolation of sick animals, grazing management, keeping barns clean, proper carcass disposal of dead animals, proper supplementary feeding, and prevention from heat stress.

Most of the farmers (27.6%) often depend on both modern veterinary medicine and traditional treatment and beliefs for prevention and control of small ruminant diseases. However, only a few farmers seem to use other health management activities (**Figure 5**).

From the focus groups, 13% reported traditional treatment practices like drenching with herbal preparations of onion (*Allium cepa*), ginger (*Zingiber officinale*), tobacco leaf (*Nicotiana tabacum*), black cumin (*Nigella sativa*), "Feto" (Lepidium sativum L.); fumigation with "kebericho" (Echinops kebericho) through nose and mouth of sick animals; and using holy water for treatment and control of pasteurellosis. For treatment of coenurosis, remedies like drenching a local alcoholic drink and tobacco leaf juice through the nose, hot iron branding of the forehead or immersing the infected sheep in well water and fumigating with burned plastic were mentioned. For treatment of diarrhea, drenching of herbal preparations made of ginger (*Zingiber officinale*) and "Feto" (*Lepidium sativum* L.) and mineral soil locally called "Bole" were used. Topical applications or dressing lesions with honey, butter, pepper powder and salty water were practiced to treat sheep and goat pox.

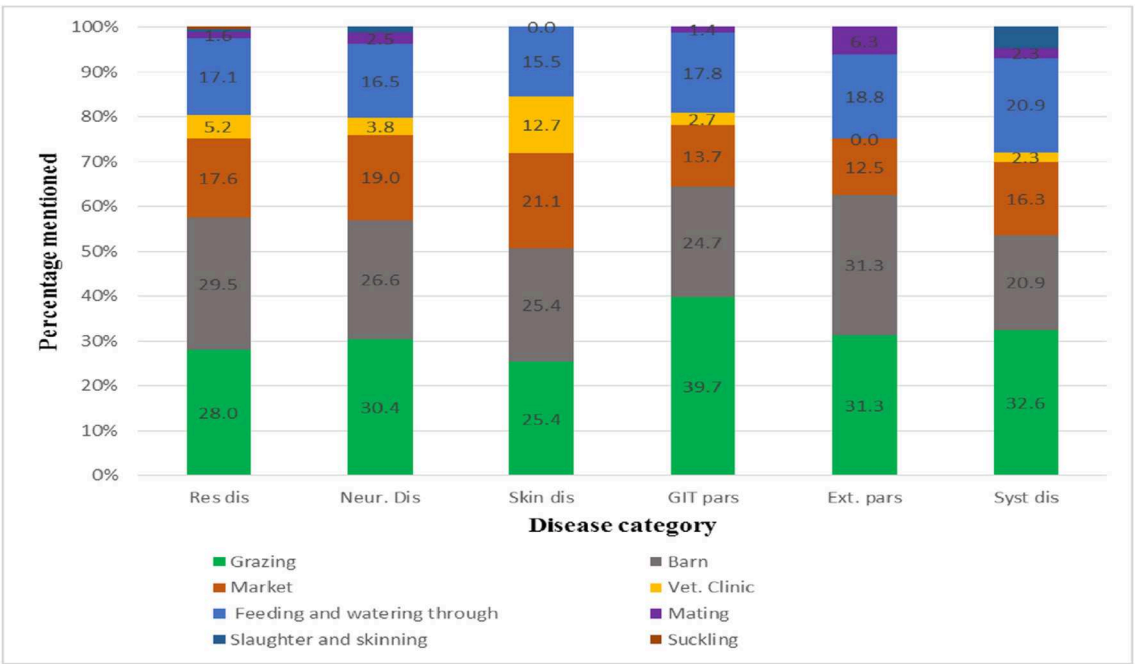

FIGURE 2 | Major disease transmission pathways identified by farmers.

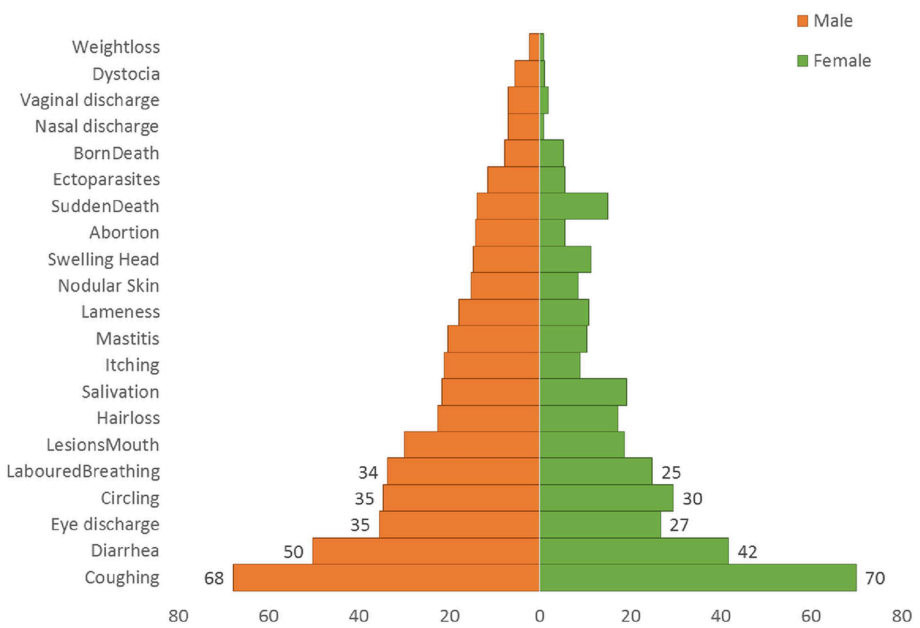

FIGURE 3 | Frequency percentage of clinical signs mentioned by gender.

The strategies to reduce economic loss during vulnerable times included sale of animals at lower price, slaughter of animals for home consumption, contractually own (rent) flock from other people/relatives, shifting to other business activities, buying new flock by selling cattle or crop, and searching for financial support from relatives/government/NGOs.

DISCUSSIONS

This study identified disease priorities as perceived by farmers and provided insights into why the diseases were considered important. The findings show that priorities of national disease control programs do not fully match priorities of

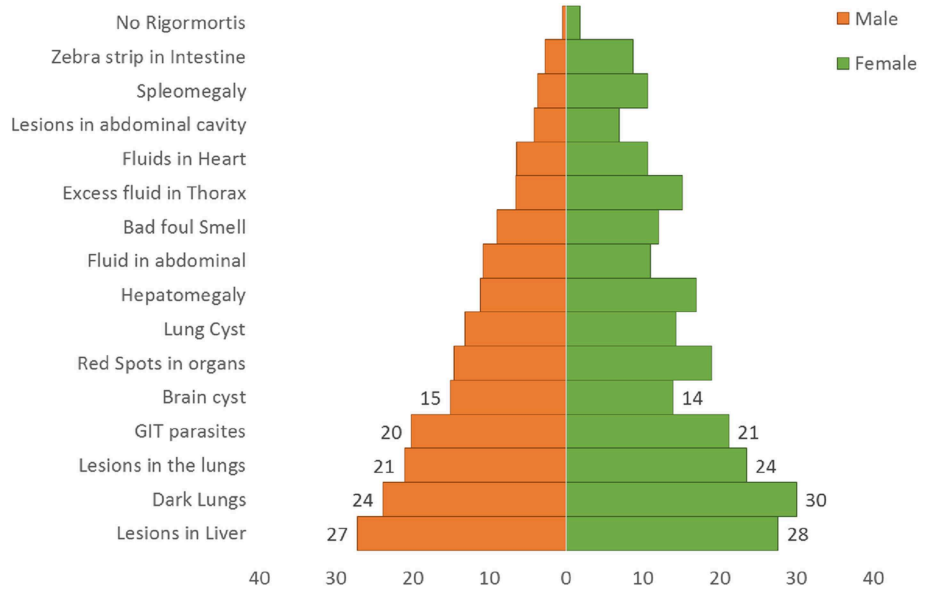

FIGURE 4 | Frequency percentage of postmortem signs mentioned by gender.

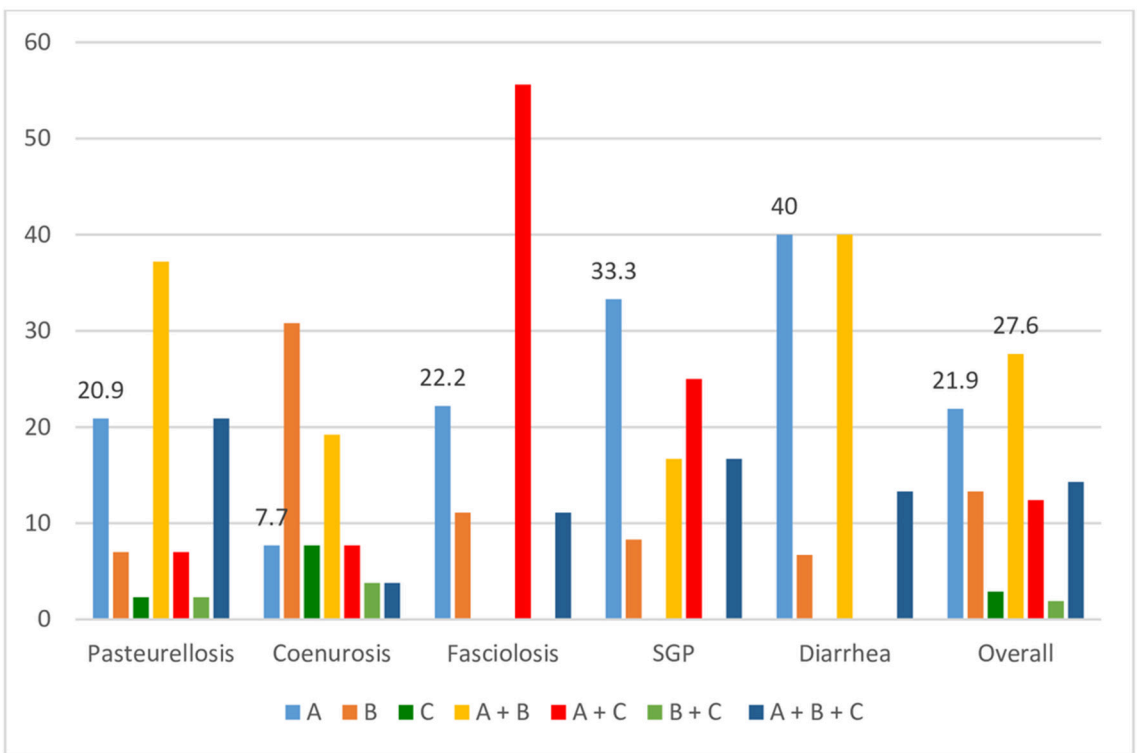

FIGURE 5 | Percentage of respondents (FGD) using different coping mechanisms against the five top small ruminant disease (A, use modern veterinary service; B, traditional treatment and beliefs; C, health management activities. +indicates combination).

farmers. The study used mixed research methods combining FGDs and household survey. This approach provided a better understanding of disease priorities than either approach alone could. The FGDs helped understand the nature of the data and the meaning of what participants said, which vary across the study area and across groups of smallholders. This influenced data analysis and interpretation. The household survey allowed for a deeper exploration of the qualitative data from FGDs to

build a fuller picture of the impact of diseases, the actions taken by smallholders when facing small ruminant diseases and insights into possible reasons for these actions.

The Government of Ethiopia recognized the importance of livestock health as a priority in the Ethiopian livestock master plan (LMP) (27). The LMP considers Peste des petits ruminants (PPR), sheep and goat pox (SGP), and CCPP as important small ruminant diseases based on their impact on rural households and their livelihoods, intensification pathways and implications for international trade.

In this study, livestock owners listed several typical multifactorial production diseases as priorities, such as pasteurellosis, coenurosis, and GIT parasites. However, these diseases are largely neglected in disease control efforts. Similarly, Gari et al. (28) documented sheep and goat diseases in two districts of Afar regional state representing the lowland pastoral and agro-pastoral production systems and found respiratory syndrome/CCPP, sheep and goat pox, diarrhea and tick- and tick-borne diseases as highly ranked health problems. They also described lung worms, pneumonia and septicemia pasteurellosis as the most suspected respiratory diseases and called for conventional surveillance in the future.

This study emphasizes a disparity between community priorities and government priorities. Likewise, it is widely agreed that national and global surveillance systems should focus on transmissible diseases affecting international trade or have importance from a public health point of view. However, production diseases are often not perceived to be a serious enough threat to gain attention. Not surprisingly, inaccurate disease surveillance reports are common, and even if targeted by national surveillance systems, disease occurrence is likely to be underreported. Being off the radar of any surveillance programs also means that there is no other investments in support of prevention and control of these diseases. Hence our study highlights the need for control programs and access to veterinary inputs that meet needs of smallholders.

Impact of the diseases presented here can be reduced by proper health management at herd or community level. But this study also found that such prevention and control measures are rarely implemented and farmers rely on veterinary inputs or traditional treatments. This is clearly an area where improved capacity of farmers is needed, which can be achieved by adequate extension systems or other animal health service provision that support and facilitate prevention. Farmers mentioned a range of coping mechanisms and/or strategies against small ruminant diseases. Traditional practices play a big role in these coping mechanisms. While their positive impact has been previously described and can be used to provide economical solutions to improve productivity of animals and reduction in poverty of the poor farmers (29), traditional methods are not always the best or most effective for treating infectious diseases. What these practices reflect though, is the need and willingness of farmers to do something in response to disease occurrence.

Catley and Mohammed (30), described livestock disease scoring method and used "importance indicators" and "difference indicators" for pair-wise comparisons to understand what herders thought were the most important livestock diseases to cause economic and production losses. In this study, livestock owners provided a range of specific reasons for ranking diseases. The reasons included the resulting economic and production loss (high mortality, high morbidity, market price devaluation, inedible meat, unmarketable skin, no treatment, and recovery); characteristics of the diseases (high transmission rate, acute and fatal); and occurrence (frequent occurrence or endemicity).

Farmers attributed high mortality to systemic diseases like PPR and anthrax, respiratory diseases like pasteurellosis and CCPP and GIT diseases caused by liver fluke. Their reasoning for priority was coherent with knowledge on how these diseases affect infected animals (see the specific diseases mentioned on Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 2019).

Farmers mentioned lack of available treatment as the main reason to prioritize neurological diseases such as coenurosis. Achenef et al. (31) also declared that no single satisfactory treatment method has been devised under field conditions, although trephining has been advocated for coenurosis. Dogs, which are part of the coenurosis transmission cycle, play an important role for herders in pastoral and agro-pastoral areas and may explain the high incidence, as the presence of freely roaming dogs on grazing land greatly contributes to the existence of the disease (32).

Catley et al. (3) indicated gender analysis tools that can be tailored to animal health activities. These include livestock keeping household activity profiling; livestock activity, access to and control over resources profiling; livestock resources and benefits index; and a practical and strategic gender needs in livestock management index.

In the design of this study, we paid great attention to capture the views of men and women by conducting separate FGDs and by ensuring women-headed households were included in the household survey. There were no major differences in men and women households and focus groups in identifying and scoring priority disease constraints. Similarly, the knowledge of men and women farmers were similar in describing the clinical signs in live animals, as well as their observation in slaughtered animals. The findings of Galie et al. (33) also showed that both women and men were involved in cattle health management in Tanzania and had similar knowledge of diseases. With the participant selection, there might have been introduction of potential "elite bias" (34) in our study. The traditional healers included in the men FGD tended to be the most knowledgeable and articulate members of the group, which may have affected comparison of men and women FGDs. However, the primary objective of the study to identify the major small ruminant disease would not be affected, and any bias introduced through the non-random selection of participants seemed minimal.

The study also found that farmers have good knowledge about disease transmission pathways between animals but that both men and women farmers had low awareness of zoonotic diseases. In this regard, it is important to note that none of the focus groups mentioned impact of zoonotic diseases on human health as reason for prioritizing diseases. However, considering the high prevalence of zoonoses and the close interactions of people and animals in these production systems, their importance cannot be

overstated. Kinati et al. (35) provided insights into the role of division of labor related to small ruminant health management in the same cohort targeted in our study and showed different levels of contact/involvement of different household members in possible transmission pathways. This is clearly another issue that should be addressed through advisory and extension services by involving both men and women.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ILRI Institutional Research Ethics Committee (ILRI IREC), ILRI IREC is accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BW, BA, HD, AM, and WK conceived and designed the study and followed up and monitored data collection. BA analyzed the data. BA, BW, and SG conceptualized and drafted the paper. All authors read, commented, and approved the final 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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Development of Reporting Guidelines for Animal Health Surveillance—AHSURED

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With the current trend in animal health surveillance toward risk-based designs and a gradual transition to output-based standards, greater flexibility in surveillance design is both required and allowed. However, the increase in flexibility requires more transparency regarding surveillance, its activities, design and implementation. Such transparency allows stakeholders, trade partners, decision-makers and risk assessors to accurately interpret the validity of the surveillance outcomes. This paper presents the first version of the Animal Health Surveillance Reporting Guidelines (AHSURED) and the process by which they have been developed. The goal of AHSURED was to produce a set of reporting guidelines that supports communication of surveillance activities in the form of narrative descriptions. Reporting guidelines come from the field of evidence-based medicine and their aim is to improve consistency and quality of information reported in scientific journals. They usually consist of a checklist of items to be reported, a description/definition of each item, and an explanation and elaboration document. Examples of well-reported items are frequently provided. Additionally, it is common to make available a website where the guidelines are documented and maintained. This first version of the AHSURED guidelines consists of a checklist of 40 items organized in 11 sections (i.e., surveillance system building blocks), which is available as a wiki at https://github.com/SVA-SE/AHSURED/wiki. The choice of a wiki format will allow for further inputs from surveillance experts who were not involved in the earlier stages of development. This will promote an up-to-date refined guideline document.

Keywords: animal health surveillance, output-based standards, reporting guidelines, harmonization, expert elicitation

INTRODUCTION

Surveillance of disease has an essential role in protecting the health and welfare of animals and humans. While, human health surveillance commonly relies on notifiable disease reporting and analysis of secondary data, animal health surveillance (AHS) places a stronger emphasis on collecting primary data via active sampling of animal populations. For example, such active surveillance will support the objective to fulfill trade requirements and ensure food safety (1). A review of the surveillance systems currently present in some European countries (2) and an investigation of the availability of surveillance information (3) showed that design and achievements of AHS are generally not well-documented in Europe, especially for non-notifiable diseases. There seems to be a lack of detail, consistency, transparency, and open access in both private and public sectors. Also, there is a limited use of output-based surveillance standards, which prescribe what the surveillance must achieve rather than how surveillance activities must be carried out (4). Output-based standards have been endorsed over recent years to provide more flexibility in surveillance planning and thus allow for more efficient surveillance systems. However, they require transparent and consistent sharing of information about surveillance design and achievements in order to enable assessments of equivalence.

During 2012–2015, the EU project RISKSUR (http://www.fp7-risksur.eu/) developed a series of decision support tools for the design of cost-effective risk-based AHS systems. The need for systematic documentation of design decisions was considered during the development of one of the tools: the design framework (5) (https://survtools.org/wiki/surveillance-design-framework/doku.php). The conceptual idea was that by moving toward output-based standards for surveillance and allowing greater flexibility in surveillance design, there will be an increased need for transparency about design features as well as how the activities are actually implemented. However, although the RISKSUR design tool is comprehensive, there is still a need for guidance on (i) what information is truly critical for assessing the quality of surveillance evidence and (ii) how to report the necessary information in a useful manner to decision makers and stakeholders. This challenge was partly addressed within the SANTERO project (http://santero.fp7-risksur.eu/), where the first steps toward producing a set of reporting guidelines to facilitate consistent and credible reporting of surveillance activities and their outcomes were taken. The work was subsequently followed up in the HOTLINE project (https://www.thehotlineproject.org/), funded by the European Food Safety Authority (EFSA), which aimed to facilitate harmonized assessment and reporting of disease occurrence information (6).

The overall goal of AHSURED is to produce reporting guidelines that support communication of surveillance activities in the form of narrative descriptions. This should not be confused with reporting of notifiable diseases according to certain procedures defined by a competent authority or international body like the World Organization for Animal Health (OIE).

The concept of reporting guidelines evolved from the field of evidence-based medicine and serves to improve consistency and quality of information reported in scientific journals. A few reporting guidelines directly relevant to the veterinary field are available, such as REFLECT (https://meridian.cvm.iastate.edu/reflect) and STROBE-Vet (https://meridian.cvm.iastate.edu/strobe). These recommendations target randomized controlled trials and observational studies reported in veterinary scientific literature. They are based on guidelines already developed for studies in humans (CONSORT and STROBE). Several more are available for studies in the medical field. For an overview, see https://www.equator-network.org/. However, none of these is directly applicable to AHS activities. This is possibly due to the limited use of active surveillance in human health. It can also be related to the different perspective in the need for reporting guidelines. Currently available guidelines aim at enhancing the consistent reporting of scientific studies in peer reviewed journals, and therefore have an academic/editorial journal perspective. In contrast, reporting of disease surveillance is usually carried out by governmental bodies and have a more policy-oriented perspective.

The aim of this paper is to present the first version of the Animal Health Surveillance Reporting Guidelines (AHSURED) and the process by which they have been developed. The adoption of AHSURED will promote a more consistent approach to communication of AHS activities and their outputs, for the benefit of stakeholders, trade partners, decision-makers and risk assessors.

MATERIALS AND METHODS

Reporting guidelines usually consist of (i) a checklist of items to be reported; (ii) a description/definition of each item, (iii) an explanation and elaboration document (including examples of well reported items; and, optionally, (iv) a website where the guidelines are maintained and made available. As part of the process of having the guidelines endorsed by journal editors, it is common to publish the guidelines as well as a description of the process by which they have been developed. Thus, the current manuscript outlines the development process that led to the AHSURED guidelines. An overview of the process is provided in **Figure 1**.

Provisional Checklist

Building upon the outcomes of the aforementioned projects (RISKSUR and SANTERO), a list of items that describe the building blocks of surveillance systems was initially identified. These represent elements that should be thought of and from which decisions are made during the design of a surveillance system. According to the definitions proposed by Hoinville et al. (7), a *surveillance system* was defined as a collection of various *surveillance components* which are all aimed at "describing the health-hazard occurrence and contributing to the planning, implementation, and evaluation of risk-mitigation actions."

Mirroring the structure of the RISKSUR design tool, the identified descriptive items were grouped into building block sections. That could be either at the surveillance system level, e.g., the context in which the surveillance system specifically operates, or at surveillance component level, e.g., the target population of

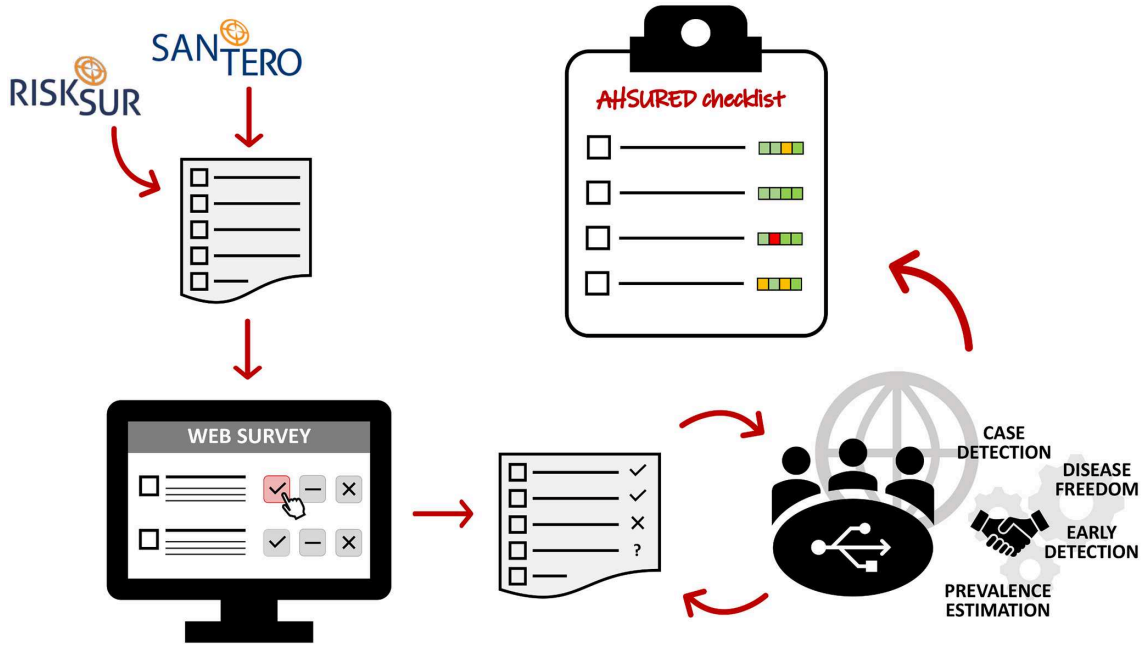

FIGURE 1 | Overview of the development process of the animal health surveillance reporting guidelines (AHSURED).

a specific surveillance component. The items to be included in each section evolved as part of the development process and are described in the results.

The RISKSUR design tool does not provide recommendations on how to present outcomes of surveillance activities. Therefore, four items were added under a "Results" section: (a) *Number of epidemiological units investigated (per stratum)*, (b) *Test results (per stratum)*, (c) *Surveillance outcomes (objective dependent)*, and (d) *Findings in relation to historical knowledge, or trend*. Item (c) means an assessment of the outcome of surveillance in relation to its objective. An example might be, for prevalence estimation, prevalence with an accompanying confidence interval. Furthermore, two sections with only one item each, titled "Interpretation," providing the conclusions about the status of the population, and "References," were added. The provisional checklist included 55 surveillance items organized in 15 sections and is available in **Supplementary Material 1**.

Survey Among Surveillance Experts

The provisional list of surveillance items was subsequently presented to international AHS experts, who were identified through the distribution list of the third International Conference on Animal Health Surveillance (ICAHS, held in New Zealand in 2017) and contacted by email. In total, 621 invitations were sent out; of these, 115 were rejected by the mailing system and 506 were delivered.

The experts were consulted through a web-based survey and asked to indicate which items should be considered critical, optional or irrelevant to report when describing animal health surveillance designs and outputs. A critical item was defined as a piece of information that is deemed very important to report in order to understand and interpret surveillance outputs. An optional item is an additional piece of information that might enhance the interpretation of surveillance results if reported, but that will not hinder such interpretation if not reported. Items deemed irrelevant usually do not pertain to a specific surveillance objective. For example, items related to timeliness of detection might be unnecessary to report when demonstrating disease freedom.

The survey included 55 questions about the proposed surveillance items, 15 note fields to document any comment and/or suggestion (e.g., insertion or deletion of surveillance items) within each section, and five additional questions describing the background of the respondent. One last question asked whether the respondent was willing to further participate in the development of the guidelines. The outline of the survey can be found in **Supplementary Material 2**.

The survey was online between 31 October and 30 November 2018, and experts could provide their answers either as individuals or as collective groups of colleagues.

Refinement and Consolidation

Starting from the provisional checklist and taking into account the insights gathered by the survey, the AHS reporting guidelines were finalized through consensus-oriented consultations limited to the group of international experts who had expressed willingness to contribute further to the process (further referred to as the core group of experts). These consultations were conducted through four thematic webinars, where the descriptive items were contrasted against example texts describing surveillance for different objectives (i.e., demonstrate

freedom, early warning, detect cases, and estimate prevalence) and disease situations (i.e., absent, sporadically present, and endemic). A fictional example of the relation between the items of a checklist for surveillance reporting guidelines and a narrative text aiming at reporting surveillance activities and their outcomes is provided in **Figure 2**.

During each consultation, the checklist was iteratively revised in the light of a new specific surveillance objective. The emphasis of the discussion was on those items where <70% of the respondents of the survey agreed on the relevance of the item, i.e., if it was critical, optional or irrelevant to report. Flexibility was provided in the process to add or modify checklist items as necessary. The items' descriptions were carefully revised, and a new objective-specific assessment of their relevance was carried out by the core group of experts. The same applied to any new item suggested by the respondents to the survey. The four thematic webinars were held between 5 February and 1 April 2019.

RESULTS

At the end of the survey period, 33 web surveys were completed by 30 individuals and 3 groups of 5–10 co-workers. The respondents were geographically distributed across several parts of the world, although the majority worked in Europe (**Table 1**). Most of the respondents had more than 10 years of experience in animal health surveillance and mainly worked for State Authorities. The competence of the respondents was broad, ranging from design, implementation and evaluation of surveillance systems, to the analysis of surveillance data and risk assessment and risk management (**Table 1**).

At least 70% of survey respondents agreed on the relevance of 40 of the 55 checklist items, which were all deemed to be critical to report. In particular: *Hazard under surveillance*; *Criteria for identification of suspicions;* and *Target unit level (unit of interest)* were judged critical by 97% of the respondents. In contrast: *Enhancements in place; When/how often are samples transferred; Who has performed the analyses; References;* and *Any other testing protocol details* scored almost 50:50 between critical and optional. Only 15 items were considered irrelevant by at least one respondent. Among these, *Institution involved and financing* was the item with the highest proportion of votes as irrelevant: 9% of the respondents. Details about the number of respondents that judged each item of the provisional list critical, optional or irrelevant can be found in **Supplementary Material 3**.

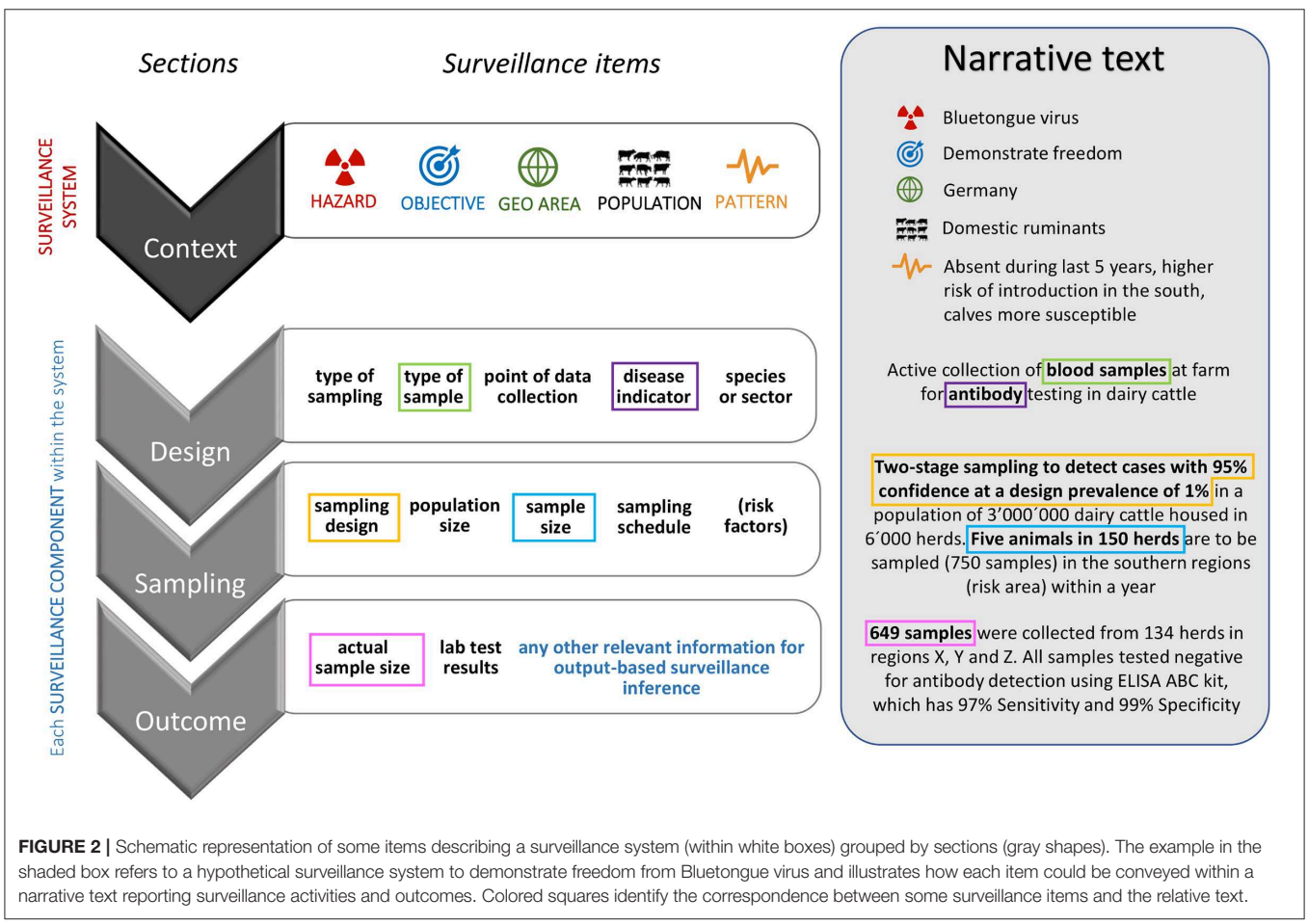

FIGURE 2 | Schematic representation of some items describing a surveillance system (within white boxes) grouped by sections (gray shapes). The example in the shaded box refers to a hypothetical surveillance system to demonstrate freedom from Bluetongue virus and illustrates how each item could be conveyed within a narrative text reporting surveillance activities and outcomes. Colored squares identify the correspondence between some surveillance items and the relative text.

TABLE 1 | Background of the respondents to the online survey.

	Individuals	Groups	Percentage of total
Total number of respondents	30	3	
From what areas of the world do you mostly have work experience?			
Africa	5	0	15%
Asia	2	0	6%
Europe	18	2	61%
Oceania	1	0	3%
North America	3	1	12%
South America	1	0	3%
What sector do you currently work for?			
Public health	0	0	0%
Ministry/government	14	3	52%
Academia	8	0	24%
Private company	3	0	9%
Other	1	0	3%
What is your current involvement in animal health surveillance? (multiple choice)			
Design of surveillance activities	18	3	64%
Evaluation of surveillance performance	16	2	55%
Reporting surveillance results at national/international level	15	3	55%
Risk assessment	12	1	39%
Risk management	7	1	24%
Implementation of surveillance activities	16	3	58%
Secondary use of data (e.g., for research purposes)	16	2	55%
Other	0	0	0%
How long have you been working with animal health surveillance?			
Less than 5 years	2	0	6%
Between 5 and 10 years	7	1	24%
More than 10 years	21	2	70%

During the thematic webinars, 10–15 international surveillance experts evaluated the various items in the light of four surveillance objectives. These were: demonstrate freedom, early warning, detect cases, and estimate prevalence. They then re-assessed the relevance of those with inconclusive results from the survey through an iterative process of active discussion and (re)voting, until simple majority was obtained. Outcomes from the discussions included the improvement of item descriptions, the addition of one item that was suggested from the survey (i.e., *Identification of surveillance components*), deletion/grouping of some items deemed redundant, and combination of some sections. This iterative process led to a consolidated checklist consisting of 40 items organized in 11 sections, which is summarized in **Table 2**. The full AHSURED checklist with the detailed item descriptions is available as a wiki at https://github.com/SVA-SE/AHSURED/wiki. The choice of a wiki format was motivated by the fact that the collegial discourse leading up to the inclusion of certain items does not end with this manuscript and refinements and improvements of the guidelines are both expected and welcomed.

DISCUSSION

The development of animal health surveillance reporting guidelines was initiated in response to the increasing need for more transparent and systematic documentation of surveillance activities and their outputs (2). The development process involved the inputs from international AHS experts, identified through the distribution list of the third ICAHS conference. While, the mailing list included more than 500 experts, the number of respondents was low, suggesting the outcome of the questionnaire may not be representative of the opinion of the entire AHS community. In addition, although the respondents were geographically distributed across all habited continents and most of them had extensive experience in the field, the gathered view mainly reflects the AHS practices in industrialized countries, primarily Europe. This could possibly overlook the reality in developing and in-transition countries, where community-based management of disease suspicions and intersectoral collaborations play a stronger role (8). Nevertheless, the possibly constrained perspectives introduced by the non-representative sample of experts involved in the development process is mitigated by publishing the reporting guidelines as a wiki. In this way, the aim is to gather further inputs from surveillance experts who were not reached in the earlier stages, and to maintain the guidelines as a living document. In fact, the currently presented guidelines are to be considered only the first version of a document that will be hopefully expanded to account for additional surveillance strategies, like for instance participatory approaches (9).

The survey as the first step of the development highlighted that some items were prone to ambiguous interpretation of their relevance, as they scored almost 50:50 between critical and optional. This could be due to an unclear description of such items or, more likely, to a real duality of their relevance. For instance, *Findings in relation to historical knowledge* could be either critical or optional to report, depending on whether surveillance activities aim at documenting disease freedom or at detecting cases of disease, respectively. To disentangle ambiguities in views, a thematic approach was successfully adopted in the second step of the development by consensus-oriented consultations. Each thematic webinar focused on one of the four main objectives of surveillance and relative disease situations (i.e., absent, sporadically present, and endemic), thus allowing a deeper and more specific assessment of the relevance of each surveillance item.

The collection of information on AHS activities is often driven by requirements from national and international reporting bodies. Such requirements often have a strong influence on how AHS is developed and implemented (4). However, there is little harmonization in this respect, which hampers the ability to assess the equivalence of information and to benchmark surveillance activity (2, 10). Notably, there is a lack of metadata standards for AHS information, which is desirable when making these data not only publicly available but also useful and unambiguously interpreted. AHSURED will not solve this issue but may inform such standards since the AHSURED guidelines can be seen as a form of metadata definition, albeit more free in their format.

TABLE 2 | Consolidated checklist* of the surveillance reporting guidelines (A detailed description of each item is provided in the actual AHSURED guidelines, available at https://github.com/SVA-SE/AHSURED/wiki).

Item \ Surveillance objective	Disease freedom	Case detection	Prevalence estimation	Early detection
1. Background				
1.1. Hazard	Critical	Critical	Critical	Critical
1.2 Geographical area	Critical	Critical	Critical	Critical
1.3. Susceptible population	Critical	Critical	Critical	Critical
1.4. Historical situation	Critical	Critical	Optional	Optional
1.5. Surveillance purpose	Critical	Critical	Critical	Critical
1.6. Surveillance objective	Critical	Critical	Critical	Critical
1.7. Risk characteristics (level, aspect of risk)	Critical	Critical	Optional	Critical
1.8. Legal requirements and actions taken as a result of findings	Critical	Critical	Critical	Optional
1.9. Institutions involved and financing	Optional	Optional	Optional	Optional
1.10. Identification of surveillance components	Critical	Critical	Critical	Critical
1.11. Population strata not covered	Critical	Critical	Critical	Optional
2. Surveillance component description				
2.1. Data collection point	Critical	Critical	Critical	Critical
2.2. Surveillance approach	Critical	Critical	Critical	Critical
2.3. Type of hazard indicator	Critical	Critical	Critical	Critical
2.4. Type of material collected	Critical	Critical	Critical	Critical
2.5. Case definition	Critical	Critical	Critical	Critical
3. Target population				
3.1. Target criteria	Critical	Critical	Critical	Critical
3.2. Population coverage	Critical	Critical	Critical	Critical
4. Enhancements				
4.1. Enhancements	Optional	Optional	Optional	Optional
5. Testing protocol				
5.1. Pooling	Critical	Critical	Critical	Critical
5.2. Screening/first test	Critical	Critical	Critical	Critical
5.3. Confirmatory/second test	Critical	Critical	Critical	Critical
5.4. Accuracy of the testing protocol	Critical	Critical	Critical	Critical
6. Study design				
6.1. Sampling design	Critical	Critical	Critical	Critical
6.2. Number of target units in the target population	Critical	Critical	Critical	Critical
7. Sampling strategy				
7.1. Sampling at the primary sampling unit level	Critical	Critical	Critical	Critical
7.2. Sampling at the secondary sampling unit level	Critical	Critical	Critical	Critical
7.3. Sample allocation at the primary and secondary levels	Critical	Critical	Critical	Critical
7.4. Risk-based allocation	Critical	Critical	Critical	Critical
7.5. Sample size	Critical	Critical	Critical	Critical
7.6. Sample collection timeline	Critical	Critical	Critical	Critical
7.7. Who collects the samples	Optional	Critical	Optional	Optional
8. Timeliness				
8.1. Time from sampling to report	Irrelevant	Optional	Optional	Optional
8.2. Time from confirmation to action	Irrelevant	Optional	Irrelevant	Irrelevant
9. Results				
9.1. Number of epidemiological units investigated (per stratum)	Critical	Critical	Critical	Critical
9.2. Test results (per stratum)	Critical	Critical	Critical	Critical
9.3. Surveillance outcomes (objective dependent)	Critical	Critical	Critical	Critical
9.4. Findings in relation to historical knowledge, trend	Critical	Optional	Critical	Optional
10. Interpretation				
10.1. Surveillance interpretation	Critical	Critical	Critical	Critical
11. References				
11.1. References	Critical	Critical	Critical	Critical

Please note that the consolidated checklist is shorter than the one initially identified. Therefore, some items mentioned in the text are not present in this table, as they have been removed or modified during the refinement process. The initial provisional checklist of 55 surveillance items can be found in* ***Supplementary Material 1**.*

Unlike existing tools promoting structured ways to design or evaluate AHS [e.g., RISKSUR design and EVA tools (5, 11), SERVAL (12), SurF (13)], AHSURED does not involve any assessment of surveillance performances, but rather aims at documenting how surveillance activities were designed and carried out. The focus of AHSURED is really on communication, through the systematic description of how the output of surveillance have been generated. In that way, the reader is informed about aspects that influence the weight that can be put to the evidence in question.

The indication of whether an item on the checklist is critical, optional or irrelevant to report for a particular surveillance objective is not prescriptive but rather a suggestion on how important that piece of information is considered to be for understanding and interpreting the outputs of surveillance. Of course, any surveillance item can be reported, regardless of its objective-specific relevance. The adoption and ongoing refinement of AHSURED will improve a concise, transparent and consistent documentation and communication of surveillance activities and their outputs. This is crucial for the benefit of stakeholders, trade partners, decision-makers and risk assessors.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

ALi and AC conceived and designed the study and wrote the first draft of the manuscript. AC implemented the web survey and summarized the results. ALi moderated the webinars. JG, GS, HS, JP, FE, JD, ALo, LE, MH, FC-A, AJ, MM-A, YV, and S-EA contributed to guidelines' refinement and consolidation. AC set up the wiki and all authors approved its content. All authors contributed to manuscript revision, read, and approved the submitted version.

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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.2019.00426/full#supplementary-material

Supplementary Material 1 | Provisional checklist of surveillance items.

Supplementary Material 2 | Outline of the web survey.

Supplementary Material 3 | Results of the web survey.

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The reviewer JS declared a past collaboration with one of the authors FC-A.

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Designing a Syndromic Bovine Mortality Surveillance System: Lessons Learned From the 1-Year Test of the French OMAR Alert Tool

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Between May 2018 and 2019, a syndromic bovine mortality surveillance system (OMAR) was tested in 10 volunteer French *départements* (French intermediate-level administrative unit) to assess its performance in real conditions, as well as the human and financial resources needed to ensure normal functioning. The system is based on the automated weekly analysis of the number of cattle deaths reported by renderers in the Fallen Stock Data Interchange Database established in January 2011. In our system, every Thursday, the number of deaths is grouped by ISO week and small surveillance areas and then analyzed using traditional time-series analysis steps (cleaning, prediction, signal detection). For each of the five detection algorithms implemented (i.e., the exponentially weighted moving average chart, cumulative sum chart, Shewhart chart, Holt-Winters, and historical limits algorithms), seven detection limits are applied, giving a signal score from 1 (low excess mortality) to 7 (high excess mortality). The severity of excess mortality (alarm) is then classified into four categories, from very low to very high, by combining the signal scores, the relative excess mortality, and the persistence of the signal(s) over the previous 4 weeks. Detailed and interactive weekly reports and a short online questionnaire help pilot *départements* and the OMAR central coordination cell assess the performance of the system. During the 1-year test, the system showed highly variable sensitivity among *départements*. This variability was partly due not only to the demographic distribution of cattle (very few signals in low-density areas) but also to the renderer's delay in reporting to the Fallen Stock Data Interchange Database (on average, only 40% of the number of real deaths had been transmitted within week, with huge variations among *départements*). As a result, in the pilot *départements*, very few alarms required on-farm investigation and excess mortality often involved a small number of farms already known to have health or welfare problems. Despite its perfectibility, the system nevertheless proved useful in the daily work of animal health professionals for collective and individual surveillance. The test is still ongoing for a second year in nine *départements* to evaluate the effectiveness of the improvements agreed upon at the final meeting.

Keywords: syndromic surveillance, cattle, mortality, OMAR project, France, field test

INTRODUCTION

In France, as in many countries, animal disease control is based on a combination of active (planned) and passive (event-driven) surveillance programs and control measures, in order to achieve freedom or controlled status for the main regulatory diseases. In a context of very low prevalence or absence of disease, these traditional surveillance measures are reaching their limits in terms of effectiveness and cost/benefit ratio. In addition, in view of the increased risk of emergence, the effectiveness of traditional surveillance programs in detecting emerging diseases or the introduction of exotic diseases is questionable, as they have not been designed for this purpose. On the other hand, the evolution of agricultural practices in recent decades with the systematic recording of livestock data and the development of powerful tools for the management and analysis of large databases allows for the cost-effective use of regularly recorded information for livestock monitoring. This context has been favorable to the development of syndromic surveillance (SyS) in animal health (1).

SyS is usually defined as the real-time (or near real-time) and automated collection, analysis, interpretation, and dissemination of health-related indicators, to reveal early changes in the health status of a population or to identify the impact (or absence of impact) of potential human or veterinary public health threats that require effective public health action (2, 3). This non-specific, data-driven surveillance system is developed as a more cost-effective and earlier warning system than traditional systems (passive and active surveillance systems), but can also complement them (4–6).

Nevertheless, although SyS data can be a valuable tool for public and animal health practices, few systems are currently operational in animal health (7). Despite the substantial number of methodological developments involving SyS, there are few fully operational SyS systems and few field feedback experiences about SyS systems especially in animal health (8–11). This contradiction reveals the difficulties in (i) convincing public or private entities to fund the evaluation of these systems in the field, (ii) finding the business model for operational deployment, and (iii) designing these systems in such a way that they are not limited to large health services that have sufficient expertise and staff to review alarms. Evaluations of human SyS systems have shown that the usefulness of the results depends highly on the expertise of human resources locally (10, 12, 13). When designing SyS systems, little attention is generally paid to how easily local health professionals can exploit the results, particularly because they may have different levels of expertise. The ease of result interpretation is a limiting factor in the value of SyS systems, because national or regional animal health services are often too far removed from the local level for satisfactory use of the results.

In France, we are developing a SyS system for bovine mortality, called OMAR (*observatoire de la mortalité des animaux de rente*), to identify significant excesses of deaths in the bovine population potentially linked to emerging diseases, epidemiological changes in the pattern of enzootic diseases, or other health events. We designed a pilot application of the system within a collaborative, multi-stakeholder working group of the French platform for epidemiological surveillance in animal health (ESA Platform, https://www.plateforme-esa.fr/). We paid particular attention to its use in the field, adapting the frequency and format of reports to the organization of animal health management units, their expectations, human resources, and expertise.

Since 2018, we have implemented a 1-year pilot phase to (i) test, in real-life situations, the system's design; (ii) assess its global performance (sensitivity, specificity, precocity, utility); (iii) evaluate the human and financial resources required; and (iv) identify any changes needed in terms of regulatory support to ensure the normal functioning of the system. The purpose of this article is to describe the methodology and design of our SyS system and to present the results of the 1-year test performed in real conditions. We also discuss the advantages and limitations of the system from the point of view of local and national animal health services.

MATERIALS AND METHODS

We use Toad for MySql 7.5.0.966, MySQL, R 3.3.3, and RStudio 1.1.383, to manage and analyze the data, and Perl 5.26.1 and the Windows tasks planner for automated task.

Organization of the 1-Year Test Period

A national call was launched in January 2018 to select a limited number of volunteer *départements*. These pilot *départements* had to demonstrate the mutual commitment of the three main local animal health services [the departmental animal health office (*service vétérinaire départemental*, or DDecPP), departmental farmers' animal health service (*Groupement de défense sanitaire*, or GDS), and departmental technical grouping of vets association (*Groupement Technique Vétérinaire*, or GTV)] to facilitate field investigations, if needed. In addition, regional voluntary services could participate in support of departmental services. Ten departments expressed interest in participating and met the criteria: *Corrèze* (number code 19), *Côtes-d'Amor* (22), *Creuse* (23), *Eure-et-Loire* (28), *Indre* (36), *Jura* (39), *Saone-et-Loire* (71), *Haute-Savoie* (74), *Vendée* (85), and *Yonne* (89) (**Figure 1**). Three of them were involved in the Omar project since its inception (*Corrèze*, Côtes-d'Amor, and *Yonne*) (14).These *département*s represented the various cattle breeding contexts in France: (i) low, intermediate, and high cattle density areas (**Figure 2**); (ii) traditional and intensive breeding practices (including mountain pasturing); and (iii) dairy and beef production types (15).

The national OMAR coordination cell (OMAR-NC) organized a 1-day meeting in April 2018 to present the system, the objectives, and expectations of the test phase and to train the health professionals (report reading method, extraction of main information, etc.). About 40 people from the three local animal health services in each volunteer department and associated regional levels attended the meeting. The coordination and organization of the various services within each *département* was unregimented, but a leader was designated in each *département* as the main departmental coordinator and in charge of reporting to the OMAR-NC. A general scheme for report interpretation (**Figure 3**) and a short online questionnaire

(**Supplementary Material S1**) helped standardize the weekly report. A mid-term meeting was held in November 2018 to present and discuss local organizations (who, how, time spent, etc.), uses of the results (how, for what, etc.), and limitations in each *départements*. This meeting was also used as an opportunity to adapt and improve the system during the period of test, if necessary. A final meeting was held in June 2019, to discuss the statistical results, the feedback, and improvements needed and to conclude on the interest (or not) in continuing to test the system.

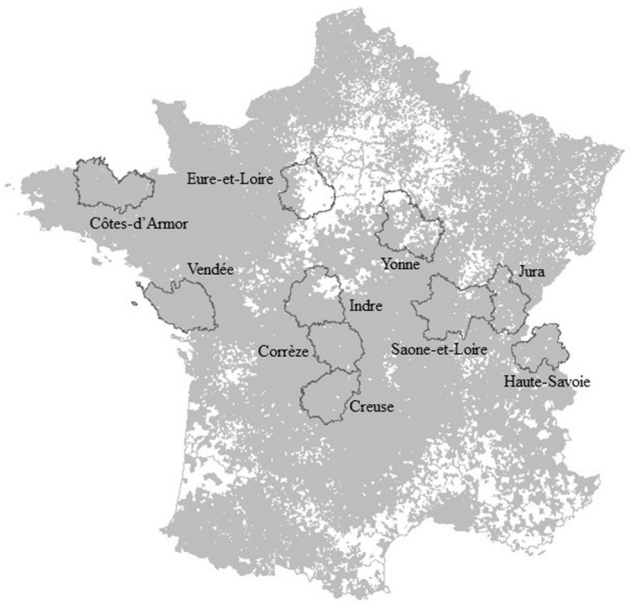

FIGURE 1 | Code number and names of the 10 pilot *départements* (dark gray line) and surveillance areas (gray) analyzed during the 1-year test period.

Data Sources

The Fallen Stock Data Interchange (FSDI) database forms the basis of the system. This database centralizes the information of the removal visits transmitted daily by each of the 56 renderer collection points in mainland France. The centralization has been all-inclusive since January 2011. We used information on

- the identification number of the holding for which the removal is requested and its location at the *commune* scale (smallest French administrative unit, hereafter referred to as "municipality");
- the number of cadavers, their identification number(s), age group, weight, and location (municipality); and
- the date of the removal request (considered as the date of death) and date of removal.

This information is of high quality because it is used for payment of rendering fees by the French dead-on-farm animal service.

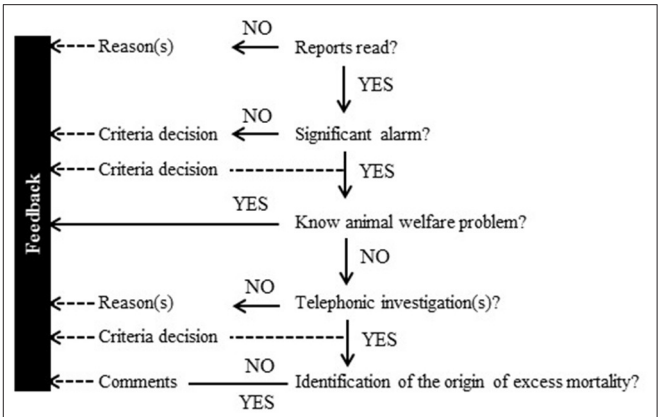

FIGURE 3 | General scheme for report interpretation and feedback.

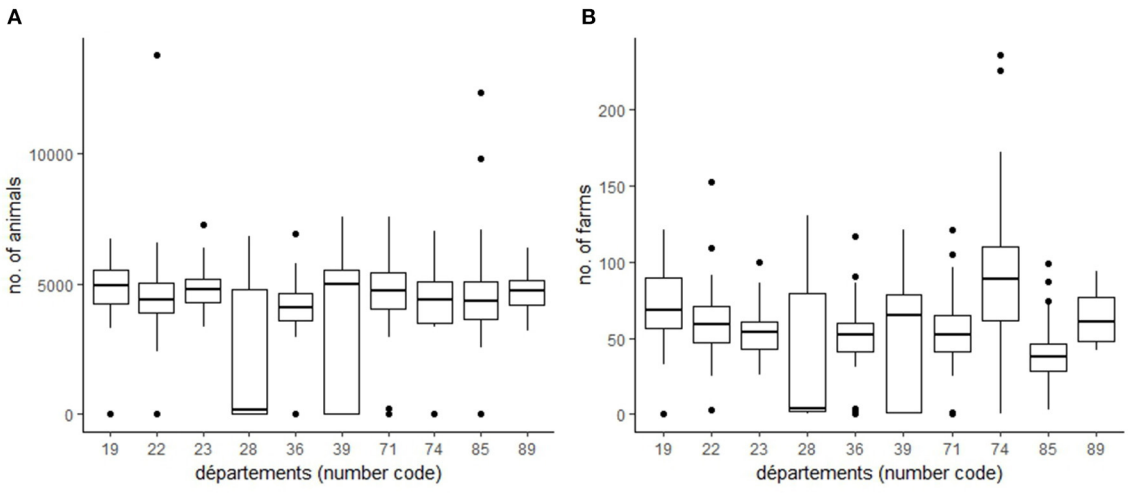

FIGURE 2 | Distribution (boxplot) of the annual number of animals **(A)** and farms **(B)** of surveillance areas in each pilot *département*.

Our SyS also uses the National Cattle Register (NCR) database because it contains all the information about the holdings (identification number, location, species kept) and animals (sex, breed, movement). It also provides demographic information (number of holdings, average number of females 2 years and older, number of cattle slaughtered, and births) for each farm and municipality (15). It also serves to cross-check the FSDI database because the two databases are not interconnected, and data such as farm identification numbers are sometimes not updated in the FSDI database.

Spatial and Temporal Units

To manage the heterogeneity of cattle density and because the precise geolocation of farms is not available, we merged the 35,756 French municipalities into 11,377 larger spatial units. This pooling scheme ensures a sufficient number of animals and deaths in each area to limit statistically random variation due to small sample sizes. It also homogenizes, as much as possible, the number of animals between spatial units to obtain an almost identical alarm probability for each spatial unit and for an equivalent health event. Our algorithm merges contiguous municipalities with cattle until each new spatial unit reaches an annual average population of at least 3,000 animals (equal to the average number of females 2 years and older and number of cattle slaughtered, calculated from the NCR) and/or there are no more municipalities or units to merge. The algorithm primarily merges municipalities within the same *département* but does not limit the merging to within *département*. Some of the spatial units thus occupy several departments. The algorithm uses the geographical file from the French National Institute of Geographic and Forest Information and demographic information from the NCR. It iteratively constructs a new file with a new data table of demographic information and list of the municipalities in each new spatial unit. Detailed aggregation algorithm rules are available in **Supplementary Material S2**.

Although the data are available daily, our SyS system operates on a weekly basis to ensure a sufficient number of deaths in each surveillance area and to be consistent with the human resources that can be assigned to the system at departmental and national levels. We aggregate the number of deaths by ISO week, from Monday to Sunday, using the date of the removal request.

Analysis

The OMAR system is designed to identify sudden increases in mortality (a one-time significant difference between observed and predicted deaths) as well as slow deviation, i.e., systematic deviation from the expected value, which is not significant over a week but significant over several consecutive weeks. To do so, we use five detection algorithms: the Holt-Winters algorithm (16), "historical limits" algorithm from the Center for Disease Control (17), and Shewhart algorithm for sudden increases and the exponentially weighted moving average (EWMA) and cumulative sum (CUSUM) algorithms for slow deviations (18).

Every Thursday of week w_{+1}, our OMAR tool evaluates the mortality in the immediately previous week w. The analysis is scheduled for 00:30 am, and the results are generally available around 8:00 am. The analysis follows classical steps for SyS systems: (i) preparing the data and time series (TSs), (ii) cleaning the TSs from historical anomalies, (iii) predicting the expected values, and (iv) comparing expected and observed values to detect potential excesses.

The particularity of our system is that we record all the elements (timetable, data, and results of the analysis), in an S4 object oriented system, adapted from R codes and the approach used in the Vetsyn package developed by Dorea et al. (19). Details of our S4 object are provided in **Supplementary Material S3**.

Step 1: Data Processing

Data on cattle removals from January 2011 to week w are extracted from the FSDI database and prepared for the analysis according to the following steps:

- Exclusion of attempted removals (cadaver not found, i.e., number of removal animals and weight equal to 0)
- Cross-checking of holding identification with the NCR database and identification of holdings that are not farms (veterinary clinics and schools, laboratories, slaughterhouses, etc.) to distinguish them
- Re-coding of age groups into four categories (under 21 days, 21 days–under 6 months, 6 months–under 24 months, 24 months and over)
- Correction of the removal municipality identifier to ensure perfect consistency with geographical files due to regular administrative changes in French municipalities
- Coding municipalities into new surveillance area
- Coding the date of the removal request into its ISO week

The number of deaths is then aggregated by ISO week and surveillance area as defined in the *Spatial and Temporal Units* section. There were as many TS as there were surveillance areas each week.

To gain calculation time, only TSs having at least one death recorded in the FSDI over the last 4 weeks (w_{-3}, w_{-2}, w_{-1}, w) are kept for analysis.

From this point, each TS_w is decomposed into four sub-times series (STS): $STS_{w-3} = t_0$ to t_{w-3}, $STS_{w-2} = t_0$ to t_{w-2}, $STS_{w-1} = t_0$ to t_{w-1}, and $STS_w = t_0$ to t_w, with t_0 = 2011-W01 for the historical limit and $t_0 = w - (5 * 52)$ for the other algorithms.

Step 2: Creation of Cleaned Baselines

To clean the STS, the observed values are compared to the value predicted by a general linear model (GLM) and those above the 95% CI are lowered to this value.

For each STS, the GLM is fitted on the entire TS (i.e., from 2011-W01). Formulae tested includes *a minima* a sinusoidal annual seasonality. Linear trend and autoregressive components over the last 4 weeks are also tested. The selection of the best formula is based on the Akaike information criterion or quasi-Akaike information criterion depending on the GLM family selected. To determine the GLM family, the model is first fitted to a Poisson family and the best formula is tested for the over dispersion. In case of over dispersion, a generalized Poisson family is tested; if there is a convergence problem, a quasi-Poisson GLM is tried, and if there is still a convergence problem, a negative binomial GLM is used.

Step 3: Calculation of the Expected Mortality for Week x (x = w_3…w)

Three methods are applied to calculate the expected mortality, depending on the detection algorithm:

- For the control charts (EWMA, CUSUM, and Shewhart algorithms), the expected mortality is estimated from the predicted value of the GLM using the family and formula selected in the cleaning step (Step 2). The model is rerun on the cleaned baseline of the last 5 years. A 4-week guard is applied to limit contamination of the past by potential recent health events.
- For the Holt-Winters algorithm, a 5-year baseline with a 4-week guard is also used. The values of the smoothing parameters for weight (α), trend (β), and seasonality (γ) are determined first testing the optimizer implemented in the Holt-Winters function of the stats R package (20); in case of failure, β is set to 0.1 or removed if no trend is detected by the GLM, and α and γ are estimated by the optimizer; in case of failure again, α, β, and γ are set to 0.1.
- For the historical limits algorithm, the expected value for the last 4 weeks is calculated from the average values observed over 12-week periods (three blocks of 4 weeks) centered on the index of the week of interest (for index week x, the period is x – 7 to x + 4) over the last Y complete years ($Y \geq 5$) (21).

Step 4: Detection of Excess Mortality in Week x (x = w_3…w) and Signal Scoring

For each STS and algorithm, the observed value in week x is compared to the upper confidence limit predicted by the algorithm at the threshold T. Seven upper confidence limits are used to obtain seven thresholds T and score the signal from low (1) to high (7) excess mortality.

For the Holt-Winters and historical limits algorithms, the observed value is compared with the upper confidence limit predicted by the algorithm at the threshold T. Seven values of SD are used from 1.65 to 3.25 to obtain seven thresholds T and score the signal from low to high excess mortality.

The control charts are applied to the residuals of the GLM due to the seasonality of mortality on French farms linked to birthing seasons and farming practices. The Shewhart method is parameterized with the default value of the qcc R package with k (the number of SDs allowed above the average), set to 2 and a classical calculation of the SD. The EWMA algorithm is parameterized to detect low increases in mortality with the constant adjustment λ set to 0.2 to give more weight to the oldest values. For the CUSUM method (h the amount of shift to detect in the process) measured in SEs is set to 1. For the three control charts, seven values of SD from 2.33 to 3.75 are used to obtain seven thresholds.

The system gradually compares the observed mortality value with each threshold of each algorithm and increase its score by +1 at each exceeded threshold. At the end of the process, each algorithm has a signal scored from 0 (no statistical excess mortality = no signal) to 7 (the observed value exceeds the seventh upper limit).

Steps 2′, 3′, and 4′ for TS With a Median Number of Deaths Under One

The system makes use of the location of death, which is not always the location of the holding, because some of the surveillance areas do not have a (permanent) bovine population. In some of these areas, mortality cases are rare, but monitoring them may be of great interest to detect, for example, seasonal health events that cause grouped cases of mortality, such as anthrax in mountain pastures. We use a very basic system to detect excess mortality for STSs with a majority of zeros (median under one): no cleaning or any detection algorithm is applied, but the observed data are compared to set values ranging from 3 to 9 to score the signal from 1 to 7.

Step 5: Alarm Classification Severity

To limit the sensitivity of the analysis, increase its specificity, and help identify excess mortality (alarm) that requires follow-up or investigation, the OMAR system classifies the severity of the excess mortality according to

- the number of algorithms with a score above 0: high consistency between the results of the different algorithms indicates high likelihood of the alarm (i.e., the excess mortality);
- the signal scores for week *w* for each algorithm and type of algorithm: increases in the global scores indicate increases in excess mortality;
- the change in signal scores over the last 4 weeks to identify excess mortality in the process of worsening; and
- the level of excess mortality and its change over time to identify worsening excesses and/or very significant mortality excesses.

We thus evaluated 25 non-exclusive criteria based on a 2-year retrospective analysis of real data in five representative *départements* (**Table 1**). We classified them into four categories, considering that the less frequent the criterion, the more serious the alarm. We obtained 3 low severity criteria, 6 medium severity criteria, and 10 high severity criteria (**Table 1**). Three criteria were not included because they were too rare. All other results were considered very low severity. For each area with an alarm, when different severity criteria were present, we reported the severity of the alarm corresponding to the highest severity criterion.

Reports

Each week, the system produces one national and one departmental report for each pilot *département*, regardless of the results of week *w* (with or without alarms). Reports are automatically uploaded to the secure https site managed by ANSES. In parallel, an email is automatically sent to a predefined list of recipients in each *département*, indicating the availability of the reports and summarizing the results (number of areas with an alarm and number of areas per alarm severity).

Départemental Report

The departmental report gives the results for the surveillance areas within the *département*, and for any overlapping areas (see the "*Spatial and Temporal Units*" section). It contains

TABLE 1 | Criteria used to classify the severity of excess mortality: results of a 2-year (2016–2018) retrospective analysis in 277 surveillance areas from five French *départements* (Côtes-d'Armor, Corrèze, Puy-de-Dome, Vosges, and Yonne).

Criteria	Average number of surveillance areas concerned per *département*	
	Per week	Per year
a. HW + HL + SH score > 14	0.9	9
b. HW + HL + SH score > 14 or EW + CU score > 7	33.1	344
c. HW + HL + SH score > 14 and EW + CU score > 7	0.1	1
d. HW and HL and SH scores > 0	2.5	26
e. EW and CU scores > 0	37.7	392
f. (HW and HL and SH) or (EW and CU) score > 0	40.1	417
g. (HW and HL and SH) and (EW and CU) scores > 0	0.4	4
h. HW + HL + SH score stable or increasing over 4 weeks	4.2	42
i. HW + HL + SH score increasing over 4 weeks	0.3	3
j. EW + CU score stable or increasing over 4 weeks	2.3	24
k. EW + CU score increasing over 4 weeks	0.3	3
l. Excess mortality ≥10% over 4 weeks	8	81
m. Excess mortality ≥20% over 4 weeks	3.2	33
n. Excess mortality ≥30% over weeks	1.7	18
o. Excess mortality ≥40% over 4 weeks	1	10
p. Excess mortality ≥50% over 4 weeks	0.5	5
q. Excess mortality ≥60% over 4 weeks	0.3	3
r. Excess mortality ≥70% over 4 weeks	0.1	1
s. Excess mortality ≥80% over 4 weeks	0.1	1
t. Excess mortality ≥90% over 4 weeks	0	0
u. Excess mortality ≥10% and increasing over 4 weeks	0.6	5
v. Excess mortality ≥20% and increasing over 4 weeks	0.1	3
w. Excess mortality ≥30% and increasing over 4 weeks	0	1
x. Excess mortality ≥40% and increasing over 4 weeks	0	1
y. Excess mortality ≥50% and increasing over 4 weeks	0	0

HW, Holt-Winter; HL, historical limits; SH, Shewhart; EW, EWMA; CU, CUSUM.

Annual average frequency per département and respective alarm severity level.

<5	*[5-25]*	*[26–50]*	*>50*	*Excluded*
High	*Medium*	*Low*	*Very low*	

information not only for assessing the severity and degree of the excesses mortality, but also for their field investigation. Detailed confidential information on cattle mortality in farms in alarm areas is provided, but access to this information is limited to authorized health services (DDPP and GDS).

The departmental report consists of three files with different levels of confidentiality.

a) Summary file

This is an interactive (leaflet) map providing a visual report on mortality in the *département* and in adjacent surveillance areas (**Figure 4A**). It shows areas color-coded by alarm severity (green = very low, yellow = low, orange = medium, red = high) and also provides summary information in display boxes that appear when the map is clicked on the following:

- The departmental display box gives the total number of areas in the *département*; number of areas with an alarm during the weeks *w*, w_{-1}, w_{-2}, and w_{-3}; number of areas per alarm severity for week *w*; and highest score by algorithm type (fast/slow) for week *w* (**Figure 4B**).
- For areas within the *département* with an alarm during week w, the display box provides the area identifier (ID), number of cattle farms, number of cattle farms with mortality during week *w*, number of cattle deaths during week *w* (total and per age groups), and score per algorithm type for weeks *w*, w_{-1}, w_{-2}, and w_{-3} (**Figure 4C**).
- For areas within the *département* that had an alarm during the previous 3 weeks and no longer had a signal during week *w*, the display box indicates the score per algorithm type for weeks w_{-1}, w_{-2}, and w_{-3}. These areas are shaded in gray on the map (**Figure 4A**).

Because this file does not contain confidential information, it is accessible to all animal health stakeholders involved in the testing phase in each pilot *département*.

b) Detailed file

Containing confidential information, the access to this Excel file is restricted to authorized animal health services in each pilot *département*. It gives detailed information on cattle mortality per area and farm during the last 4 weeks, in six sheets (**Supplementary Material S4**):

- The "Alarm w" sheet gives information on areas with an alarm for week w. We detail the alarm identification number (ISO week + area identification number); area identification number; number of animals and farms; number of farms with mortality; number of deaths (total and per age group); criteria that led to the alarm classification severity for week *w*; number of algorithms with a score over 0 for each weeks *w*, w_{-1}, w_{-2}, and w_{-3}; percentage and minimum and maximum number of the excess mortality; and score for each detection algorithm for weeks *w*, w_{-1}, w_{-2}, and w_{-3}.
- The "Alarm −1 to −3" sheet provides information on the area for which an alarm was detected over the previous 3 weeks w_{-1}, w_{-2}, or w_{-3} during the reanalysis in week *w*; information is the same as that of "Alarm w" sheet, except for the severity criteria which are not calculated.
- The "Farms w" sheet provides information on farms with mortality in area with an alarm during week *w* to help identify the source of the excess mortality. For each farm having requested a removal, we detail the alarm and area identities; the identification number, name, municipality, and group type (15) of the farm as recorded in the NCR database; the identification number, name, and municipality of the farm as recorded in the FSDI database; the municipality of removal; the standardized mortality ratio calculated for the last 1-year period (from the NCR database); average number of animals in the last 1-year period (from the NCR database); number of removals during week *w* (total and by age group); number of removals during the last 4 weeks and for the same 4-week period but the previous year; and identification number of

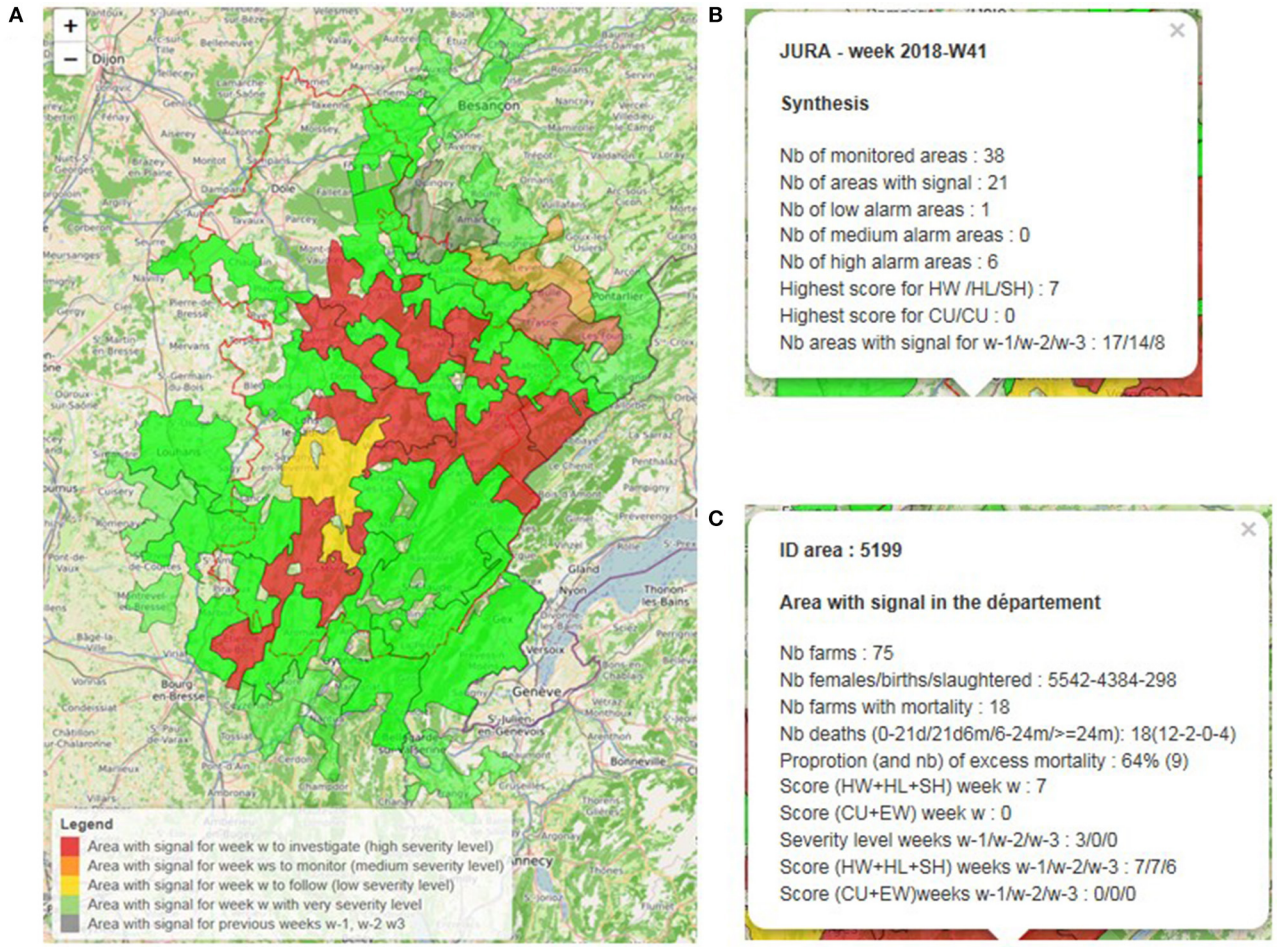

FIGURE 4 | Example of a departmental summary report: results for Jura (39) for week 2018-W41 showing global map **(A)**, departmental display box **(B)**, and area display box **(C)**.

the health veterinary (veterinary in charge of the control of regulatory diseases in the herd).

- The "Farms −1 to −3" sheet provides the same information as the "Farms w" sheet, but for farms in areas with an alarm during the previous 3 weeks w_{-1}, w_{-2}, and w_{-3} upon reanalysis. The mortality per 4-week blocks is not provided.
- The "Area info" and "Municipality info" sheets give information as well as links between areas (spatial units obtained from the aggregation algorithm) and municipalities (real administrative unit).

c) Follow up file

This interactive html map (**Figure 5**) facilitates the follow-up of the alarm area over time. It is updated each week with the result of week w and shows week by week the alarm areas per severity. A toolbar is available to scroll manually or automatically through each week. It helps animal health services to monitor the spatio-temporal dynamics of excess mortality and to identify worsening patterns (increase in severity over time, progressive grouping of alarm areas, and excess mortality spatial shifts through the *département,* etc.). It is accessible to all animal health stakeholders involved in the testing phase of each *département*.

National Report

The national report is similar to the follow-up report of the departmental file (html map, **Figure 6**) but extended to the national level. It provides an overview of the spatio-temporal changes in bovine mortality week by week across all of mainland France. It is made available to the OMAR-NC and national rendering companies, so that they can have an overall quantitative view of mortality and validate their observations.

RESULTS AND DISCUSSION

The 1-year test period (hereafter called "test period") began on 21 May 2018 (week 2018-W21) and ended on 19 May 2019 (week 2019-W20).

Before the beginning of the test, we checked the time it took for data to be reported in the FSDI database to assess

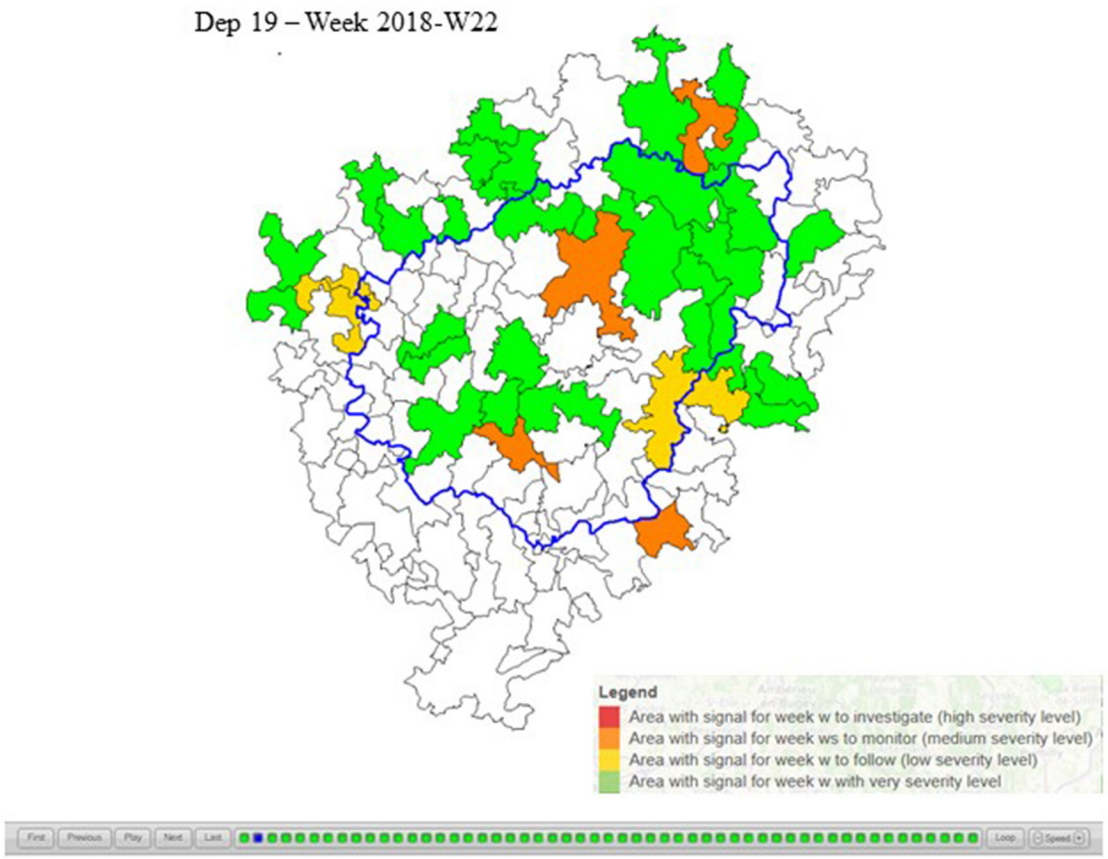

FIGURE 5 | Departmental follow-up report for Corrèze: image for week 2018-W22.

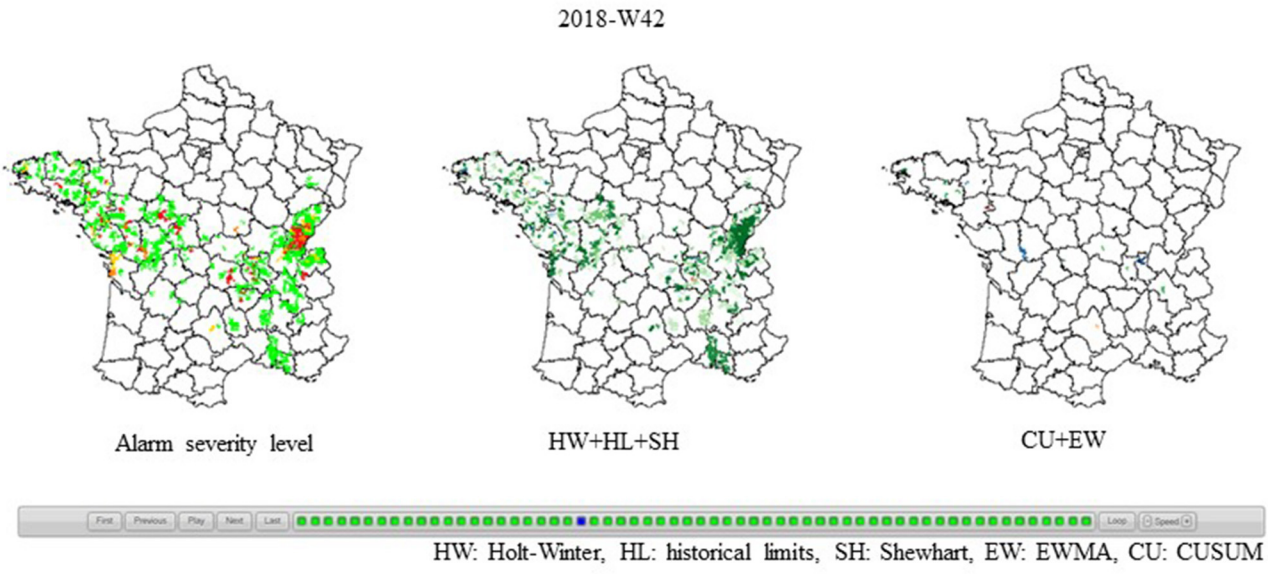

FIGURE 6 | Example of the national report: results for ISO week 2018-W42, per alarm severity (**Left**), global score [from 1 (light green) to 21 (dark green)] for algorithms detecting sudden increases (**Middle**), and global score [from 1 (light green) to 14 (dark green)] for algorithms detecting slow increases (**Right**).

the reality of "real-time" monitoring and determine the best day of week for analysis. Legally, the renderers have 2 full days after the request to collect the cadavers and a maximum of 7 days after the removal visit to report the information to the FSDI. Nevertheless, the analyses revealed longer delays in full data reporting with high variation among the collection centers and thus *départements* (generally, one or two different centers operate in each *département*) (delay distribution is available in **Supplementary Material S5**). We present and discuss the results in light of this situation.

Results From Data and Statistical Point of View

We reanalyzed every week of the previous 4 weeks to take into account the ongoing updating and correction of data in the FSDI, as well as delays in data transmission. After discussion, we chose Thursday as the best compromise between reporting time and human resources.

Among the 11,377 surveillance areas defined by the spatial aggregation of municipalities, 8831, including 435 with cattle, had no mortality recorded over the period, and were not monitored. This high percentage of areas without animals (74%) is the due to our merging method (municipalities without animals cannot merge and stay small) and the heterogeneity of cattle density in France (**Figure 1**).

Over the test period, we analyzed 137,056 TS including 26,995 TS in the pilot *départements*. These TSs involved 2932 surveillance areas including 157 areas without animals or farms, and 553 areas located in pilot *départements*. Among the TSs, <5% had a median number of deaths under one. The number of TS analyzed each week was relatively constant over time, between 2,586 and 2,707, and the majority of surveillance areas showed mortality recorded every week (85%, $n = 2{,}492$). Similar results were observed in pilot *départements*, but the number of TSs weekly analyzed varied greatly among them due to the heterogeneity of the number of surveillance areas, linked to cattle and farm density (**Figure 2**) and mortality observed at the time of analysis (**Table 2**).

Due to our inclusion criteria in the analysis process (TSs with mortality over the previous 4 weeks), 46% of TSs analyzed ($n = 63{,}239$) had no mortality recorded for week w at the time of analysis (**Table 2**). Among the 73,817 TSs having mortality during week w, no excess mortality was detected for 86% of them ($n = 63{,}536$). We identified very low excess mortality severity for 11% of TSs ($n = 8{,}308$), low severity for 1% ($n = 766$), medium severity for 0.5% ($n = 384$), and high severity for 1% ($n = 823$) (**Table 3**). Very low and low level severity alarms mainly originated from historical limit algorithm, known to be very (too much) sensitive (17). Nevertheless, this algorithm has proven its interest in monitoring major diffuse mortality phenomena with very low local excess mortality in France (Sala C, personal communication). **Table 1** provides an overview of the global high sensitivity of the system. The number and proportion of TSs per severity level varied weekly (**Figure 7**). Similar results were observed in pilot *départements*, but the weekly number of areas with an alarm varied greatly among *départements* (**Table 3** and **Figure 7B**). We detected excess mortality only once in Eure-et-Loire (28), but in Creuse (23), Saone-et-Loire (71), and Indre (36), we observed a high number of TSs with an alarm (20%).

We first assumed that the difference in results among the pilot *départements* was due to the heterogeneity of demographics (more alarms in areas of high cattle density) and data transmission (fewer alarms in areas with longer reporting time), but this hypothesis was not strong enough to explain the low number of alarms obtained in Eure-et-Loire. When preparing the final meeting, we re-extracted the mortality from the FSDI database from the beginning of the test period to obtain the real mortality, to compare it with the mortality available at the time of the analysis (hereafter called "observed mortality"). This comparison revealed that the delay in data reporting involved not only time but also the number of deaths, leading to significant under-reporting of the mortality at the time of analysis (**Table 2**). Although real mortality was relatively constant over time, the amount of information available at the time of analysis varied greatly among areas and weeks (**Supplementary Material S6**). We thus observed very high variability between pilot *départements*, and no data had been reported at the time of analysis in Eure-et-Loire. This explains the absence of signals during the test period in this *département*. In fact, the data reporting delays had a major influence on the detection of excess mortality and alarm severity, especially for high severity (**Figure 8**). Nevertheless, the amount of data available in pilot *départements* was generally higher than that observed at the national level.

TABLE 2 | Distribution of the percentage of deaths reported at the time of analysis at the national and departmental levels (n = number of surveillance areas).

	France ($n = 2932$)	***Départements number* codes* ($n = 553$)**									
		19	**22**	**23**	**28**	**36**	**39**	**71**	**74**	**85**	**89**
Minimum	0	0	0	0	0	0	0	0	0	0	0
1st quartile	0	0	60	63	0	50	23	50	0	56	0
Median	31	29	80	89	0	80	67	82	73	80	0
Mean	41	38	75	80	3	73	58	71	58	76	13
3rd quartile	83	71	100	100	0	100	92	100	92	100	17
Maximum	800	400	775	300	100	200	162	229	233	600	133

**19 Corrèze, 22 Côtes-d'Amor, 23 Creuse, 28 Eure-et-Loire, 36 Indre, 39 Jura, 71 Saone-et-Loire, 74 Haute-Savoie, 85 Vendée, 89 Yonne.*

TABLE 3 | Global results over the test period at the national level and per pilot *départements*: number of times series (TS) analyzed and repartition per alarm severity (percentage among TS with mortality).

	France	*Départements* number codes**									
TS*		**19**	**22**	**23**	**28**	**36**	**39**	**71**	**74**	**85**	**89**
Total	137,056	2,763	4,592	3,484	783	2,361	1,766	4,829	1,192	4,851	1,196
No mortality	63,239	1,118	127	73	737	133	397	515	352	172	813
No alarm	63,536 (86)	1,479 (90)	3,933 (88)	2,646 (78)	45 (98)	1,785 (80)	1,157 (85)	3,395 (79)	685 (82)	4,235 (91)	332 (87)
Very low severity	8,308 (11)	147 (9)	457 (10)	602 (18)	1 (2)	347 (16)	160 (12)	612 (14)	132 (16)	401 (9)	38 (10)
Low severity	766 (1)	11 (1)	32 (1)	70 (2)	0 (0)	39 (2)	19 (1)	97 (2)	8 (1)	19 (0)	3 (1)
Medium severity	384 (1)	5 (0)	7 (0)	29 (1)	0 (0)	18 (1)	6 (0)	70 (2)	2 (0)	7 (0)	4 (1)
High severity	823 (1)	3 (0)	36 (1)	64 (2)	0 (0)	39 (2)	27 (2)	140 (3)	13 (2)	17 (0)	6 (2)

**Some time series are common to several départements.*

***19 Corrèze, 22 Côtes-d'Amor, 23 Creuse, 28 Eure-et-Loire, 36 Indre, 39 Jura, 71 Saone-et-Loire, 74 Haute-Savoie, 85 Vendée, 89 Yonne.*

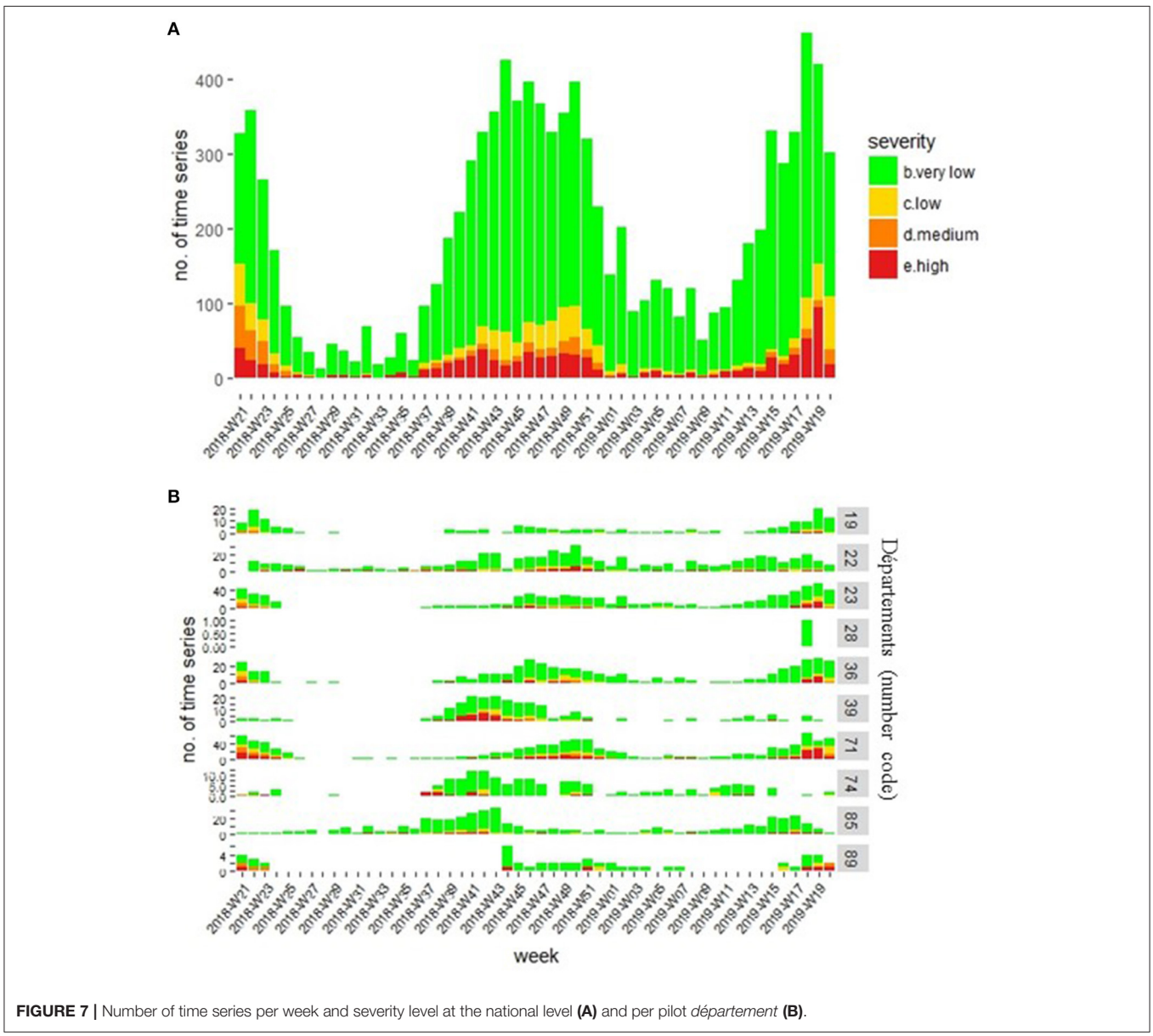

FIGURE 7 | Number of time series per week and severity level at the national level **(A)** and per pilot *département* **(B)**.

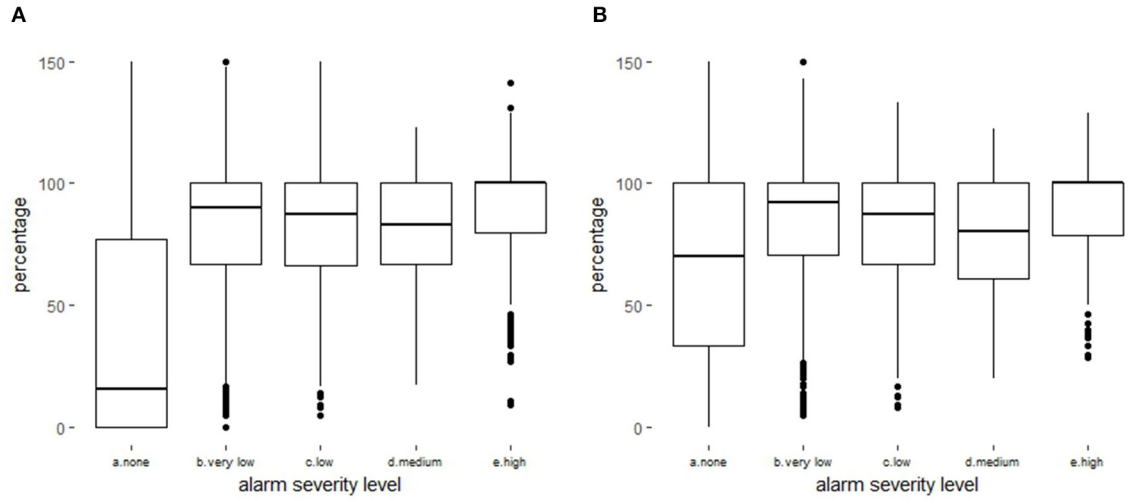

FIGURE 8 | Distribution (boxplot) of the percentage of the deaths available at the time of analysis according to the severity of the alarm at national level **(A)** and in pilot *départements* **(B)**.

Finally, we observed that real reporting times had a limited impact on the number of TSs analyzed. We calculated that only 729 TSs (0.5%) were not analyzed due to the absence of observed mortality.

Results and Experience From Animal Health Services

The objectives were to draw up a general flowchart, to evaluate the effectiveness of the reports, and the advantages, drawbacks, and performance of our SyS system. An important point was the estimation of the time to consult and interpret the reports for the calculation of the financial resources required by the system. Due to insufficient human resources at the local level and the lack of financial and legal support, we limited the work of animal health professionals to consulting and interpreting reports, telephone surveys on alarms of interest (if possible), and weekly feedback via a short online questionnaire. During meetings, we also asked for feedback on the local organization, difficulties, and the usefulness of the system.

Organization at the local level was similar in the 10 pilot *départements*, with one person in charge of interpreting the report, carrying out the surveys and responding to the questionnaire. In the absence of the leader, an alternate did the work. In most *départements*, the GDS was the leader, possibly alternating with the DDecPP. Weekly results were discussed with all the other animal health stakeholders, as necessary, to identify the source of the excess mortality.

Between 4 June 2018 and 29 May 2019, 343 of the 520 questionnaires expected were filled out, with high variability among *départements* (**Figure 9A**). The lack of feedback was attributed to unexpected limitations in human resources (sick leaves), high workloads with variable local human resources, and the requirement to complete the questionnaire immediately, whereas the interpretation of the results and investigations could take several days.

The 343 responses indicated that the reports were consulted in their totality (summary and detailed reports) in 87% of the cases. The two reasons for not consulting the reports were the absence of alarm areas (56%) and the low level of the severity of the alarm (44%). Lack of time was never mentioned, but the lack of time to complete the questionnaire may have biased the responses. As initially estimated, the animal health service needed <30 min to analyze the reports in 90% of cases (60% <10 min) (**Figure 9B**). The time to interpret the reports appeared correlated with the number of medium and high severity alarms, with an analysis time of <20 min in the absence of alarms at these levels.

Over the test period, the analysis of the reports led to 44 telephone investigations, mainly with veterinarians ($n = 33$) but also with GDS ($n = 6$), DDecPP ($n = 8$), farmers ($n = 7$), and others ($n = 3$). In the majority of cases (91%; $n = 40$), these investigations took <30 min. Three investigations took 1–2 h and one more than 2 h. In four cases, further investigations would have been necessary in light of the severity of the alarm, the excess mortality, and the first telephone investigations. In 33 cases, the results and initial investigations did not reveal whether further investigations would have been useful. The motive to carry out an investigation was essentially based on the criteria of alarm severity (50%), the number of alarm areas (26%), and the distribution of the mortality by age group (17%). The type of alarm (sudden or gradual), proportion of excess mortality, and number of farms with mortality rarely inspired investigation. When no investigation was conducted, the main reasons were the lack of relevance of the excess mortality (78%; $n = 197$), knowledge of the origin of the mortality (welfare or known health problems) (17%; $n = 43$), and also lack of time (12%; $n = 31$). During the field test, despite the incompleteness of data reporting, the system generated many alarms overall,

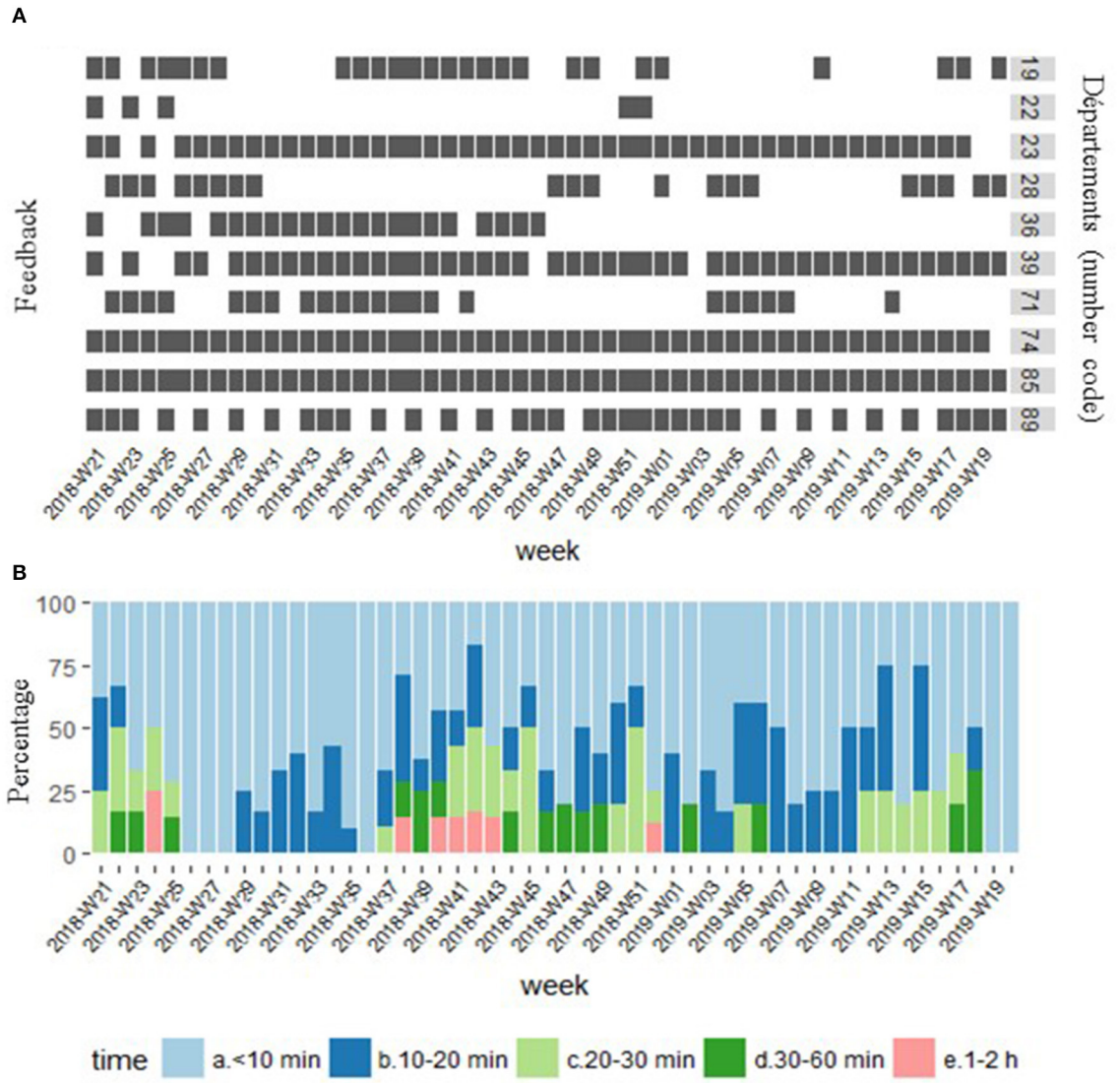

FIGURE 9 | Feedback per pilot *département* and week (gray box indicates response from *département* leader) **(A)** and distribution of time dedicated to reading the report each week **(B)**.

few of which required investigation. These results concord with previous feedback from human SyS systems on the high sensitivity of SyS algorithms (16, 17). Nevertheless, in our case, this sensitivity was acceptable as long as a correct method was implemented for interpreting the results and the organization among animal health services was effective.

Beyond the initial objectives [identification of (re)emergence(s)], our SyS system assists animal health services in their daily missions. It contributed to the monitoring of seasonal diseases, such as influenza or the quantification of the (absence of) impact of bluetongue. It also identified an atypical excess mortality that would otherwise have gone undetected, such as a grouped mortality due to lightning strikes in mountain pastures and the impact of a local salmonellosis problem. In addition, by providing mortality data at the farm level, the system helps health professionals identify or monitor, in real time, farms with welfare or health problems that may require inspection or other support. Finally, feedback indicated that this type of system helps maintain the link between animal health stakeholders, providing an additional opportunity to work together. The lack of reactivity of the system (up to +10 days) was not perceived by users as a limitation. As observed in human SyS systems (12, 18), health professionals are more interested in the follow-up and quantification of the effect of health events rather than early detection.

The main limit identified during the test period was the low amount of reported deaths available at the time of analysis. This low data availability strongly affected the results and the usefulness of the system in two *départements*. Although the high number of alarms in *départements* with the highest data reporting

rate first required an adaptation period to find the right analysis method and organization, the main difficulty was handling the frequency of the reports, even though no additional human resources were involved. Another difficulty was the management of alarms in areas overlapping with neighboring non-pilot *départements* when mortality involved livestock outside the *départements*. We encountered this situation especially in *départements* with low cattle density, where many municipalities were merged with municipalities in neighboring *départements* with higher cattle density. Finally, the NC-OMAR could not fully play its role due to insufficient human resources (less than one person for scientific and technical support and coordination). This lack of personnel limited the use of national and departmental results, due to insufficient support for pilot *départements* in interpreting results and reporting. On the other hand, the lack of overall coordination led to a lack of knowledge of the system. Therefore, veterinarians and farmers have little knowledge of the system, which prevents their active participation in the SyS system.

PERSPECTIVES

Despite the limitations and the lack of dedicated resources, nine *départements* decided to continue to assess the effectiveness of the improvements discussed during the mid-term and final meetings. Regarding data reporting, in addition to the discussions between the three national rendering companies and the NC-OMAR, local health services will interact with the collection centers to try to improve data reporting in their *départements*. In addition, data analysis will be delayed 1 week ($w+2$ instead of $w+1$) to work on more complete information, and obtain more realistic results, because responsiveness is not a priority for animal health services at present, due to the current lack of resources needed to react quickly. In the current situation, we hope that in the event of a rapid increase in mortality, local health services will be alerted by farmers, veterinarians, and/or renders, especially as they are used to communicating and working together. This context explains that the current priority is the quality of information to support health services in their daily work and to provide a qualitative quantification of the effect of known events.

To improve the performance of the system, we will add additional seasonality to the GLM, extend the number of weeks guard, and remove the lowest thresholds of the detection algorithms if the increase in data reporting rate leads to a strong increase in alarm areas. To facilitate work in low-bovine-density *départements* (Eure-et-Loire and Yonne), manual spatial aggregation will be set up based on the advice of the local animal health services to test the system under appropriate conditions in these *départements*. Finally, a farm-level analysis will be implemented, to identify farms with abnormally high mortality rates compared to their usual mortality. This monitoring will supplement the current analysis carried out at the area level. It will be helpful to monitor mortality in near-real time in each farm, to better identify farms in difficulty or with health problem when no signal is detected at the area level. It will also help guide investigations to prioritize farms to investigate in alarm areas. This part of the system is eagerly awaited by health professionals and has been delayed due to lack of coordination resources.

CONCLUSION

To our knowledge, our bovine SyS system, OMAR, is unique in its organization. It is designed with and for its users in the field and so meets the expectations of stakeholders. This likely explains its success despite the lack of resources and the time that local health services needed to adapt to this new type of surveillance system. Unlike in other SyS systems for which information is available, the interpretation of results is not done at the central level but is based on the expertise on the field (9, 10, 22). This is probably the first time that a SyS has been evaluated in the field in a continuous and systematic way, allowing statistical results to be compared with the actual situation week after week. This feedback, which is an integral part of monitoring systems (23), is rarely carried out because it is time-consuming and organizationally demanding. In our case, feedback revealed unexpected uses of the system by animal health services consistent with their health and surveillance missions. For example, the individual farm mortality data provided in detailed reports are used to monitor the mortality in farms where health control plans have been implemented to assess their effectiveness and ensure that the situation is under control. This demonstrates the usefulness of the system beyond SyS. These unexpected uses are very important to maintain the effectiveness of the system "in peacetime" and the motivation of health services. The 1-year test, in the absence of significant health events, did not provide sufficient precise information on human and financial resources and the main challenge remains to ensure regular working time. It is expected that this evaluation will take several years. Nevertheless, although the demonstrated interest in the system initially compensated for the limited resources, the system is reaching its limits and additional human, financial, and legal resources are now needed to ensure the sustainability of OMAR.

DATA AVAILABILITY STATEMENT

The data used by the project can be requested from the French Ministry of Agriculture.

AUTHOR CONTRIBUTIONS

CS wrote the article, managed, and analyzed the data and co-coordinates the system. J-LV managed the data and automated the analyses. FP and YL are members of the OMAR coordination office and participated in discussions. DC was a member of the OMAR coordination office and coordinated the ESA Platform. CD is a member of the coordination office, coordinates the ESA Platform, and participated in discussions. EG and AT participated in discussions and co-coordinated OMAR.

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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.2019.00453/full#supplementary-material

Supplementary Material S1 | Structure of the short online questionnaire for the feedback.

Supplementary Material S2 | Rules of the spatial aggregation algorithm.

Supplementary Material S3 | The S4 object.

Supplementary Material S4 | Detailed report.

Supplementary Material S5 | Distribution of the time elapsed between the removal request (death of the animal) and data reporting to the FSDI during the test period for each collection point (based on 1,470,422 removals).

Supplementary Material S6 | Weekly distribution of the percentage of the deaths reported in the FSDI at the time of analysis in the surveillance areas in each pilot *département*.

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Correlation of Anti-*Salmonella* Antibodies Between Serum and Saliva Samples Collected From Finisher Pigs

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Saliva samples obtained by using absorptive devices, can provide an alternative diagnostic matrix to serum for monitoring disease status in pigs. The aim of this study was to investigate the correlation of anti-*Salmonella* antibodies between serum and saliva samples collected from pigs. Twenty individual paired serum and saliva samples were collected from a single farm. Anti-*Salmonella* IgG was detected in individual serum samples using a commercial *Salmonella* ELISA kit, validated for sera. The same kit was used with a protocol modified by extending incubation time and increasing temperature to test individual saliva samples. Anti-*Salmonella* IgG antibodies in pig saliva were always detected at a lower level than in the matching serum samples. A correlation ($rho = 0.66$; $p = 0.002$) and a moderate agreement ($K > 0.62$ $p = 0.003$) was found between individual *Salmonella* IgG in serum and saliva samples. Both correlation and the agreement levels are moderate. The size of this investigation was small, and further studies are necessary to further confirm these findings. The results of this work provide some evidence that saliva samples have the potential to be used for the diagnosis of *Salmonella* infection in pig farms.

Keywords: *Salmonella* antibody, saliva, oral fluid, serum, pigs, ELISA

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INTRODUCTION

Salmonella is an important foodborne pathogen and the consumption of contaminated pork meat is one of the major sources of human outbreaks (1). In the latest Europe-wide survey, the prevalence of *Salmonella* in United Kingdom pigs was amongst the highest in Europe (2). Surveillance in pig herds is limited by the cost-effectiveness and efficiency of sampling methods (3). Disease monitoring often involves blood sampling for serological assessment, or environmental sampling (for example floor fecal swabs) for bacteriological culture, which are costly to the farmer due to veterinary fees (blood sampling) or require several days for a result (bacteriology) (3, 4). In the last decade, oral fluid (OF) diagnostic technology has been rapidly gaining interest for veterinary medicine as a convenient and rapid diagnostic measure of disease status in pigs (5, 6). Oral fluid is composed of saliva and a transudate that originates from oral capillaries, particularly gingival crevicular fluid that leaks from the crevices between teeth and gum (7). This transudate is a product

of the circulatory system and consequently contains many of the components found in serum, including antibodies (8–10).

Collecting OF samples from pigs using cotton ropes hanging in pens is an easy and welfare-friendly sampling method, relying on their natural chewing behavior and exploratory motivation (11, 12). The use of oral fluid is also attractive because sample collection does not require special training which makes samples easy to obtain. Moreover, the physical and biological risks associated with blood sampling are eliminated (13). Pigs chew the cotton ropes which absorb the OF. A rope thus contains a pooled sample, although the contribution of individual animals to the pool is unknown. Samples can then be assayed for the presence of specific antibodies indicating exposure to pathogens (14, 15). White et al. (15) showed that results obtained from a rope hung for 30–60 min in a pen 25/28 pigs were representative of 75% of the animals.

As there is a range of collection methods available, it is important to accurately describe the resulting samples using standardized terminology. Following the guidelines outlined by Atkinson et al. (16), *whole saliva* is defined as "the fluid obtained…by expectoration" and *oral fluid* as "the fluid obtained by insertion of absorptive collectors into the mouth." Samples can be collected under stimulated and unstimulated conditions depending on the method of collection, or use of chemical stimulants to induce salivary flow (17). Samples collected with absorptive materials are often considered "stimulated" by masticatory action whereas samples obtained via expectoration or drooling are called "unstimulated" (16, 17).

The OF is collected under stimulated conditions, while the saliva is collected under unstimulated conditions.

Use of OF as an alternative to blood for the diagnosis and surveillance of important pathogens is of great interest in veterinary medicine due to the relative ease with which they can be obtained (11, 13). However, in order to be used as a routine surveillance tool, any developed or modified sample types need to be validated against current gold standard methods.

There are a range of commercially available ELISA kits for detection of exposure to bacterial pathogens, most of which are validated for use with serum, or meat juice (18). Such assays have the potential to be adapted to detect antibodies in oral fluid (19). When the test medium differs to that which the test kit was originally designed for, changes to the test protocol (for example, sample dilutions, incubation times and temperature) may be necessary to optimize the performance of the assay (20).

Several countries use serological surveillance to establish the prevalence of *Salmonella* pig herds as part of their national control programs (21, 22). ELISAs to detect anti-*Salmonell*a antibodies in serum and meat juice are used as an indicator for the degree of *Salmonella* burden in pig herds (23).

In this study, we adapted a commercial *Salmonella* ELISA kit (IDEXX Laboratories, Westbrook, ME USA) for use on pig saliva and OF samples. In order to evaluate the potential of oral fluids and saliva samples as alternative sample types, anti-*Salmonella* antibody responses in individual and pooled saliva and pen-based OF samples were compared with serum samples collected from the same animals. The results obtained from serum samples were used as a gold standard.

MATERIALS AND METHODS

Sample Collection

This study was carried out in the United Kingdom in a farrow-to-finish farm consisting of approximately 500 sows and gilts, 2,000 weaners, 2,000 growers, and 2,000 finisher pigs. The farm involved in this study had experience of clinical disease in young animals associated with *Salmonella* serovar Typhimurium for many years. Individual paired blood and saliva samples (five samples from 20 pigs per pen, representing 25% of the pen population) were collected from four pens (A, B, C, and D; 10% of the total finisher boxes) of finisher pigs (Large White breed, approximately 17 weeks of age and 60–70 Kg). In addition, pooled OF samples were also collected from each pen by hanging a three-strand, twisted cotton rope following the method described by Prickett et al. (6). Cotton ropes were left in pens and collected after 30–40 min to allow approximately 75% of animals in the pen to chew the rope (15). No attractant was used.

Prior to sampling, pigs were marked in order to match the individual saliva and blood samples throughout the sampling process. Matched saliva and blood samples were taken from five pigs from each of the four pens. Blood samples were taken for veterinary diagnostic purposes, and any remaining serum was stored for use in this study. Individual saliva samples were collected from the buccal cavity using a cotton sponge (Salivette®, Sarstedt, Nümbrecht, Germany). Sponges were fixed to a sterile plastic rod and held in the mouth of the pigs until thoroughly moistened. After collection, the saliva sponges were placed in sterile tubes and chilled on ice for transport to the laboratory (<4 h). In order to gather a sufficient amount of saliva from each animal, two sponges were collected. The volume obtained from the two sponges was pooled and the saliva samples were first tested individually and then the remaining volume was used to create a pool from the five animals sampled in each pen.

To prevent cross-contamination, a new plastic rod and clean pair of gloves were used for each sample taken. At the laboratory, tubes containing saliva samples were centrifuged at 3,000 × g for 10 min and the supernatants stored at −80°C until testing (24, 25).

At the same time as the serum and saliva samples were collected, samples of pen-based (pooled) OF were collected from the same four pens. A three strand cotton rope of 12 mm of thickness and 50 cm long (RopeServices UK, Houghton Le Spring, UK) was suspended in each pen and left in place for 30–40 min. After being chewed by the pigs, each rope was manually squeezed and the OF placed in 50 mL sterile tubes and transported back to the laboratory in a cool box. All the OF samples were centrifuged (1,500 g for 10 min) and the supernatants stored in aliquots at −80°C until use (20).

For pen-based testing, pooled OF samples (cotton ropes samples) were collected with stimulation (by masticatory action) while individual saliva samples were collected without stimulation (no exogenous gustatory, or mechanical stimulation). Data from a previous bacteriological investigation of the farm's, reported 60% *Salmonella* prevalence in weaners pigs. Accordingly the pen-based sample size was calculated to detect *Salmonella* infection considering a minimum expected

prevalence of 50% and 95% confidence level (26, 27). In addition to the farm samples collected, five individual saliva and three OF samples were collected from *Salmonella*-free sows housed in biosecure pens at the Animal and Plant Health Agency to serve as negative controls.

Detection of *Salmonella*-Specific Antibodies by ELISA in Saliva, Serum, and of Samples

A commercial ELISA kit (IDEXX Swine *Salmonella* Ab Test, IDEXX Laboratories, Westbrook, ME, USA) validated for serum and meat juice samples was used to evaluate the presence of *Salmonella*-specific IgG antibodies in serum, saliva and OF samples.

Saliva and serum samples were tested individually and in pools. Saliva and serum pools were created using equal volumes of sample from each of the five animals sampled within a pen, resulting in four pools.

Individual/pooled serum samples were tested in duplicate, according to the manufacturer's protocol. Briefly, ELISA plates containing 100 μl samples diluted 1/20 were incubated for 30 min at 24°C, washed three times with wash buffer, then incubated for 30 min with 100 μl anti-porcine IgG conjugate. Plates were washed three times before incubation with 100 μl 3.3',5,5'-tetrametilbenzidine (TMB) substrate for 15 min. The reaction was then stopped by addition of 100 μl of stop solution. For each assay, positive and negative kit control samples were used. The absorbance values were read with a plate reader at 630 nm and the OD values converted into ELISA sample-to-positive (S/P) ratios to determine positive/negative result.

According to the manufacturer's instructions, samples with a S/P ratio above 1.00 were considered positive for *Salmonella*-specific IgG.

Individual and pooled saliva samples and pooled OF samples were also tested using the IDEXX ELISA kit. All samples were tested in duplicate using a modified protocol. Following a preliminary study using a range of dilutions (neat−1:8, results not shown), individual and pooled saliva samples and OF samples were diluted 1:1 in the dilution buffer. This dilution was the most effective in detecting differences between animals using minimum volumes of individual and pooled saliva samples and pooled OF samples.

Briefly, samples were diluted 1:1 and 50 μl added to wells which were incubated for an incubation time of 2 h at a temperature of 37°C. After this step, the protocol followed the one detailed for serum samples for completion of the assay. The five negative saliva samples and the three OF collected from *Salmonella*-free pigs were, respectively, pooled and included on each plate as a negative control. S/P ratio was calculated using the negative control serum of the kit.

Statistical Analysis

Statistical analysis was performed using SPSS 25.0 (IBM SPSS Statistics, NY, US). Correlation analyses between ELISA S/P in saliva and serum (individual and pool) samples were performed using Spearman's *rho* ranked coefficient test. The positive or negative status of the individual saliva samples was compared to that of the matched serum samples. Cohen's *Kappa* coefficient was calculated to assess the agreement between saliva and serum samples. Values of $p < 0.05$ were considered statistically significant.

The receiver operating characteristic (ROC) curve was used to assess the optimal cut-off values for S/P) ratios interpretation of the saliva and OF results. Sensitivity (Se) and specificity (Sp) against the gold standard (ELISA examination of the sera) were calculated. The Kolmogorov–Smirnov test for goodness of fit was used to verify normality of the sample distribution, and, on the basis of the results of this test, the Mann–Whitney *U*-test and the Kruskal-Wallis H test were used to compare S/P values in sera and saliva samples at pen level and herd level, respectively.

RESULTS

Individual saliva samples were more difficult to obtain and needed to be collected in duplicate to obtain sufficient volume for testing. Sponges only yielded volumes of 467.2 ± 102 μl (mean ± SEM). One pig from pen A was omitted from testing as the saliva sponges yielded an insufficient sample. The volumes of two other saliva samples collected from pen A animals were only sufficient for testing individually and could not be used to contribute to a pool.

The volume of OF collected from hanging cotton ropes ranged from 3 to 8 ml per pen.

Significant differences were observed between S/P values in sera and saliva samples at herd level (all data together) and pen level.

At the herd-level the ELISA S/P ratio values for saliva samples were significantly lower than S/P values of the corresponding sera ($U = 0.00$; $p < 0.001$) (**Figure 1** and **Table 1**). Similarly, significant differences were observed between S/P values of serum and saliva samples in each of the 4 boxes, with S/P values in sera always greater than the S/P values in the saliva samples ($U = 0.00$ $p = 0.03$; $U = 0.00$ $p = 0.01$; $U = 0.00$ $p = 0.01$; $U = 0.00$ $p = 0.01$ in pen A, B, C, D, respectively). No significant differences in S/P values for serum or S/P values saliva samples were observed between the four pens ($H = 5.94$; $p = 0.12$ and $H = 2.87$; $p = 0.41$, respectively).

However, when the results of the two sample types were compared using Spearman's *rho* ranked coefficient, a positive correlation was observed ($rho = 0.66$; $p = 0.002$) (**Figure 1**).

The ROC curve analysis showed that the best correlation (Area under the curve, AUC: 90.0%) between saliva and serum ELISA results occurred when the saliva S/P ratio threshold was ≥0.03. Using the S/P ratio threshold ≥0.03 saliva samples had a Se and Sp of 86% (95%CL: 57–98) and 80% (95%CL: 28–99), respectively when compared with ELISA results obtained from individual serum samples (**Table 2**).

Using Cohen's *Kappa* coefficient, a moderate agreement ($K > 0.62$ $p = 0.002$) was found between ELISA results for serum which represents the gold standard (positive if S/P ratio > 1.00) and saliva individual samples (positive if S/P ratio > 0.03). Only

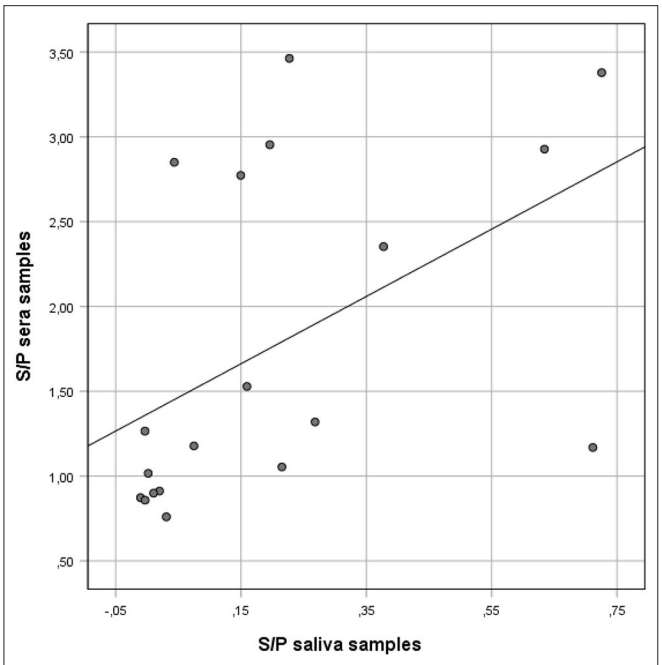

FIGURE 1 | Correlation between anti-*Salmonella* ELISA IgG S/P ratio values of individual serum and matching S/P ratio saliva samples collected from finisher pigs. *Salmonella* IgG was detected on saliva and serum samples using a commercial ELISA kit validated for serum and meat juice.

two seropositive pigs had saliva samples that yielded negative ELISA results.

In three of the four pens involved in this study, when individual samples were pooled the saliva and serum pools gave positive results even when positive samples were pooled with negative samples (pens C and D) (**Table 1**).

However, for pen A, only two individual samples, one positive and one negative (serum and paired saliva), were available to make the pool. In this case, saliva and serum pools were both negative.

Based on the sample size, a pen was defined as having a *Salmonella* seroprevalence ≥50% if at least one of the individual sera taken from that pen tested positive by ELISA.

Pen-based (pooled) OF data were analyzed and considered to be positive when the pen seroprevalence was ≥50%. Three of the four pens had a high proportion (>50%) of ELISA-positive sera and correspondingly OF collected from these pens tested positive for anti-*Salmonella* antibodies. In Pen D, despite the majority of the individual serum samples being negative, the OF sample collected from that pen was positive by ELISA.

DISCUSSION

In this study we modified the protocol of a commercial ELISA kit validated for serum and meat juice in order to test individual and pooled saliva samples (from oral sponges) and pen-based OF samples (from cotton rope chews) for the presence of anti-*Salmonella* antibodies in finisher pigs.

TABLE 1 | Anti-*Salmonella* ELISA IgG OD values of individual and pool samples of serum and saliva and pen-based OF.

Pen	Animal	Individual serum			Individual saliva			Pool of serum			Pool of saliva			Pen based OF		
		OD	S/P ratio	Pos/Neg	OD	S/P ratio	Pos/Neg	OD	S/P ratio	Pos/Neg	OD	S/P ratio	Pos/Neg	OD	S/P ratio	Pos/Neg
A	1	3.23	2.95	Pos	0.27	0.20	Pos									
A	2	1.25	1.17	Pos	0.76	0.71	Pos	0.79	0.67	Neg	0.09		Neg	0.35		Pos
A	3	1.15	1.05	Pos	0.29	0.22	Pos									
A	4	0.78	0.91	Neg	0.08	0.02	Neg									
B	6	2.82	2.85	Pos	0.10	0.04	Neg									
B	7	2.75	2.77	Pos	0.22	0.15	Pos									
B	8	3.39	3.46	Pos	0.30	0.23	Pos	2.63	2.45	Pos	0.32		Pos	0.54		Pos
B	9	1.26	1.18	Pos	0.15	0.07	Pos									
B	10	3.20	2.93	Pos	0.73	0.63	Pos									
C	11	0.98	0.87	Neg	0.07	0.00	Neg									
C	12	1.39	1.32	Pos	0.34	0.27	Pos									
C	13	3.32	3.38	Pos	0.83	0.73	Pos	2.19	2.03	Pos	0.33		Pos	0.35		Pos
C	14	1.59	1.53	Pos	0.23	0.16	Pos									
C	15	1.11	1.02	Pos	0.06	0.00	Neg									
D	16	0.87	0.76	Neg	0.09	0.03	Neg									
D	17	0.96	0.86	Neg	0.08	0.00	Neg									
D	18	1.00	0.90	Neg	0.09	0.01	Neg	1.37	1.23	Pos	0.28		Pos	0.38		Pos
D	19	1.25	1.26	Pos	0.05	0.00	Neg									
D	20	2.36	2.35	Pos	0.46	0.38	Pos									

Positive samples are chosen in accordance with the S/P >1 for the serum and S/P>0.03 for saliva and OF samples. Serum and saliva pools of pen A were prepared by pooling only samples from animal 3 and animal 4.

TABLE 2 | Number of porcine serum and saliva samples positive and negative for anti-Salmonella IgG antibodies.

		ELISA results in saliva (%)		
		Positive	**Negative**	**Total**
ELISA results in serum	Positive	12 (85.7)	2 (14.3)	14
	Negative	1 (20.0)	4 (80.0)	5
	Total	11 (57.9)	8 (42.1)	19
		Se: 86% (95% CL: 57–98)	Sp: 80% (95% CL: 28–99)	*K*: 0.62

Cohen's kappa coefficient showed a substantial agreement (K = 0.62).

Although IgA is the predominant isotype present in OF (8, 9), several studies reported that IgG antibodies are a better target for determining exposure to specific pathogens (5, 28, 29). Compared with IgG, the IgA concentration seems to be more variably influenced by stress to the animals and by the rope material used for collection (5, 29). A previous study showed a lack of sensitivity for IgA detection in OF compared with the IgG isotypes (5). Therefore, only IgG levels where assessed in the current study.

Using a modified protocol (extended incubation time and increased temperature), we demonstrated that the IDEXX ELISA was able to detect anti-*Salmonella* antibodies in pig OF and saliva samples. Modifications to the sample dilution, incubation time and incubation temperature have significant effects on ELISAs to detect antibodies in OF (19, 30). Modifications of the original manufacturer's protocol were made to account for the lower concentration of antibody in OF and saliva samples. For this purpose, a decrease sample dilution was used and a longer sample incubation at high temperature was set up to allow potential antibody within the saliva and OF sample to bind to the antigen-coated on the ELISA plate. Modification of the ELISA was assessed, and Se and Sp were estimated at 86 and 80%, respectively, against the gold standard test (**Table 2**). Our study showed a moderate correlation between saliva and the corresponding serum results. This positive correlation indicates that the increase in S/P values of serum samples was correlated with an increase in S/P values saliva samples. These results suggest that individual saliva samples can represent a suitable alternative to blood samples for the detection of anti-*Salmonella* antibodies at an individual pig level.

Anti-*Salmonella* antibody levels in pig sera were always higher than in the matching saliva samples in all samples tested ($p > 0.05$). It has been reported that the IgG concentrations in OF are approximately 800 times lower than in serum (29). Therefore, pigs whose sera are only just above the ELISA cut-off could have saliva IgG levels below the limit of detection. Despite the substantial agreement found between individual serum and saliva samples, two seropositive pigs had saliva samples that yielded negative ELISA results in this study. These two negative results are not unexpected considering that the corresponding sera had S/P ratios only just above the ELISA kit cut-off, and similar variability has been found for meat juice when compared with serum (31).

By using pooled samples, a large number of animals may be analyzed for a reduced cost. However, it is important that the analytical performance of the assays remains high. Three pools were positive by ELISA, even when the pools consisted of positive and negative individual samples. However, for one pen (pen A) the dilution effect of pooling samples led to a loss of sensitivity, leading to a negative ELISA result. This could be due to the fact that for this pen only two of the five samples contributed to a pool. The risk of diluting positive samples with negative fluid to such an extent that the specific antibody concentration gives a negative ELISA result is a problem with pooling samples, but pooled samples are still suitable for herd screening unless the test sensitivity is very low (32–34). The effects of dilution depend on the relative concentrations of target antibodies in each sample.

Pen-based OF sampling using hung cotton ropes is another cost-saving strategy. The four OF samples collected by cotton ropes represented a pool of a higher number of animals compared with the five saliva samples collected individually.

Pen-based OF that originated from pens that had a high *Salmonella* seroprevalence (≥50) resulted to be ELISA-positive (**Table 1**) (26, 27). Even when the majority of the individual serum samples were negative (Pen D), the resulted OF sample tested positive for anti-*Salmonella* antibodies. This is presumably due to high levels of specific antibodies in the individual samples that were positive.

Despite the study was limited to one farm and a low number of samples were tested, to the best knowledge of the Authors, this is the first field study describing anti- *Salmonella* antibodies in pigs' OF. Although the results of this work should be evaluated with caution, we proved that the modification of the ELISA kit protocol allowed the detection of *Salmonella* IgG in saliva samples, emphasizing that this specimen has the potential to be used for the diagnosis of *Salmonella* infection in pig farms. Our work has demonstrated that individual saliva samples have the potential to be used for the diagnosis of *Salmonella* infection using the IDEXX ELISA with a modified protocol. Furthermore, pooled and oral fluid sampling using cotton ropes may have the potential for use in the detection of anti-*Salmonella* antibodies in field conditions.

Further studies are necessary to confirm and expand upon our findings. In particular, the effects of pooling, which is highly dependent on the dilution effect of mixing positive with negative samples, need to be fully understood. If there is great variability in antibody levels within the pen population, the strategy may lead to unreliable results. Furthermore, repeat sampling could lead to very different results.

The current study was carried out on a limited number of animals on a single farm. It is therefore recommended that further, larger scale studies are carried out in order to provide better evidence on the use of OF and saliva as a diagnostic samples for *Salmonella*.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the animals sampled in this study were undergoing veterinary investigation for a respiratory disease. The serum samples were collected by a veterinary surgeon on farm for diagnostic purposes, and therefore the collection was not a regulated procedure under the Animals (Scientific Procedures) Act (ASPA, 1986). The animals sampled were chosen for diagnostic purposes and the requirements of this study did not influence the selection of the animals or the volume of blood withdrawn. In this study excess serum was used if any was left after the sample had been used for diagnostic purposes. The collection of saliva samples or oral fluid samples does not constitute a regulated procedure under ASPA.

AUTHOR CONTRIBUTIONS

This study experiment was designed by AD, FO, and FM. AD: co-wrote paper and developed the study. SC: provided serological expertise at the laboratory level, co-wrote paper. RD and RS: provided critical review and revising of paper. CB: provided serological expertise and co-wrote paper. FO: performing statistical analysis and revising of paper. FM: developed study, and critically revised the manuscript. All authors read and approved the final 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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The Influence of Sociodemographic Factors on the Engagement of Citizens in the Detection of Dead Corvids During the Emergence of West Nile Virus in Ontario, Canada

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West Nile virus (WNv) was introduced into North America in 1999, and by 2002 was identified in most regions of Ontario, Canada. Surveillance of WNv included testing of corvids found dead and reported by citizens across Ontario, which at the time was a novel citizen science application for disease surveillance. While this surveillance program was successful for timely identification of WNv as it emerged and spread across the province, it is important to consider the influence of non-disease factors on surveillance data collected by the public. The objective of this study was to examine associations between rates of citizen phone reports of dead corvids and sociodemographic factors within the geographic areas where the reports were obtained. The data were grouped by forward sortation area (FSA), a geographical area based upon postal codes, which was linked with census data. Associations between the weekly rate of citizen reports and FSA-level sociodemographic factors were measured using multilevel negative binomial models. There were 12,295 phone call reports of dead corvids made by citizens in 83.3% of Ontario FSAs. Factors associated with the weekly rate of phone reports included the proportion of high-rise housing, the proportion of households with children, the proportion of seniors in the population, the proportion of citizens with no knowledge of either official language and the latitude of the FSA. There were higher rates of citizen phone reports in FSAs with <80% high-rise housing and greater proportions of households with children. A positive and negative association in the rate of calls with the proportion of seniors and latitude of the FSA, respectively, were moderated by the proportion of the population with knowledge of official language(s). Understanding the sociodemographic characteristics associated with citizen reporting rates of sentinels for disease surveillance can be used to inform advanced cluster detection methods such as applying the spatial scan test with normal distribution on residuals from a regression model to reduce confounding. In citizen-derived data collected for disease surveillance, this type of approach can be helpful to improve the interpretation of cluster detection results beyond what is expected.

Keywords: West Nile virus, surveillance, citizen science, arbovirus, wildlife disease surveillance

INTRODUCTION

West Nile virus was first identified in 1937 in Uganda (1), and until the mid-1990's was associated with sporadic outbreaks of mild illness in humans in Africa, the Middle East, and Europe (2). Subsequently, strains of WNv appeared to cause higher morbidity and mortality in humans and horses in parts of Europe, North Africa and the Middle East (3). A strain of WNv implicated in outbreaks of mortality in domestic geese in Israel was homologous to the strain that emerged in New York State, United States (U.S.) in 1999 (4). These outbreaks were associated with more severe neurologic disease and higher mortality in humans, horses and birds. Members of the corvid species, e.g., American Crows (*Corvus brachyrhynchos*), Black-billed Magpies (*Pica hudsonia*), Common Ravens (*Corvus corax)*, Blue Jays (*Cyanocitta cristata*), and Canada Jays (*Perisoreus canadensis)* were thought to be particularly susceptible to WNv infection, with American Crows, Black-billed Magpies and Blue Jays experimentally demonstrating high levels of viremia and mortality (5). In Canada, surveillance activities for the detection and monitoring of WNv were initiated in 2000, which included mosquito trapping and testing, sentinel surveillance using chickens, collection and testing of corvids found dead by the public, as well as reporting of human and equine cases (6). In addition, a database of all dead corvid phone reports in Ontario was created, which included the location of all corvid carcasses across Ontario, including those which were not tested for WNv and the test status of carcasses that had been collected (6). In 2001, the disease was first identified in Ontario in a found dead corvid, and subsequently, cases in corvids and humans were found across the province (and most of North America) through the summer and fall of 2002 (7).

Our previous research demonstrated that the citizen-derived data on dead corvids collected and tested provided timely identification of West Nile virus activity in public health units across Ontario, and demonstrated a high sensitivity for WNv detection (8). This was more evident during the early years after the incursion of WNv into Ontario, when public interest was high and naïve populations of corvids were highly susceptible to the disease. Furthermore, these data were useful for predicting where human cases would later occur, especially after adjusting for underlying sociodemographic and geographic factors associated with human cases (9).

There is increasing interest in citizen-derived data for scientific study (i.e., "citizen science"), including for the surveillance of wildlife diseases (10) and emerging vector-borne infections like Lyme disease (11) and Zika virus (12). Opportunistic citizen reporting can be a cost-effective option for data collection over wide geographic and temporal scales, including on private land (10). However, citizen-derived data can be biased by a number of factors including non-random distribution of effort and detection probability, which can influence their spatiotemporal distribution (13). The citizen phone reports of dead corvids during the initial emergence of WNv across Ontario in 2002 have not been explored for their associations with underlying area-level sociodemographic factors that may have influenced citizen participation in the detection and reporting of carcasses found in the environment. It is important to consider these potential confounding effects when advanced statistical methods such as cluster detection techniques are utilized for identification of higher-than-expected morbidity or mortality. While other potential explanatory factors such as other causes of mortality or varying underlying spatial distribution of corvids cannot be controlled, differences in the likelihood of citizens to report dead corvid carcasses when they are present may also bias these data if they are to be used for cluster detection. Specifically, adjusting cluster analyses for potentially confounding variables related to public participation can improve the identification of epidemiologically meaningful clusters. Understanding associations between citizen participation in data collection can also shed light on the potential level of citizen engagement among different population demographics during future disease emergence events, which can be used to improve communications soliciting citizen engagement and hence, their participation. Thus, the objective of this study was to examine the citizen phone reports of dead corvids in relation to area-level sociodemographic factors, in order to understand inherent biases in these particular data and to inform future research and communication strategies regarding the use of sentinel indicators for disease surveillance when participation by the public is requested.

METHODS

Data Sources and Management

A dataset containing all citizen phone reports of found dead corvids across Ontario during 2002 was provided to the researchers by the Canadian Wildlife Health Cooperative (CWHC). This dataset included the date the dead corvid was found, whether the caller was a member of the public or from an organization, the street address, town/city and postal code, or geographic coordinates in latitude and longitude where the corvid carcass was located. The most consistently accurate level of spatial location for these entries at the highest geographic resolution available was the postal code information. In Canada, the first 3 characters of the postal code, i.e., the Forward Sortation Area (FSA), represents a geographic area within a major region or province/territory, based on mail distribution zones (14) and can be linked to Canadian Census data. Cartographic boundary files (14) and 2001 FSA Census data (14) were obtained from Statistics Canada. Phone reports which contained the latitude and longitude coordinates rather than street address and postal code were linked to the FSA within ArcMap v10.2.1 (ESRI, Redlands, CA, USA).

The latitude and longitude of the FSA centroid was determined using the Calculate Geometry function in ArcMap. Sociodemographic variables and human population size within the FSAs were obtained from the 2001 Canada Census (14). While the Census contains many potential explanatory variables, the sociodemographic variables chosen were based on the hypothesized non-overlapping biological or sociological influence on distribution of phone reports. The included the following variables: the proportion of high-rise homes (defined by Statistics Canada as a dwelling, owned or rented, in a building

with 5 or more stories), the proportion of new homes (defined by Statistics Canada as those built between 1996 and early 2001 when the Census was collected), the proportion of households with children, and the proportion of low income households (defined by Statistics Canada as the percentage of economic families or unattached individuals who spend 20% or more of their income than average citizens on food, shelter, and clothing) (14). We also obtained the following Census sociodemographic variables concerning the proportion of the population that were: seniors (i.e., 65 years of age or older), in the labor force, had obtained a Bachelor's degree or higher education level, and had no knowledge of either official language (i.e., English and French) at the FSA level (14). Weekly phone reports of dead corvids within FSAs were calculated using Microsoft Excel (2016). These data were linked to the FSA centroid coordinates and FSA-level sociodemographic variables in a common dataset using Stata/SE version 14.0 (StataCorp, College Station, TX, USA).

Statistical Analyses

Descriptive Statistics

The dead corvid reports were summarized by proportion of species reported (i.e., American Crows, Common Ravens, Blue Jays, Gray Jays (renamed Canada Jays), and non-corvid species). The phone call reports were summarized by number of calls per FSA, including those FSAs with no calls received over the study period.

Univariable Analyses

The dependent variable for this study was the FSA-level weekly phone call reporting rate, based on the number of weekly phone calls about dead corvids within each FSA. Because the outcome was rate-based and the data were over-dispersed, univariable negative binomial models were fit using Gaussian-Hermite quadrature (using the "menbreg" command in Stata). The models included an offset, which was the natural log of the number of people residing in the FSA, and a random effect with an independent covariance structure for FSA to control for clustering due to repeated observations within FSAs. Linearity was assessed using locally-weighted regression scatterplot smoothing (lowess) curves (15) between the FSA-level weekly rate of phone call reports and the following FSA-level continuous independent variables: FSA centroid latitude and longitude, the proportion of high-rise homes, the proportion of new homes, the proportion of households with children, and the proportion of low income households, as well as the proportions of the population that were seniors, in the labor force, had obtained a Bachelor's degree or higher education level, and had no knowledge of either official language. Any continuous variables found to have a non-linear relationship with these phone call reporting rates were categorized, if an appropriate transformation could not be found or the relationship could not be modeled with the addition of a quadratic term (15). Spearman's rank correlation coefficients (rs) were examined for each pair of independent variables to assess potential collinearity in subsequent multivariable modeling. If any pair of variables was found to be strongly correlated, using a cut-point of $rs \geq |0.8|$, the variable considered most informative from a biological perspective was included in the proceeding models.

Multivariable Analyses

Multi-level negative binomial models were next fit by Gaussian-Hermite quadrature to explore the associations between the weekly citizen phone call reporting rates and FSA-level sociodemographic factors, in addition to the FSA centroid latitude and longitude. As for the univariable models, a random effect with an independent covariance structure for FSA was included to control for clustering due to repeated observations within FSAs and the natural log of the number of people residing in the FSA was the offset.

Based on the univariable models, variables that demonstrated an association with weekly citizen phone call reporting rates at a liberal significance level of alpha = 0.20 were further evaluated in multi-variable models using a backward step-wise elimination process. Associations and pair-wise interactions between variables considered for inclusion in the multi-variable model were assessed using two-tailed likelihood ratio (LR) tests at a significance level of alpha = 0.05. Assessment of confounding was performed by removing each non-intervening variable from the model and evaluating whether its removal resulted in a change in any coefficient by 20% or more. Coefficients were exponentiated and reported as incident rate ratios (IRR). Predicted curves were used to interpret interaction effects involving continuous variables. Fixed quantities were estimated for interaction terms on phone call reporting rates, while holding all other variables constant at their mean values.

Multi-variable model fit was evaluated by assessing the assumptions of normality and homogeneity of variance for the best linear unbiased predictors (BLUPs), graphically using a normal quantile plot and by a scatterplot of the BLUPs vs. the predicted outcome, respectively (15). Pearson and deviance residual plots were also examined to identify potential outliers (15). The final multi-level negative binomial model was also compared to a multi-level Poisson model and zero-inflated negative binomial model, using Akaike's Information Criterion (AIC) to identify the better fitting model for the data.

RESULTS

Descriptive Statistics

There were 12,886 citizen phone call reports in Ontario concerning sightings of dead birds in 2002. The majority of bird species were classified as American Crows (95.64%), with the remainder classified as Blue Jays (0.99%), Common Ravens (0.53%), Gray Jays (0.02%), European Starlings (0.01%), Gulls (0.01%), and "other birds" (2.80%). There were 122 phone reports with either missing dates or location information (including postal codes), which were excluded from further analyses. Of the remaining records, the number of phone call reports about dead corvids within each FSA ranged from 0 to 407, with a mean of 21.8 and median of 6 calls. There were 85/510 (16.7%) FSAs where no citizen or organization reported dead corvids in 2002.

Univariable Models

Based on univariable analyses, none of the variables were highly correlated with another variable. Those that were associated with phone call reporting rates based on a liberal p-value ($p < 0.20$) are displayed in **Table 1**. The following variables meeting these criteria were considered for inclusion in the multivariable model: FSA centroid latitude, the proportion of high-rise homes (categorized into two groups based on a cut-point of 80% high-rise), the proportion of new homes, the proportion of households with children, the proportion of the population with low income (modeled as a quadratic relationship), the proportion of seniors in an FSA, and the proportion of the population with no knowledge of either official language.

Multivariable Models

Significant associations were identified ($p < 0.05$) between phone call reporting rates and the proportion of high-rises and the proportion of households with children (**Table 2**). There were interactions between the proportion of seniors and the proportion of people having no knowledge of either official language (**Table 2**), and the proportion of seniors and the latitude of the FSA (**Table 2**). The final fitted multi-level model demonstrated that, after controlling for other covariates, FSAs with >80% of households characterized as high-rise were associated with lower reporting rates (**Table 2**). Phone call reporting rates increased with the proportion of households with children (**Table 2**). Generally, as the proportion of seniors in the population increased, so did the rate of phone call reports about dead corvids. However, this increase was less rapid in FSAs where the proportion of the population speaking an official language was lower (**Figure 1**). For FSAs located at higher latitudes (i.e., further north), the predicted rate of phone call reports decreased, and there was a sharper decline in the rate of reports in FSAs where the proportion of people speaking either official language was lower (**Figure 2**).

The best-fitting model based on AIC values was the negative binomial model with a random effect for the FSA. The evaluation of the residuals identified several outliers, but removal of these observations did not change the model estimates. The BLUPs for the random intercept for FSA were also normally distributed and demonstrated homogeneity of variance.

DISCUSSION

When West Nile virus appeared in North America, the reporting of dead corvids by citizens in various jurisdictions in North America provided a unique approach toward monitoring the spread of a vector-borne disease. This was made possible due to the high public concern about the implications of human West Nile virus infections, high mortality rates among corvid species infected, and their widespread distributions. The impetus for this study was to explore how human sociodemographic factors influenced rates of phone calls reporting corvids found dead by citizens in Ontario during 2002, when WNv first spread across the province.

We found that there was a high volume of citizen phone call reports of dead corvids received from most areas of Ontario during the spring, summer and fall of 2002. The majority of birds were classified by citizens as American Crow, which is consistent with previous studies on WNv-positive species distribution (16). Few birds were reported as non-corvid species, indicating the public was well-informed about which species to report, although most reports were not validated since the majority of specimens were not collected for testing (8). The citizen reports would have been influenced by a number of geographic and sociodemographic factors related to the likelihood of detecting corvid carcasses in the environment given their presence, and social factors related to an individual's knowledge of the WNv surveillance program requesting reports of dead crow carcasses and their willingness to report by phone any dead crows found. The number of households within FSAs may have also influenced these data if each phone call was placed by a single household, however, the population size of the FSA was used instead as an offset since phone call reports were placed by individuals, to control for the likely influence of FSA population size on number of phone call reports received. Household-level sociodemographic variables, available from the Census, were included to account for sociodemographic features of the small areas represented by FSAs.

Due to the ecological nature of this study, one must be cautious interpreting our findings from the community level (i.e., FSA) to the individual caller level. Small areas such as FSAs in Ontario, being more homogenous in their population structure can limit the effect of ecological bias (15). Furthermore, the latitude and longitude of the FSA were considered in relation to the weekly phone call reporting rates as a coarse measure of the geographic distribution of corvids, although we could not completely control for differential underlying distribution of corvids across the province, which would be influenced by the seasonal distribution patterns related to breeding, migration, and locations of roosting sites. Corvid distribution data are collected via other citizen participatory approaches, such as the North American Breeding Bird Survey (BBS) (17) and Project FeederWatch (18), but these point data are collected at a single timepoint along roadside survey routes (BBS) or during winter (Project FeederWatch), and were primarily developed for temporal trend analyses over a number of years. However, since corvids are widely distributed across the province and known to cohabit in proximity to humans (19), the following results suggest certain sociodemographic factors within small areas influenced the reporting of dead corvids by citizens in Ontario, given their presence in the surrounding environment.

We found that there were fewer phone call reports in areas with a high proportion of high-rises, in comparison to low-rise settings. This finding seems contrary to previous research which has shown that the American Crow (19) and *Culex* mosquitoes (20) are found in greater abundance in urban vs. rural areas. Also, research by Ward et al. (21), who used crow decoys to compare urban and rural differences in citizen detection and reporting rates and scavenging rates by other animals in a county in Georgia, U.S.A., found that detection

TABLE 1 | Univariable[+] associations between sociodemographic factors and rates of citizen phone call reports of dead corvids in Ontario during the West Nile virus outbreak of 2002.

Sociodemographic variables	Incidence rate ratio (IRR)	95% confidence interval	*P*-value
Area with >= 80% high-rise dwellings Referent: area with <80% high-rise dwellings	0.37	0.27–0.50	<0.001
Proportion of households with children	1.06	1.05-1.07	<0.001
Proportion of seniors	1.14	1.11–1.16	<0.001
Proportion of citizens with no knowledge of either official language	0.92	0.86–0.98	0.013
Forward sortation area (FSA) centroid latitude	0.68	0.61–0.75	<0.001
Proportion of new homes	1.02	1.01–1.03	<0.001
Proportion of population with low-income	1.01*	0.95–1.07	0.76
Proportion of population with low-income squared	0.997*	0.996–0.999	0.004

+Univariable mixed-effects negative binomial models. Forward sortation area (FSA) was included as a random intercept. Dependant variable, weekly number of phone call reports of dead corvids within the FSA. Offset, natural log-transformed number of people residing within the FSA. Only variables associated with phone call reporting rates based on a liberal p-value ($p < 0.20$) are displayed.
**Exponentiated coefficients for squared terms and their main effects rather than true IRRs.*

TABLE 2 | Multivariable[+] associations between sociodemographic factors and rates of citizen phone calls reporting dead corvids in Ontario during the West Nile virus outbreak of 2002.

Sociodemographic variables	Incidence rate ratio (IRR)	95% confidence interval	*P*-value
Area with >= 80% high-rise dwellings Referent: area with <80% high-rise dwellings	0.54	0.40–0.72	<0.001
Proportion of households with children	1.03	1.01–1.05	0.004
Proportion of seniors	1.09	1.05–1.13	<0.001
Proportion of citizens with no knowledge of either official language	3822.5*	16.73–873155.9	0.003
Forward sortation area (FSA) centroid latitude	0.74*	0.66–0.82	<0.001
Proportion of citizens with no knowledge of either official language × FSA centroid latitude	0.83*	0.73–0.94	0.003
Proportion of citizens with no knowledge of either official language × Proportion of seniors	0.97*	0.95–0.98	<0.001

+Multi-variable mixed-effects negative binomial models. Forward sortation area (FSA) was included as a random intercept. Dependant variable, weekly number of citizen reports of dead corvids within the FSA. Offset, natural log-transformed number of people residing within the FSA.
**Exponentiated coefficients for interaction terms and their main effects rather than true IRRs.*

and reporting was significantly higher in urban vs. rural areas, and carcass removal by scavengers occurred more quickly in rural parts of the country. It is intriguing then, that FSAs with a high proportion of high rises (as a surrogate measure for urbanicity) were associated with lower reporting rates in our study. Perhaps the cut-point of 80% high-rise density used in this study which was made based on the linear relationship with reporting rates, captured a different relationship in very high-density urban environments compared with all other regions. For example, areas with high density housing likely have fewer trees and may be further from woodlands for roosting, and this result may reflect a lower density of corvids. Lower abundance of mosquitoes and less transmission of WNv to corvids may provide another explanation, or anthropogenic factors related to the human population characteristics could explain the reduced likelihood of citizen detection and reporting of dead crows in these settings. For example, perceived safety and pleasurability of surrounding outdoor spaces has been found to be lower in high density neighborhoods, with a concurrent negative association with time spent outdoors (22).

We also found higher rates of phone call reports were associated with areas in which there were higher proportions of households with children. This finding may reflect the geographic features of areas with higher proportions of households with children, like suburban areas, being more suitable to corvid populations. It is also possible that citizens from households with children generally spend more time outdoors and are more engaged with public health messaging. Canadian children spend greater amounts of time outdoors relative to all other age groups (23), and other research has shown that children interact more directly with the natural environment in comparison with adults (24). Thus, this age group may be more likely to identify dead birds in their surrounding environment.

The association found between higher sightings rates and areas with higher proportions of seniors may reflect features of these FSAs promoting larger corvid populations since the majority of senior Canadians live in urban metropolitan areas (25). A national survey-based study does not support the presumption that Canadian seniors spend greater amounts of time outdoors in comparison with other Canadians (23), nor have they been found to participate more often than younger people in survey-based studies (26). Further research would be needed to better understand the underlying mechanisms behind higher reporting rates among FSAs with more senior residents.

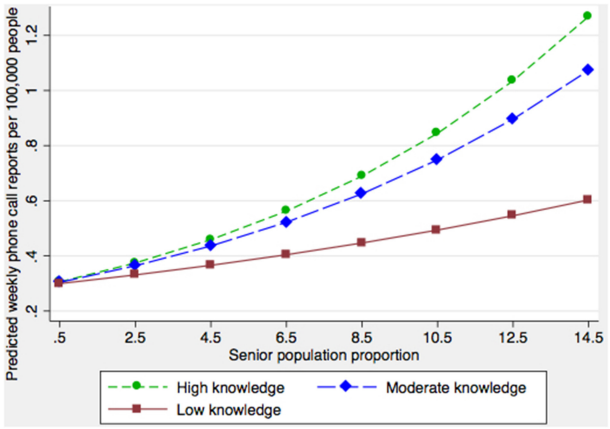

FIGURE 1 | Model-adjusted predicted citizen call rates concerning dead corvids by forward sortation area (FSA) senior population proportion at three levels of official language knowledge. "High," "Moderate" and "Low knowledge" classifications refer to the 25, 50, and 75th percentiles based on the proportion of the population within FSAs having no official language knowledge.

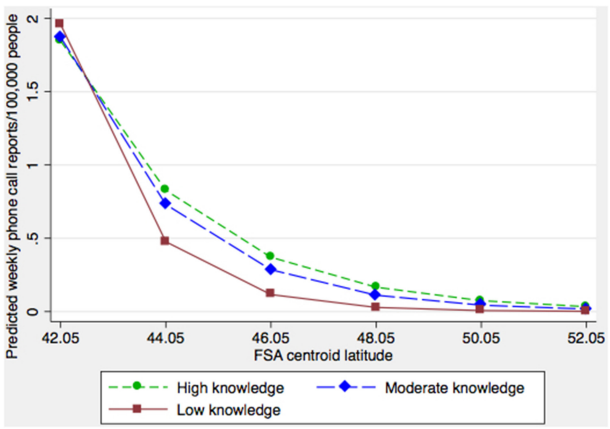

FIGURE 2 | Model-adjusted predicted citizen call rates concerning dead corvids by FSA latitude at three levels of official language knowledge. "High," "Moderate" and "Low knowledge" classifications refer to the 25, 50, and 75th percentiles based on the proportion of the population within FSAs having no official language knowledge.

The reduction in phone calls reports about dead corvids with increasingly northern, cooler latitudes likely reflects lower rates of WNv-infected crows due to mosquito-dependant WNv replication, amplification and transmission cycles. Further, FSAs in northern Ontario have generally lower human population densities, more open space, and likely more opportunities for scavenging of corvid carcasses by wildlife. Previous studies of anthropogenic causes of bird mortality have shown that the removal of bird carcasses (by scavengers) before an observer has a chance to detect them is one of the largest biases in estimating mortality rates using citizen-derived data (27, 28).

We also found that the associations between phone call reporting rates and the proportion of seniors in the population, as well as phone call reporting rates and the latitude of the FSA, varied by the level of knowledge of official languages within FSAs. It may be that in areas with higher proportions of new Canadians, there was lower awareness of West Nile virus risk and/or understanding of public health messaging requesting the reporting of found dead corvids. Other social and cultural barriers may have reduced the likelihood of citizens finding and/or calling to report dead corvids in areas where higher proportions of new Canadians reside. The finding that FSAs with higher proportions of seniors were associated with higher phone call reports is likely related to a number of reasons including having more available time and higher interest in, and knowledge of birds, although this effect was modified by the proportion of the population with knowledge of an official language.

It is likely that some citizens reporting dead corvids may have misclassified the species involved, since it may be difficult for average citizens to differentiate American crows, ravens and magpies from various other black species of birds (e.g., grackles and starlings). Geographic differences in bird species awareness among Ontario citizens may have produced unmeasurable biases in the data. In the current context, a mobile phone application could be developed to aid in identification of different species (29), and a photograph of the specimen could be uploaded by the user to verify the species and evaluate carcass quality for testing. There were also a high proportion of location entries with small errors that precluded the use of exact coordinates for analyses. Since we investigated associations at a small area level this was not a serious problem for the purpose of this study. Current widespread use of digital technologies, including smartphone mobile applications and GPS capabilities, would allow for relatively easy dead crow reporting, verifiable with respect to exact location and species identification. These technological advancements may improve reporting rates for future studies and surveillance programs and reduce some of the biases in citizen-derived wildlife data.

Citizen reporting of dead corvids in Ontario during the 2002 emergence and spread of WNv across the province depended on citizens having the knowledge and willingness to make phone reports, and on their likelihood of detecting the corvid species of interest if they were present. While the program was very successful at timely and sensitive identification of WNv in Ontario, the dead corvid reports collected from citizens, were non-random samples requiring careful handling in order to make valid spatial inferences about risk. Here, we identified sociodemographic factors related to the different rates of dead corvid reports among small areas in the province which may have been related to the likelihood of reporting, given the presence of the disease. Measures of WNv risk based on citizen reports of dead corvids should consider these potential factors influencing reporting, since statistical measures can be implemented to control for their confounding effects in epidemiological risk-based studies and cluster analyses (30, 31). Hence, it is important to consider underlying biases for any studies which utilize valuable citizen-derived data.

DATA AVAILABILITY STATEMENT

The datasets for this manuscript are not publicly available from the authors due to privacy restrictions. Requests for the caller data can be made to the CWHC. Census data are available from the Government of Canada.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Ethical review and approval was not required for the animal study because Citizen-acquired phone calls about deceased corvids did not require ethical review and approval by the University of Guelph.

AUTHOR CONTRIBUTIONS

The study design and manuscript revision were completed by AT-B, DP, EP, and OB. Data analysis, interpretation and drafting of the manuscript were completed by AT-B.

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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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Improving the Utility of Voluntary Ovine Fallen Stock Collection and Laboratory Diagnostic Submission Data for Animal Health Surveillance Purposes: A Development Cycle

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There are calls from policy-makers and industry to use existing data sources to contribute to livestock surveillance systems, especially for syndromic surveillance. However, the practical implications of attempting to use such data sources are challenging; development often requires incremental steps in an iterative cycle. In this study the utility of business operational data from a voluntary fallen stock collection service was investigated, to determine if they could be used as a proxy for the mortality experienced by the British sheep population. Retrospectively, Scottish ovine fallen stock collection data (2011–2014) were transformed into meaningful units for analysis, temporal and spatial patterns were described, time-series methods and a temporal aberration detection algorithm applied. Distinct annual and spatial trends plus seasonal patterns were observed in the three age groups investigated. The algorithm produced an alarm at the point of an historic known departure from normal (April 2013) for two age groups, across Scotland as a whole and in specific postcode areas. The analysis was then extended. Initially, to determine if similar methods could be applied to ovine fallen stock collections from England and Wales for the same time period. Additionally, Scottish contemporaneous laboratory diagnostic submission data were analyzed to see if they could provide further insight for interpretation of statistical alarms. Collaboration was required between the primary data holders, those with industry sector knowledge, plus veterinary, epidemiological and statistical expertise, in order to turn data and analytical outcomes into potentially useful information. A number of limitations were identified and recommendations were made as to how some could be addressed in order to facilitate use of these data as surveillance "intelligence." e.g., improvements to data collection and provision. A recent update of the fallen stock collections data has enabled a longer temporal period to be analyzed, with evidence of changes made in line with the recommendations. Further development will be required before a functional system can be implemented. However, there is potential for use of these data as: a proxy measure for mortality in the sheep population; complementary components in a future surveillance system, and to inform the design of additional surveillance system components.

Keywords: surveillance, fallen stock, ovine, mortality, syndromic, diagnostic submission, fasciolosis, existing data

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INTRODUCTION

In the last two decades the surveillance of animal, especially livestock, populations has become an increasingly discussed topic. "Surveillance" has emerged as a discipline in its own right, rather than just another field within the epidemiologist's province (1). Systems, methods, data and designs have been reviewed and evaluated [(2–9), amongst others]. This activity has been driven by a number of parties for a variety of reasons. These include: government officials, who have to mitigate the effects of disease outbreaks and do not wish to be surprised by either the occurrence of specific threats (such as incursions, or outbreaks, of exotic or zoonotic diseases) or by the (re)emergence of known diseases, while operating in an environment; industry bodies, who want to know how much disease is out there, estimate the losses incurred and identify what they can do to improve the health and the productivity of their sector, and researchers themselves, keen to explore new arenas.

The use of automated bio-surveillance systems for outbreak detection and syndromic surveillance (SynS) in the human field (10–14) stimulated studies within the veterinary sphere, reviewed in Dupuy et al. (15). They found that, in 2013, although there were 27 veterinary syndromic surveillance systems in 12 European countries, most of these did not yet have the statistical wherewithal to adequately analyse the data. Subsequently, there have been additional studies [e.g., (16–22)] and increased momentum within the animal health surveillance sector. Although there is evidence of further development of digital surveillance systems for animal populations (23), they have not yet matured into fully functional, digital, automated bio-surveillance systems providing outbreak detection, syndromic surveillance, monitoring of trends and situational awareness in animal populations.

A major constraint is the lack of computerized, automatically collected data (2). This apparent scarcity may be driven more by a lack of accessibility and availability, plus issues of data management (e.g., poor data quality, absence of core data needed for analysis), design (e.g., coverage) and documentation rather than an absence of data sources (9, 24, 25). However, in the United Kingdom (UK), there are continued demands to make use of available health-related information, from alternative existing data sources that were not designed for surveillance purposes, as surveillance intelligence within a wider surveillance system (26, 27). Elsewhere the potential to use national, or regional, statutory, or mandatory, centralized registers of cattle, pig, or equine identification, movements and fallen stock to provide measures of excess mortality has been investigated (28–35). The attractiveness of these registers arises from the tranche of European legislative requirements to record births, movements and deaths of individual animals and to dispose of livestock that die without passing through slaughterhouses, by appropriate means (https://eur-lex.europa.eu/). The existence of single systems for registration, or centralized collation of data from a number of rendering plants, or collection centers, facilitates this; although analyses can still be challenging and careful interpretation is required. These challenges are compounded when recording of death is not necessarily at an individual animal level, when there is no centralized collation of electronic data, and when a free market system operates i.e., farmers can choose from multiple fallen stock collection providers, who themselves can chose the level of service provision. This is the case with the British fallen stock collection system, especially for the ovine population. Attempts to use such data result in yet another "particularly interesting situation for epidemiologists," despite this being a small, rather than Big Data, scenario (36).

The overall aim of these studies was to assess whether data from a fallen stock scheme with voluntary membership had potential for use for livestock health and disease surveillance purposes.

The initial objectives of this study were: firstly, to describe the mortality experienced by British sheep, as measured by collection of ovine material from National Fallen Stock Company (NFSCo) members; secondly, to determine if there were any temporal (i.e., seasonal or annual) trends and/or spatial clusters associated with mortality and thirdly, to investigate the application of an aberration detection algorithm. The focus of the study was the utility of the data, rather than the statistical methods i.e., Could these objectives be achieved when the data collection was not designed with these purposes in mind? In the process, any limitations of the data would be identified, as would ways that they might be addressed. Analysis of the initial available dataset raised additional questions of relevance to the development of a functional surveillance system component. One of these was whether an additional existing data source—laboratory diagnostic submissions data collected as part of a passive surveillance system—could be utilized in parallel to the fallen stock collections data, in order to provide insight and facilitate interpretation of any statistical alarms that might be raised in the latter. The study was therefore extended with the addition of an objective to explore the utility of these data, using an exemplar diagnosis. After all, the definition of animal health surveillance implicitly includes action, or at least the inclusion of an action plan, with regard to the implementation of interventions that aim to mitigate the identified risk (37, 38).

MATERIALS AND METHODS

There were four developmental stages. These were: Stage 1—an exploration of the utility of 2011–2014 Scottish ovine fallen stock data, leading to initial results and initial recommendations; Stage 2—extension of the analyses to 2011–2014 England and Wales ovine fallen stock data, leading to initial results and further recommendations; Stage 3—an exploration of the utility of 2011–2014 Scottish ovine fasciolosis diagnostic data, specifically to see whether it could contribute to interpretation of the Stage 1 outputs; Stage 4—an update using 2015- mid-2018 Scottish plus England and Wales ovine fallen stock data, leading to updated results due to increased interest being shown by third parties.

Available Data

Fallen Stock Collection in Great Britain and the National Fallen Stock Company (NFSCo)

In accordance with EU Animal By-Product regulations (39), livestock that dies on British farms must be collected, identified and transported away as soon as is reasonably practical, by an approved transporter. Disposal must be via an approved,

registered animal by-products premise (40). Exceptions are made for pet animals and horses, remote areas including large parts of the Scottish Highlands and Islands (41) and in cases of natural disaster. Livestock keepers can make arrangements themselves directly with one of the many companies that deal with collection and disposal, or the process can be facilitated by becoming a member of the National Fallen Stock Company (NFSCo) (40). A collection service or a disposal premise may operate privately and/or as a member of NFSCo.

NFSCo CIC is a not for profit Community Interest Company that acts as an intermediary between the farming community and fallen stock collectors across Great Britain (GB) (www.nfsco.co.uk). It provides a service; sets standards; promotes high levels of bio-security; creates competition; facilitates payments and simplifies invoicing and cash flow. Membership is voluntary and free. Data are, therefore, collected for business needs. When a collection is made from a members' property, receipts are provided for monthly invoicing purposes. The number of Species Service Units (SSUs) that are collected on date X, from postcode Y, belonging to member Z, are recorded.

The definition of "Fallen Stock" for NFSCo is "animals which were killed (euthanasia with or without definite diagnosis) or have died (including stillborn and unborn animals) on farm and which were not slaughtered for human consumption. This includes animals killed by routine culling as part of normal production arrangements, where no government support is applied and animals lost during events that would ordinarily be covered by existing insurance arrangements e.g., fires and road accidents." (www.nfsco.co.uk). NFSCo collections are, therefore, an indication of the mortality experienced in the livestock populations that belong to NFSCo Members.

British Postcodes

A full British postcode consists of two parts of up to eight specific alpha-numeric characters (i.e., **AA**00 00BB). The first two alphabetic characters define a postcode area e.g., IV—Inverness, CH—Cheshire. There are 124 postcode areas in the United Kingdom of Great Britain and Northern Ireland (UK); they do not equate to country boundaries. The unit code (i.e., the last two letters, AA00 00**BB**) part of the full postcode identifies a postal route (or walk) of up to 100 addresses, for delivery purposes. A property may, therefore, have its own unique full postcode, or there may be multiple properties per full postcode (42). Thus, in an area of high livestock holding density, or where the area that the unit code applies to is large, more than one collection point may have the same postcode. They may, or may not, belong to different members and members might have multiple collection points.

Available NFSCo Datasets

Stages 1 and 2: Separately for each of the three countries, Scotland, England and Wales, two datasets were provided: a membership file and a Species Services file for sheep.

Stage 4: Two Species Services files for sheep were provided; one for Scotland and one for England and Wales together.

Membership files

These files provide an anonymized snapshot of the entire NFSCo membership, for each of the three countries, as of the date that the file was extracted. For Scotland this was "as of 31st October 2014," whereas for England and for Wales it was "as of 31 July 2015." Each member record had a unique identifier, a user status and postcodes for each of the addresses belonging to that member. There were no join, start, or stop dates and no other attribute data such as livestock species belonging to the member, livestock numbers, management type, or species collected from a member.

Species service file—sheep

The sheep Species Services files contain aggregated Species Service Unit data (SSU).

Types of SSU: In the Stages 1 and 2 files up to 11 types of SSU were recorded, although not all types were present in every country's file (**Table 1**). In Stage 4, there were a small number of additional SSUs, mainly in the England and Wales dataset. These mainly occurred infrequently apart from Dolav® sheep per kilo. This refers to a type of industrial plastic pallet box that can be used to store material.

Time periods and aggregation:

In the original datasets (Stages 1 and 2), the time period available was January 2011 to December 2014, inclusive, for Scotland and January 2011 to July 2015 inclusive, for each of England and Wales. Only the period to the end of December 2014 was analyzed initially. SSUs were aggregated by full postcode and by calendar month. This level of aggregation was defined by the data providers.

In Stage 4, the time period provided was January 2015 to June 2018, inclusive, for both the Scotland and combined England and Wales datasets. SSUs were aggregated by a unique ID representing the membership number, full postcode and by date of collection. These data were combined with the Stages 1 and 2

TABLE 1 | The 11 original SSUs recorded in the Sheep services files and what they consist of.

Species service unit	Explanation 1 unit equals…
Lambs 0–1 month[c]	1 unit = 1 lamb, between birth and 1 month of age
Lambs 2–12 month[c]	1 lamb, between and including 2–12 months of age
Sheep over 12 month[c]	1 sheep, over 12 months of age
10 + bags of lambs[c]	10 or more bags of lambs, under 12 months of age
10 + Lambs 2–12 month[c]	10 or more lambs, between and including 2–12 months of age
10 + Sheep over 12 month[c]	10 or more sheep, over 12 months of age
Sheep/lambs per 10 kg[c]	10 kg of dead ovine
Container/bag for lamb[c]	one container or bag for putting lambs in
10 + lambs 0–1 month[a]	10 or more lambs, between and including 0–1 months of age
Sheep/lambs per 10LTR[b]	One 10 liter volume unit of mixed ovine material
Unweighed Skip – Sheep[b]	One unspecified size skip of adult ovine material

[a] In English data files only; [b] In English and Welsh data files only; [c] In all three data files.

data to produce a dataset for the full period 2011 to mid-2018 for Scotland and for England and Wales.

SRUC Veterinary Services Fasciolosis Data (Stage 3)

The SRUC Veterinary Services (VS) Disease Surveillance network consists of eight centers (DSCs) located around Scotland. They receive diagnostic submissions from livestock keepers, in conjunction with their veterinarians. These submissions are made to confirm, or elucidate, a diagnosis in cases where diagnostic uncertainty exists based on clinical examination. A submission may consist of single or multiple samples from single, or multiple animals. All submissions are recorded in a laboratory information management system (LIMS). For diagnostic samples, when a diagnosis is reached according to specific criteria then a standardized Veterinary Investigation Diagnosis Analysis (VIDA) code is applied to that submission. There are two fields in which such a diagnosis can be recorded (VIDA1 and VIDA2). These may refer to a primary and secondary diagnosis in the same animal, or to two primary diagnoses in two different animals included in the same submission.

Acute fasciolosis is often fatal in sheep (43). For exploration in parallel with the descriptive spatio-temporal analysis of fallen stock data, the interest in fasciolosis diagnoses is as a potential explanatory factor for statistical alarms indicating increased mortality. For sheep, the most appropriate submissions should be those diagnosed as VIDA CODE 372 Acute fasciolosis (VIDA372). Chronic fasciolosis is recorded as VIDA CODE 373 (VIDA373) (**Table SM1**).

All ovine diagnostic submission records between January 2011 and December 2014 inclusive that had VIDA372 or VIDA373 recorded as a diagnosis were extracted from LIMS. These submissions consisted of carcase, liver, and/or feces samples appropriate to the VIDA CODE assigned (**Table SM1**).

Data Transformations

Assumptions Made in Order to Convert SSUs to Animal Units (AUs)

The values for the SSUs "container/bag for lamb" were excluded as they record a charge for the provision of containers, not for ovine material collected.

There are three age groups of interest: lambs from 0 to 1 month of age; lambs from 2 to 12 months of age, and sheep over 12 months of age. Some assumptions were needed to convert the SSUs in the datasets into animal units (AUs). For the SSU 10 + [age group], an estimate of the AUs collected can be made by multiplying the number of these SSUs by 10. This will be a consistent under-estimate, as this is the minimum number for this SSU. It was assumed, based on the frequency distributions over time of SSU collections and on industry knowledge, that bags of lambs would be most likely to consist of lambs between 0 and 1 months of age, usually in lambing periods and that there would be a minimum of four lambs per bag, The number of collections of 10+ bags of lambs was thus multiplied by 40, to give an estimate of the number of 0–1 month lamb animals units that they represented.

The unit by weight (Sheep/lambs per 10 kg) occurred as integers (i.e., 1, 2, 3 etc.), with each unit equating to 10 kg of dead ovine material. More than half of the collections were five units (50 kg) or less with the peak period contemporaneous with peaks for numbers of Lambs 0–1 month and co-incident with the peak period for numbers of Sheep over 12 months (data not shown). It was assumed that, firstly, an average weight for a dead lamb would be 5 kg and, secondly, the weight of a dead sheep over 12 months of age is likely to be similar to, or lower than average breed live-weights (kg). As the latter vary considerably depending on breed, it was assumed that a minimum weight for a sheep over 12 months of age would be more than 45 kg. These assumptions led to formulae for conversion of by 10 kg collections into AUs (**Table 2**).

Records for Sheep/lambs per 10LTR collections and for Unweighed Skip—Sheep collections were rare. Records for Dolav® sheep per kilo started in 2015 and were more frequent, although the approximate numbers of collections annually were consistent for England and Wales for the period 2015–2018. No clear assumptions could be made as to what these SSUs represented in terms of AUs. They were therefore excluded from further analysis.

Validation of assumptions for conversion of fallen stock data to animal units

The assumptions were tested for the Scottish data analyses, by consultation with a Sheep Research-Industry Interface Group and a Veterinary Advisory group.

SRUC VS Submissions Data

The VS submission records were aggregated into postcode areas from the full postcode provided and into calendar month from the submission date (day/month/year), in order to be comparable with the fallen stock data.

TABLE 2 | Relationship between Sheep/lambs per 10 kg and animal units (AUs) in Stages 1, 2, and 4.

Species service unit	Number of animal units
Sheep/lambs per 10 kg—<5 units	Less than 50 kg of dead ovine = number of units*10 = kg At 5 kg a lamb = Kg/5 = number of 0–1 month lambs
Sheep/lambs per 10 kg—5 or 6 units	Number of units*10 = kg = 50–60 kg Count as 1 sheep over 12 months
Sheep/lambs per 10 kg—7 or 8 units	Number of units*10 = kg = 70–80 kg Treat as a mixture—one adult sheep of 45 kg and the rest is made up 0–1 month lambs so the remaining weight in kg/5 = number of lambs of 0–1 month
Sheep/lambs per 10 kg—9 to 12 units	Number of units*10 = kg = 90–120 kg Count as 2 sheep over 12 months
Sheep/lambs per 10 kg—more than 12 units	Number of units*10 = kg = > 120 kg Kg/45 = number of sheep over 12 months—the rest is made up 0–1 month lambs so the remaining weight in kg/5 = number of lambs of 0–1 month

Only two of the age groups in the fallen stock data, lambs of 2–12 months of age and sheep over 12 months of age, are of relevance for fasciolosis due to the life cycle of the liver fluke. Additional data fields were used to categorize the submissions into the relevant age groups.

A VIDA diagnosis (i.e., submission records, hereafter also referred to as a "case") was used as one unit of analysis. However, although a diagnosis is applied at submission level, a submission may consist of single or multiple samples from single, or multiple animals. Additional data fields were used, therefore, to attempt a conversion to AUs. This was used as a separate unit of analysis. Where data were missing an assumption of 1 AU for each submission was made.

Analytical Methods

Spatial Aggregation

Country and postcode area were the spatial units of analysis used for the Scottish data. While country was used for both England and Wales in Stage 2 and England and Wales combined in Stage 4, postcode area was not feasible for use in England and Wales. This was due to a combination of factors. Postcode areas were therefore aggregated into larger spatial areas across both England and Wales, as regional units specifically for the purposes of these analyses. These regions were given descriptive assigned names that do not directly correlate with other regional classification systems. In Stage 3, the VS data were analyzed at country level only.

Descriptive Summaries

Fallen stock membership

Stages 1 and 2 membership data were briefly described on a country basis to provide information on the levels of membership, duplication of collection points per member and per postcode area (Scotland) or by new regional areas (combined England and Wales).

Fallen stock species service and animal units

In Stages 1 and 2 only, the number of units of each of the SSUs collected, per month, was plotted over the studied time period. This assisted in determining the assumptions for conversion into AUs, described above.

In Stages 1 and 2, to examine whether or not the effect of omission and the potential for mis-classification (when converting SSUs to AUs) made a substantial alteration to the outputs all AU analyses were run without any of the multiple SSUs included and without the ovine material (kg) contributions and then the outputs were compared. With each of the multiple units and ovine material (kg) contributions included sequentially, the new estimates of AU (i.e., re-categorized variables) were then analyzed and compared. Consequently, the variables that included the estimated AU from both multiple units and the kg conversion were used for each of the three age groups: All lambs 0–1 month; All lambs 2–12 months; All sheep over 12 months. These are the outputs reported in this manuscript and they were the AU categories that were used for each age group in Stage 4 (January 2011 to June 2018 inclusive).

Stages 1, 2 and 4: The number of AUs were summarized and described: per age group, by country, by postcode area or regional area, by year and by month and combinations thereof.

SRUC VS fasciolosis data

Stage 3: The number of VIDA372 (acute fasciolosis) and VIDA373 (chronic fasciolosis) diagnoses, in terms of submissions assigned a diagnosis (cases) and as estimated AUs in those submissions, were summarized and described for the country as a whole, and by postcode area, by age group and by time in a manner comparable to that used for the fallen stock data.

Time-Series Analysis

Descriptive time-series analyses were used to investigate and visualize temporal patterns in all of the datasets (fallen stock and VS). The latter was restricted to only the diagnostic submission records of Scottish origin with primary fasciolosis acute fluke (VIDA372) diagnoses.

The monthly time-series, seasonal pattern, annual totals, and average annual trend were plotted. Seasonal patterns were represented as monthly boxplots, to depict both the seasonal pattern and reflect differences in the average monthly number of events/units—where an event is the relevant unit of analysis—and variations across months. The monthly seasonal effects (indices) were then computed by, firstly, eliminating the trend from the data by dividing each monthly observation by their yearly average. This quotient is then averaged for each month across all the years. This gives an indication of how the value in a particular month differs from what is expected per month on average. The average annual trends were plots of the average yearly events, where average could be either mean or median number of events in a given year. The default was usually the mean; the median was used for data with strong outliers in order to minimize the influence of the outliers on the shape of general trend. These analyses were performed at country level for each stage, by age group.

For the fallen stock data, time-series plots were also plotted for each postcode area (Scotland) and region (England and Wales), by age group. Differences in average annual trends, seasonal patterns and variations across these spatial areas were captured in these plots. These area time-series data were later used to fit temporal alarm detection algorithms (TADA).

Aberration Detection

The datasets were scanned using the classic (original) Farrington method (44) that allows for analysis of counts without adjustments. Data were modeled as over-dispersed Poisson counts. A subset of the available data was used as a reference period to inform the models and account for seasonality. The 12 months of the first year (2011) were used in Stages 1, 2, and 3. The generalized linear models were used to predict what would be expected to occur i.e., to construct an upper prediction interval, or threshold, at 0.01 level of uncertainty. These threshold values are compared with the observed value at each time point from January 2012 to December 2014. If the actual value exceeds the threshold, an alarm is raised. The same algorithm was used for analyses of Stage 4 data but the first 24 months (2011 and 2012)

were used as the reference period values to reflect the longer time-series and to improve the model performance.

All analyses were done in R statistical package (45) and the TADA was fitted using the surveillance package (46).

RESULTS

Data

Lack of Denominator Data

There were no suitable denominator data available for either the fallen stock or the VS data. Due to lack of mutual farm or premise identifiers, these two data sources could not be linked and no alternative data sources could be linked with the fallen stock datasets to augment them and provide a denominator dataset, or additional attribute data. Therefore, subsequent analysis had to use count data.

Spatial Aggregation

Stages 1 and 4: Fourteen mainland Scottish postcode areas were represented in the fallen stock collections data (**Figure 1** and **Table SM2**). An additional one, the Outer Hebrides, was present in the Scottish membership file.

Stage 2: There were more than 90 postcode areas in the English membership file and nine in the Welsh one. Four of the postcode areas in the English file also appeared in the Welsh file because these postcode areas extend across the border. When aggregated into larger units across both England and Wales, there were 18 designated regional areas (**Figure 1** and **Table SM3**).

Stage 3: Acute fasciolosis cases came from 13 of the 14 mainland Scottish postcode areas, with no diagnoses from Dundee, nor from the Outer Hebrides or Shetland island postcode areas. All 14 of the mainland Scottish postcode areas had at least one chronic fasciolosis case, as did the two additional island areas, Outer Hebrides and Shetland.

Available Datasets—Analysis

Not all of the background data and figures from which the following results are drawn are included in this manuscript. They are available on request from the corresponding author.

Fallen Stock Membership

Numbers of members were similar in Scotland and Wales, with approximately three times as many in England. The majority (90–96%) of active members had only one collection point (CP); however a small proportion (0.06–0.1%) had more than 10 collection points. In some full postcodes, there were multiple active CPs, with a maximum of 10. In Scotland, more than one in five of the full postcodes that contained active CPs, had at least two located within them. This was similar in Wales, whereas in England it was less, at just over one in 10. The spatial distribution of members roughly approximated to expectations, given livestock holding density and size/shape of postcode or regional areas, except for the Outer Hebrides where representation was low.

Fallen Stock Animal Units (AU)

The total number of AUs collected each year, stratified by age group varies (**Table 3**).

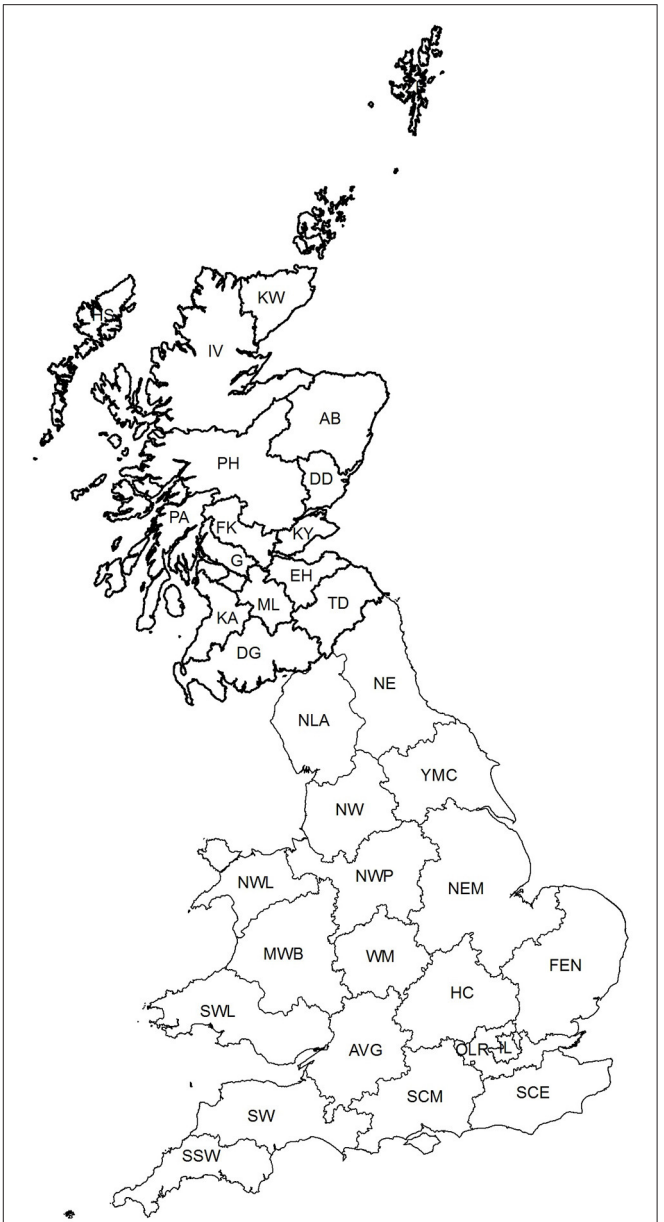

FIGURE 1 | The location of the aggregated spatial units used in the analyses—16 postcode areas in Scotland and 18 regional areas in England and Wales (see **Tables SM2, SM3** for details). Ordnance Survey data © Crown copyright and database right 2017; Royal Mail data © Royal Mail copyright and database right 2017 and National Statistics data © Crown copyright and database right 2017.

Stage 1—Scotland 2011–2014

In the subset of analyses that built up to the re-categorized "all animal" unit variables, for each of the three age groups: Lambs 0–1 month; Lambs 2–12 months; Sheep over 12 months, no alarms were raised when only the single AUs were used. As components were added, there were slight effects on the shape of the seasonal patterns and the aberrations detected, with more marked effects on the magnitude of the annual trends.

TABLE 3 | The estimated total number of (all) animal units (AUs) collected from each country by year and age group.

Animal units		Year							
Country	Age group	2011	2012	2013	2014	2015	2016	2017	2018 (Jan–June only)
Scotland	Lambs 0–1 month	15,436	14,547	18,977	8,980	27,150	31,183	28,210	40,001
	Sheep over 12 months	78,066	64,565	96,891	60,724	65,080	74,120	82,378	94,737
	Lambs 2–12 months	32,678	26,741	30,506	21,096	22,170	29,153	38,092	23,264
England and Wales	Lambs 0–1 month	45,423	48,494	74,059	48,747	32,042	28,229	27,268	29,123
	Sheep over 12 months	115,327	119,722	142,126	118,313	124,962	12,7961	134,585	102,461
	Lambs 2–12 months	27,367	33,683	37,789	32,533	30,959	36,709	39,630	17,870

For the estimated "all AU" variables, in all three age groups, seasonal patterns with an annual cycle were seen. These were similar for Lambs 0–1 month and Sheep over 12 months but differed for Lambs 2–12 months. This similarity was reflected in the seasonal patterns (**Figures 2A,C,E**) and seasonal effects (**Figures 3A,C,E**).

For both Lambs 0–1 month and Sheep over 12 months, the seasonal pattern peaks in April and is at its lowest in August and September, respectively (**Figures 2A,E**). The seasonal effects are above 1 in the period March to May, with the effect in March higher than in May for Sheep over 12 months and vice versa for Lambs 0–1 month (**Figures 3A,E**).

The seasonal effect in April is ∼5 times the expected monthly average mortality for Lambs 0–1 month and ∼2.7 times that for sheep over 12 months. However, the seasonal patterns and effects for Lambs 2–12 months are very different (**Figures 2C**, **3C**). These were at their lowest in May and highest in the late autumn/winter months October to January.

The average annual trend was similar for all three age groups although the magnitudes of the peaks and troughs differed slightly (**Figures 4A–F**). These dropped sharply from 2011 to 2012, rose in 2013 and dropped again in 2014.

At a country level, the predicted threshold for the TADA was exceeded and an alarm triggered in April 2013 for both Lambs 0–1 month and Sheep over 12 months (**Figures 5A,E** and **Table 4**), whereas no alarm was triggered throughout the study period for the Lambs 2–12 months (**Figure 5C** and **Table 4**).

There were variations by postcode area in the overall number of collections, SSUs, use of bulk collections and thus AUs, with higher numbers predominantly in the central and southern areas of Scotland that encompass higher sheep and sheep-holding densities. Annual trends also varied by postcode area (data not shown).

At a postcode area level, the April 2013 alarm was apparent in five postcode areas for Lambs 0–1 month, of these four also had alarms in April 2013 for Sheep over 12 months. Four additional postcode areas had April 2013 alarms for Sheep over 12 months (**Figure 6B**). Additional alarms were triggered in other months, in other postcode areas for both age groups, while a number of alarms were triggered, in a variety of postcode areas for the Lambs 2–12 month age group (e.g., **Figure 6A** for January 2013—other data not shown).

Validation of assumptions for conversion of fallen stock data to animal units. Both working groups agreed that bags of lambs would be most likely to be used at peak lambing periods for peri- and neonatal deaths of 0–1 month lambs; that four would be considered a minimum for lambs in a bag and that 5 kg was possibly a little on the low side for the lamb weight. They also suggested that dead ewes weighed less than one would anticipate. No better method of allocation and classification was proposed.

Stage 2—England and Wales 2011–2014

As for Stage 1, subset analyses were completed to confirm that the multiple units needed to be included to provide an overall picture. This was the case in all analyses. The pattern of use of the multiple units varied between the two countries and years—using the full combination of "all animal" unit variables, for each of the three age groups, produced results that were comparable.

As in Stage 1, in all analyses, for all three age groups seasonal patterns with an annual cycle were seen.

England (data not shown). The seasonal pattern and seasonal effects were again similar for English Lambs 0–1 month and Sheep over 12 months but differed for Lambs 2–12 months. The former had a peak between February and May with between 4 and 4.5 times the expected monthly average mortality in March and April for Lambs 0–1 month and ∼2.5 times that for Sheep over 12 months. The February-May peak was more sharply defined for the Lambs 0–1 month than the Sheep over 12 months age group. The seasonal patterns and effects for Lambs 2–12 months were similar to those for this age group in Scotland being at their lowest in May and highest in the late autumn/winter months October to January. However, the English data did not show a plateau in the autumn period as seen in the Scotland data, while in England there was a monthly average mortality above 1 in September.

The average annual trend was similar for all three age groups although the magnitudes of the peaks and troughs differed slightly between them. They differed from the average annual trends in Scotland, being consistently upwards overall from 2011 to a peak in 2013, with 2014 being similar to 2011.

At a country level, the predicted threshold was exceeded and some alarms triggered in different age groups at a number of time points (**Table 4**).

Wales (data not shown). The seasonal pattern and seasonal effects were similar to those seen in the English data for all three age groups. As per the average annual trend in the Scotland and England data, these rose to a peak in 2013 for all three age

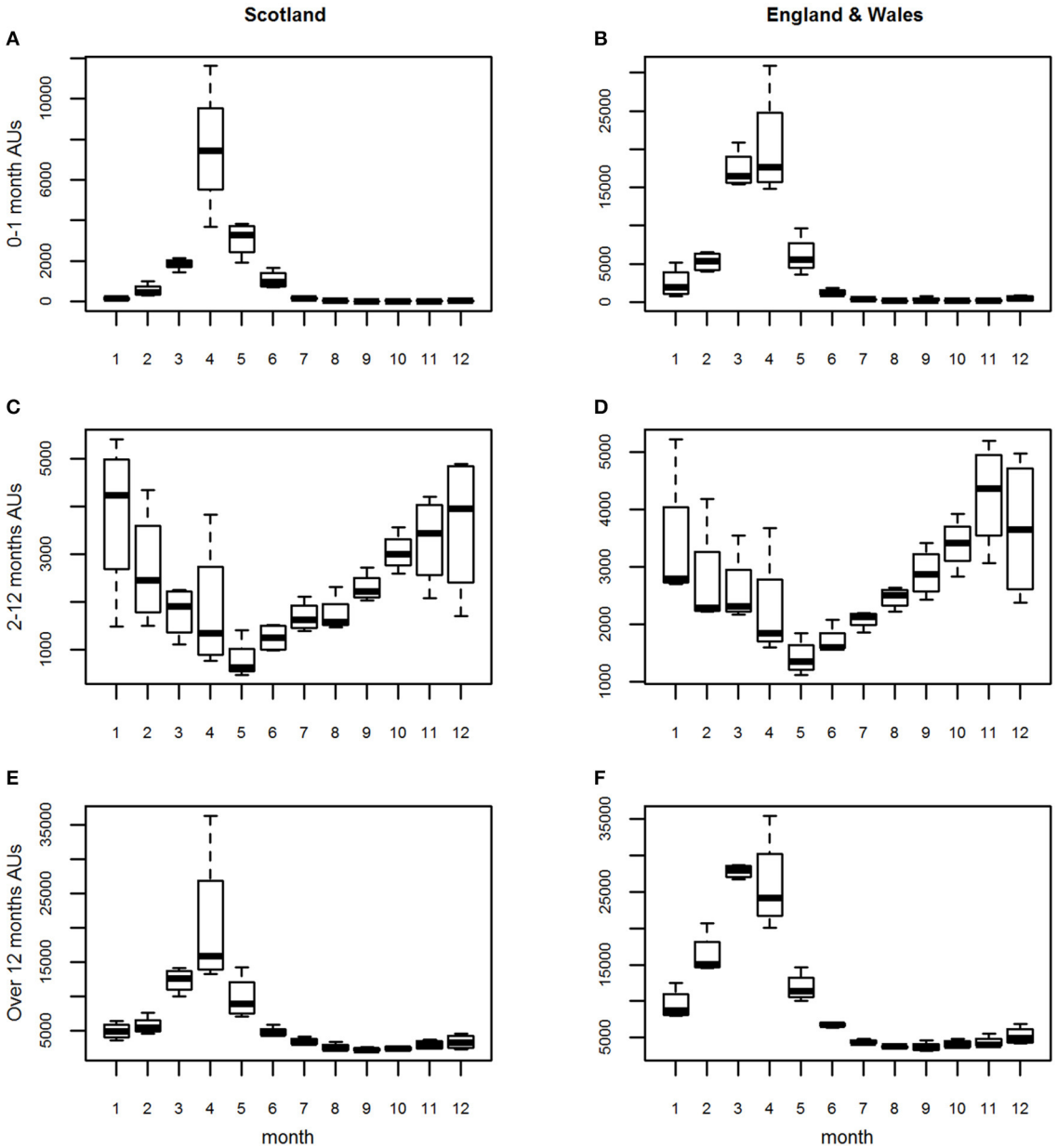

FIGURE 2 | (A–F) The seasonal patterns (i.e., distribution of the monthly counts) for the three age groups of estimated animal units (AUs) by country using the 2011–2014 fallen stock datasets.

groups. However, while for Sheep over 12 months and Lambs 2–12 months overall 2014 was similar to 2011, for Lambs 0–1 month there was a decline, similar to the pattern observed in Scotland, but different from the rise observed in England. Again a number of alarms were triggered in different age groups (**Table 4**).

England and Wales combined. The seasonal patterns, seasonal effects and annual trends (**Figures 2**, **3**, **4B,D,F**) for each age group were subtly different from those in the Scottish dataset (**Figures 2**, **3**, **4A,C,E**).

By combining the England and the Wales datasets, it became possible to look at spatio-temporal patterns on a regional area basis. Over the study period, the majority of the estimated Lambs 0–1 month AUs are from two regional areas (HC and NLA) with the majority of Sheep over 12 months AUs coming from sheep-dense regional areas (MWB, NLA, NWL, and SW). The majority of the estimated Lambs 2–12 month AUs are also from four of these areas (NLA, MWB, and SW with NWL being replaced by NW).

The average annual trends vary between regional areas and age groups (data not shown). For Lambs 0–1 month, in several regional areas the estimated number of AUs collected increases on an annual basis, with an additional peak in 2013. This is particularly apparent in four areas. In some regional areas there is

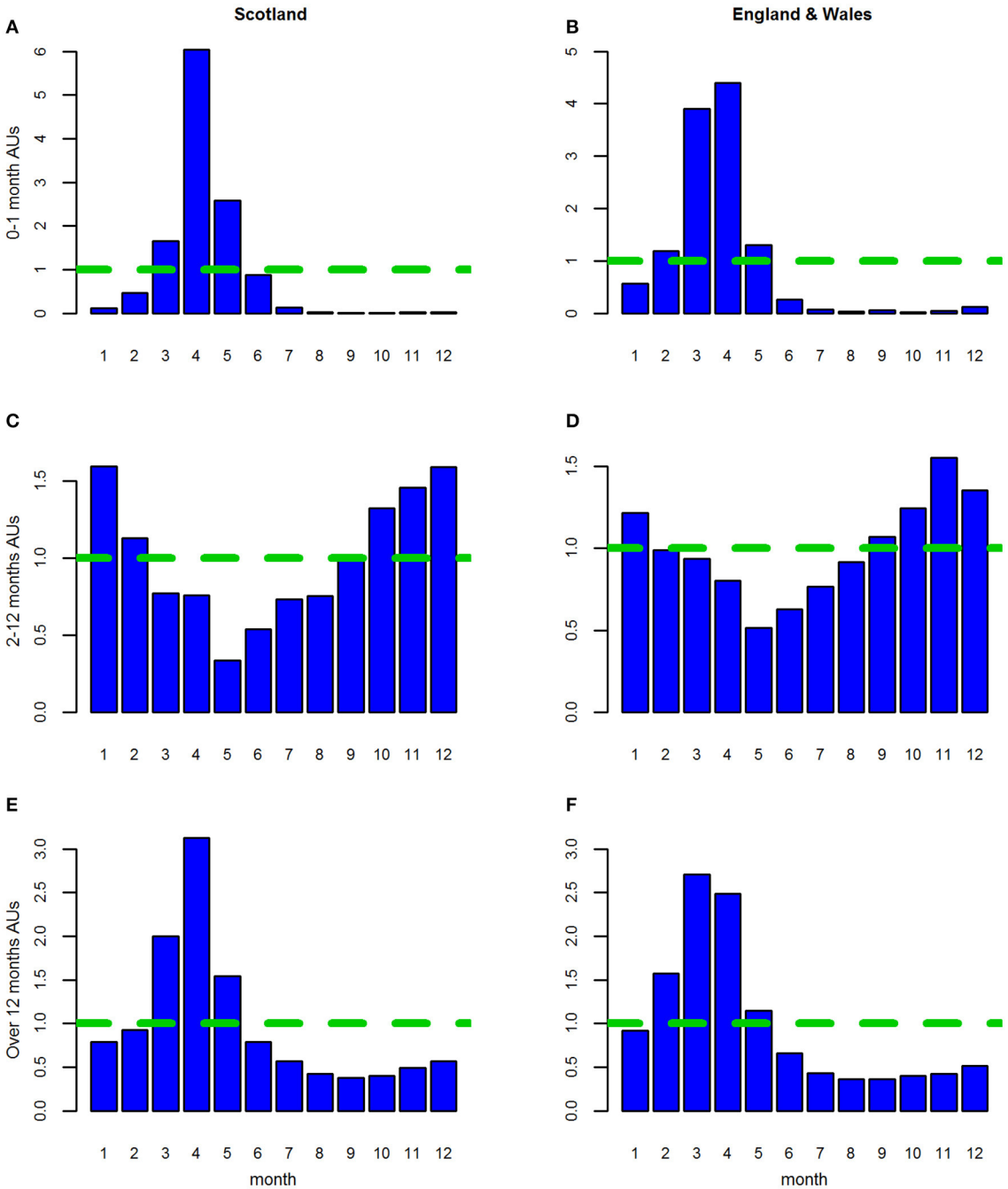

FIGURE 3 | (A–F) The seasonal effects for the three age groups of estimated animal units (AUs) by country using the 2011–2014 fallen stock datasets.

a fall in 2014, while in one the number drops from the reference year of 2011 and is much lower in all of the next 3 years. For Sheep over 12 months, on an annual basis, the number collected from most of the regional areas is fairly steady, although several regional areas saw an increase in numbers in 2013. In two regional areas, the estimated number of AUs collected increased on an annual basis, while in one, apart from 2013, there is a decline over the time period. For Lambs 2–12 months, the estimated numbers of AUs collected are again generally fairly steady, or gradually increase, sometimes with indications of peaks in 2013. However, in one regional area, the number collected decreased from 2011; in two there were increased numbers collected in both 2012 and 2013, while in another one there was a steady year on year increase.

A number of alarms were raised in various regional areas and age groups over the time period (see **Figures 6A,B**, for

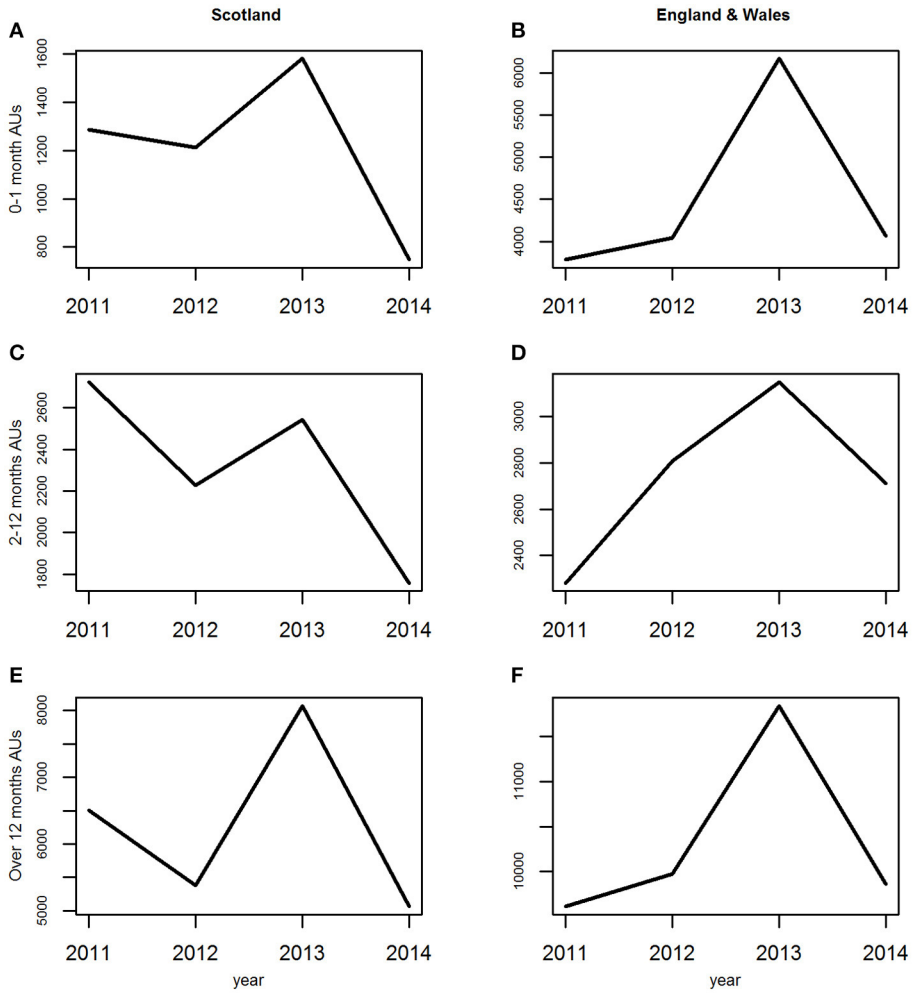

FIGURE 4 | (A–F) The average annual trends for the three age groups of estimated animal units (AUs) by country using the 2011–2014 fallen stock datasets.

examples). Some contribute to the overall England and Wales alarms e.g., the Lambs 0–1 month September 2013 overall alarm appears to arise from a single regional level alarm that month; the October 2012 Sheep over 12 month overall alarm is reflected by regional alarms in this age group and month in three areas. It is also possible to identify areas where alarms were raised in a number of age groups, either coincidently, or in subsequent months e.g., The alarm for Lambs 2–12 months, seen in the SW in November 2013, follows alarms in the same area in September and October 2013 for Lambs 0–1 month and Sheep over 12 months, respectively (data not shown).

Recommendations from Stages 1 and 2

Twenty-one recommendations arose from Stages 1 and 2 (**Table SM4**). These included suggestions for improvement of the data, other actions required to facilitate use for surveillance purposes and for further investigations were made to the data-providers.

SRUC VS Fasciolosis Data 2011–2014

SRUC VS fasciolosis data- descriptive analysis

There were 1,036 eligible ovine diagnostic submission records in the 4 year study time period i.e., cases of either VIDA372 (acute) or VIDA373 (chronic). Almost three-quarters had a primary diagnosis of chronic fasciolosis, while just under one in five (17%) had a primary diagnosis of acute fasciolosis. Some submissions were not of Scottish origin (**Table SM5**).

The 178 Scottish primary acute fasciolosis cases represented an estimated 249 AUs. Just over half of these were from sheep, or groups of sheep, that could be categorized as over 12 months of age ($n = 98$, 55%), over a third were from lambs between 2 and 12 months of age ($n = 67$, 38%) while the rest, less than one in 10, could not be categorized into one of these two age groups, given the available information ($n = 13$, 7%).

Approximately a third of these acute cases, at both submission and AU level, came from Dumfries and Galloway (DG) postcode area. Four out of five came from the five southern postcode areas

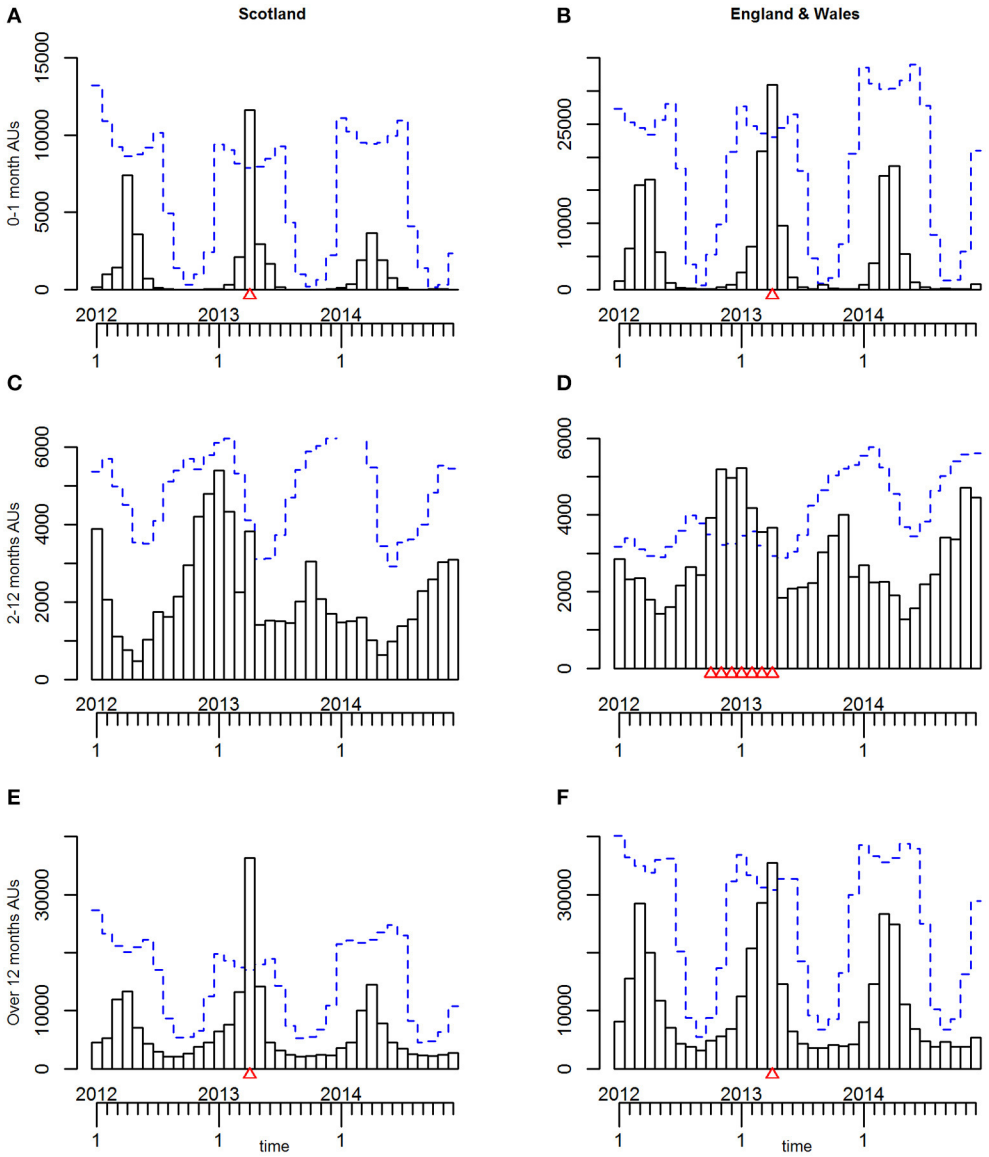

FIGURE 5 | (A–F) The time-series plots and temporal aberration detection (TADA) for the three age groups of estimated animal units (AUs) by country, using the 2011–2014 fallen stock datasets, with 2011 as the reference period.

(DG, TD, KA, ML, EH) that are high density areas in terms of the number of breeding ewes. Within each of these five areas, the number of cases (as AUs) was greatest in 2012.

The 702 Scottish primary chronic fasciolosis cases represented an estimated 2,150 AUs. Just over half of these diagnoses were from sheep, or groups of sheep, that could be categorized as over 12 months of age ($n = 373$, 53%), while just over a tenth were from lambs between 2 and 12 months of age ($n = 74$, 11%). The rest—over a third—could not be categorized into one of these two age groups, given the available information ($n = 254$, 36%). As for acute fasciolosis, just under a third of the chronic fasciolosis diagnoses came from the DG postcode area, although elsewhere a more even distribution across postcode areas was seen than with the acute fasciolosis diagnoses.

Unlike the acute fasciolosis cases, where there was a peak in 2012 in many postcode areas, the pattern of numbers per year, or annual effects, for chronic fasciolosis case varied widely, by postcode areas. These data were excluded from the time-series analysis due to the more indirect link with mortality in sheep than with acute fasciolosis.

SRUC VS fasciolosis data—time-series analysis

The majority of acute fasciolosis cases occurred between October 2012 and January 2013. At an animal level (AUs) there is a similar pattern to that of the submission-level cases, although there is an apparent additional peak in the former during the autumn of 2013 (data not shown).

TABLE 4 | Time-points at which country level statistical alarms were raised by the application of the Farrington TADA to the datasets, by country, dataset and age group.

		Age category		
Time period	**Country area—dataset**	**Lambs 0–1 month**	**Sheep over 12 months**	**Lambs 2–12 month**
2011–2014 reference = 2011	Scotland—fallen stock (**Figures 5A,C,E**, respectively)	April 2013	April 2013	None
	England—fallen stock		October 2012	October 2012 to
		April 2013		April 2013 inclusive bar March 2013
		September 2013		
	Wales—fallen stock	October 2012	October 2012	October 2012 to April 2013 inclusive
		March 2013	April 2013	September 2013
	England and Wales combined—fallen stock (**Figures 5B,D,F**, respectively)		October 2012	October 2012 to
		April 2013	April 2013	April 2013 inclusive bar March 2013
		September 2013		
	Scotland—VIDA372 Animal Units	n/a	January 2012	January 2012
			March 2012	
			October 2012 to February 2013 inclusive	October 2012 to January 2013 inclusive
			May 2013	
	Scotland—VIDA372 Submission-level assigned diagnosis units	n/a	January 2012	January 2012
			October 2012 to February 2013 inclusive	October 2012 to January 2013 inclusive
2011-mid 2018 Reference = 2011 + 2012	Scotland—fallen stock (**Figures 11A,C,E**, respectively)	April 2013	April 2013	
		April and May 2015		
		April and May 2016		
				September 2016 to January 2017
			April 2017	
				August, September, and November 2017
		April 2018	April 2018	February to April 2018
	England and Wales combined—fallen stock (**Figures 11B,D,F**, respectively)	April 2013	April 2013	January to April 2013 inclusive
			August 2015	
				March 2016
				October and November 2016
				November 2017
				March 2018

The seasonal effect for acute fasciolosis cases is <0.5 for the months of March to July with a rise starting in August (**Figure 7A**). This rise continues with increased seasonal effects being seen for each consecutive month until November, then declining again. The effect is similar at an AU level except that the October seasonal effect is larger than for cases.

Over the study period, the number of acute fasciolosis cases for Lambs 2–12 months was broadly similar to, but did not entirely mirror, that of the Sheep over 12 months of age group (**Figure 7B**). There are indications, for both age groups, of a seasonal pattern with an annual cycle that varies from year to year. The autumn 2013 peak in Sheep over 12 months is higher when AU level units, rather than submission-level cases, are considered.

Overall, using cases, the seasonal pattern has a peak between October and January (**Figure 7C**). At an AU-level, by age group the peak is similar but with subtle differences between the age categories. The average annual trend rose sharply from 2011 to 2012, and then dropped to lower than 2011 levels in 2014 overall (**Figure 7D**) and for both age groups (data not shown).

The TADA were only run at a country level due to the low number of data points. TADA alarms were raised at the same time points for both AUs and submission-level cases, using all primary VIDA372 records. These were in: January and March 2012; months between October 2012 to January 2013 inclusive and in May 2013. The TADA alarms at country level for Sheep over 12 months were the same as those for the all age analysis when using AUs, except for an extension of a month into February 2013 (**Table 4**). While with submission-level cases, for Sheep over 12 months, the March 2012, May 2013 and February 2013 alarms did not get triggered (**Figure 8B**). However, for the Lambs 2–12 months group, while similar to the all records analysis, the March 2012 and May 2013 alarms did not occur with either unit of analysis (**Figure 8A** and **Table 4**).

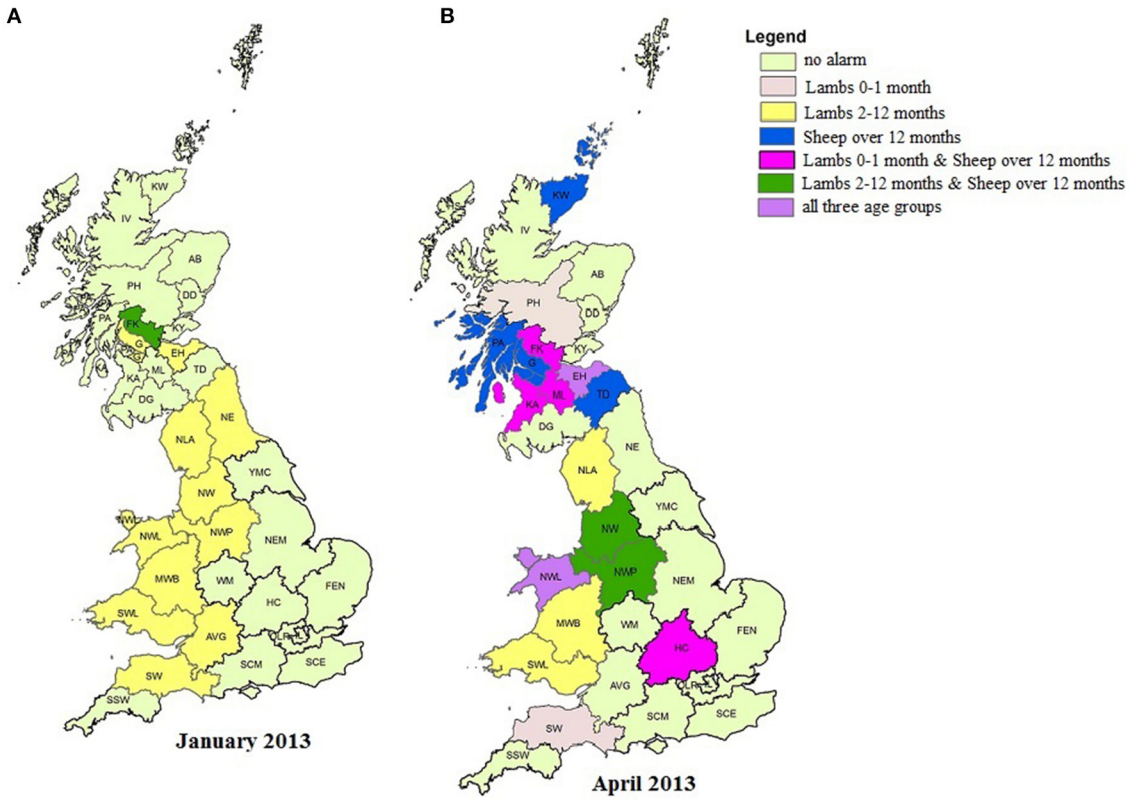

FIGURE 6 | (A,B) Postcode area (Scotland) and regional area (England and Wales) alarms raised by the temporal aberration detection algorithm (TADA), by age group for the 2011–2014 fallen stock datasets for two calendar months: January 2013 **(A)** and April 2013 **(B)**. Ordnance Survey data © Crown copyright and database right 2017; Royal Mail data © Royal Mail copyright and database right 2017 and National Statistics data © Crown copyright and database right 2017.

Recommendations from Stage 3

Six recommendations arose from Stage 3 (**Table SM4**). These included suggestions for improvement of the data and other actions required to facilitate use for surveillance purposes and for further investigation.

Fallen Stock Animal Units (AUs)

Stage 4—2011- mid-2018

As in Stages 1 and 2 subset analyses were completed to confirm that the multiple units needed to be included to provide an overall picture. This was the case, so the outputs from the categories including all the multiple units are presented here, as in previous sections.

Scotland. As in the shorter dataset, for all three age groups, in AUs, seasonal patterns with an annual cycle were seen. These were broadly similar for Lambs 0–1 month and Sheep over 12 months but differed for Lambs 2–12 months. The seasonal patterns (**Figures SM1A,SM1C,SM1E**) and effects (**Figures 9A,C,E**) varied slightly from those seen in the Stage 1, although the period where the seasonal effect is above 1 remained the same for each of the three age categories (**Figures 9A,C,E**). For Lambs 0–1 month, the peak was still in April (5x); however the effects were higher in May (3x) and March than previously (**Figure 9A** cf. **Figure 3A**). The peak for Sheep over 12 months was also still in April (∼2.8x) and was higher in March than in Stage 1 (**Figure 9C** cf. **Figure 3C**). For both these age groups the seasonal effect was now lowest in October. The seasonal patterns and effects for Lambs 2–12 months were still lowest in May and highest in the late autumn/winter period although there are subtle differences in individual months between the two time periods (**Figure 9C** cf. **Figure 3C**).

As previously, the average annual trend over the extended time period differed between the three age groups (**Figures 10A,C,E**). For Lambs 0–1 month following the 2013 peak and 2014 decline the numbers rose substantially above 2013 levels in 2015 to 2017, with a further dramatic rise in 2018 (**Figure 10A**). For Sheep over 12 months there was a low gradual increase from 2014 to 2017, with a subsequent dramatic rise in 2018 (**Figure 10C**); whereas for the Lambs 2–12 months, there was a consistent rise in trend year on year from 2015 to 2018, inclusive (**Figure 10E**).

At a country level, alarms were triggered in April 2013 for both Lambs over 0–1 month and Sheep over 12 months. There were additional statistical alarms triggered in the spring of 2015, 2016, and 2018 for Lambs 0–1 month and in 2017 and 2018 for Sheep over 12 months, the alarms (**Figures 11A,E** and **Table 4**). Alarms were triggered for Lambs 2–12 months in the autumn and end of

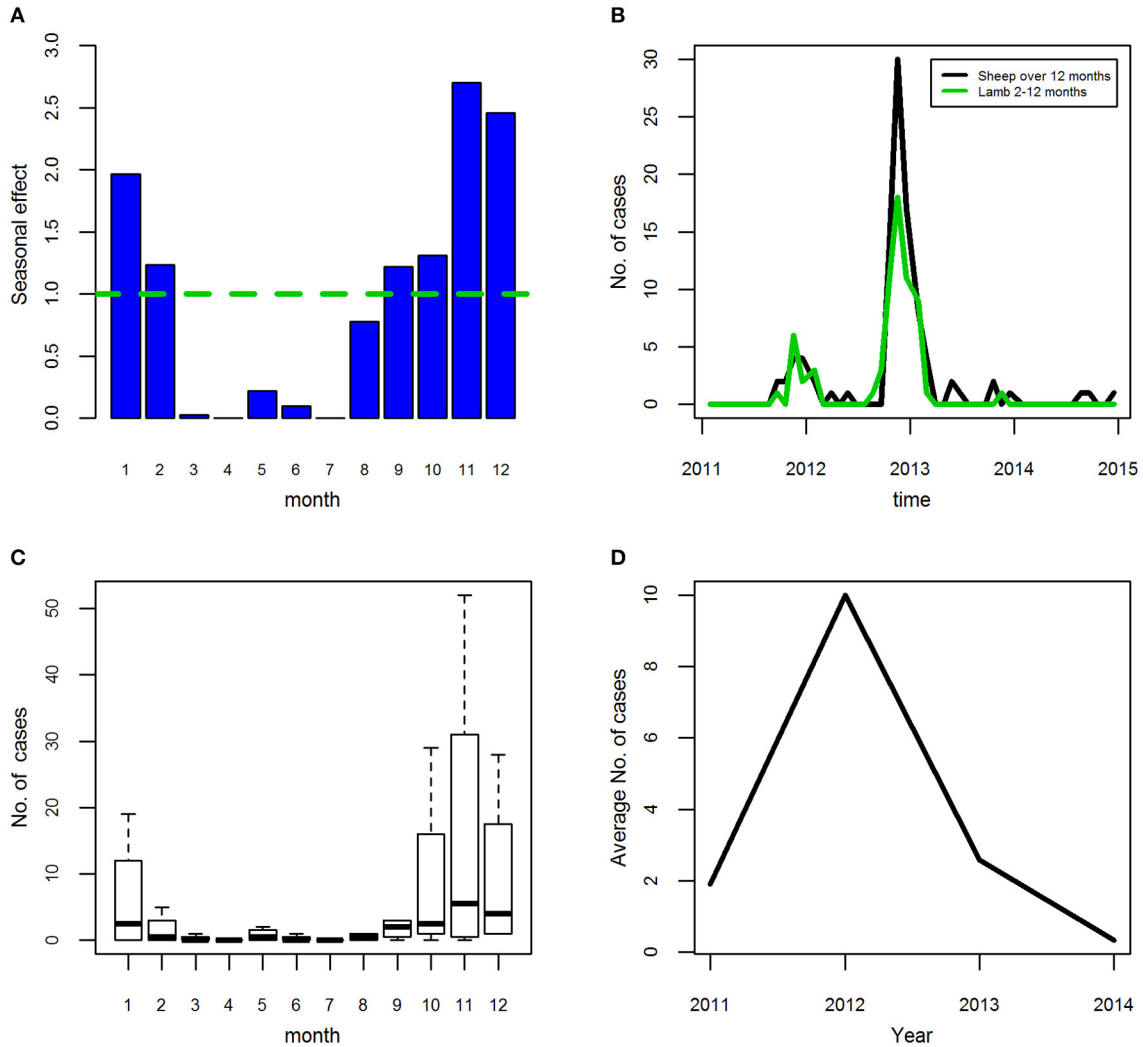

FIGURE 7 | (A–D) Seasonal effects **(A)**, time-series **(B)**, seasonal pattern **(C)**, and annual trend **(D)** for ovine acute fasciolosis (VIDA372) diagnoses assigned at a submission-level (i.e., cases) in Scotland, 2011–2014 inclusive.

the year 2016, again in some months of the same period in 2017 and the first third of 2018 (**Figure 11C** and **Table 4**).

As with the earlier dataset, there were variations by postcode area in the overall number of AUs and annual trends (data not shown). The most notable of these being an absence of any AUs from one area in 2018 and a 20x increase in the annual numbers of Lambs 0–1 month from one postcode area in 2015–2018 compared to the period 2011–2014. A corresponding increase in the magnitude of Sheep over 12 months from this area during 2015–2018 was not seen.

At a postcode area level with the new reference period, the April 2013 alarm was now apparent in six postcode areas for Lambs 0–1 month and 11 for Sheep over 12 months. Additional alarms were triggered in other months, in other postcode areas for both age groups. The spring 2015 and 2016 Lamb 0–1 month alarms were not confined to the postcode area with the increase in numbers noted above but were also raised in five and two other postcode areas, respectively. Nine areas had alarms for Lambs 0–1 month in April or May 2018, and eight for Sheep over 12 months in March or April 2018. Again, a number of alarms were triggered, in a variety of postcode areas for the Lambs 2–12 month age group (data not shown).

England and Wales combined. As per the shorter time period in Stage 1, the seasonal pattern (**Figures SM1B,SM1D,SM1F**) and effects (**Figures 9B,D,F**) were broadly similar for Lambs 0–1 month and Sheep over 12 months but differed for Lambs 2–12 months. The seasonal effects for the first two age groups remained similar to those seen before (**Figures 3B,F** c.f. **Figures 9B,F**), whereas both the seasonal patterns and effects for Lambs 2–12 months have changed slightly. While the effects are still lowest in May, they are now above 1 for October through to March, inclusive rather than September to February and the November peak effect, seen earlier, has been moderated (**Figure 9D** c.f. **Figure 3D**).

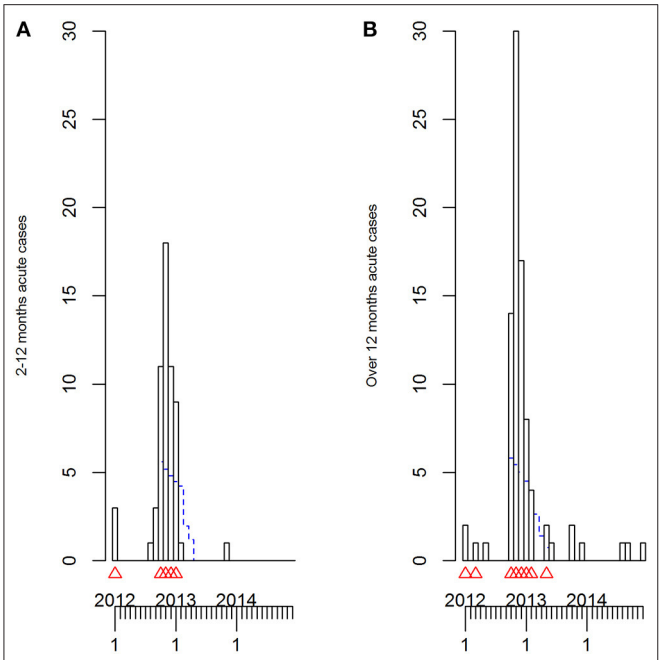

FIGURE 8 | (A,B) The time-series plots and temporal aberration detection (TADA) for all ovine acute fasciolosis (VIDA 372) diagnoses assigned at a submission level (i.e., case) in Scotland, 2011–2014 inclusive for the two age groups: lambs 2–12 months **(A)** and sheep over 12 months **(B)**.

The average annual trend over the extended time period differed between the three age groups to an even greater degree than that observed in the Scottish data. For Sheep over 12 months the trend is similar to that seen in this age group in the Scottish data (**Figures 10E,F**), whereas, Lambs 0–1 month differ (**Figure 10B** c.f. **Figure 10A**). The average annual trend for Lambs 2–12 months in the combined England and Wales dataset also differs from that seen in this age group in Scotland (**Figure 10D** c.f. **Figure 10C**).

For the combined English and Welsh dataset, overall, only the April 2013 alarm remains for Lambs 0–1 month and Sheep over 12 months, while there is an additional alarm in August 2015 for the latter (**Figures 11B,F** and **Table 4**). For the Lambs 2–12 months there are still alarms in the first third of 2013, with additional alarms at a number of spring and late autumn periods in a number of years. Some of these are coincident with alarms in Scottish Lambs 2–12 months, others differ (**Figures 11C,D** and **Table 4**).

When looking at the annual numbers by regional area, the most notable variations are a substantial reduction in the numbers of Lambs 0–1 month per annum from one area from 2016 on and double the usual numbers of Sheep over 12 months from another area in both 2015 and 2017 (data not shown).

A number of alarms were raised in various regional areas and age groups over the time period. Some contribute to the overall England and Wales alarms and some don't e.g., seven areas have a Spring 2013 alarm for the Lambs 0–1 month, either just before, just after, or in April, while six areas have an alarm in this age group in March or April of 2018. For Sheep over 12 months, nine areas have a Spring 2013 alarm, there are scattered alarms in this age group in the late months of 2017 and early months of 2018 and only two regional areas with an August 2015 alarm, while there are scattered alarms elsewhere in neighboring months. In the Lambs 2–12 months, seven regional areas have alarms raised in either February or March 2018, while all but two regions have alarms raised in one or two of the months between January 2013 and April 2013 inclusive.

DISCUSSION

Key Findings

In this study, an existing data source—business operational data from a voluntary fallen stock collection service—was investigated, to determine if these data could be used as a proxy for the mortality experienced by the British sheep population, in the absence of any other appropriate data source for this species. Despite various limitations (discussed below), these data could, with appropriate domain expertise, be converted into a useable format. They did reflect the seasonal pattern expected from knowledge of the British sheep production-year calendar (47); the spatial distribution of the sheep population of GB (48, 49) and the slight variations across Britain associated with the different sheep management systems and its stratified nature (50). Statistical aberrations were detected in relevant areas and age groups at the time of a known extreme weather event that occurred during March and April 2013 at peak lambing time (51). Similarly, the effects of a more widespread weather event slightly earlier in the spring of 2018 were detected (52). In addition a number of statistical alarms were raised in regions at specific time-points early in the study period that potentially reflected other known challenges. This prompted the investigation of the SRUC Veterinary Services fasciolosis diagnostic data. The primary aim here was to determine if it could be utilized in parallel i.e., to see if it precedes, coincides or assists in the explanation of specific mortality alarms that have been detected and thus facilitate interpretation; albeit based on an *a priori* hypothesis that fasciolosis contributed to the mortality. These data brought their own limitations (also discussed below), one of which is the sparsity of the data, although they do reflect both the annual life cycle of liver fluke and the sensitivity of this parasite to changing weather conditions from year to year (53).

Based on the limitations identified, a number of recommendations were made for each data source. These recommendations were framed in the context of development of these data to facilitate their use for animal health surveillance purposes, either as a surveillance system component (38) or as contextual background i.e., "intelligence" (23, 27). Most would require improvements to data collection and provision. While the re-analysis in Stage 4 addressed the need for a longer temporal period [Recommendation (R) 13, **Table SM4**], the updated dataset also provided evidence of changes that addressed two other recommendations; changes which facilitate the interpretation of any statistical alarms (R4, **Table SM4**) and which provide potential for future analysis at a more relevant time-scale (R3, **Table SM4**).

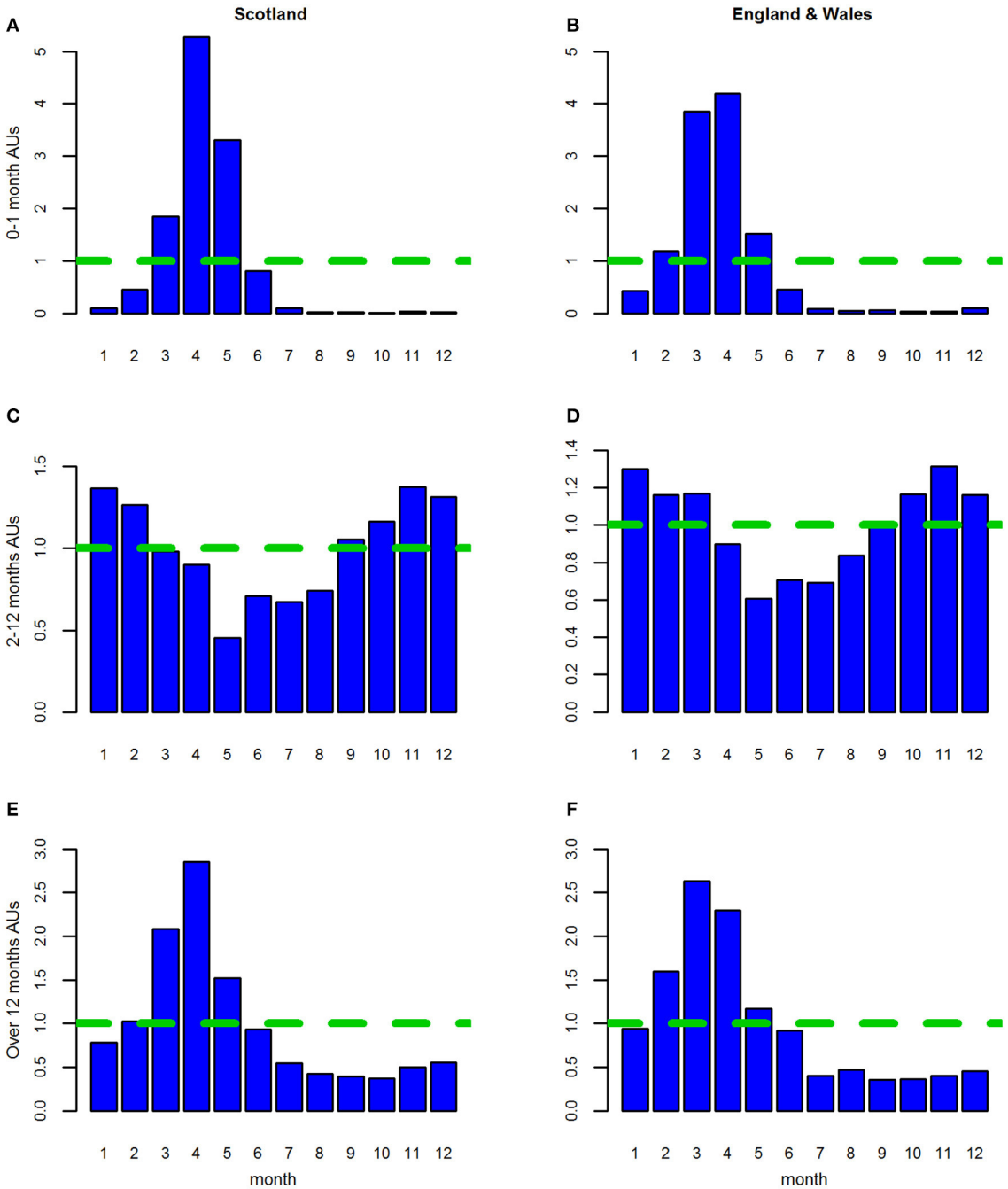

FIGURE 9 | (A–F) The seasonal effects for the three age groups of estimated animal units (AUs) by country using the 2011 to mid-2018 combined fallen stock datasets.

Challenges; Limitations and Interpretation

For both data sources, one of the primary challenges was the lack of suitable denominator data. While the initial fallen stock membership files provided some insights into aspects of the data that would assist interpretation of patterns, trends and alarms, they were not appropriate for use as denominator data. This was due to the lack of information on the livestock species, or numbers, at a Collection Point and the "snapshot" nature of the data. The unique identifier for a member was specific to this system, therefore collection records could not be linked to other data sources such as the June Agricultural Census or December Sheep and Goat Inventory to acquire additional information. Using the total number of sheep holdings [as defined by County/Parish/Holding (CPH) numbers] in either of these data sources would not be appropriate due to a number of factors. These include: the voluntary nature

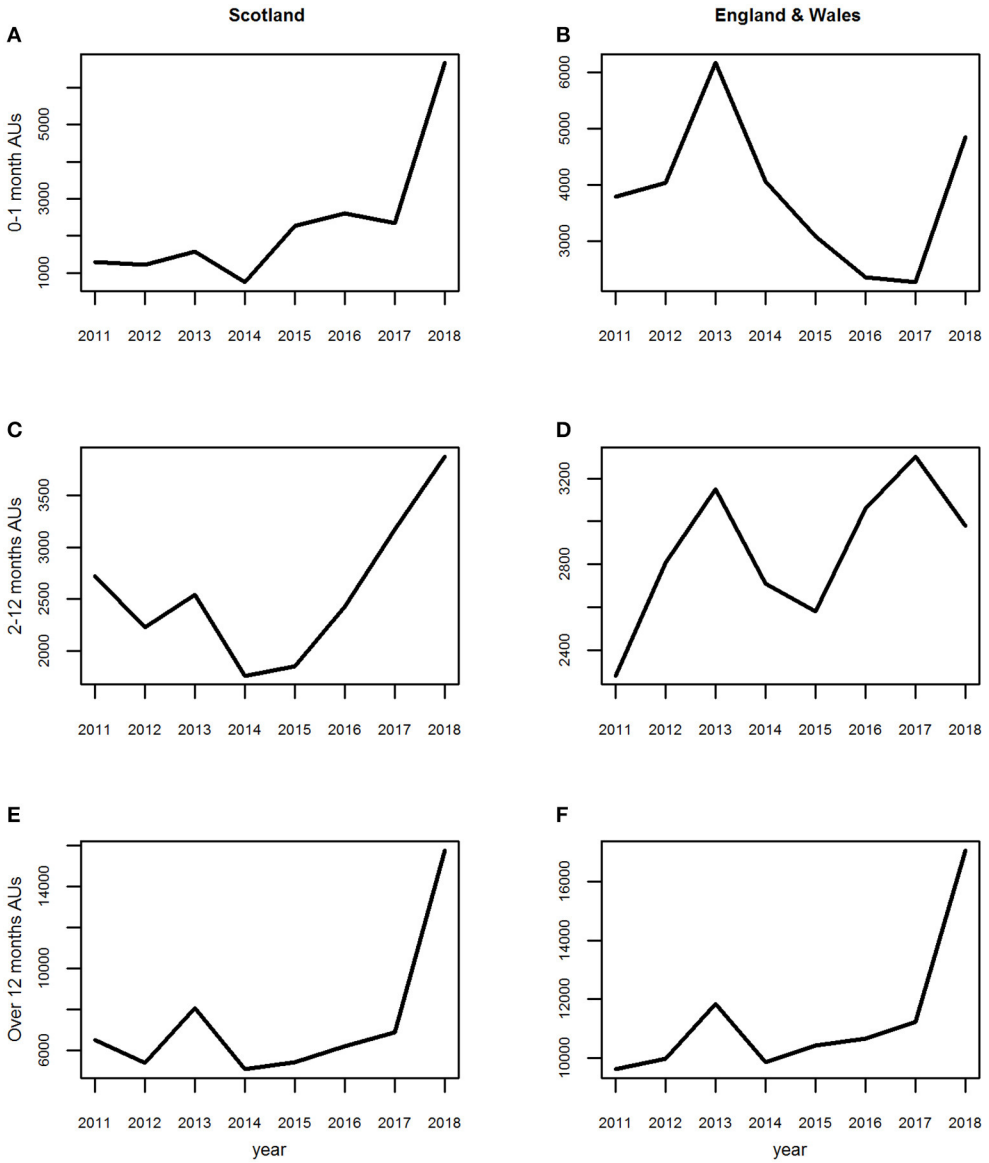

FIGURE 10 | (A–F) The average annual trend for the three age groups of estimated animal units (AUs) by country using the 2011 to mid-2018 combined fallen stock datasets.

of membership—not all farms/holdings with sheep will be members; the availability of alternative collection services—not all collectors will be members; variability in the use (if and when available) of these alternative collection services; associated drivers for changes in membership and use of NFSCo collectors, plus the existence of and variability in uptake of the derogations for remote areas (40). The latter particularly applies to the Scottish Highlands and Islands (41). This is evident in the data and means that significant contributions from these areas might be missed and events go undetected. It is essential that the potential for changes in any, or all, of these factors need to be taken into account first when interpreting outputs.

In the VS fasciolosis dataset, CPH numbers were available for the majority, but not all, of the diagnostic submission records. While CPH is not a perfect identifier and new systems are in development (54), it is (probably) the best that currently exists. However, it is known that not all holdings would submit to the VS network (3). Thus, in addition to the requirement to analyse the VS data in a comparable manner to the fallen stock data, again neither of the two statutory demographic data sources would be a suitable denominator.

For the fallen stock data, the second challenge was the conversion of the relatively standardized recording unit used for business purposes (SSUs) into something that more appropriately measured the mortality experienced, either at a member, flock,

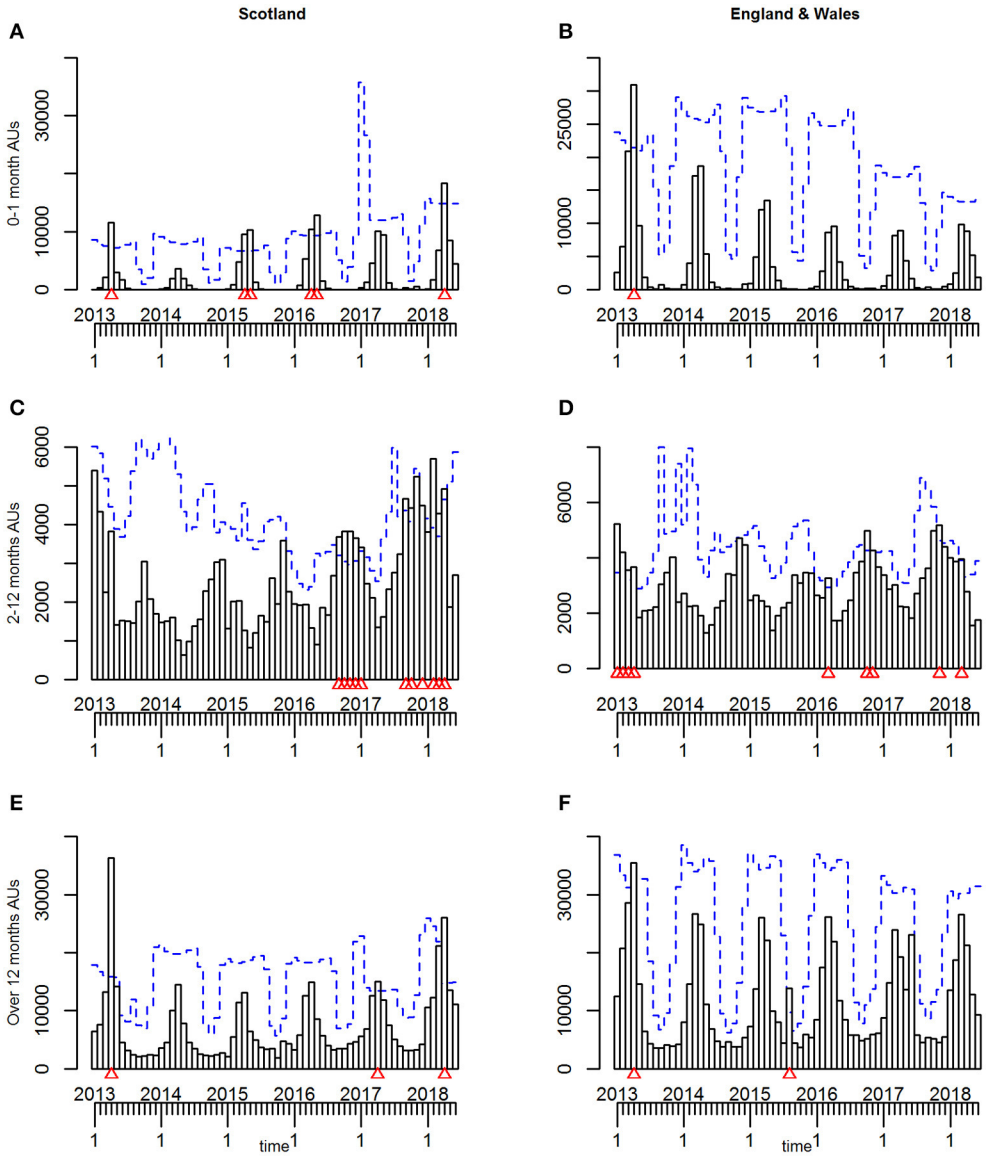

FIGURE 11 | (A–F) The time-series plots and temporal aberration detection (TADM) for the three age groups of estimated animal units (AUs) by country, using the 2011 to mid-2018 fallen stock datasets, with 2011–2012 inclusive as the reference period.

collection point, or animal level. The former three were not feasible, given the aggregation of the data and lack of denominator information. Simple multiplication was used to change the 10+ animals of a stated age group into what is a recognized under-estimate; the other multiple units; bags, kg, liters, skips presented more of a problem. The solution was to plot frequencies and assess the impact of inclusion, exclusion on the analyses, in conjunction with discussion with those who have indirect and direct experience of using such services. Exclusion of liters and unweighed skips due to the infrequency of their use, plus exclusion of Dolav® sheep per kilo based on their specific but relatively consistent use, was strengthened by the additional likelihood that the time of collection for these SSUs did not necessarily correlate as closely with the time of death of the animals that are within the collection. It would, however, be advisable to monitor usage of such bulk SSUs. If these changed in frequency substantially, this in itself could raise an alarm to be investigated (R14, **Table SM4**). Reasons for use may vary from needing a larger container due to a true increase in mortality to specific preferences for storage of fallen stock by members, or provision of services by collectors. Either way their increased use could result in corresponding reductions in use of other SSUs, or vice versa, their decreased use might result in increased usage of other SSUs, with the potential for production of false-positive alarms. One such change was observed in one of the Scottish postcode areas, although it involved the Sheep/lambs per 10 kg

SSU. From 2015 on there was a huge increase in the number of Lambs 0–1 month SSUs. There was a coincident decrease to almost 0 in the use of the Sheep/lambs per 10 kg SSU. Alarms were raised in this postcode area for the Lamb 0–1 month age group during spring 2015 and 2016; however, they were not confined solely to this area.

The frequency distribution of the 10+ bags of lambs over time was very similar in shape to that of the single lambs 0–1 month age group, in the Stages 1, 2, and 4 datasets. SAC Consulting Farm Business Services colleagues confirmed that this was the age group for which bags would be used and provided the initial estimate of a minimum of four lambs per bag. This was validated as a likely under-estimate by two other industry groups. As all of these groups were Scottish based, it would be worthwhile ensuring that this assumption holds for England and Wales (R10, **Table SM4**). The addition of the AUs resulting from the 10+ Lambs 0–1 month and the 10+ bags of lambs did not alter the general shape of the time-series, trends and patterns for this age-group but did alter the magnitude of effects. It was, therefore, necessary to ensure that these multiples were included, while accepting that the assumptions used still lead to under-estimation of AUs in this age group and that it is also reliant on the assumption that bag-use behavior is consistent over time. This underestimate might lead to a potential for a reduction in the ability to detect an aberration when one exists and could be improved by additional recording of the actual number collected, rather than just "10+" (R7, **Table SM4**). More difficult was the need to incorporate the relatively frequent but very area-specific use of 10 kg of Sheep/lamb SSUs. Whilst being relatively confident from the frequency distributions over time that these are more likely not to be 2–12 months old lambs, the assumptions made based on total weight frequencies are open to challenge. The potential for variable weights of lambs and sheep over 12 month, presumed to be ewe carcases, due to both breed variations and decomposition on storage is acknowledged. This is of particular importance with regard to the country-level alarm raised in the Stage 2 analysis of the Lambs 0–1 month age group in both England and Wales for September 2013. This would not be a time of year when typically a large number of 0–1 month old lambs would be expected to be on the ground, even when this category will include stillborn and unborn i.e., aborted fetuses. Potential misclassification includes lambs of the 2–12 months age group being bagged and adult sheep contributing to kg collections. No emerging threats were noted at this time (55).

The European standard approximate average bodyweight used for adult sheep in estimating antimicrobial consumption (ESVAC) is 75 kg (56). The lower value chosen of 50 kg may be more appropriate for carcases, or a reflection of the Scottish focus of the Stage 1 analysis i.e., more hill and upland sheep, rather than lowland sheep. Again, validation of this assumption for England and Wales (R10, **Table SM4**) may be worthwhile. Sensitivity analyses with different weight distribution algorithms are also a possible approach. However, given the approximate nature of the estimates already being made with imperfect data, that may be more of an academic exercise than a beneficial use of resources. Sensible interpretation would possibly maximize reliability (57).

A number of aberration detection algorithms—Farrington (44), improved Farrington (58), Binomial CUSUM (59), Negative Binomial (60), CUSUM (61, 62) and BODA (63)—were considered. The use of count data with an initially short study time period drove the choice of the original Farrington method (44). Seasonality is taken into account by using a subset of the available data as a reference, with the added assumption that there is no aberration in the reference period. An aberration (or statistical alarm) is detected at a single time point, if the count exceeds a threshold value, however, the method does not detect sustained shifts as it does not accumulate evidence over several time points. Additionally, only a window of historical values is taken for estimation of the threshold values, with no values taken from the current year. Despite these shortcomings, it is widely used in human public health surveillance (44, 64) as it is easy to implement and tackles major issues encountered with surveillance data; adjusting for over-dispersion of data, past outbreaks, trend and seasonality.

The choice of reference year can affect the outcome, especially when the assumption that there is no aberration in the reference period may be difficult to achieve in practice. There was little potential for choice other than 2011 in the short 4 year time period of the Stages 1–3 analyses. There was concern that this might not be a "typical" year, especially for Scotland, given the decline in average annual trend between then and 2012, although this may possibly have been driven more by membership changes than mortality effects. Despite this and the Schmallenberg incursion in the South-East of England in 2012 (55), the alarms seen in Lambs 0–1 month and Sheep over 12 months, in Scotland and England and Wales for April 2013 were observed in both the shorter and longer temporal period analyses, as were the alarms in the first quarter of 2013 for the 2–12 months lambs in England and Wales, suggesting a degree of stability.

The initial aggregation of the fallen stock data at a monthly level was sufficient to explore the utility of the data. It may also be sufficient for the provision of context, i.e., situational awareness and to evidence anecdotal observations, or to answer policy questions (22, 23). There is always going to be an initial delay between fatalities and collection and then collation, provision and analysis of data, as observed in cattle data (32, 65). This is likely to be longer in a non-statutory system but may be improved with the introduction and increased usage of digital technologies. The finer timescale of the Stage 4 data (R3, **Table SM4**) aids interpretation of observed alarms at the monthly aggregation-level and raises the possibility of further analyses. The most appropriate time-scale to use needs to be investigated to optimize the noise-signal ratio. The mortality experienced in a population would fit the definition of syndromic surveillance -"Surveillance that uses health-related information (clinical signs or other data) that might precede or substitute for formal diagnosis" (37, 38) if a semi-real time system could be implemented. For many disease conditions it would be hoped that existing surveillance systems would pick up incidents earlier. However, new and re-emerging threats might come from a variety of sources. These include factors such as: management changes; economic factors; food and forage prices, availability and nutritional content; extreme weather events; climate change; changes in welfare etc. These may

come in a variety of different forms; be they insidious, sporadic, repetitive and intermittent, or continuous. Death is one of the few certainties of life, although the cause may vary. Mortality within a population may be affected by many of these potential threats, therefore, an increase in mortality may be an indicator of reduced health of a stable population and vice versa. It is also possible to visualize conceptually and theoretically how different data sources may be brought together for syndromic surveillance, using "syndromes" made up of multiple components, of which mortality could be one, particularly in a species such as sheep that have a propensity to be "found dead" (43). Of course, to extrapolate the fallen stock mortality experience from NFSCo members to the national sheep flock would require the assumption that the mortality experience for non-members is the same as that for members. This would be a dangerous assumption to make without further investigation and understanding of the drivers behind membership and a better understanding of the attributes of members' flocks, stock numbers and type at collection points. Integration with data from other fallen stock companies could, potentially, provide increased coverage and improve representativeness of the mortality experience of British livestock populations. This would require either unique identifiers, or sufficient, standardized identifiers in each data source to facilitate the linkages. It would also require a degree of standardization of the collection process and data recording of the animal units collected.

While many potential threats will not respect man-made boundaries, given the lack of point location data and concerns about confidentiality, plus the variation in the distribution density of livestock holdings and the British sheep population (48, 49), some degree of spatial aggregation and regionalization is required. The choice then becomes what scale is appropriate and what boundaries to use. Here this was driven by the recording and structure of the data provided. The Scottish Government's Agricultural regions (66) are broadly similar to the postcode areas. By using the latter no further data manipulation was required. Due to the much larger numbers and border peculiarities of the English and Welsh postcode areas, application at this level was not possible and new regional areas based on multiple postcode areas had to be generated. The advantage is that they were formed using domain expertise; taking into consideration the density distribution of the sheep population and holdings, likely differences in management systems, urban and suburban areas, natural barriers and east-west/north-south weather influences, so as to have some meaning in this context. The disadvantage is that they could be considered to be arbitrary and subjective. Nomenclature of Territorial Units for Statistics (NUTS) codes (67) at level 1 provide only 10 areas, with no distinction between North and South Wales, whereas the NUTS 2 areas would be too fine (35 areas). Additionally, further data processing would be needed and they may not have been possible to assign. Time-series analysis is required at both the larger country level and at the smaller regional level, run in parallel, to facilitate understanding of the underlying processes and aid interpretation of any alarms raised. If analyzed only at a country level, there is the danger of missing anomalies that affect specific areas with insufficient magnitude to trigger a countrywide alarm i.e., that would be "washed-out" in the larger picture. However, it is plausible that a country-wide alarm might be triggered by small regional increments that are not detected by the regional analysis but that add up to an incident overall. At a regional level the disadvantage is that the data is being split further and, especially in areas with low numbers of AUs, it may thus result in more false-positive alarms. As statistical alarms are only indicators, raising alerts of the need for further investigation, this then becomes a matter of resource availability and allocation and signal optimization (14).

Many of the limitations and challenges identified with the VS fasciolosis data mirror those in the fallen stock data, with the added issue of the paucity of data points. Some could be addressed through improved data quality and management. Others will require discussion and determination of what is most appropriate to use in order to answer the specific surveillance question that is postulated. The ones of most importance—for these types of time-series and aberration analyses are accurate capture and recording of the: number of animals included in a submission; number of animals in the submission that were allocated the specific diagnostic code; ages of animals (where that has a bearing on management, frequency, pathogenesis, or disease process and control strategies); first or repeat submission from an epidemiological unit and the flock/holding identifier for linkage to other data sources. Many of these are being addressed via the introduction of a new LIMS. However, as the primary purpose of the laboratory network is to identify and mitigate the effects of disease outbreaks, so recording of number of animal affected or examined is not of primary importance to reach a diagnosis at flock level. Therefore, the optimum units to use for the types of analysis investigated here will need to be agreed; whether it is animal unit or submission record, herd, flock, or holding level. This may need to be done at an individual diagnostic code, or syndrome level. For acute fasciolosis there was only a slight difference between estimated AUs and submission level analysis. This is likely to be due to the types of sample submission that lead to such a diagnosis i.e., found dead, or wasting submission of single animal carcase; rather than the submission of multiple feces samples for a chronic fasciolosis diagnosis. It was the timeliness of mortality associated with acute fasciolosis in sheep and the knowledge that 2012/13 had been a particularly bad year for livestock fasciolosis (55) that drove the choice of this as the exemplar condition for investigation. Alternative methods of analysis will need to be trialed to optimize the use of VS diagnostic data, in order to facilitate their use as surveillance intelligence. A first line, in parallel with mortality from fallen stock data, could be monitoring species-specific carcase submission levels for post-mortem, followed by cause-specific diagnostic code analyses when warranted. However, recent changes to the Disease Surveillance Center network (68, 69) may mean that existing data are no longer valid for reference. A better understanding of the drivers of the number of animal units per submission would be useful, although this may also be affected by the network changes.

Advance and the Future

Despite the stated limitations, for the first time (in the authors' knowledge), population-level ovine mortality trends from fallen stock have been investigated. These studies have demonstrated that there is potential for imperfect data—voluntary fallen stock data collections—to contribute to surveillance intelligence provided the question is clearly defined and an informed approach is used for interpretation. The need to make better use of such data has been recognized for some time (4) and re-iterated recently (27). There is really no justification for the collection of data if it is not converted into information. However, when data are originally collected for a different purpose then not only the resources but a range of skills and subject-specific knowledge are required, working collaboratively with those involved in collecting and collating the original source data, to convert them into useful information (36). If it is to be of value and sustainable, operational implementation of any surveillance intelligence system will require cross disciplinary interactions and adequate resources; both for development and thereafter, as well as sufficient planning both of how to respond to statistical alarms and how decisions are made to deploy further investigative resources and/or apply mitigation measures (57). Although data and the technology to exploit them exist, human aspects will have to be factored in for meaningful progress to be made (70).

The fallen stock data could also be used to improve animal health surveillance in other ways. For example, if the delivery of collected fallen stock is known and if this is relatively stable i.e., collections from points A usually go to fallen stock center B; then it should be possible to inform and optimize the sampling design for targeted surveys using fallen stock material to estimate specified conditions (71, 72). Alternatively, the aggregated, population level, estimates of the frequency of occurrence of conditions identified emerging from farmer and/or veterinary practitioner requested, post-mortem (PM) of fallen stock at fallen stock centers (73) currently suffer from a large range of challenges and bias. To interpret this data meaningfully one needs to understand the relationships between the outputs, the data collection methods, the sampled population, the source population and national livestock populations. Analyses that aid understanding of the spatial distribution of fallen stock collection data could facilitate its use to guide improved interpretation of fallen stock acquired PM data (72). Furthermore, the recording of a standardized, categorized, member-derived "reason for death" at collection (R12, **Table SM4**) could only improve the interpretation of any observed changes in trends, patterns and any alarms with syndromic information. Whatever the direction of any future use of these existing data, these studies provide a foundation, or proof of concept; further development will be required before a functional system can be implemented. However, there is potential for use of these data as: a proxy measure for mortality in the sheep population; complementary components in a future surveillance system, and to inform the design of additional surveillance system components.

DATA AVAILABILITY STATEMENT

Data have been obtained from a third party. The data analyzed in this study were obtained from National Fallen Stock Company and from SRUC Veterinary Services. Requests to access these datasets should be directed to the corresponding author for forwarding to the appropriate contact.

AUTHOR CONTRIBUTIONS

ST conceived, designed, and led the studies, with contributions from JE (All stages), CC-G (Stages 1 and 2), and FB (Stage 3). FB contributed to collation and extraction of the SRUC Veterinary Services data (Stage 3). On receipt JE prepared all datasets and performed the statistical analysis. ST and JE contributed to the interpretation of all analyses, CC-G to interpretation of Stages 1 and 2, and FB to Stage 3. GG internally peer-reviewed the Stage 1 project report and helped secure Strategic Research Programme funding. With contributions from JE and CC-G, ST planned, drafted, and wrote the manuscript except for the statistical analysis sections, which were written by JE. CC-G, JE, and FB contributed to manuscript revision. All authors read and approved the submitted version.

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R. Riberio [SRUC Epidemiology Research Unit (ERU)] assisted with the production of maps in initial reports to data providers, for which GG, Dr. A. Reeves, and Dr. H. Auty (SRUC ERU) provided internal peer-review. A preliminary version of the Stages 1–3 studies was presented as a poster at the International Conference for Animal Health Surveillance (74).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00487/full#supplementary-material

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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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One Health in Practice: Using Integrated Bite Case Management to Increase Detection of Rabid Animals in Tanzania

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Rabies is a neglected zoonotic disease that causes an estimated 59,000 human deaths worldwide annually, mostly in Africa and Asia. A target of zero human deaths from dog-mediated rabies has been set for 2030, and large-scale control programs are now advocated. However, in most low-income endemic countries surveillance to guide rabies control is weak and few cases of rabies are recorded. There is an urgent need to enhance surveillance to improve timely case detection and inform rabies control and prevention, by operationalizing a "One Health" approach. Here we present data from a study piloting Integrated Bite Case Management (IBCM) to support intersectoral collaboration between health and veterinary workers in Tanzania. We trained government staff to implement IBCM, comprising risk assessments of bite patients by health workers, investigations by livestock field officers to diagnose rabid animals, and use of a mobile phone application to support integration. IBCM was introduced across 20 districts in four regions of Tanzania and results reported after 1 year of implementation. Numbers of bite patient presentations to health facilities varied across regions, but following the introduction of IBCM reporting of bite patients at high-risk for rabies more than doubled in all regions. Over 800 high-risk investigations were carried out, with 49% assessed as probable dog rabies cases on the basis of clinical signs, animal outcome, and rapid diagnostic testing. The status of a further 20% of biting animals could not be determined but rabies could not be ruled out. Livestock field officers reported that use of rapid diagnostic tests (RDTs) were useful for confirming rabies occurrence. Overall, our study provides further evidence that IBCM is a practical approach that can improve rabies detection in endemic countries, and be used to monitor the impact of mass dog vaccinations, including potential to verify rabies freedom. However, the main challenges to implementation are limited training of health workers in rabies, perceived burden of real-time recording and limited

resources for livestock field officers to undertake investigations. Nonetheless, IBCM dramatically improved case detection and communication between sectors and we recommend further implementation research to establish best practice and applicability to other settings.

Keywords: case detection, domestic dog, dog-mediated rabies, elimination, patient management, post-exposure prophylaxis, surveillance, zoonosis

INTRODUCTION

Rabies is a zoonotic disease caused by a virus transmitted through the bite of an infectious animal (1). Around 59,000 people die of rabies each year, with over 99% of these cases occurring in Low- and Middle-Income Countries (LMICs) (2). Yet the disease is entirely preventable through vaccination of dogs to eliminate infection in the reservoir population and by prompt administration of post-exposure prophylaxis (PEP) to people exposed to the virus (1, 3). Control of rabies requires collaboration between public health and veterinary sectors (a "One Health" approach) to manage risks in humans and interrupt transmission in dogs (4). An example of One Health is the Tripartite [World Health Organization (WHO), Food and Agricultural Organization of the United Nations (FAO), and World Organization for Animal Health (OIE)]. These international organizations have united to confront the problem of rabies (5). Nonetheless, the practical coordination of One Health activities by frontline public health and animal health workers remains challenging and this is exemplified by the implementation of rabies surveillance.

Surveillance is essential to control and ultimately eliminate infectious diseases (6). Effective disease surveillance involves the systematic collection and analysis of disease data and timely dissemination of results to guide planning and implementation of control strategies (7–9). Routine analysis of surveillance data can identify changes in disease incidence, including disease outbreaks and should inform public health professionals so as to improve the implementation of interventions and evaluate their impact (10). For rabies, surveillance could include data on persons bitten by rabid animals that are seeking PEP as well as human rabies deaths and on diagnosed animal rabies cases; data that needs to be shared between sectors to inform control measures like dog vaccination campaigns and prevention through provisioning of PEP. Yet, in many rabies endemic countries there are no formal systems used for reporting bite patients, and even if bite patients are reported, information on the risk of rabies is not reported (11). Moreover, limited operationalization of One Health means that the veterinary or public health sectors rarely ever receive information from the other sector to guide their control and prevention activities.

In LMICs where rabies is endemic, there is an urgent need to strengthen health systems and develop effective surveillance tools and response systems (8). Surveillance capacity in both the animal and human health sectors is limited and disease detection is hampered by inadequate laboratory facilities and difficulties in submitting samples to laboratories from rural areas (7, 12, 13). These limitations also render national epidemiological data unreliable with substantial underreporting of both human and animal rabies cases (14) and underestimation of the mortality burden of rabies and its economic impact (15, 16). This leads to rabies control not being prioritized by decision makers against other competing public health concerns.

Integrated Bite Case Management (IBCM) is an approach for rabies surveillance that directly and formally links workers in public health and veterinary sectors to assess the risk of rabies among animal bite patients and biting animals, respectively (17). IBCM has been promoted to increase rabies case detection (17), improve the administration and cost-effectiveness of PEP (18), and as a potential surveillance strategy for verifying freedom from rabies (19). The objective of this study was to determine whether IBCM could be implemented in Tanzania and what potential impact this could have. Here we present results from piloting IBCM in four regions of Tanzania, describing the challenges to implementation and the perceived benefits.

METHODOLOGY

Study Area

The study was undertaken across 20 districts in 4 regions in Southern, Central, and Northern Tanzania. IBCM was introduced into Mtwara and Mara regions in June 2018, Lindi region in July 2018 and into Morogoro region in August 2018 (**Figure 1**). The total human population within these regions was estimated at 7,100,000 in 2019, projected from the 2012 Population and Housing census survey (20). The average human: dog ratio (HDR) in these settings was estimated at 30:1 (21), but varied across districts, giving a dog population in 2019 of around 250,000. The study areas comprise a range of cultural settings with mainly agro-pastoralists in Mara region, agro-pastoralists and farmers in Morogoro region, while farming and fishing dominate in Southern Tanzania.

IBCM Framework in Tanzania

Figure 2 illustrates how an IBCM approach was developed for integration within the existing health and veterinary sectors in Tanzania. The introduction of IBCM involved training health workers to undertake risk assessments and Livestock Field Officers (LFOs), a paraprofessional cadre working within the Ministry of Livestock and Fisheries, to undertake animal investigations. IBCM involves undertaking risk assessments for all patients who present to health facilities with animal bites to determine whether they were bitten by potentially rabid animals or normal healthy animals and to ensure that PEP is correctly

administered to exposed individuals to prevent the onset of rabies. Epidemiological investigations should be conducted for animals that bit people to diagnose animal rabies cases. Through these investigations, other exposed individuals may be identified and referred to health facilities that offer PEP.

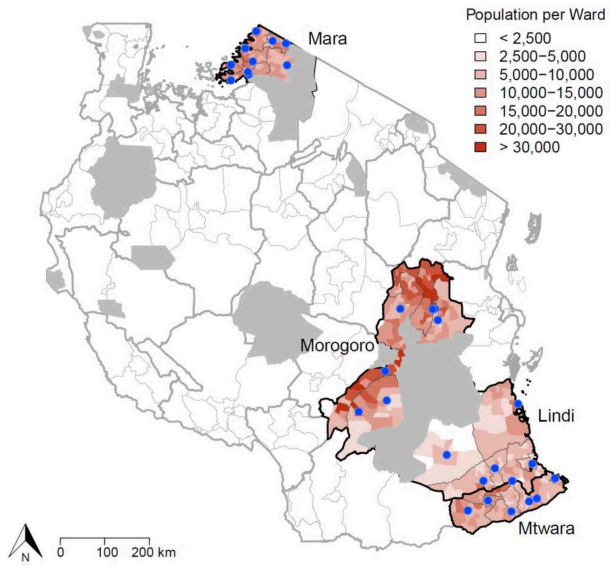

FIGURE 1 | Study area in Tanzania where IBCM was introduced. The blue dots indicate health facilities/hospitals in the districts implementing IBCM where PEP is provided ($n = 35$). The population density of each ward is also illustrated, with wildlife protected areas shown in gray. Human activities are prohibited in wildlife protected areas and these areas are uninhabited.

The IBCM Application

A mobile phone-based surveillance system for rabies previously developed and set up in southern Tanzania (22) was adapted as the basis for an IBCM application (app) for android phones with a web-based interface (dashboard). The app included risk assessment forms for completion by health workers (**Appendix 1**) and epidemiological investigation forms for LFOs that also cover sample collection (**Appendix 2**). The forms use mainly multichoice selections to minimize free-text data entry. The dashboard was developed to monitor submitted records and is accessible via the app or a password protected website. The app was developed using Waterfall development methodology starting with requirement solicitation followed by design, testing, deployment, and maintenance (23). The app is hosted on Google Playstore and has been updated to fix bugs and add new features as required. Data can be accessed via the dashboard by government stakeholders including regional and district veterinary and health officers, who provide feedback to their respective health workers and LFOs on the data being collected.

In February and March 2019, additional functionality was added to the app so that "high-risk" bites were identified following risk assessments by health workers. In response to a high-risk bite being identified, an automated alert would be sent to designated LFOs to trigger an investigation. A "high-risk" investigation was triggered if one of the following criteria were met: (i) a person was bitten by an animal that displayed at least one sign suggestive of rabies (e.g., excessive salivation, paralysis—see **Appendix 1** for indicative signs), (ii) a person was bitten

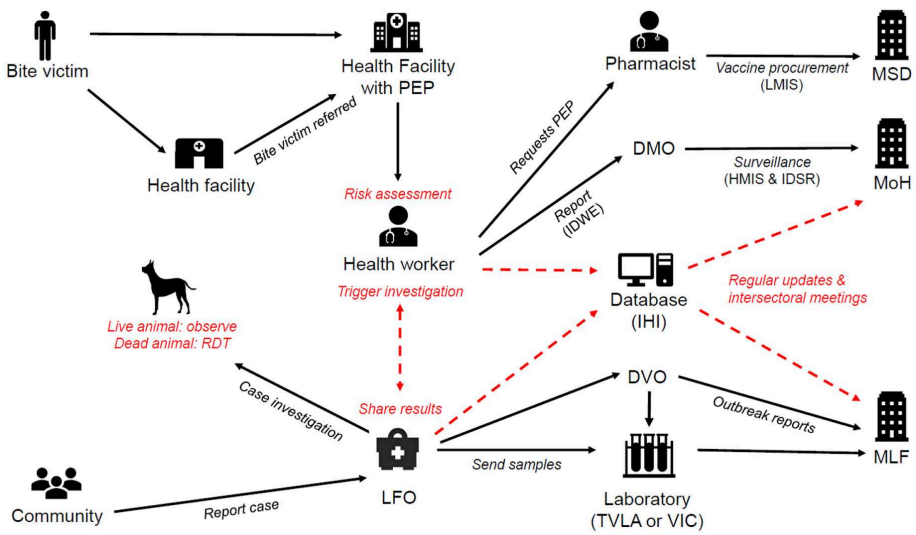

FIGURE 2 | IBCM framework in Tanzania. Red text and arrows indicate interventions introduced as part of IBCM. The existing health systems and reporting structures under the Ministry of Health (MoH) and Ministry of Livestock and Fisheries (MLF) are shown in black and include: the Medical Stores Department (MSD), District Medical Offices (DMO), District Veterinary Offices (DVO), Veterinary Investigation Centres (VIC), the Tanzania Veterinary Laboratories Agency (TVLA), Livestock Field Officers (LFO), the Integrated Disease Weekly Ending (IDWE) surveillance and reporting system, the Logistic Management Information System (LMIS), the Integrated Disease Surveillance and Response system (IDSR) and the Health Management Information System (HMIS). Ifakara Health Institute (IHI) hosts the database server for the IBCM. RDTs are Rapid Diagnostic Tests.

by an animal that subsequently disappeared or died or was of unknown origin, (iii) a person was bitten by a wild animal, (iv) a person presented to a health facility with symptoms of rabies. If one of these conditions were met, an automated alert would be triggered when the health worker submitted the risk assessment form. The generated message for the LFO contained the patient ID, their name and location details, including their village and phone number to facilitate the investigation. Automated alerts to LFOs were only generated on a bite patients first visit to a health facility and not for their subsequent visits. Bite patients were given a vaccination card on receipt of their 1st PEP dose and were required to present the card on subsequent visits. The card contained the patient name, age, village and district, date bitten, and PEP dates following the newly recommended 1-week ID regimen (24). If a bite victim sought care but PEP was unavailable, a patient ID was still generated and an alert sent to trigger the investigation. The victim was advised to travel to another health facility for PEP and was given a vaccination card containing their ID and other details indicating that they required PEP. This enabled the next facility to provide PEP to the victim without triggering another investigation.

Training of Government Personnel

At least one government health facility offers PEP in each district, with a few districts having more than one government facility providing PEP (**Figure 1**). In each district in the study area, two health workers from each government facility that offers PEP and one LFO were chosen and trained to be focal rabies personnel. The trained health workers were from the immunization departments of each district hospital. To ensure all bite victims who presented to the hospital for treatment were captured, all health and medical attendants working at the Out Patient Department (OPD) were also informed by the respective hospital authorities to refer bite victims to the immunization departments. A joint on-job training was held with LFOs brought to health facilities in their districts and together with health workers they were trained in IBCM. Specifically health workers were trained to undertake risk assessments of bite patients, while the LFOs were trained on how to conduct epidemiological investigations. To maintain implementation of IBCM monthly phone credit was provided to all focal persons (1 GB per month) and reimbursement or advance payment to LFOs for fuel to undertake investigations (typically 10,000–20,000 Tsh per investigation).

The protocol for LFOs involved first conducting a phone consultation with either the animal bite victim or a relative of the victim whose phone number was recorded during the health worker's risk assessment. In scenarios where the biting animal's information could not be obtained through phone consultation, LFOs were advised to visit the household of the animal owner. If multiple people were attacked by the same animal the LFO was required to record their names, patient ID (if the person had sought care), village, and PEP status on the investigation form. If the biting animal was vaccinated against rabies and did not appear sick, no further investigation was undertaken, however the owner was instructed to observe the animal for 10 days following the bite incident and immediately inform the LFO if any health or behavioral changes were observed. If the investigation revealed the animal was suspected to be rabid, the LFO was advised to check within the community to determine whether any other persons or animals had been exposed. LFOs were trained to collect samples from animals that had been killed or died, and were provided with BioNote rapid diagnostic test (RDT) to test for rabies where possible (25). Following investigations, LFO were advised to inform the health worker of the investigation result. Consent was not sought from patients for undertaking risk assessments or for animal investigations, as both activities are considered part of government duties. However, patients were informed that their data was being recorded electronically to inform an investigation of the biting animal.

Six months after IBCM was first introduced between June and August 2018, a proficiency questionnaire was administered to health workers to assess their knowledge of the clinical signs of rabies and whether they could distinguish rabid from healthy biting animals whilst attending animal bite victims. This was done in February 2019 in Lindi and Mtwara regions, in March 2019 in Morogoro region and in April 2019 in Mara region, and was immediately followed by refresher training and a post-training assessment examining two rabies risk scenarios.

To quantify baseline incidence of bite patient presentations, prior to the introduction of IBCM, we collected paper records from health facilities in the study from the 1st of January 2018. To determine the impact of introducing IBCM, we analyzed records from the IBCM database up until the 1st of August 2019, providing 1 year of data following the introduction of IBCM. We used a chi-squared test to investigate differences in risk classifications pre- and post- implementation of IBCM.

RESULTS

Bite Patient Risk Assessments

Prior to the introduction of IBCM, an average of 55.7 (range: 15–86) new bite patients presented per month in these regions, with only 26.9% indicating a risk of rabies by the health worker who completed the record (**Figure 3**). Following the introduction of IBCM, an average of 92.2 (range: 15–174) bite incidents were reported per month, with 64.9% assessed by health workers to be by suspect rabid animals. Overall bite patient presentations corresponded to an incidence of 17.4 bites per 100,000 persons per annum over the study period from January 2018 until August 2019 (from 1 to 64.9 among districts), but a risk of 12.0 rabies exposures /100,0000/year (from 1 to 62.2 among districts) under IBCM (from June 2018 until August 2019), assuming that the health workers risk assessments provide a more accurate indicator of rabies than routine records of bite patients (vs. 4.1/100,0000/year pre-IBCM from January 2018 until June 2018) before the introduction of IBCM (**Table 1**).

Of the bite victims that presented to health facilities following the introduction of IBCM (between July 2018 and August 2019; $n = 1,291$), most were due to bites from domestic dogs (93.0%) with only a few being bitten by wild animals (Lindi, $n = 3$; Morogoro, $n = 1$, Mtwara, $n = 14$, and Mara, $n = 7$). Most bite patients were recorded with scratches or minor wounds (78.0%, $n = 1,007$), while 19.5% ($n = 252$) had more severe wounds and 1.2% ($n =$

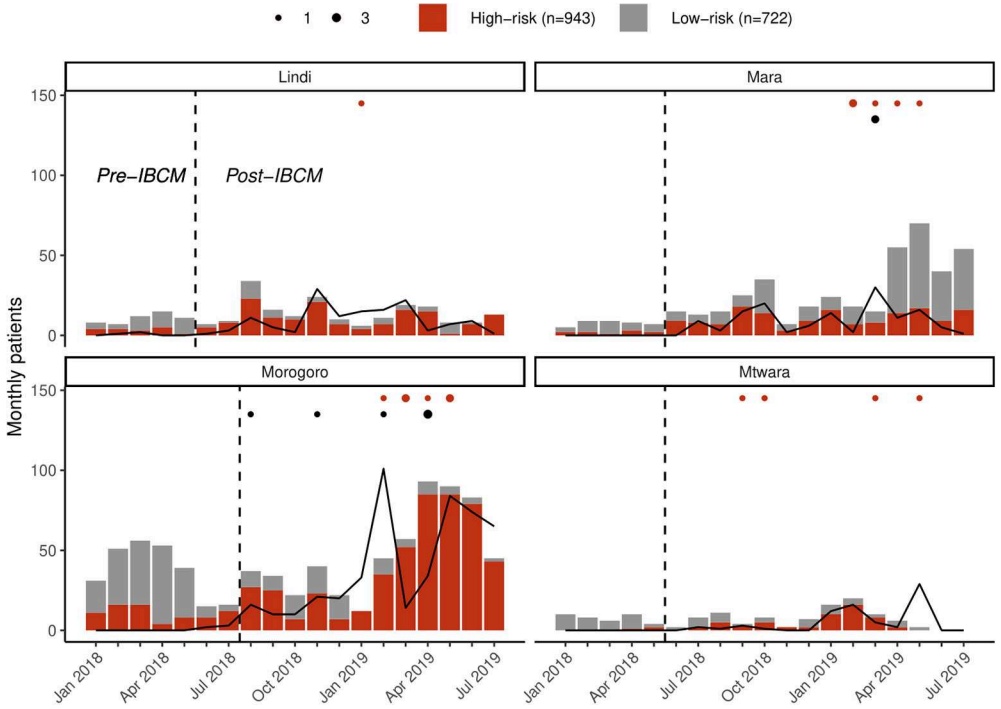

FIGURE 3 | Regional reporting of bites assessed as high risk (red) vs. low risk (gray) and investigations of biting animals (lines) in the study regions. The dotted line indicates when IBCM was implemented in each region; red dots indicate the number of human deaths ($n = 16$) attributable to rabies; black dots indicate the number of positive animal rabies cases ($n = 8$) confirmed through rapid diagnostic tests.

TABLE 1 | Patient presentations in study regions before and after the introduction of IBCM.

Region	Pre-IBCM		Post-IBCM	
	Patient presentation per 100,000 persons per annum	% high-risk	Patient presentation per 100,000 persons per annum	% high-risk
Lindi	15.0	31.5	19.4	76.0**
Mara	5.2	26.3	20.1	39.1
Morogoro	28.1	28.8	22.6	82.9**
Mtwara	7.2	7.9	6.7	59.0**

*Significant differences in the proportions of high-risk patients pre- and post-IBCM are indicated by * < 0.05 and ** < 0.001, as detected by a chi-squared test.*

16) required hospitalization due to broken bones or infection. One child (aged 2) died as a result of bite injuries. Throughout the study regions, PEP was unavailable for 74 bite patients (5.7%) upon presentation to a health facility, during the period of IBCM implementation. Only 63 of these bite patients were referred to other facilities for PEP with 43 assessed as being suspect rabies exposures. Sixteen human deaths due to rabies were reported within the IBCM study districts between July 2018 and August 2019 (**Figure 3**) from: Kilwa (1) in Lindi region; Bunda (4) and Serengeti (1) in Mara region; Morogoro Urban (3) and Ulanga (3) in Morogoro region; and Mtwara Rural (1) and Newala (3) in Mtwara region. These deaths were also confirmed through the investigations done by LFOs after the health worker's alert.

Animal Investigations

Prior to the introduction of IBCM, investigations were not carried out as standard by LFOs but were only carried out on an *ad hoc* basis. However, since IBCM began in the study area, 823 investigations have been conducted by LFOs. Seven hundred and seventy-seven investigations were conducted following an alert of a potentially high-risk bite while 46 investigations were carried out following community reports of sick, dead, or biting animals (**Figure 3**). The number of investigations undertaken following the introduction of IBCM differed between regions, with LFOs investigating an average of 10.3 cases/month in Mara, 9.7/month in Lindi, 40.2/month in Morogoro, and 7.9/month in Mtwara (**Figure 3**). An outbreak of rabies that began in February 2019 resulted in a surge of investigations in Morogoro region (**Figure 3**). Out of all the investigations, 157 were carried out in person, and 666 were completed via a phone consultation. From the 157 in-person investigations, 13 samples (8.3%) were collected between August 2018 and August 2019 (**Figure 4**) and 10 of these were tested with a RDT.

From the investigations, 49.1% (404/823) of biting animals showed at least one clinical sign consistent with rabies and/or were positive following a RDT ($n = 8/10$; one specimen tested negative and one test was inconclusive), while 21.5% (177/823) were determined to be healthy and 4.9% (40/823) to be sick from other causes that were not rabies. The remaining 24.5% (202/823) were classified as unknown status due to insufficient evidence. 20.9% (172/823) of the animals investigated were alive at the time of investigation, allowing for observation of clinical signs; the remaining 79.1% (651/823) had either disappeared (66.6%; 548/823) or were already dead (12.5%; 103/823) at the time of investigation, with a large proportion killed by community members ($n = 62$) or their owner ($n = 11$). Almost all domestic dogs are owned in rural Tanzania, but also almost all domestic dogs roam freely. Therefore, investigations were difficult to resolve if the owner of the biting dog was not known and the dog disappeared following the bite, but such circumstances were assumed to be high-risk and potentially indicative of rabies.

Veterinary and Health Data Combined (One Health)

The high-risk bites and animals assessed as suspect for rabies were generally widespread across the study regions (**Figure 5**). Both health workers and LFOs reported similar criteria about biting animals that they assessed to be suspect rabid. Health workers considered suspect rabid animals to show unprovoked aggression (including attempting to bite and grip people, animals, or objects without feeding; 45.2%), excessive salivation (10.4%), restlessness (6.4%), and/or abnormal vocalization (6.9%). In 23.1% (298/1,291) of bite patients, the health worker did not report any clinical signs for the biting animal, yet still classified 117 them as suspect rabies, apparently because the animal was unknown or the attack unprovoked. On investigation of high-risk bites, LFOs also reported animals displaying unprovoked aggression (49.5%), abnormal vocalization (16.8%), restlessness (9.8%), and/or excessive salivation (8.7%). LFOs did not report any clinical signs in 14.2% (117/823) of investigations, but considered 36 of these animals to be suspect rabid on the basis of other unreported information.

Assessment of Health Worker Knowledge

The proficiency testing indicated that all health workers could identify at least three clinical signs in animals consistent with rabies. Excessive salivation (94%), restlessness (86%), unprovoked aggression (86%), and abnormal vocalization (74%) were the most commonly identified signs, but a few respondents also identified paralysis (34%), and diurnal activity amongst nocturnal wildlife (24%) as well as a lack of fear among wildlife (16%) as clinical signs. However, only 66% of respondents considered a bite from an unknown animal as suspicious for rabies.

During proficiency testing most health workers stated that the wound severity would affect their recommendation for PEP and whether they would inform LFOs to investigate. Health workers reported that they were most likely to classify an exposure as high-risk and recommend PEP when treating severe wounds (wounds requiring hospitalization 90%, large wounds 96%, minor wounds 86%, and scratches 62%) and were also more likely to request LFOs to investigate severe bites (fatal wounds 78%, wounds requiring hospitalization 90%, large wounds 88%, minor wounds 70%, and scratches 56%). Only 78% of health workers indicated they would inform an LFO if they received a patient presenting with clinical signs of rabies. Following the refresher training, 74% of health workers were able to correctly recommend PEP to a patient bitten by an unknown suspected dog who had been delayed in seeking treatment.

DISCUSSION

Implementing IBCM demonstrated important public health impacts of rabies in Tanzania and the need to improve PEP access to prevent human rabies deaths as well as mass dog vaccination to control the disease at source. Reports of bites by suspected rabid dogs more than doubled under IBCM, and a large proportion of biting animals were identified as probable rabies cases upon investigation. Over half of patients presenting to clinics were assessed to have been bitten by suspect rabid dogs and therefore urgently required PEP. But, shortages of PEP occurred and human rabies deaths were reported from every region. Although it was possible to implement IBCM across this large geographic area, some activities were challenging, including recognition of indicative signs of rabies by health workers and investigations leading to sample collection by LFOs. Extended training could go some way to addressing these difficulties but limited resources are a constraint. Nonetheless, IBCM shows considerable promise for improving case detection and communication between sectors, and further implementation research is warranted.

IBCM showed promise as a tool to support rabies surveillance. Specifically, IBCM increased case detection, and generated data from health facilities that is much more useful for assessing the impact of PEP than numbers of bite patients alone, which may often not reflect rabies incidence directly (11, 26–28). The use of the mobile phone application was generally successful and both health workers and LFOs were enthusiastic about how IBCM improved intersectoral collaboration and understanding of the rabies problem, with LFOs particularly positive about using RDTs to confirm rabies. This was most evident during the response to a rabies outbreak in the first half of 2019 in Morogoro region, where several deaths occurred and dog cases were confirmed. The incidence of bite patients, suspect rabies exposures and deaths identified through IBCM in Morogoro was similar to numbers reported from the investigation of a previous outbreak in the region in 2007 (29), whereas incidence in the other districts was relatively low, likely because of previous dog vaccination campaigns.

Limitations of our study restricted the conclusions we were able to draw. For example, we introduced IBCM to the government designated hospital in each district that offers PEP, but private referral hospitals, such as St Francis in Kilombero District or Maneromango in Nachingwea district, were not included in the study, though they also offer PEP. Bite victims who directly attended these facilities (sometimes due to PEP stockouts elsewhere) were therefore not captured by IBCM. In

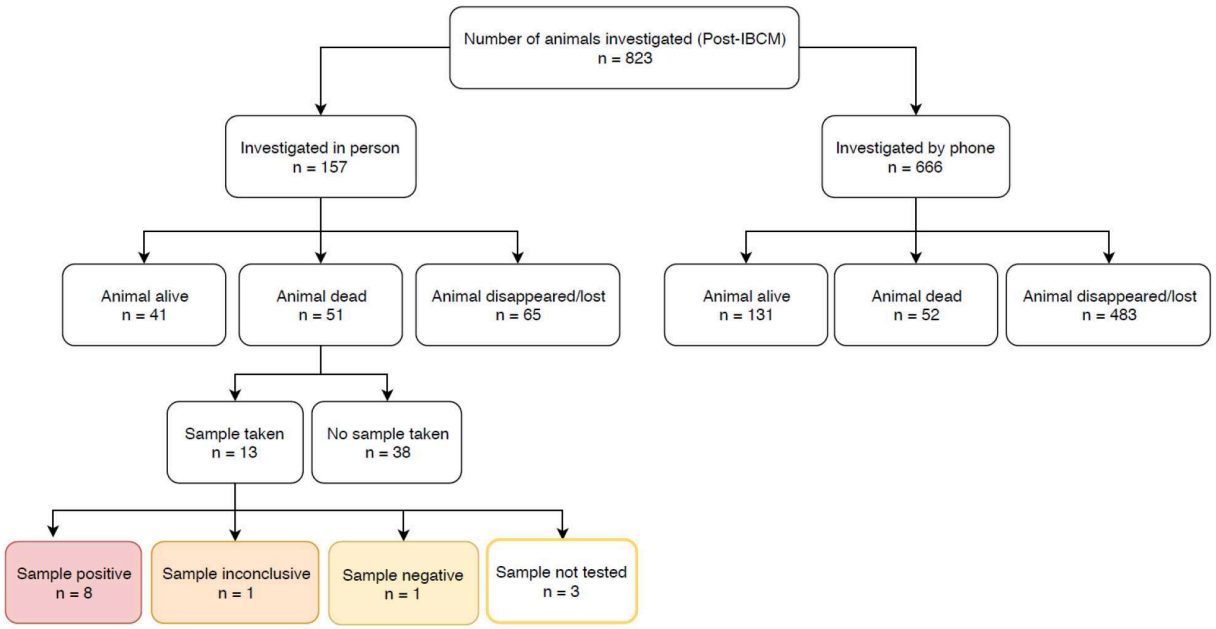

FIGURE 4 | Number of investigations carried out by LFOs between August 2018 and May 2019. The outcomes of rapid diagnostic tests are highlighted.

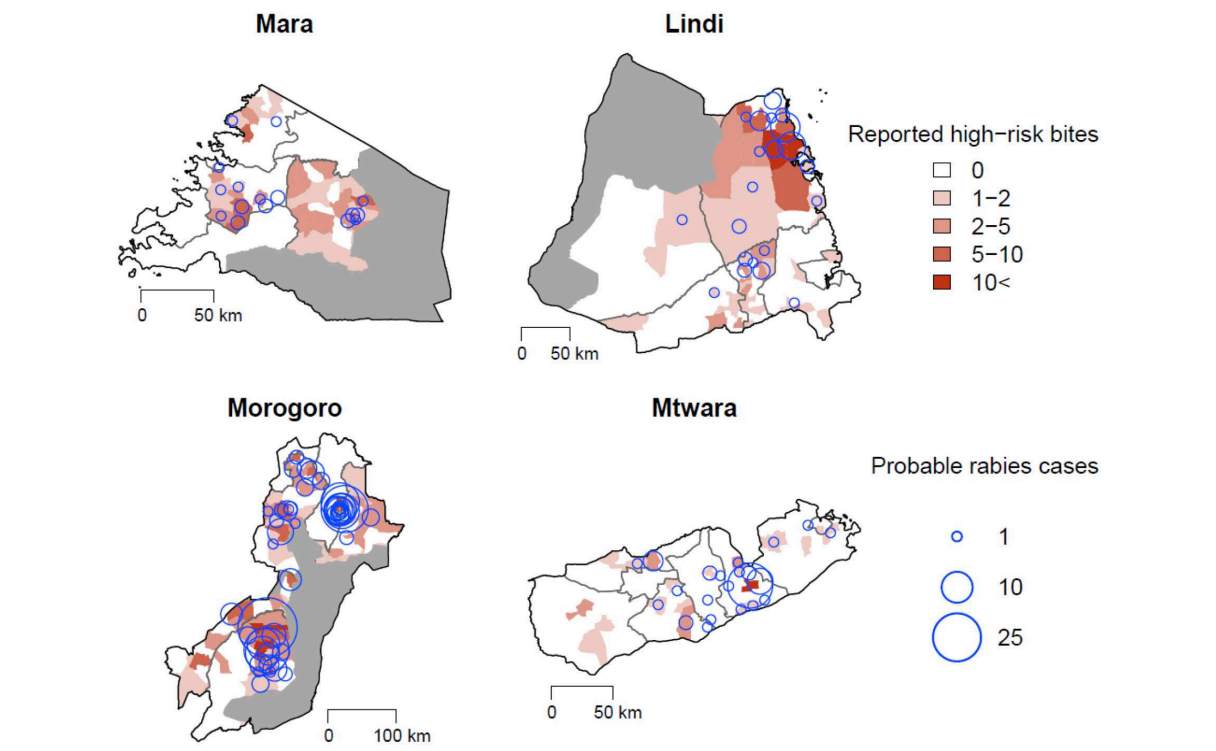

FIGURE 5 | High-risk bites per ward reported through health facilities (red polygons) and probable cases confirmed through LFO investigations (blue circles). Protected areas are overlaid in gray.

addition, bite victims who never attended any of the health facilities and developed rabies and died at home were not captured by either IBCM or routine surveillance, leading to underestimation of the disease burden. Integration of private facilities may be needed in future if Tanzania is to bring rabies under control and IBCM is used to verify rabies freedom.

Training was given to government workers and follow up provided by the research team, with one assistant remotely supporting all four regions. Without such technical support it may be difficult for the government to scale up IBCM to other parts of the country. More generally, district councils may vary in terms of follow up, levels of staff training and availability of funds that affects the quality of operations. These points likely apply to other LMIC settings and should be considered if efforts are made to improve PEP access and introduce IBCM (30). Nonetheless, once trained, both health workers and LFOs were able to fully implement IBCM independent of the research team, and IBCM activities were mostly adopted and integrated within routine duties. Further work will be required to fully understand sustainability of IBCM.

Trained practitioners are indispensable to an effective health system and this applies directly to IBCM. Two or three health workers were trained to implement IBCM in each facility. This increased the workload of these health workers and they felt deserved extra payment. Some health workers were also re-assigned to other facilities or departments, which required the recruitment of replacements who were trained remotely via phone. The level of knowledge and familiarity with smartphones also differed between users (both health workers and LFOs), and for a few using an app was challenging. In some facilities in Southern Tanzania only very few bite victims presented, and as a result health workers in these areas (7 out of 63 across the study) needed reminding to conduct risk assessments, and were encouraged to use the IBCM guide provided during their training to recall procedures. Generally, health workers receive only limited professional training in rabies in Tanzania. The proficiency training we provided aimed to boost their ability to recognize signs of rabies, because of the difficulty that health workers showed in fully understanding rabies risks and indicative clinical signs in animals. Providing regular incentives such as training outside their workplace or in monetary terms could potentially help improve their performance, but is a challenge for sustainability. Government supported training to reinforce IBCM, particularly over time and with staff turnover, could benefit sustainability. But it is likely that an ongoing support person would be required for troubleshooting, ideally a designated government employee.

From the animal health side, obtaining samples for diagnosis was difficult. Timely investigations are critical for confirming cases as well as for detecting other exposures, in both animals and people. Delays compromise sample collection opportunities and heighten risks for those who have not sought care. However, many cases that require investigation are far from district headquarters and the focal LFO responsible for sample collection. It is difficult for LFOs with limited resources and inadequate transport to reach these cases. Nevertheless, our experience suggests that sample collection could improve, with emphasis on timely submission of risk assessments by health workers, additional training of LFOs based in more remote areas. Unfortunately at the start of the study we were unable to fully equip LFOs with RDTs and so not all samples were collected and tested, but feedback from LFOs suggested that the ability to test samples was also a strong incentive for collection. One Health is widely promoted (31) and is highly recommended for rabies control and prevention (12). IBCM represents a formal means of practicing intersectoral collaboration. We suggest that further joint discussions about surveillance findings amongst practitioners, including engagement with the regional and council health management teams, could help reinforce IBCM and ultimately promote better implementation of One Health and rabies control and prevention activities. Surveillance investments typically focus on laboratory diagnostics and infrastructure, but resourcing health workers to conduct risk assessments and LFOs to carry out investigations would be a critical first to improve rabies case detection.

Vaccination of all persons exposed to a suspected rabid animal is an effective approach to protect people from rabies (5, 32). However, rabies vaccines in Tanzania are in short supply, so unnecessary use can also limit availability for those most in need (33–35). While risk assessments indicate some potential for more judicious use of PEP in patients bitten by clearly healthy animals, the number and proportion of those presenting due to healthy animal bites is small compared to some settings, particularly in Asia and the Americas (27, 36). Risk assessments to determine PEP decisions needs to be both sensitive and specific. PEP should always be recommended if there is any doubt concerning the risk of rabies, and therefore risk assessments with low sensitivity could lead to human rabies cases if PEP is either not initiated or delayed in a genuine rabies exposure, whilst risk assessments with low specificity could lead to people receiving PEP unnecessarily, incurring expenses and potentially limiting supply for those in need. A challenge for judicious PEP administration is that bite victims may demand PEP for bites from healthy animals, particularly in areas with recent rabies cases, and will cover any costs required or demand PEP and associated costs be covered by dog owners. Our findings suggests that there is quite limited scope for more prudent PEP use in Tanzania, and that increasing PEP access should be the first priority. Nonetheless, the use of IBCM in such highly endemic settings could sensitize practitioners to the risks of rabies, and given limited diagnostic capacity and PEP availability, may be useful to guide PEP recommendations and prevent unnecessary overuse, particularly with a view to progressing toward elimination (18, 19, 33, 37).

CONCLUSION

In Tanzania, animal disease surveillance falls under the Ministry of Livestock and Fisheries, while the Ministry of Health deals with bite victims. An intersectoral programme, such as the One Health Coordination Unit, under the Prime Ministers' office encourages both sectors to work together in a practical way, but coordinating rabies prevention and control between the two sectors has always been a challenge. IBCM has helped to integrate these sectors and generates more accurate surveillance data that can guide policy decisions and public health measures. IBCM improved intersectoral communication,

helped to identify rabies-exposed bite victims requiring PEP, facilitated follow up of cases, and encouraged LFOs to rapidly test cases during investigations. Surveillance is crucial to guiding effective patient management decisions, disease control interventions and for verifying disease elimination. A well-established surveillance system will be essential to evaluate the impact of mass dog vaccination programmes and to ensure rapid responses to outbreaks. IBCM appears to be a practical and promising approach to improve case detection and was extremely useful during the outbreak of rabies in Morogoro region. Whether IBCM can confirm the interruption of disease transmission will depend on implementation. Until now, many practices for rabies control and prevention are still weak in LMICs with endemic rabies (9), and will need strengthening to achieve the zero by 30 goal. We encourage greater use of IBCM and recommend further implementation research to develop best practice for IBCM in different settings, and evaluate its potential to support the goal of elimination of dog-mediated rabies.

DATA AVAILABILITY STATEMENT

Anonymized data and code to reproduce the results, including the figures and tables are available on the GitHub repository: https://github.com/LushasiK/IBCM_TZ_Frontiers.

ETHICS STATEMENT

The study was approved by the Medical Research Coordinating Committee of the National Institute for Medical Research of Tanzania, with approval number NIMR/HQ/R.8a/vol.IX/2788, the Ministry of Regional Administration and Local government with the reference number AB.81/288/01, and the Institutional ethical review board of Ifakara Health Institute with approval number IHI/IRB/No: 22-2014.

AUTHOR CONTRIBUTIONS

KL and KH conceived the study. KL, ZM, and KH designed the study. KL, HH, FM, ZM, and JC collected the data. KL, RS, and KH analyzed the data. KL reviewed the literature and wrote a first draft. RS, KH, and FL redrafted the manuscript. EM, HN, DH, JB, and NG critically reviewed and approved the final manuscript for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2020.00013/full#supplementary-material

Appendix 1 | Risk assessment form for Health Workers.

Appendix 2 | Investigation form for Livestock Field Officers.

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ORIGINAL RESEARCH
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Near Real-Time Monitoring of Clinical Events Detected in Swine Herds in Northeastern Spain

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Novel techniques of data mining and time series analyses allow the development of new methods to analyze information relating to the health status of the swine population in near real-time. A swine health monitoring system based on the reporting of clinical events detected at farm level has been in operation in Northeastern Spain since 2012. This initiative was supported by swine stakeholders and veterinary practitioners of the Catalonia, Aragon, and Navarra regions. The system aims to evidence the occurrence of endemic diseases in near real-time by gathering data from practitioners that visited swine farms in these regions. Practitioners volunteered to report data on clinical events detected during their visits using a web application. The system allowed collection, transfer and storage of data on different clinical signs, analysis, and modeling of the diverse clinical events detected, and provision of reproducible reports with updated results. The information enables the industry to quantify the occurrence of endemic diseases on swine farms, better recognize their spatiotemporal distribution, determine factors that influence their presence and take more efficient prevention and control measures at region, county, and farm level. This study assesses the functionality of this monitoring tool by evaluating the target population coverage, the spatiotemporal patterns of clinical signs and presumptive diagnoses reported by practitioners over more than 6 years, and describes the information provided by this system in near real-time. Between January 2012 and March 2018, the system achieved a coverage of 33 of the 62 existing counties in the three study regions. Twenty-five percent of the target swine population farms reported one or more clinical events to the system. During the study period 10,654 clinical events comprising 14,971 clinical signs from 1,693 farms were reported. The most frequent clinical signs detected in these farms were respiratory, followed by digestive, neurological, locomotor, reproductive, and dermatological signs. Respiratory disorders were mainly associated with microorganisms of the porcine respiratory disease complex. Digestive signs were mainly related to colibacilosis and clostridiosis, neurological signs to Glässer's disease and streptococcosis, reproductive signs to PRRS, locomotor to streptococcosis and Glässer's disease, and dermatological signs to exudative epidermitis.

Keywords: swine health, endemic diseases, monitoring, data mining, web application, endemic-epidemic multivariate time-series model

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INTRODUCTION

The prevention and control of diseases are essential to ensure efficient and sustainable swine production. Getting updated information on the health status of the target swine population in near real-time can facilitate the implementation of efficient measures by swine stakeholders, veterinary practitioners, and government. Innovative surveillance methods based on the analyses of various types of data, which may serve as indirect health indicators, are under development (1–3). The ability to collect data in a cost-effective and timely manner from a wide range of sources, the use of data mining techniques and time series analyses, and the possibility of generating dynamic reproducible reports, has led to the development of new ways of conducting surveillance in near real-time (4, 5).

In recent years, the Spanish swine sector has grown significantly, with over 50% of the pig herds concentrated in Catalonia and Aragon (regions located in the North East of the country). In those areas, the number of sows in large-scale operations has increased, and an important proportion of facilities are part of integrated industries with highly specialized farrowing, post-weaning, and finishing sites (6). In this context of swine production, it is essential to maintain a good sanitary status.

The Porcine Sanitation Group of Lleida, Spain (GSP) is a non-profit association that brings together pig owners, independent breeders, and companies associated to the swine sector in Northeastern Spain. The GSP aims to improve the swine health in farms and collaborates closely with the official animal health authorities carrying out actions related to disease surveillance, prevention, and control. In 2012, the GSP decided to carry out a near-real time monitoring system in Aragon, Catalonia and Navarra to gather data on clinical events detected by practitioners. The GSP hypothesized that, by monitoring, targeting, and reporting clinical signs and presumptive diagnoses, it would be possible to reveal in near real-time the occurrence of endemic diseases that are not notifiable. This information might help assess the spatiotemporal distribution of such diseases in these populations, identify subpopulations at high risk and factors that influence disease presence. Practitioners and swine stakeholders would benefit from this information to plan and take more efficient control measures.

It is important to highlight that the initial intention of this monitoring tool was not associated with a pre-defined control plan against a specific disease. The main aim of the tool was to gather data from swine herds in near real-time and provide accessible and regularly updated information to veterinary practitioners and swine stakeholders. The system aimed to visually track the spatiotemporal distribution and spread of endemic diseases and support the decision of where and when actions were necessary. Moreover, the system aimed to enhance the communication and cooperation within the swine sector in Northeastern Spain. This work aims to evaluate the functionality of this system developed to monitor the frequency of endemic diseases in the swine population at region and county level, and discusses the advantages and limitations related to its implementation.

Abbreviations: GSP, Porcine Sanitation Group; app, web application; REGA, National official farm identifier; APP, *Actinobacillus pleuropneumoniae* infection or porcine pleuropneumonia; PRRS, porcine respiratory reproductive syndrome.

MATERIALS AND METHODS

To illustrate how the GSP monitoring system operates, we analyzed the data of clinical events reported voluntarily by veterinary clinicians from swine farms of the Catalonia, Aragon, and Navarra regions (Northeastern Spain) between January 2012 and March 2018.

Development of a Web Application to Report Clinical Events Detected in Swine Farms

The researchers and technicians of GSP, in collaboration with many swine stakeholders and veterinary practitioners, developed a web application (app) to collect and store data on clinical events detected by veterinarians during their visits to farms. Before launching the system, all the practitioners and representatives of the swine industry of this zone were convened to a recruitment meeting. Afterwards, twice a year the participants were convened to a meeting for promoting their continuous participation. The veterinarians that participated worked for large integrated companies as well as small individual farms. Several meetings with representatives of the swine sector and veterinary practitioners took place to define and agree what data fields to include in the app, which could be executed by desktop computer, smartphone, tablet, or other mobile device. Data from farms was supplemented with diagnostic test results if samples had been submitted to the official laboratory. A program was created to analyze the data automatically and report the health status of the swine population to veterinary practitioners that participated. This app is currently accessible using a user code and password through the link: http://www.gsplleida.net/es/content/app-del-gsp. **Figure 1** shows the app interface with the fields to be filled out by a user detecting a clinical outbreak in a pig farm.

Data Source, Types and Preparation

Data were mainly sourced from veterinarians who routinely visited the pig farms. If a veterinarian detected pigs with clinical signs during a visit to a farm, he/she registered the following variables in the app for each clinical event at farm level: severity of the clinical event, date of the visit, official identification of the farm, company to which the farm belonged, location of the farm, type of animal (i.e., sows or pigs), category of age affected, body system affected, lesions observed during necropsy (if applicable), vaccines applied, and presumptive diagnosis of the disease. The veterinarian identified the affected body system according to the clinical signs observed in swine, distinguishing between respiratory, digestive, neurological, locomotor, dermatological, and reproductive system. In the event of detecting multiple disorders in the same farm (e.g., respiratory and digestive), each sign could be recorded individually.

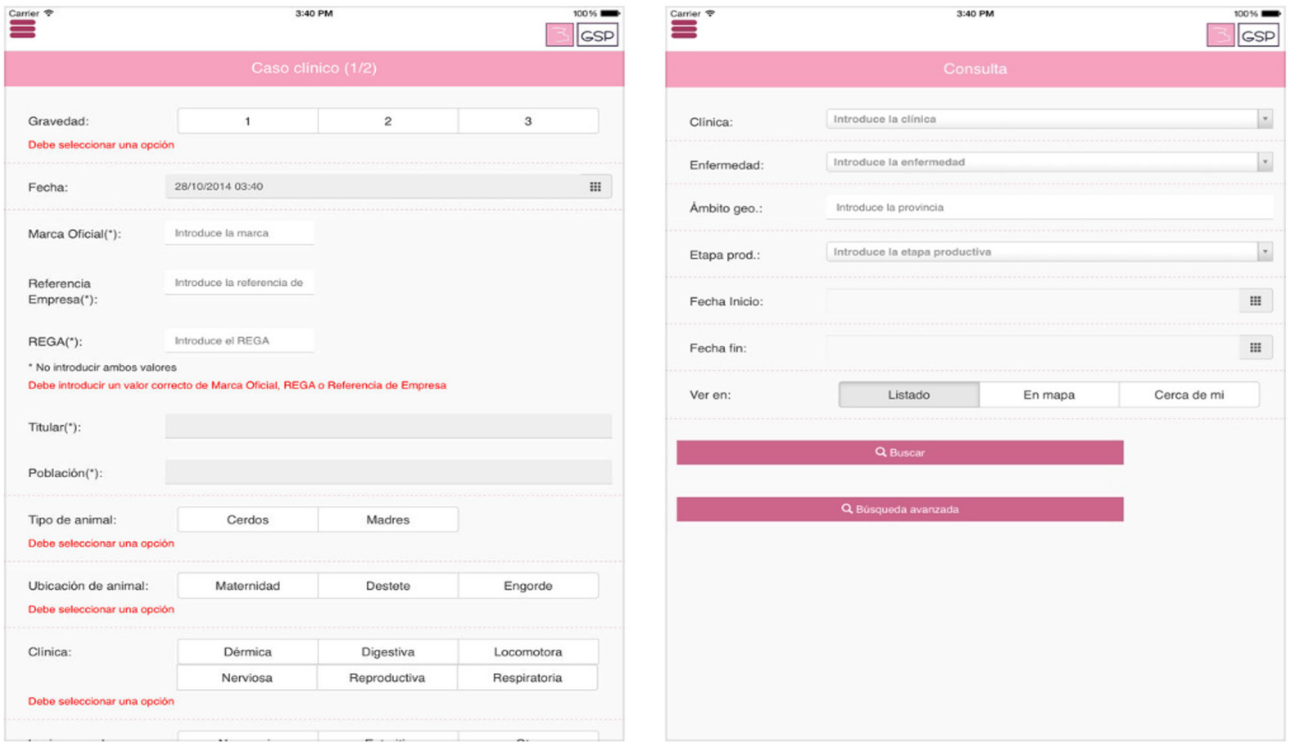

FIGURE 1 | Interface of a web application to record clinical event data from swine herds in Northeastern Spain. Fields written in Spanish. English translation: Case (*Caso clínico*), Severity (*Gravedad*), Date (*Fecha*), Regional official farm identifier (*Marca Oficial*). Company reference (*Referencia empresa*), National official farm identifier (*REGA*), Owner (*Titular*), Municipality (*Población*), Type of animal (*Tipo de animal*), Piglets or fattening pigs (*Cerdos*), Sows (*Madres*), Farm Location of the affected animals within the farm (*Ubicación del animal*), Maternity (*Maternidad*), Nursery (*Destete*), Fattening (*Engorde*), Clinical signs (*Clínica*), Dermatological (*Dermatológica*), Digestive (*Digestiva*), Locomotor (*Locomotora*), Neurological (*Nerviosa*), Reproductive (*Reproductiva*), Respiratory (*Respiratoria*), Presumptive disease (*Enfermedad*), Geographical situation (*Ámbito geográfico*), Productive phase (*Etapa productiva*), Date of clinical onset (*Fecha inicio*), Date of clinical end (*Fecha fin*), See (*Ver en*), List (*Listado*), Map representation (*En mapa*), Closest clinical cases (*Cerca de mí*), Search (*Buscar*), Advanced search (*Búsqueda avanzada*).

Moreover, the veterinarian indicated the most plausible presumptive diagnosis based on his/her clinical experience. The presumptive diagnoses comprised a closed list of endemic diseases that included: porcine pleuropneumonia (APP- due to *Actinobacillus pleuropneumoniae*), porcine circovirus associated disease (due to Porcine Circovirus type 2), clostridiosis (due to *Clostridium* spp.), unspecific diarrhea (when the microorganism involved was unknown), swine dysentery (due to *Brachyspira hyodisenteriae*), colibacillosis (due to *Escherichia coli*), exudative epidermitis (due to *Staphylococcus hycus*), streptococcosis (due to *Streptococcus suis*), Glässer's disease (due to *Haemophilus parasuis*), swine influenza (due to Swine Influenza virus), ileitis (due to *Lawsonia intracellularis*), leptospirosis (due to *Leptospira* spp.), mycoplasmosis (due to *Mycoplasma hyopneumoniae*), any swine parasitosis, pasteurellosis (due to *Pasteurella multocida*), rectal prolapse, matrix prolapse, porcine respiratory reproductive syndrome (due to PRRSV), atrophic rhinitis (due to *Bordetella bronchiseptica* and/or *Pasteurella multocida*), salmonellosis (due to *Salmonella* spp.), and gastric ulcers. The app allowed reporting of several presumptive diagnoses during a single visit. However, this situation was very unusual, since the clinician usually indicated a unique presumptive diagnosis. Finally, the veterinarian also categorized the severity of a clinical event as mild, moderate or severe taking into account his/her own clinical experience and considering the rates of mortality and morbidity and the negative impact of the event on productive performance.

The second source of data was the GSP official laboratory for swine diseases. If practitioners submitted clinical samples from a reported affected herd, the laboratory carried out diagnostic testing to confirm or rule out a suspected endemic disease. The type of test used and the results obtained at farm level were then recorded to the app. Data of clinical cases and laboratory confirmation testing were integrated at farm level using a relational database built by the IT services of GSP. The elapsed time between the report of a clinical event and its laboratory confirmation ranged between 24 h and a week depending on whether the diagnosis was performed by PCR, serology or microbiology.

The third data source was the official census of the active swine farms in the regions of study (i.e., Aragon, Catalonia, and Navarra) (7). This census contained the following fields: a unique identifier of the farm, the company to which the farm belonged, the municipality, the county, the province, the number of adult sows/boars, the number of fattening pigs, the number of piglets in nursery, the type of production, and the UTM coordinates (x, y). The data registered by the veterinarians during their visits, and by the official laboratory were pre-processed and integrated with the census data in order to get a final data set that could be analyzed.

Coverage Assessment

The initial aim of the GSP monitoring system was to gather data on clinical events occurring in any swine farm of Catalonia, Aragon and Navarra. The swine population of these three regions totals 6,741 active pig farms, 79% of which were located in Catalonia, 18% in Aragon and 3% in Navarra. Around 90 swine practitioners routinely visited these farms, the majority of which were located in 12 of the 62 counties of the regions. Half of the farms belonged to 20 integrated swine companies.

Over the study period, the implementation of the monitoring system was partial, as not all the veterinarians used the app to report clinical events when visiting the swine farms. Initially, to evaluate the coverage achieved by the GSP system, it was assessed from which counties the swine practitioners reported clinical events. This set of counties corresponded to the accessible population. Then, the coverage was also analyzed by type of production. The comparison allowed identification of those swine farms not participating in the monitoring system and inference of results solely to the participating population.

Spatiotemporal Analyses and Modeling of Clinical Signs and Presumptive Diagnoses

Several descriptive analyses were conducted to summarize the frequencies of clinical events with different clinical signs and presumptive diagnoses, and visualize if any spatiotemporal pattern emerged from the data.

An initial exploration was carried out to describe the trend of clinical events monitored by week. The clinical signs and the respective presumptive diagnoses were grouped and summarized in tables, maps, and bar plots. The severity of clinical signs, the types of production and the age categories of the affected animals were also analyzed to characterize the subpopulations affected. The analysis was complemented by the diagnostic testing results from the laboratory.

Next, to evidence possible patterns over time and space, the number of events with different clinical signs and presumptive diagnoses were explored at low spatiotemporal granularity (i.e., by county and week). The counts of clinical signs and presumptive diagnoses reported weekly were represented with multiple surveillance time series. These series showed the pattern of each clinical sign for each one of the 33 counties included in the population of study between January 2012 and March 2018. Moreover, the cumulative counts of clinical events were mapped monthly and yearly at county level.

Spatiotemporal Modeling Illustrated by Clinical Events of Porcine Pleuropneumonia as Presumptive Diagnosis

Counts of some clinical events grouped by clinical sign or presumptive diagnosis evidenced an overall trend and/or annual seasonality over time (e.g., clinical events such as porcine pleuropneumonia as presumptive diagnosis). To get a better understanding of the observed patterns for different groups, endemic-epidemic multivariate time series models for infectious disease counts were used (8–13). This approach considers that the incidence reported over time can be additively decomposed into two components: an endemic component (or baseline rate of cases with a stable temporal trend) and an epidemic (or autoregressive component). The endemic component includes several terms to represent the reference number of cases as the intercept, the trend and the possible seasonal variation over time. Added to these parameters, these endemic-epidemic multivariate time series models also allow the inclusion of a neighbor-driven component and random effects to explain their influence on the clinical events.

A basic formulation of the endemic-epidemic multivariate time series models can be expressed as:

$$\mu_{it} = e_i \upsilon_t + \lambda Y_{i,t-1} + \varphi \sum_{j \neq i} \omega_{ji} Y_{j,t-1} \quad (1)$$

$$\log(\upsilon_t) = \alpha^{(\upsilon)} + \beta_t t + \gamma \sin(\varpi t) + \delta \cos(\varpi t) \quad (2)$$

where the mean incidence of clinical events in each county i at week t (μ_{it}) depends on two components.

(1) An endemic component (υ_t) multiplied by an offset that corresponds to the accessible population fraction located in each county (e_i). Here, υ_t is incorporated as log-linear predictor that includes an overall trend β_t and sine-cosine terms to represent an annual seasonal variation with a wave frequency $\omega = 2\pi/52$.

(2) An epidemic component split into two parts: an autoregressive part that reproduces the incidence within county i ($\lambda Y_{i,t-1}$), and neighborhood effects that represent the transmission from other adjacent counties j ($\varphi \sum_{j \neq i} \omega_{ji} Y_{j,t-1}$). These epidemic parameters $\lambda = \exp(\alpha^{(\lambda)})$ and $\varphi = \exp(\alpha^{(\varphi)})$ are assumed homogeneous across geographical units and constant over time.

The multivariate count time series defined at different spatiotemporal units can be fitted to a Poisson model, or a negative binomial model if we need to account for overdispersion. In the case of a negative binomial model, the conditional mean (μ_{it}) remains the same, but the conditional variance increases to $\mu_{it}(1+ \mu_{it}\psi_i)$ with additional unknown overdispersion parameter $\psi_i > 0$.

These models are very flexible and allow the inclusion of covariates, estimated transmission weights, and random effects to eventually account for unobserved heterogeneity of the units.

In this study, to model the spatiotemporal patterns of clinical events of porcine pleuropneumonia as presumptive diagnosis, different models were evaluated adding diverse sequential extensions. Initially, a basic model was evaluated accounting for endemic and epidemic parameters with annual seasonality variation and overall trend. Then, other covariates, such as county neighborhood effect or population fraction of each county, were tested on the endemic or epidemic parameters, and finally random effects were tested to eventually account for unobserved heterogeneity of counties.

The most appropriate model was selected by comparing the values obtained from the Akaike Information Criterion and choosing the lowest one (14, 15).

Reporting Information in Near Real Time

The GSP application allowed not only recording and integration of data on clinical events, but also immediate feedback to veterinary practitioners on trends and spatiotemporal evolution of events at county and regional level.

In addition, reproducible documents were created in "pdf" format to report the updated information extracted from the analyses of data. All the stakeholders and veterinarians who collaborated in the network received these reports with detailed results related to reported clinical events and spatial and temporal evolution of patterns of clinical signs and presumptive diagnoses.

It is important to notice that these reports did not show raw information. In order to protect the privacy of the participating stakeholders, the information was summarized by region or county without giving exact details on individual farms.

Software Used for the Development of the Web Application and the Implementation of Analyses

The application of GSP was developed using the following software: HTML5 (16), Java script (17), and Angular 2 (18), and the working environment of IONIC 4 (https://ionicframework.com).

The analyses of this study were carried out using the statistical software R (19) jointly with RStudio as a development environment (20). The plotting of multiple time series, the mapping and the modeling were performed using the "surveillance" package. This package has been broadly used to monitor public health data in diverse European institutions (16). In addition, other R packages were used to make calculations and graphs: "doBy" (21), "gdata" (22), "ggplot2" (23), "lattice" (24), "psych" (25), "maptools" (26), "rgeos" (27), "foreign" (28), "plotrix" (29), "sp" (30), "rgdal"(31), and "spdep" (32).

The reproducible reports were built in Latex format (33) and compiled with RStudio (20).

RESULTS

Coverage Assessment

Between January 2012 and March 2018, a total of 55 practitioners out of 90 volunteered to report clinical events (i.e., veterinary

TABLE 1 | Coverage and number of swine farms reporting by production type and number of clinical events recorded by the GSP monitoring system between January, 2012 and March, 2018.

Production type	Coverage (%)	No. farms in the target population (%)	No. farms reporting (%)	No. clinical events (%)
Fattening	25	4,677 (69)	1,494 (88)	9,014 (85)
Sow farm	16	664 (10)	103 (6)	947 (9)
Farrow-to-finish	8	647 (1)	50 (3)	504 (5)
Nursery	10	253 (4)	20 (1)	45 (0.4)
Gilt development unit	6	177 (3)	10 (0.6)	107 (1)
Continuous flow finisher	4	173 (3)	6 (0.4)	24 (0.2)
Multiplication	11	85 (1)	9 (0.5)	12 (0.1)
Boar stud	0	34 (0.5)	0 (0)	0 (0)
Boar development unit	0	17 (0.2)	0 (0)	0 (0)
Continuous flow nursery	11	9 (0.1)	1 (0.1)	1 (0.01)
Others	0	5 (0.1)	0 (0)	0 (0)
Total	–	6,741 (100)	1,693 (25.1)	10,654 (100)

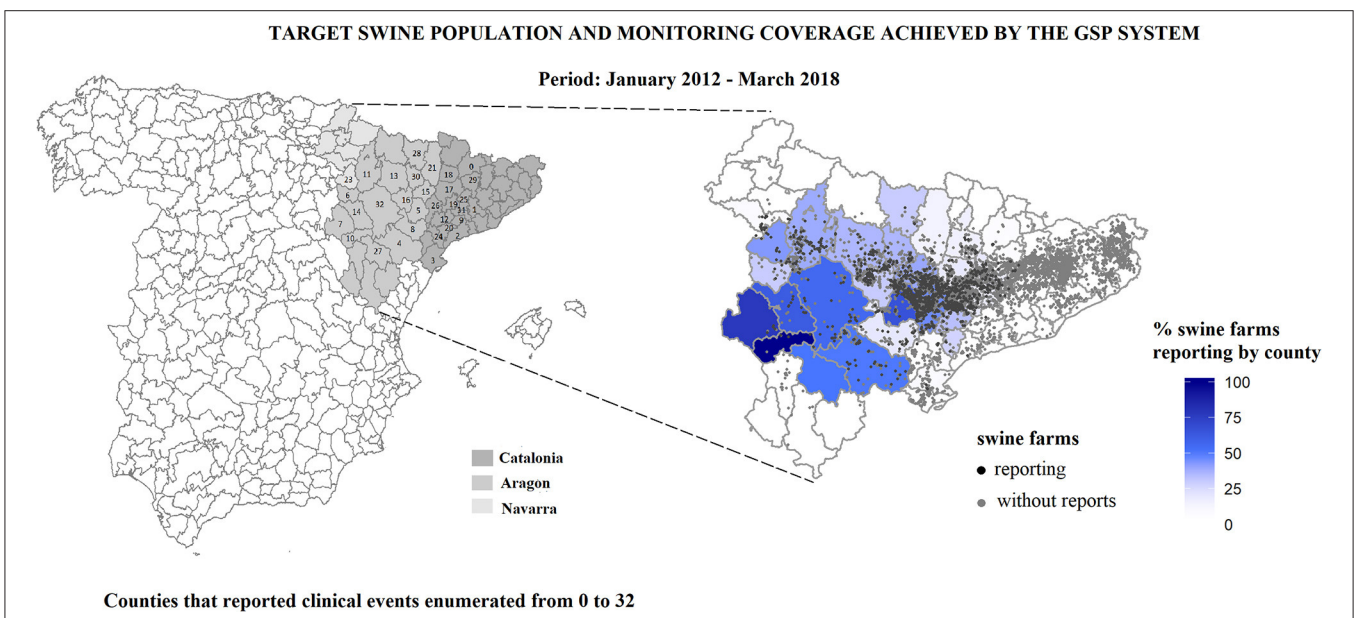

FIGURE 2 | Location of the target swine population and the coverage achieved by the GSP system by county between January, 2012 and March, 2018 in Aragon, Catalonia and Navarra.

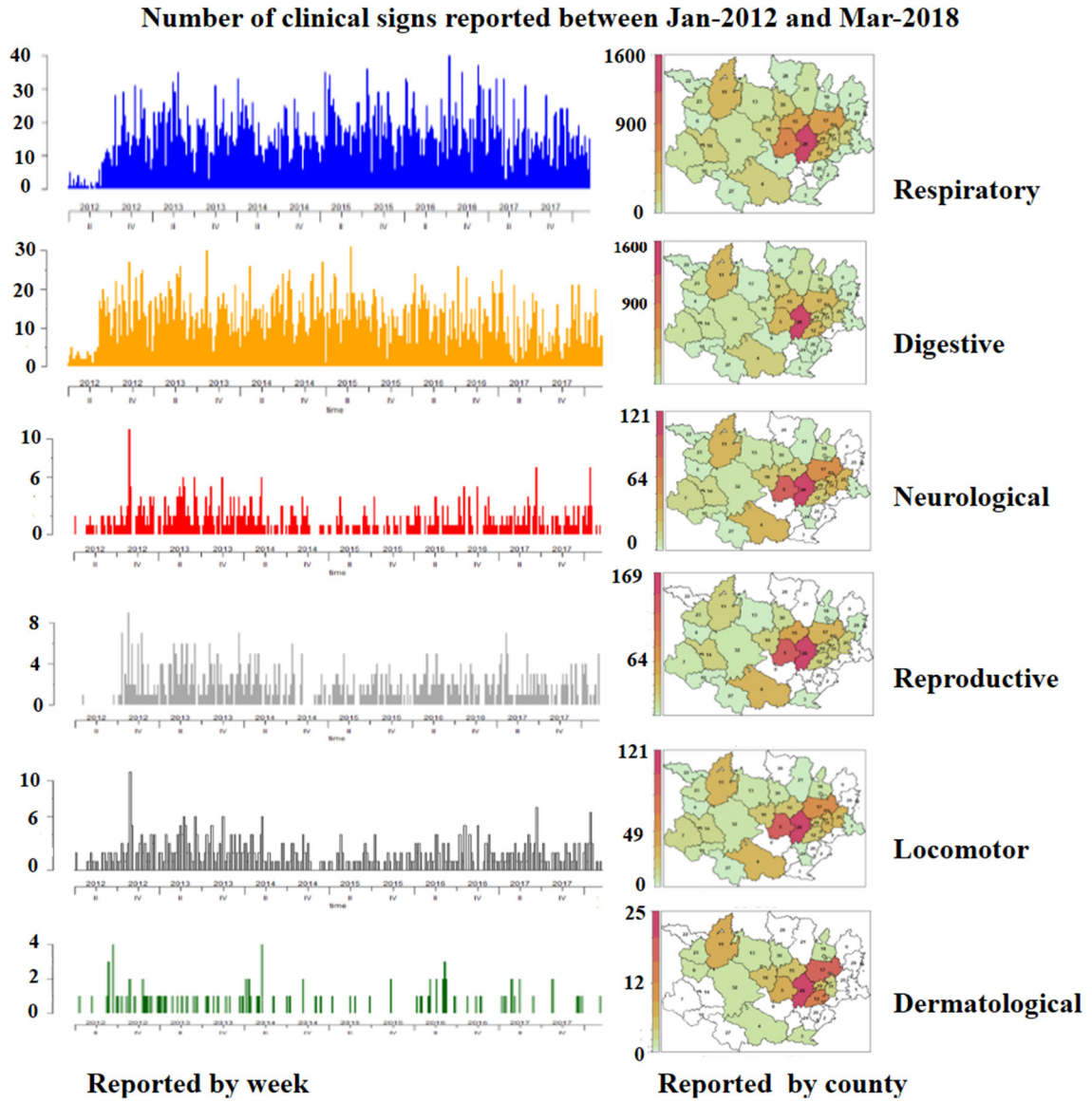

FIGURE 3 | Frequency of clinical signs with their spatiotemporal distribution reported by week and by county in swine farms of Northeastern Spain between January, 2012 and March, 2018.

participation of 61%). These practitioners covered 33 counties of the 62 existing counties and reported a median of 5 clinical events by farm with a range between 1 and 136. The 33 accessible counties comprised 4,207 swine farms, which represented 62% of 6,741 farms of the target swine population. The counties of Western Catalonia and Aragon were the most represented. Over this period, 10,654 clinical events were reported from 1,693 farms (i.e., 25 and 40% of target and accessible swine population, respectively). **Figure 2** shows the location of the target swine population and the coverage achieved by the GSP system by region and county. **Appendix** lists the numerical county codes with their corresponding names.

Most of the clinical events were reported in fattening farms (88%), followed by sow farms and farrow-to-finish farms with 8 and 4%, respectively. The composition of the swine target population was slightly different and comprised 11 production types in which the fattening farms were the most abundant (69%), followed by sow farms (10%), and farrow-to-finish farms (10%). Moreover, it is interesting to remark that the median size of farms that reported clinical events were larger than in the target population, mainly in fattening, continuous flow finisher, and sow farms. This demonstrates that farms from integrated large-operations with highly specialized facilities were more likely to report problems to the system.

Table 1 summarizes the coverage and number of swine farms and clinical events by production type reported by the GSP monitoring system.

TABLE 2 | Summary of clinical signs and presumptive diagnoses differentiating growing pigs and sows (period: January, 2012–March, 2018).

Type of clinical signs	Type of affected animal	No. of affected farms	No. of clinical signs	No. of counts by week (median and range)	Degree of severity	Presumptive diagnoses	No. of presumptive diagnoses	No. of confirmed diagnoses
Respiratory	Growing pigs 88%	1,231	5,698	17 (1–41)	75% mild,	Swine Influenza	1212	19
					23% moderate,	Mycoplasmosis	1029	3
					2% severe	Pasteurellosis	731	3
						PRRS	875	14
						Porcine pleuropneumonia	678	8
						Glässer's disease	680	1
						Rhinitis	31	0
						Porcine circovirus associated disease	6	2
	Sows 12%	118	751	4 (1–12)	1% mild,	Swine Influenza	67	2
					96% moderate,	Mycoplasmosis	12	0
					3% severe	PRRS	82	15
						Porcine pleuropneumonia	80	2
						Glässer's disease	189	1
						Rhinitis	0	0
						Porcine circovirus associated disease	1	1
Digestive	Growing pigs 87%	1,115	4,256	13 (1–31)	73% mild,	Colibacilosis	1231	7
					25% moderate,	Unspecific diarrhea	599	0
					2% severe	Clostridiosis	670	2
						Swine dysentery	440	14
						Salmonellosis	314	4
						Ileitis	137	1
						Ulcers	71	0
						Any parasitosis	33	0
						Rectal prolapse	4	0
	Sows 13%	126	662	3 (1–14)	4% mild,	Colibacilosis	315	3
					93% moderate,	Unspecific diarrhea	42	0
					3% severe	Clostridiosis	1	2
						Swine dysentery	73	12
						Salmonellosis	93	4
						Ileitis	6	3
Neurological	Growing pigs 82%	708	1,778	5 (1–16)	75% mild,	Glässer's disease	584	0
					23% moderate,	Streptococcosis	226	2
					3% severe			
	Sows 18%	32	379	3 (1–9)	98% moderate	Glässer's disease	157	0
					2% severe	Streptococcosis	217	0
Reproductive	Growing pigs 84%	328	575	2 (1–10)	92% mild,	PRRS	573	57
					5% moderate,	Porcine circovirus associated disease	0	0
					2% severe			
	Sows 16%	71	90	1 (1–3)	17% mild,	PRRS	87	57
					60% moderate,	Porcine circovirus associated disease	2	1
					23% severe	Leptospirosis	1	0
Locomotor	Growing pigs 98%	429	654	2 (1–11)	67% mild,	Streptococcosis	618	0
					32% moderate,	Glässer's disease	56	0
					1% severe			

(Continued)

TABLE 2 | Continued

Type of clinical signs	Type of affected animal	No. of affected farms	No. of clinical signs	No. of counts by week (median and range)	Degree of severity	Presumptive diagnoses	No. of presumptive diagnoses	No. of confirmed diagnoses
	Sows 2%	9	11	1 (1–2)	91% moderate, 9% severe	Streptococcosis	11	0
Dermatological	Growing pigs 96%	95	112	1 (1–4)	83% mild, 15% moderate, 2% severe	Exudative epidermitis	77	0
						PRRS	1	0
	Sows 4%	5	5	1 (1–1)	60% moderate, 40% severe	Exudative epidermitis	5	0

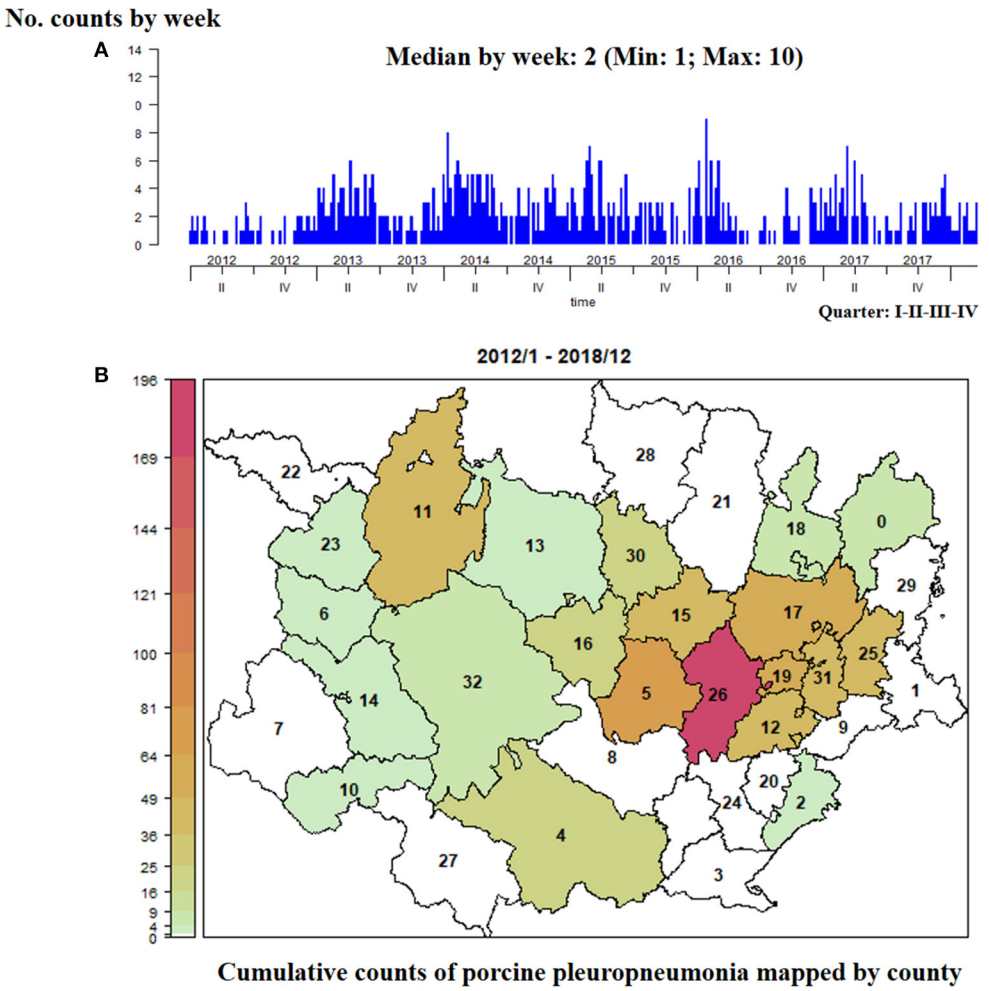

FIGURE 4 | Number of clinical events with porcine pleuropneumonia as presumptive diagnosis (January, 2012–March, 2018). **(A)** Counts reported by week. **(B)** Cumulative counts mapped by county.

During the first semester of 2012 the number of reports was relatively low, but in the second semester of that year the level of reporting increased substantially being subsequently sustained throughout the whole study period (see **Figure 3**).

Spatiotemporal Descriptive Analyses of Clinical Signs and Presumptive Diagnoses

From 10,654 clinical events a total of 14,971 clinical signs were reported, most of them in growing pigs (85%). In general, the degree of severity of clinical events detected in growing pigs

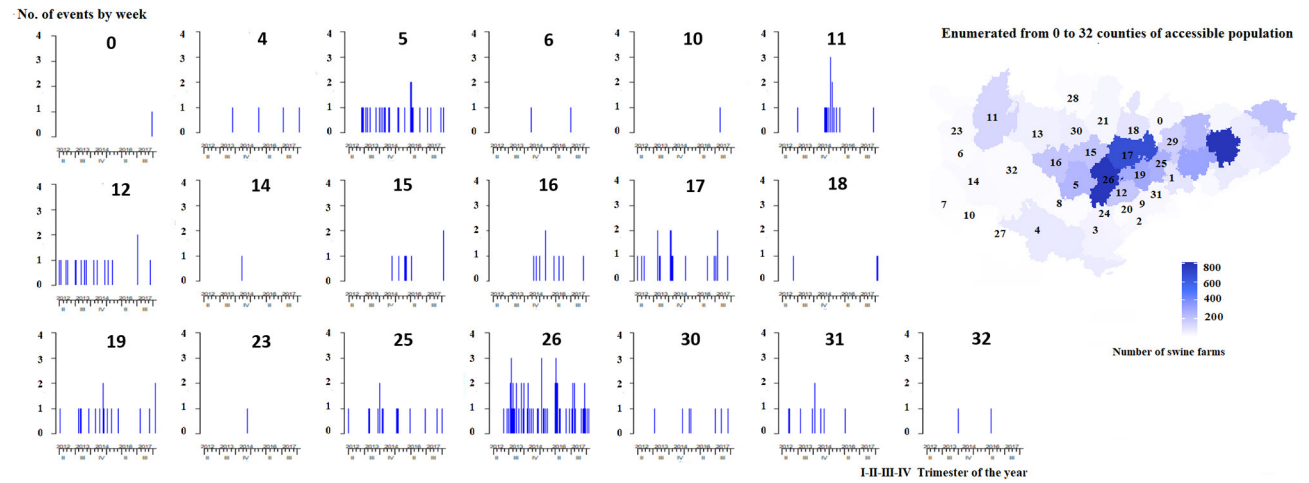

FIGURE 5 | Clinical events of porcine pleuropneumonia as presumptive diagnosis reported by week and by county in Northeastern Spain between January, 2012 and March, 2018.

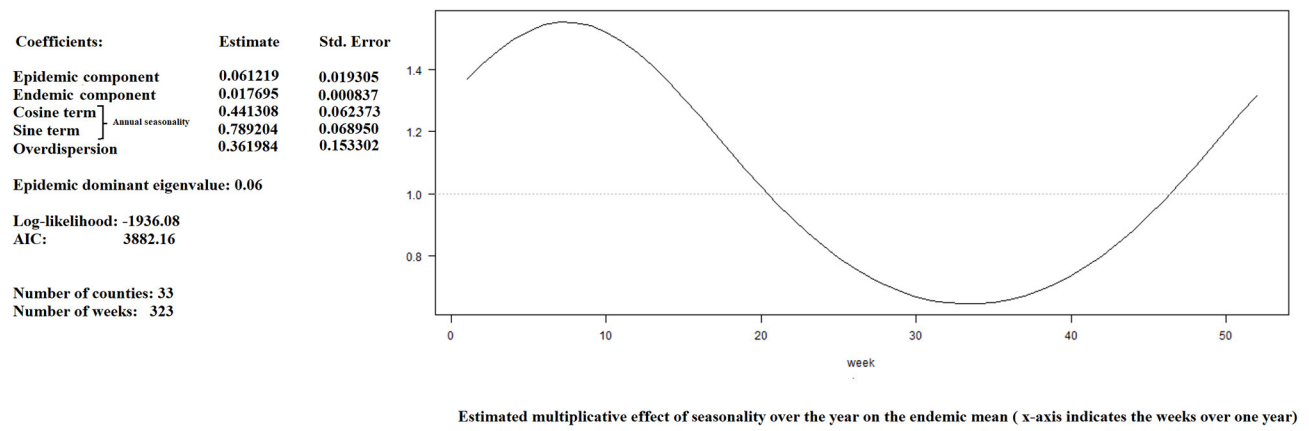

FIGURE 6 | Summary of the fitted model to data of suspected clinical events of porcine pleuropneumonia recorded by the GSP system between January, 2012 and March, 2018: coefficients and resulting multiplicative effect of seasonality on the endemic component.

farms (nursery and fattening pigs) was milder than in sow farms (sows and/or nursery pigs). In both growing pigs and sow farms, respiratory clinical signs were the most frequent, followed by digestive, neurological, locomotor, reproductive, and finally dermatological signs. A combination of several clinical signs was observed in 30% of these events (n = 3,182). The most frequent combination was digestive and neurological signs (7%), followed by respiratory and neurological (5%), respiratory and locomotor (4%), and respiratory and reproductive (4%). **Figure 3** summarizes the frequency of all clinical signs reported with their respective spatiotemporal distribution by county and week.

The main presumptive diagnoses associated with respiratory clinical signs were diseases that belong to the porcine respiratory complex (such as swine influenza, PRRS, and mycoplasmosis), followed by Glasser's disease, pasteurellosis, and porcine pleuropneumonia. The most frequent presumptive diagnosis reported in clinical events with digestive signs was colibacilosis, while the most frequent suspicion for neurological signs was Glässer's disease, PRRS for reproductive signs, streptococcosis for locomotor signs, and exudative epidermitis for dermatological signs. Less than 1% of those presumptive diagnoses were confirmed by laboratory diagnosis. **Table 2** summarizes the clinical signs by type of affected animals (i.e., growing pigs or sows), the number of affected farms, the degree of severity and the associated presumptive and confirmed diagnoses.

Next, using porcine pleuropneumonia as example of presumptive diagnosis, we illustrate how the clinical events grouped by each presumptive diagnosis were represented spatiotemporally. It is important to note that the clinical signs of porcine pleuropneumonia are quite pathognomonic, and thus clinical suspicions were a useful measure of the pattern of disease. The disease was suspected by the veterinarians on 758 occasions, most often in pigs on fattening farms (88%).

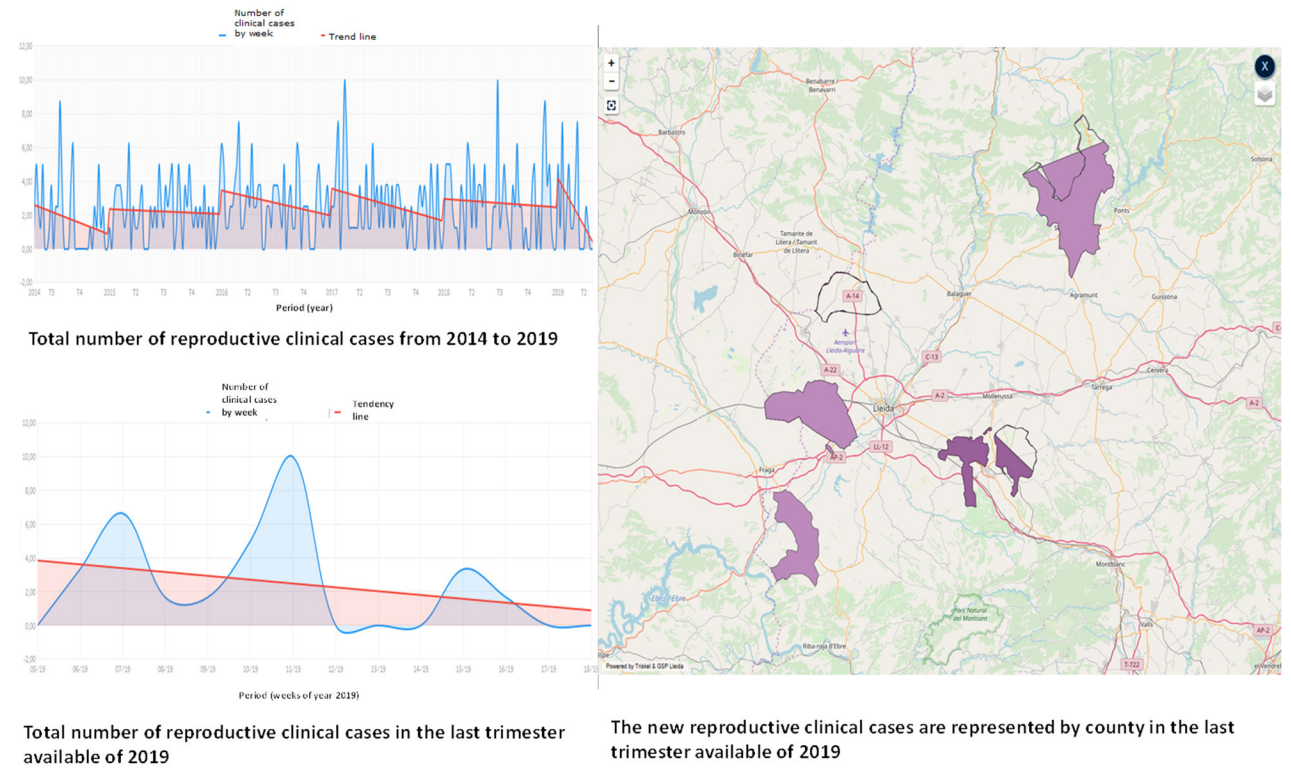

FIGURE 7 | Illustration of layout of the GSP web application to visualize the area and the trend of reported reproductive clinical events.

Figure 4 illustrates the time series of the number of clinical events reported by week and the cumulative counts of events reported by county (see **Figure 4**).

Moreover, using multivariate surveillance time series, trend at county level can be visually assessed and compared among counties (see **Figure 5**).

Figures 4, **5** show that, except for 2012, throughout the whole period of study the overall trend of reporting was quite stable with a seasonal increase each winter. From the accessible population 19 out of the 33 counties reported at least one suspicion of porcine pleuropneumonia. Although the number of weekly counts by county were relatively low (i.e., maximum 3), those counties of Western Catalonia and Aragon that had more swine farms also reported more consistently suspicions of porcine pleuropneumonia (e.g., county labeled as 26).

Spatiotemporal Modeling for Porcine Pleuropneumonia as Presumptive Diagnosis

The data on porcine pleuropneumonia as presumptive diagnosis was fitted using a negative binomial model. This model included an endemic component with a marked seasonality that increased between January and March and an epidemic component. In this case, the influence of random effects structures could not be assessed due to the lack of convergence. The coefficients of the fitted model and the resulting multiplicative effect of seasonality on the endemic component for the suspected clinical events of swine pleuropneumonia are shown in **Figure 6**.

Reporting Information in Near Real Time

Finally, with the aim of providing a continuous feedback to stakeholders and veterinarians, and communicate information on the health status of the population in near real time, the system produced different reports. Directly from the web application the user could get the area where the clinical events were reported during the last 3 months and the trend (see **Figure 7**).

Moreover, every month the stakeholders received a brief report summarizing the information of the clinical events reported by age, type of farms, and counties. Each year they also received a very detailed report of the monitoring conducted and the results of the models that evaluate the pattern of some clinical signs and presumptive diagnoses.

DISCUSSION

Traditionally, in Spain, the reporting of many endemic swine diseases through passive surveillance has been very disperse and scarce, and the information required for making proper decisions in health management at population level has often been poor (4). The development of new user-friendly and

standardized digital methods to report and analyze data on clinical events can help to determine the frequency and the evolution of diseases at population level (5, 34). Recent initiatives have been carried out to monitor some endemic diseases in swine populations using these methods. Although many authors have pointed out the potential for near real-time monitoring, system development faces several technical, social and communication challenges in order to define data standards and data-sharing agreements (1–3, 5). Implementation involves a multidisciplinary approach and the participation of the swine sector. This was the case with the GSP system, built in collaboration with researchers in swine diseases, lab technicians, computer scientists, practitioners, epidemiologists and with the support of producers of Northeastern Spain, all of whom were fully committed to the initiative.

The coverage assessment of the GSP system allowed identification of the accessible population under monitoring. This study, based on data collected over more than 6 years, shows that implementation was gradual during the first year (2012), while in subsequent years the practitioners registered clinical events on a regular basis. The spatial coverage of the target swine population was partial and varied between counties (see **Figure 2**) and types of production (see **Table 1**). Despite this limitation, the monitoring system achieved the collection of data from an important proportion of fattening farms from integrated large operations in 33 counties, mainly concentrated in Western Catalonia and Aragon. In total 55 out of 90 swine practitioners in Northeastern Spain volunteered to participate. Veterinarians usually worked in a specific area and this could lead to spatiotemporal clustering of reporting of clinical events. In each county, the clinical events were reported by veterinarians from different companies so, to ensure standardized reporting, the same training was provided to all the system participants.

To improve the coverage in areas where the app was not used, our suggestions are to hold more meetings explaining the benefits of the information provided by this kind of monitoring and try to sort out the problems that prevent practitioners from participating. On the other hand, since this system did not allow differentiating if a specific farm was not visited or the veterinarian did not detect any clinical event, we recommended adding a field in the application to record all visits of the practitioner, even when no disease was observed. We believe that the recording of all the clinical inspections carried out would help improve the assessment of the coverage of the system and serve to demonstrate the absence of endemic diseases.

The spatiotemporal descriptive analyses from clinical events reported at farm level allowed the identification in near real-time of the most frequent clinical signs and presumptive suspicions at county and regional level. This easily accessible and current information could be useful to veterinary clinicians and stakeholders for decision making. For example, if a practitioner knew that the incidence of an endemic disease had increased in neighboring farms, he/she could decide to implement or modify preventive measures (e.g., vaccination) or take samples in other swine farms to confirm the presence or absence of infection.

In addition, the spatiotemporal descriptive analyses of retrospective data from clinical events allowed assessment of the evolution of different clinical signs and endemic diseases, comparison of different subpopulations and identification of groups of farms, areas, or periods with higher incidence of specific problems. This long-term monitoring could help to determine the baseline frequency of clinical signs or endemic diseases, assess the influence of different factors on disease presence, and predict clinical events. In our study, the results of these analyses showed that the most frequent clinical signs reported from the accessible population were respiratory, followed by digestive and neurological. Moreover, as example of a more detailed analysis, by combining data of clinical signs and presumptive diagnoses, we observed that the practitioners mainly associated these signs with diseases of the respiratory complex (such as swine influenza, mycoplasmosis, or PRRS), followed by pasteurellosis, porcine pleuropneumonia, and Glässer's disease.

However, it is important to note that due to the small number of samples (<1% of events), most of these presumptive diagnoses were not confirmed by the laboratory. The main reason why swine practitioners did not take samples was that they believed that the laboratory confirmation would not change the medical interventions to undertake at farm level; and thus, they preferred to avoid extra-costs and logistical difficulties. The monitoring of presumptive diagnoses without laboratory confirmation could result in false alerts being raised. To minimize this limitation, we suggest identifying subpopulations frequently affected by clinical signs or endemic disease suspicions, communicating the information to practitioners and recommending submission of samples for laboratory confirmation. Furthermore, the reporting of presumptive diagnoses was defined as a closed list of possible endemic diseases and the option to report other endemic diseases (not included in the list) or exotic diseases was not considered. To improve this reporting, we suggest including the option of "other suspicion" within an open field, where the veterinarian could record other diagnoses or findings.

The reproducible reports created by the GSP system provided updated and continuous information to the practitioners and swine stakeholders who participated. These reports showed visually the frequency of health problems at county and regional level, allowed the identification of their spatial distribution and progress, and helped the decision-making of where and when actions were necessary. Practitioners and swine stakeholders benefited from sharing information of clinical events occurring in the neighboring areas to plan control measures against these infections at farm level. Moreover, this system facilitated communication within the swine sector in Northeastern Spain and promoted co-operation. However, at this initial stage, the interventions to undertake in the event of alert at population level had not been agreed upon by different practitioners and private stakeholders, so the system was not ready to be used to plan specific actions. A future potential use of this system would be as surveillance system in order to detect outbreaks or aberrations. Nevertheless, for directing effective control actions, we still need to gradually build more trust in the current monitoring and achieve a better consensus and commitment from the whole swine sector.

CONCLUSIONS

Overall, we believe that the kind of monitoring system described in this study provides very useful information to detect and monitor the trend of the most frequent endemic diseases, identify specific health problems and to enhance communication within the swine sector. A consensual and broad implementation of the system on the whole target population could shorten the response time to prevent and control certain diseases, decreasing productive, and sanitary losses.

Further research could be directed at identifying disease characteristics and modeling other covariates of interest at company or county level to further benefit endemic disease control within the swine industry.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The approval of our study was not required as per the local legislation. This research did not involve any specific clinical study using animal experimentation. This work has been written in agreement with the veterinary clinicians and stakeholders who provided data. All data have been analyzed in aggregate form guaranteeing their privacy and security.

AUTHOR CONTRIBUTIONS

AA-C conceived the research, performed the statistical analyses, developed the reproducible reports, and wrote the article. EA conceived the research, designed the app, obtained the data, and provided the layout to show the results in the app. VT and JB conceived the research, built, coordinated, and motivated the network of veterinary clinicians that participated in the GSP system. EN conceived the research, performed the laboratory tests, and interpreted the results. SN interpreted the laboratory test results and wrote the article. LF conceived the research, reviewed the statistical analyses and reproducible reports, guided the study, and wrote the article.

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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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APPENDIX

TABLE A1 | List of numerical county codes and corresponding names covered by the GSP monitoring system.

Codes	County names
0	ALT URGELL
1	ANOIA
2	BAIX CAMP
3	BAIX EBRE
4	BAJO ARAGON
5	BAJO CINCA
6	BORJA
7	CALATAYUD
8	CASPE
9	CONCA DE BARBERA
10	DAROCA
11	EGEA DE LOS CABALLEROS
12	GARRIGUES
13	HOYA DE HUESCA
14	LA ALMUNIA DE DONA GODINA
15	LA LITERA
16	MONEGROS
17	NOGUERA
18	PALLARS JUSSA
19	PLA D'URGELL
20	PRIORAT
21	RIBARGORZA
22	RIBERA ALTA ARAGON
23	RIBERA BAJA
24	RIBERA D'EBRE
25	SEGARRA
26	SEGRIA
27	SERRANIA DE MONTALBAN
28	SOBRARBE
29	SOLSONES
30	SOMONTANO
31	URGELL
32	ZARAGOZA

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Leptospira Seroprevalence Among Ugandan Slaughter Cattle: Comparison of Sero-Status With Renal *Leptospira* Infection

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Despite evidence of both human and animal *Leptospira* exposures in Uganda, the epidemiology of the disease is still not well-investigated. Contact with animals and their environments have been pointed out as potential source of infection with *Leptospira* species in humans; and cattle may be an important reservoir in Uganda. In this cross-sectional study, we estimated the prevalence of anti-*Leptospira* antibodies by the standard microscopic agglutination test (MAT); and associated risk factors among slaughtered cattle. We also compared the performance of the MAT used in this study against a *lipL32* based real time PCR (qPCR) assay previously conducted on the kidneys and urine of the same slaughter cattle as tested in this reported study. Of 500 cattle sampled, 27.8% (95% CI 23.9–32.0) tested positive (titer $\geq$ 100) to at least one *Leptospira* serovar, with the majority of seropositive cattle reacting to serovars Tarassovi (sg Tarassovi) (11.6%), Sejroe (Sg Sejroe) (7.8%), and Australis (Sg Australis) (5.2%). Older animals had 2.8 times (95% CI 1.0–8.2, *p*-value 0.055) greater odds of being seropositive than younger ones (<1.5 years). The sensitivity and specificity of the MAT over the qPCR were 65.9% (95% CI 50.1–79.5) and 75.9% (95% CI 71.7–79.7), respectively; with a negative predictive value of 95.8% and positive predictive value of 20.9%. In conclusion, slaughter cattle in this study were significantly exposed to pathogenic *Leptospira* species of mainly the Tarassovi, Sejroe, and Australis serogroups, with seroprevalence being higher among older cattle. The high specificity and negative predictive value of MAT as used in this study when compared to the qPCR assay may imply a rather strong association between seronegativity and absence of renal *Leptospira* infection. However, MAT predictability for renal *Leptospira* infection may be interpreted cautiously since predictive values of diagnostic tests are dependent on prevalence.

Keywords: leptospirosis, microscopic agglutination test, renal *Leptospira* infection, slaughter cattle, seroprevalence

INTRODUCTION

Leptospirosis is one of the most wide spread zoonotic bacterial diseases that is endemic in subtropical and tropical countries; accounting for a global annual incidence of 1.03 million human cases and 58,900 deaths (1). The etiological agents of the disease are spirochetes of the genus *Leptospira*, comprising over 250 pathogenic serovars (2). Certain serovars are known to be regionally endemic and reserved in certain species of wild mammals and domesticated animals. These carrier animals may remain asymptomatic but capable of transmitting leptospires to other animal species (incidental hosts) and humans, via direct contact with contaminated urine or indirectly through contaminated water and soil (3). Particularly, cattle have been reported to maintain serovars Hardjo, Sejroe and at times Pomona (3–5).

Despite evidence of both human and animal leptospirosis in Uganda, the epidemiology of the disease is still not well-investigated. Seropositivity to *Leptospira* species has been described in buffaloes (6) and in dogs (7), with the first case of clinical canine leptospirosis in Uganda reported recently (8). A random survey in beef and dairy cattle herds in two districts of Uganda revealed a seroprevalence of 19% (9). Additionally, Dreyfus et al. (10) demonstrated 35% prevalence of anti–*Leptospira* antibodies in health centre patients in Hoima, Uganda; with skinning of cattle during slaughter being significantly associated with the observed seropositivity. This further implicates cattle as potential sources of *Leptospira* infections to humans. Furthermore, renal carriage and/or shedding of pathogenic *Leptospira* was recently confirmed in 8.8% ($n = 44$) of slaughter cattle from the same population as this current study (11). The aim of this study was to determine the prevalence of anti-*Leptospira* antibodies by the standard microscopic agglutination test (MAT); and establish associated risk factors for *Leptospira* serostatus among slaughtered cattle. In order to assess the usefulness of serological tests as tools for surveillance of leptospirosis in cattle herds, we compared the performance of MAT against a *lipL32* based real time PCR (qPCR) assay conducted previously on the kidneys and urine of the same slaughter cattle tested in this study.

MATERIALS AND METHODS

Study Design

We conducted a cross-sectional study between June and July 2017, in two purposively selected cattle abattoirs in Kampala, Central Uganda. The two abattoirs were Nsooba slaughter house, Kalerwe (AK) and City abattoir (LC). Selection of the two slaughter facilities was based on their large average daily slaughter volumes (162 cattle at AK and 221 at LC) and the broad regional diversity of source markets for their slaughter cattle. Cattle slaughtered at these abattoirs are gathered as individual animals (not herds) on a daily basis by various independent traders who buy them from independent farmers from geographically distinct locations of Uganda.

Sample Size and Sampling Strategy

The sample size for estimating the seroprevalence with a 95% confidence level and precision of 0.05 was determined as 237 cattle, using Epitool calculators (12), and based on an estimated 19% prevalence earlier reported in a serological survey in dairy and beef herds in Uganda by Dreyfus et al. (9). Since we sampled individual animals and not herds, we did not have to take clustering into account. Blood samples were collected from a total of 500 randomly selected slaughter cattle, following the same sampling strategy that Alinaitwe et al. (11) used to co-currently collect matching kidney and urine samples from the same cattle population tested in this study (from the same animal, kidney, urine and blood/serum were collected; and serum tested separately by MAT).

In brief, the two abattoirs were visited on alternating week days for 21 days. At abattoir LC, four of the fourteen slaughter lines were randomly selected on each visit, and samples systematically collected. At the second abattoir (AK), there were no slaughter lines; and as such the facility was virtually divided into two spaces along its width. Animals slaughtered through one virtual space were opportunistically sampled, and the collections alternated between the two virtual spaces on subsequent visits. Here, random selection of individual animals from the pre-selected virtual space depended on the slaughter process itself. It would take 15–25 min to enroll and collect samples from a single animal. During this time another 5–8 animals would be laid down, which limited the probability that animals from the same population characteristics were selected.

Sample Collection

At the time of evisceration, 4 ml of blood was collected from each randomly selected slaughter animal by cardiac puncture into a plain vacutainer (Becton Dickinson BD^{TM}). Additionally, animal demographic data was taken, and information on origin of the slaughtered cattle obtained from abattoir records or at times on consultation with the respective animal traders. The blood samples were kept on ice until delivery to the Central Diagnostic Laboratory at College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University. At the laboratory, the blood was centrifuged at 2,000 g for 5 min, and serum harvested into cryogenic tubes for storage at −20°C.

Serological Testing

The microscopic agglutination test (MAT) was used to determine presence of anti-*Leptospira* antibodies; in accordance with the OIE standards (13). A panel of 11 serovars (**Table 1**) representing 11 serogroups previously described as prevalent in Uganda (9) and those shown to be prevalent or maintained in cattle elsewhere in East Africa (14, 15) was employed. Briefly, seven day old live *Leptospira* cultures were used to screen the serum samples at an initial dilution of 1:50. Those with a positive reaction were then titrated in a serial 2-fold dilution to determine the end-point/titer (the reciprocal of the highest serum dilution at which ≥50% of the leptospires remained agglutinated). Sera with a titer ≥100 against any *Leptospira* serovar were considered positive. Sera samples that reacted to one or more *Leptospira* serovars were considered as positive for all the reacting serovars, despite any

TABLE 1 | Strains of *Leptospira* species used as live antigens in the MAT.

Genomospecies	Serogroup	Serovar	Strain
L.interrogans	Australis	Australis	Ballico
	Icterohemorrhagiae	Icterohemorrhagiae	RGA
	Pomona	Pomona	Pomona
	Canicola	Canicola	Strain Hond Utrecht IV
	Djasiman	Djasiman	Djasiman
L. kirschneri	Autumnalis	Butembo	Butembo
	Grippotyphosa	Grippotyphosa	Duyster
L. borgpetersenii	Pyrogenes	Nigeria	Vom
	Tarassovi	Tarassovi	Perepelitsin
	Sejroe	Sejroe	M84
L. santarosai	Shermani	Shermani	1342K

disparities between antibody titers detected against each of the reacting serovars.

Real-Time PCR Assay

Data on renal *Leptospira* infection (shedding and/ or carriage of pathogenic *Leptospira* species) was provided by Alinaitwe et al. (11), who had already conducted a Taqman real-time PCR (qPCR) assay on kidney homogenates and urine of the same slaughter cattle whose matching sera samples we have tested by MAT in this study. The qPCR targeted the gene *lipL32* which encodes for a major outer membrane protein, only present in pathogenic *Leptospira* species (16).

Data Analysis

We recorded the data in Microsoft Excel 2010 (Microsoft Corp, Redmond WA, USA) and analyzed it in Stata 15 (Stata Corp., USA). The overall prevalence of seropositive animals and seroprevalence by serogroup (serovar) were calculated. The association between seroprevalence and the exposure variables "source abattoir," "age," "sex," "breed," and "region of origin" of slaughtered cattle were analyzed by univariable logistic regression. Further, in a manual forward selection method, we assessed the association between these exposure variables and *Leptospira* seropositivity by multivariable logistic regression analysis. Exposure variables were each entered in the model and were kept in the model if the likelihood ratio test was statistically significant ($p \leq 0.05$) compared to the nested model. The performance of the MAT against the qPCR (the latter being the reference) was assessed based on the test's sensitivity, specificity, predictive values and level of agreement as determined by the Cohen Kappa statistic (17). Since we tested individual animals from the slaughterhouse and not herds, we did not account for clustering.

Ethical Considerations

The study procedures were approved by the Uganda National Council for Science and Technology (A565), and consent from abattoir representatives was obtained ahead of the study and also at the time of sampling.

TABLE 2 | Population characteristics of the sampled slaughter cattle ($N = 500$), *Leptospira* seroprevalence and associated risk factors as calculated by univariable logistic regression.

Variable	*n* (%)	Prev %	OR	95% CI	*P*-value
Breed					
Local	401 (80.2)	26.4	Ref		
Cross	94 (18.8)	31.9	1.3	0.8–2.1	0.285
Exotic	5 (1.0)	60.0	4.2	0.7–25.3	0.12
Sex					
Female	272 (54.4)	29.4	Ref		
Male	228 (45.6)	25.9	0.8	0.6–1.2	0.38
Age					
Juvenile (<1.5 Years)	32 (6.4)	12.5	Ref		
Adult	468 (93.6)	28.9	2.8	1.0–8.2	0.055
Region					
Central	200 (40.0)	28.5	Ref		
Western	69 (13.8)	33.3	1.3	0.7–2.3	0.45
Northern	39 (7.8)	25.6	0.9	0.4–1.9	0.72
Eastern	55 (11.0)	21.8	0.7	0.3–1.4	0.33
Across Boarder	42 (8.4)	26.2	0.9	0.4–1.9	0.76
Undetermined	95 (19.0)	27.4	1.0	0.6–1.6	0.84
Abattoir					
AK	187 (37.4)	26.2	Ref		
LC	313 (62.6)	28.8	1.1	0.8–1.7	0.538

n is the number of observations under each variable level, and Prev% is the percentage Leptospira seroprevalence by variable level (population characteristic). Ref is the parameter in a given category to which the other parameters in the same category were compared.

Local collectively refers to breeds of cattle indigenous to Uganda while exotic to those originating outside Uganda. Cross refers to an offspring of interbreeding between a local and exotic breed.

MAT, microscopic agglutination test; OR, odds ratio and CI, confidence interval.

RESULTS

Study Population Characteristics

Of the 500 slaughter cattle sampled, 468 (93.6%) were adult cattle (≥1.5 years), and there were slightly more cows (54.4%) than bulls. The majority of the cattle slaughtered at the abattoirs were sourced from the central (40.0%) and western (13.8%) regions of Uganda. Up to 8.4% of the slaughter cattle were reportedly sourced across the borders of Uganda (mainly Tanzania), while the definite origin of up to 19.0% of the cattle could not be established due to insufficient accompanying documentation from their source markets. Indigenous breeds of cattle dominated the slaughter population at the two study abattoirs (80.2%) as compared to their exotic and cross-bred counterparts (**Table 2**).

Prevalence of Anti-Leptospira Antibodies

Of 500 cattle tested, 27.8% (95% CI 23.9–32.0) ($n = 139$) tested positive (titer ≥ 100) to at least one *Leptospira* serovar. The majority of seropositive cattle reacted to serovars Tarassovi (sg Tarassovi) (11.6%), Sejroe (Sg Sejroe) (7.8%), and Australis (Sg Australis) (5.2%); with no cattle reacting to serovar Djasman (**Table 3**). Seropositivity to multiple *Leptospira* serovars was detected in 4.4% (22/500) of cattle, and up to 15.8% (22/139)

TABLE 3 | Prevalence and levels (titers) of serovar-specific anti-*Leptospira* antibodies measured by the microscopic agglutination test (MAT) among cattle slaughtered at major Ugandan abattoirs ($N = 500$).

	MAT titer							
Serovar	**100**	**200**	**400**	**800**	**1,600**	**NPos* serovar**	**% Positive**	**95% CI**
Tarassovi	10	12	21	13	2	58	11.6	8.9–14.8
Sejroe	23	13	3	0	0	39	7.8	5.7–10.6
Australis	19	6	0	0	1	26	5.2	3.5–7.6
Shermani	0	6	5	2	0	13	2.6	1.4–4.5
Grippotyphosa	4	1	1	0	0	6	1.2	0.5–2.7
Pomona	0	3	0	1	1	5	1.0	0.4–2.4
Icterohemorrhagiae	1	2	1	1	0	5	1.0	0.4–2.4
Canicola	0	2	1	1	0	4	0.8	0.2–2.2
Nigeria	1	1	1	0	0	3	0.6	0.1–1.9
Butembo	2	0	0	0	0	2	0.4	0.07–1.6
Djasman	0	0	0	0	0	0	0.0	0.09–0.9
Npos* titer	60	46	33	18	4	161		

*Npos*titer refers to the number of cattle with the various levels of antibodies (titers) and Npos* serovar to those reacting against the respective Leptospira serovars/serogroups tested. CI, Confidence interval; MAT, microscopic agglutination test.*

of the seropositive cattle had high anti-*Leptospira* antibody titers (≥800).

Risk Factors for Seroprevalence of Pathogenic *Leptospira* Species

In the univariable logistic regression model, none of the exposure variables were significantly associated with cattle having antibodies against leptospires at a $p \leq 0.05$ (**Table 2**). Nevertheless, older animals had 2.8 times (95% CI 0.98–8.24) greater odds of being seropositive than younger ones (<1.5 years), albeit with a *p*-value of 0.055. None of the exposure variables improved the model fit in the multivariable logistic regression model (data not shown).

Performance of the MAT Against the lipL32 qPCR Assay

Of the 44 qPCR positive samples reported by Alinaitwe et al. (11), matching sera from 23 were found to be positive by MAT as well. Overall there was a fair agreement between the MAT and the qPCR results (Cohen's kappa statistic 0.21; $P < 0.001$). The sensitivity and specificity of the MAT over the qPCR were 65.9% (95% CI 50.1–79.5) and 75.9% (95% CI 71.7–79.7), while the positive predictive and negative predictive values were 20.9 and 95.8%, respectively. Furthermore, the performance of MAT against the qPCR assay was explored at different MAT cutoffs. While the sensitivity and NPV decreased with increasing MAT titer cut-off, the specificity and PPV increased (**Table 4**).

DISCUSSION

Detection of a high prevalence of anti-*Leptospira* antibodies against several *Leptospira* serogroups/serovars in cattle slaughtered at Ugandan abattoirs indicates that cattle are exposed to various *Leptospira* serogroups and serovars. This result supplements already existing data from studies that previously demonstrated anti-*Leptospira* antibodies in cattle (6, 9), and one that confirmed renal *Leptospira* infection in cattle from various areas of Uganda (11). The latter study was conducted in the same abattoirs as the current study. The studied abattoirs are currently the largest in Uganda (in terms of daily slaughter volume), and source their slaughter animals from a wide geographical range, thus reducing sampling bias. Additionally, cattle slaughtered at these abattoirs were never contributed by selected herds but rather these were individual animals independently sold to various cattle traders daily and by independent farmers from geographically distinct locations of Uganda. Seropositive animals were detected among cattle sourced from all the five regions, with no significant association between seropositivity and any of the regions of origin. Therefore, we postulate that leptospirosis is endemic and widely spread in several cattle populations in Uganda. A similar level of *Leptospira* prevalence as found in this study has been reported in cattle in Tanzania (14, 18), and in Kenya (19), with serovars Tarassovi and Hardjo (Hardjo is in the same serogroup as Sejroe used in the current study) being more prevalent. Tanzania and Kenya are neighbors with Uganda and the three countries share a similar ecology. This may mean that similar factors influence the leptospirosis burden in the three countries. In addition, there is evidence of trans-boundary movement of animals including cattle between these countries (20, 21). Up to 15.8% of the seropositive cattle had high anti-*Leptospira* antibody titers (≥800), probably indicating they had recently been infected with the respective *Leptospira* species at the time of sampling. The association between seropositivity and age of cattle as observed in this study could be explained by the higher likelihood of exposure to *Leptospira* contaminated sources with increasing age, especially in endemic settings.

Previous studies in Uganda have so far demonstrated circulation of the cattle maintained serovar Hardjo (6, 11). In the current study, we found high reactivity to Sejroe, a serovar in

TABLE 4 | Performance of the MAT against the *lipL32* qPCR assay at various titer cut-offs.

	MAT titer cut-off				
Test parameter	**100**	**200**	**400**	**800**	**1,600**
Sensitivity (CI)	65.9 (50.1–79.5)	52.3 (36.7– 67.5)	31.8 (18.6–47.6)	18.2 (8.2–32.7)	4.6 (0.6–15.5)
Specificity (CI)	75.9 (71.7–79.7)	84.2 (80.5–87.4)	91.5 (88.5–93.9)	97.2 (95.2–98.5)	99.6 (98.4–100.0)
PPV (CI)	20.9 (16.8–25.6)	24.2 (18.3–31.3)	26.4 (17.5–37.8)	38.1 (21.3–58.4)	50.0 (12.6–87.4)
NPV (CI)	95.8 (93.8–97.2)	94.8 (93.1–96.2)	93.3 (91.9–94.5)	92.5 (91.5–93.4)	91.5 (91.0–92.0)

PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

the same serogroup as Hardjo. Cross-reactivity between serovars of the same serogroup has been demonstrated before (15, 22). However, the prevalence of Hardjo in Ugandan cattle seems lower than reported elsewhere in East Africa; probably indicating the role of other serovars in *Leptospira* infection in Ugandan cattle. The low seroprevalence of rodent associated serogroups/serovars, such as Icterohemorrhagiae and Grippotyphosa in this study is in agreement with previous studies conducted on cattle (9) and in humans (10) in Uganda. This raises questions on the role of rodents in maintenance and transmission of *Leptospira* species in Uganda. It remains unclear whether rodents carry different serovars from those used on the test panels in Uganda or if the level of environmental contamination by rodents is generally low to permit an indirect transmission. In this regard, we already are testing kidneys collected from rodents trapped from several ecological sites, to establish the renal carriage of pathogenic *Leptospira* and genomic identity of *Leptospira* species carried by rodents in Uganda. In the present study, we found a high prevalence of anti-*Leptospira* antibodies against serovar Tarassovi, which has also been reported in cattle (18, 23) and pigs (24, 25) in East Africa and elsewhere. Though there are not many published reports of leptospirosis in other species of animals in Uganda, we still think that the nature of animal husbandry practices may facilitate interspecies interactions and spread of *Leptospira* infection. In rural Uganda (where the majority of slaughter cattle were sourced), small scale farmers may leave their cattle to free-range with several other animals including goats, sheep, dogs, and swine, hence increasing interspecies interactions. Additionally, the free-ranging herds often drink from common open water sources that may play an important role in indirect transmission of leptospirosis. Australis, a serovar found prevalent in cattle in the current study was diagnosed in 6.1% ($n = 23$) of the 379 pigs tested in a survey conducted on rural piggery farms in South Western Uganda (unpublished data). Such interspecies interactions not only make the transmission cycle of leptospirosis more complex but may also magnify the disease burden in livestock, especially if infection occurs with non-adapted serovars. While leptospirosis may be widespread among cattle herds in Uganda, the infecting *Leptospira* serovars seem to vary regionally. For example, in two districts representing the Northern and Eastern regions of Uganda, Pomona seroprevalence was highest with 9.5% (6.4–13.7%), followed by Kenya 5.1% (2.9–8.6), Nigeria 4.0% (2.1–7.2), Wolfii 3.3% (1.6–6.3), Butembo 1.9% (0.7–4.4), and lastly Hardjo 1.5% (0.5–3.9) (9); yet Tarassovi, Sejroe (same serogroup as Hardjo) and Australis were the most prevalent in the current study. This may imply that seasonal and geographical differences have influence on the epidemiology of leptospirosis, and thus the need to institute *Leptospira* surveillance programs at a regional level.

At a herd level, direct non-invasive molecular detection of *Leptospira species* in urine could be the best approach to assess *Leptospira* infection status and associated risk of transmission to other species (including humans). However, serological testing, including the MAT is currently still more widely available and one of the most common diagnostic tools used to collect surveillance data on leptospirosis in both humans and animals. The MAT indirectly measures *Leptospira* exposure and/or infection through detection of specific antibodies against *Leptospira* serogroups/serovars. In the current study, we attempted to compare MAT output with output of a qPCR assay and only found a fair agreement of 0.21 (Cohen's kappa statistic $P < 0.001$) between the two tests. This may be expected since: 1. in carrier animals *Leptospira* infection may not induce long lasting natural immunity, yet leptospires persist in the kidneys of these animals for several months or years. 2. In an endemic setting, animals may get recurrent infections through exposures to contaminated environments (become seropositive), but only intermittently shed leptospires in urine, limiting chances of detection by molecular techniques. In addition, detectable levels of *Leptospira* antibodies may persist in cattle that have recently been treated with certain antibiotics that reduce urinary shedding. MAT as used in this study was found to be highly specific, and had a high negative predictive value when compared to the qPCR assay, implying a rather strong association between sero-negativity and absence of renal *Leptospira* infection. However, the reliability of MAT predictability for renal *Leptospira* infection should be taken with a lot of caution since predictive values of diagnostic tests depend on prevalence (assuming similar sensitivity and specificity). Therefore, the reportedly high negative predictive value of MAT in this study may change significantly under other

prevalence scenarios. Since there is already evidence of good performance of ELISA (26, 27) and point-of-care diagnostics (28, 29) for human leptospirosis, it may be necessary to validate these and more such serological assays for veterinary use. These would then serve as cheaper screening options for use in animal leptospirosis surveillance programs in low resource settings, including Uganda. Nevertheless, for confirmation of clinical cases of leptospirosis, a qPCR result of urine or MAT on paired sera is recommended.

CONCLUSIONS

Findings of anti-*Leptospira* antibodies among slaughter cattle in this study implies exposure of cattle to leptospires; with older cattle (≥1.5 years) having higher odds of being exposed than younger ones. Cattle in Uganda are commonly exposed to serovars Tarassovi (Sg Tarassovi), Sejroe (Sg Sejroe), and Australis (Sg Australis), with potential to expose humans and other species by shedding *Leptospira* species at the human-livestock-environment interface. Individuals in close contact with cattle, including abattoir workers, those involved in obstetrics, milking, and animal transportation may be at highest risk. This risk and the general leptospirosis burden should be assessed in the human population in Uganda. And if leptospirosis is shown to be a health problem in humans (what the authors strongly hypothesize), vaccination of cattle herds together with treatment of infected animals, and protection of water sources could be some of the control strategies at the animal level (farms). Other indirect measures may include sensitization of workers in risky occupations and use of appropriate personal protective equipment.

The high specificity and negative predictive value of MAT as used in this study when compared to the qPCR assay may imply a rather strong association between seronegativity and absence of renal *Leptospira* infection. However, MAT predictability for renal *Leptospira* infection may be interpreted cautiously since predictive values of diagnostic tests are dependent on prevalence.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The study procedures were approved by both the institutional review board of the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University (SBLS/REC/17/003) and the Uganda National Council for Science and Technology (A565). We also obtained consent of abattoir representatives ahead of the study and also at the time of sampling.

AUTHOR CONTRIBUTIONS

LA and AD conceived the study. LA, DN, and PP participated in the collection of data, while LA and AD conducted the statistical analyses and AD and CK oversaw the study. LA coordinated the drafting of the article and AD was the main reviewer.

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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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The Comparison of Three Statistical Models for Syndromic Surveillance in Cattle Using Milk Production Data

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Two vector-borne infections have emerged and spread throughout the north-western part of Europe in the last decade: Bluetongue virus serotype-8 (BTV-8) and the Schmallenberg virus (SBV). The objective of the current study was to compare three statistical methods when applied in a syndromic surveillance context for the early detection of emerging diseases in cattle in the Netherlands. Since BTV-8 and SBV both have a negative effect on milk production in dairy cattle, routinely collected bulk milk recordings were used to compare the three statistical methods in their potential to detect drops in milk production during a period of seven years in which BTV-8 and SBV emerged. A Cusum algorithm, Bayesian disease mapping model, and spatiotemporal cluster analysis using the space-time scan statistic were performed and their performance in terms of sensitivity and specificity was compared. Spatiotemporal cluster analysis performed best for early detection of SBV in cattle in the Netherlands with a relative sensitivity of 71% compared to clinical surveillance and 100% specificity in a year without major disease outbreaks. Sensitivity to detect BTV-8 was low for all methods. However, many alerts of reduced milk production were generated several weeks before the week in which first clinical suspicions were reported. It cannot be excluded that these alerts represent the actual first signs of BTV-8 infections in cattle in the Netherlands thus leading to an underestimation of the sensitivity of the syndromic surveillance methods relative to the clinical surveillance in place.

Keywords: veterinary syndromic surveillance, aberration detection methods, vector-borne diseases, cattle, milk production data

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INTRODUCTION

Syndromic surveillance aims at identifying unusual increases in health related events in a population based on data aggregated across the monitored population and has been used frequently in the field of public health surveillance. The field of veterinary syndromic surveillance focusses on detection of emerging diseases as well as changes in trends of endemic diseases. It has been rising during the last decade due to increasing availability of relevant data sources and interest of veterinary epidemiologists for syndromic surveillance (1, 2). Often, the first step in the detection process is the construction of temporal time series of the data that are being monitored (cases, rates, counts, etc.), to define a baseline model for the expected number of events. The next step is the

application of statistical methods to distinguish if an observed event rate is significantly different from the expected levels defined by the historical baseline. In these analyses, the modeling approach can be directed at temporal abnormalities, spatial abnormalities or a combination of both (3).

In the temporal context, time series from a region or country as a whole are inspected prospectively for abnormalities, for example in the form of statistical process control charts. Statistical process control charts are quality control methods used to monitor production processes over time to detect changes in process performance (4). Various statistical process charts have been developed since early in the twentieth century. The cumulative sum (Cusum) chart is an example of a method that is known for its ability to detect small shifts in the parameter of interest (5). The principle of the Cusum algorithm in its simplest form is that it accumulates deviations between expected and observed values (e.g., counts, percentages, rates, etc.). Generally, an arbitrary constant k is chosen to explain the variation of the mean of the baseline period (meaning that deviations smaller than k will not be summed). If the process remains "in control" the cumulative sum should fluctuate stochastically around zero. Identification of aberrations occurs when the absolute cumulative sum of differences between expected and observed values exceeds a fixed control limit (h) to be chosen by the user.

Alternatively, when focusing on detecting abnormalities in a spatial context in the data source, statistical methods are aimed at detecting regions in which the distribution of the parameter of interest within a certain timeframe is abnormally high (or low) compared to other regions. This is known as disease mapping, aiming at quantifying the amount of true spatial heterogeneity and its associated patterns to highlight areas of elevated risk (6). Disease mapping methods vary broadly from being non-parametric in nature (e.g., smoothing models) to more complex Bayesian random effects models (7). Bayesian disease mapping models treat the relative risks as random variables and specify a distribution for them, to capture the unexplained variability in the observed data that might be the result of (local) disease outbreaks. In the 1990s, Besag et al. (8) proposed to split the relative risk parameter in separate variance components, i.e., spatially uncorrelated variance heterogeneity and spatially correlated heterogeneity. This led to the introduction of Bayesian disease mapping models based on conditional autoregression (CAR), i.e., the value for any given spatial unit is estimated conditionally on the values of neighboring units. In these models, the estimated correlation structure between neighboring spatial units is used as prior distribution for the (correlated) spatial random effect. This prior distribution is then combined with the likelihood of the observed data to obtain a posterior distribution for the random effect(s), followed by inference to identify areas where the relative risk exceeds a predefined value (9).

The most widely used method in the field of spatiotemporal modeling is the space-time scan statistic as proposed by Kulldorff (10). With this method, a cylindrical window scans across all units in space and time in the data source, noting the number or value of observed and expected observations inside the window. In a prospective analysis only those cylinders that include the end of the study period are considered, hence excluding clusters from the past. A likelihood ratio statistic is then computed for each space-time window, by comparing the likelihood of the observed data (given the value or rate of events within and outside the window) and the likelihood function assuming the rate of events within and outside the window are equal (10, 11). The method adjusts for multiple testing by evaluating only the significance of the window with the maximum likelihood ratio statistic over all cylinders (i.e., the most likely cluster), using a p-value obtained from Monte Carlo simulations (12).

Two vector-borne infections have emerged and spread throughout the north-western part of Europe in the last decade: Bluetongue virus serotype-8 (BTV-8) and the Schmallenberg virus (SBV). BTV-8 emerged in August 2006 and re-emerged in July 2007 and caused important economic losses (13). In the outbreak of 2007, the most prominent clinical signs observed in affected cattle farms were fever, lameness/stiffness, conjunctivitis, nasal discharge, crusts/lesions of nose and/or mouth, redness/lesions of teats and a drop in milk yield (14). SBV emerged in north-western Europe in the late summer of 2011, causing diarrhea and drop in milk production in adult cattle (15) and congenital malformations in new-born ruminants (16). Since BTV-8 and SBV both have a negative effect on milk production in dairy cattle, routinely collected milk production records may have the potential to be used for early-warning syndromic surveillance of such pathogens. In a previous study, we assessed the value of routinely collected milk production data for the early detection of emerging vector-borne diseases in cattle using the space-time scan statistic (17). Although the results seemed promising, a number of improvements were suggested for future use of such data. For example, milk yield was calculated based on monthly "test-day" milk recording data, providing monthly observations on herd level. As an alternative, the use of bulk milk collection data (resulting in approximately three observations per herd per week) might increase timeliness and sensitivity of detection of disease outbreaks. Also, milk yield was analyzed with a single statistical method. The objective of the current study was to compare three statistical methods, the Cusum chart, Bayesian disease mapping and prospective spatiotemporal cluster analysis, to assess their potential to detect drops in milk yield during a period of seven years in which BTV-8 and SBV emerged using routinely collected bulk milk collection data.

MATERIALS AND METHODS

Data

A total of 13,330,908 bulk milk collection recordings (2–3 records per week per herd) from 21,074 dairy farms (∼96% of the Dutch dairy herds) were obtained between July 1, 2005 and December 31, 2011. These data were provided by seven dairy processors. Herd size information on monthly level was obtained from the national identification and registration (I&R) database and was used to estimate the number of lactating cows per herd per day. It was assumed that at any time point, 10% of the cows >2 years of age in a herd were in the dry period and therefore not taken into account in the number of lactating cows per herd.

FIGURE 1 | Two-digit postal districts in the Netherlands ($N = 90$).

For each herd, the average milk yield per animal per day was calculated by dividing the total amount of collected bulk milk on a given day (taking into account the number of milkings since the last collection) by the estimated number of lactating cows. Days with an average milk production >50 kg/cow were considered administrative errors and excluded prior to analysis (1% of the data). Herd location data on postal district level (2-digit postal code) was obtained from Royal GD. The 90 postal districts of the Netherlands are shown in **Figure 1**. The mean number of herds per postal district decreased from 221 in 2007 to 195 in 2011. Bulk milk data from 2005 to 2006 were incomplete for 37% of the 90 postal districts (mainly in the northern and eastern area of the country; districts 65 to 99), due to a merger of the largest dairy processor in the Netherlands in 2007. This part of the Netherlands was not affected yet by BTV-8 in 2006 (18).

Data Analysis

Construction of the Baseline Model

Time series analyses were carried using STATA/SE version 14 software (19). Due to the large amount of milk production records and to guarantee the privacy of herds, aggregation of the milk yield per cow per herd by calculating its mean at postal district-week level was done prior to statistical analyses. Time series of the mean milk yield per cow per postal district per week was constructed using a harmonic linear regression model (regress) (Equation 1). Annual seasonality was taken into account by including two sine/cosine harmonics as predictors.

TABLE 1 | Boundaries of the baseline and prediction periods for the construction of time series and corresponding week-by-week prediction of mean milk yield per cow in the Netherlands, including the mean root mean squared error of the model (RMSE) calculated over the baseline period (and its standard deviation).

Model	Baseline period	Prediction period	Mean RMSE (sd)
BTV-model	July 1, 2005–June, 30, 2006	July 1, 2006–Dec. 31, 2007 (79 weeks)	0.74 (0.06)
SBV-model	Jan. 1, 2009–Dec. 31, 2010	Jan. 1, 2011–Dec. 31, 2011 (52 weeks)	0.58 (0.02)
Control-model	Jan. 1, 2008–Dec. 31, 2009	Jan. 1, 2010–Dec. 31, 2010 (52 weeks)	0.66 (0.04)

The number of harmonics, n, was chosen according to the AIC criterion to best fit the observed milk production data.

$$y_{ij} = \beta_0 + \beta_1 district_i + \beta_2 week_j + \left(\sum_{n=1}^{2} \alpha_n \times \cos\left(\frac{2\pi^* t^* n}{52} \right) + \beta_n \times \sin\left(\frac{2\pi^* t^* n}{52} \right) \right) + \varepsilon_{ij} \quad (1)$$

where:

$E(y_{ij})$ = the expected milk yield per cow in postal district i in week j

β_0 = intercept (constant)

α_n, β_n = phase and amplitude parameters

t = the number of weeks since the first week in the dataset

n = harmonic number

ε_{ij} = random error for district i in week j.

The time series model was run in a time-periodic prospective fashion, i.e., repeating the analysis every week with an updated (moving) baseline period. In each model-run, the expected milk yield per cow in postal district *i* in week *j* was predicted using up to two preceding years (104 weeks) as baseline. By doing so, we simulated the use of the system as if it were applied in real-time on a weekly basis, i.e., on a practically feasible manner. These time-periodic analyses were carried out for three separate time periods (**Table 1**).

In the first model ("BTV-model"), the expected milk yield was estimated for each postal district from week 27 of 2006 (i.e., the first week of July) to week 52 of 2007. It was hypothesized that during parts of this period, milk production was affected as a result of the BTV-8 epidemic, which started in the Netherlands in the summer of 2006 (20) and re-emerged (more intensively) in the summer of 2007 (21). The BTV-model used only data from postal districts for which complete time series could be constructed, thus excluding the northern and eastern areas for which data from 2005 to 2006 were incomplete or missing ($N = 35$). The expected milk yield in the first predicted week (week 27 of 2006) was estimated using the preceding 52 weeks as baseline. After the first predicted week, the baseline was extended with extra week(s) to a maximum of 104 weeks up to the prediction of week 27 of 2007. For the weeks thereafter, the expected milk yield for each week was predicted using a baseline period of 104 weeks. A second model was used to investigate drops in milk production as a result of the SBV epidemic, which started in the Netherlands in the later summer of 2011 (15, 22). In this model ("SBV-model"), the expected milk yield was estimated for each postal district-week from week 1 to 52 of 2011, using data from the two preceding years (104 weeks of 2009–2010) as baseline. A third model was used as control model ("Control-model") in which drops in milk production in 2010 were investigated, a year without major epidemics in the Netherlands. The expected milk yield was estimated for each postal district-week from week 1 to week 52 of 2010, using data from the preceding 2 years (2008–2009) as baseline.

The root mean squared error (RMSE) of each week-run was calculated and averaged per model as a measure of fit of the time series models over the baseline period (**Table 1**). RMSE is a common metric used to measure accuracy for continuous variables. The observed and predicted milk yield estimates in each district-week were obtained after each run of each model. These were subsequently used for further analyses using a Cusum algorithm, a Bayesian disease mapping model and a prospective spatiotemporal scan statistic.

Cusum Analysis

Cusum analyses were carried out per postal district after each run of the moving time series analysis. Aiming to detect drops in milk production, a one-sided negative Cusum function was used to calculate the cumulative sum of differences between observed and predicted milk production (Equation 2), inspired by Lawson and Kleinman (9) and Marceau et al. (23). The algorithm that was applied to the time series of each district *i* can be described as follows:

$$Cusum_t = \min\{0, Cusum_{t-1} + (y_t - \hat{y}_t - k_i)\} \quad (2)$$

where t is the time unit in weeks, y_t is the observed milk yield in district i at week t, $\hat{y}_t$ is the predicted milk yield in district i at week t and k_i is a reference value to explain the variation of the mean of the baseline period for district i. For k, the 5th and 10th percentile of the difference between observed and predicted milk yield estimates (the residual) from the baseline period within district i was used. As these residuals fluctuate around 0, the 5th and 10th percentile of the distribution of the residuals (and thus k) have a negative value. Please note that a negative Cusum function was used, shown by the "min" in Equation 2, as we are interested in detecting drops in milk production, i.e., observed minus predicted milk yield being <0. The algorithm works as follows. At t_0, the Cusum is set at 0. Once $y_t - \hat{y}_t$ (the residual) is negative and more extreme than k, the Cusum value is changed to $(y_t - \hat{y}_t - k_i)$. If the residual is less extreme the next weeks, by being less negative than k or even positive, the Cusum value decreases and will reach 0 once $(y_t - \hat{y}_t - k_i) > 0$. By doing so, a series of Cusum-values (the "Cusum chart") is created for each district. The control limit h_i was then applied to the Cusum chart. The first week in which the Cusum value is more extreme than h was considered the alert week. Only alerts generated in the

prediction week were kept for interpretation, i.e., alerts from the past were considered meaningless. To find the optimal balance between the algorithm's timeliness, sensitivity and specificity, a number of values of h_i were explored as a function of k_i: 1.5k, 2k, 2.5k, and 3k. A threshold value of 2.5*k_i yielded best results and was therefore used as control limit h_i. A graphical example of a Cusum chart can be found in **Supplementary Figure 1**.

Bayesian Disease Mapping Analysis

Spatial disease mapping models typically assess the spatial association of case event or count data [reviewed by (9)], expressing localized variation in disease risk as relative risks. We constructed a disease mapping model that estimates residuals instead of relative risks, by adding a spatial component to the residuals per district of the baseline model (Equation 1). A purely spatial model was developed using OpenBUGS (24) to identify areas in which the probability of a lower than expected milk production exceeds a predefined threshold (Equation 3), inspired by the conditional autoregression (CAR) models described by Richardson et al. (25) and Lawson (26). The model can be described as follows:

$$sres_i = \frac{(y_i - \hat{y}_i) + u_i}{\sigma_i / \sqrt{nherd_i}} = \text{standardized residual for district } i \quad (3)$$

$$y_i = \text{observed milk production in district } i$$

$$\hat{y}_i = \text{expected milk production in postal district } i, \text{ based on the time series regression model (Equation 1)}$$

$$u_i \sim N(b_i, \tau n_i) = \text{spatial random effect for district } i \text{ where } \tau \text{ is the precision weighted by } n_i, \text{ i.e., the number of neighboring districts of district } i \text{ and } b_i \text{ is the mean of the spatial component in the set of districts adjacent to district } i$$

The model was run time-periodically, using only observed (y_i) and predicted ($\hat{y}_i$) milk yield data from week j per run. In the model, a spatially correlated random effect (u_i) was added to the residual milk yield estimates $(y_i - \hat{y}_i)$ derived from the time series regression model (Equation 1), creating a new residual. The model included a correlation structure between postal districts by specifying a weighted conditional autoregressive Gaussian distribution (CAR) as prior for the correlated spatial random effect (u_i).The new model residuals were standardized ($sres_i$) by adjusting them to the number of herds per district i ($nherd_i$). Non-informative prior distributions were given to the precision τ of the spatial random effect [gamma(0.001,0.001)]. Residual thresholds of -1 and -5 were used to calculate the posterior exceedance probabilities for each district i. Alerts were defined as district-week combinations where P($sres_i < -5$) or P($sres_i < -1$) was more than 0.99. The model was compiled with two sets of initial values. A burn-in period of 5,000 iterations was applied; conclusions are based on the next 10,000 iterations. The Brooks-Gelman-Rubin diagnostic was used in randomly chosen model runs to assure that the two chains had converged (27), inspecting the plots for the potential scale reduction factor being very close to 1. Geweke's test was used in randomly chosen model runs to detect signs of failing convergence (28).

Spatiotemporal Cluster Analysis

Model residuals at postal district-week level, comprising the complete baseline period plus the predicted week, were uploaded in SaTScan™ (29) after each model run to identify space-time clusters of low milk production. Prospective analyses were carried out using the normal probability model in SaTScan™. Model residuals were weighted by the square root of the number of herds per district-week to account for uncertainty in residuals from areas with a low cattle herd density. A circular window shape was chosen. We scanned for "low mean" clusters, i.e., lower observed milk production than would have been expected. For each window, a likelihood-ratio test statistic was calculated and the window with the maximum value was considered the cluster that is least likely to have occurred by chance. Its distribution under the null hypothesis and its corresponding p-value was obtained by Monte Carlo hypothesis testing (999 simulated random datasets). Clusters can comprise multiple districts. The maximum spatial cluster size was set at 5 and 10% of the population at risk. These relatively low cluster sizes were chosen as the spatial scan statistic was used for early detection of clusters of reduced milk production. The maximum temporal cluster size was set at 1 week. Clusters of low milk production were defined as windows with a $p \leq 0.01$.

Evaluation of Performance

To compare and evaluate the performance of the different statistical methods, sensitivity and specificity of detecting the BTV-8 and SBV epidemics were calculated during corresponding time periods for each method. A dataset with suspicions of clinical BTV-8 infections in cattle in 2006 and 2007 was obtained from the Netherlands Food and Consumer Product Safety Authority, from which the first confirmed suspicion per district was derived (referred to as "BTV-reference signals"). It must be noted that BTV-8 indicative clinical signs in affected animals were more prominent in sheep flocks than in cattle farms. Also, according to Elbers et al. (30), during the BTV-8 outbreak in 2006 clinical signs had started ∼12–17 days before a suspicion was reported to the veterinary authorities. In the Netherlands, notifying SBV suspicions was not mandatory up to December 2011. An overview of clinical SBV suspicions in adult cattle, notified voluntarily by farmers and veterinarians in August and September 2011 (when the existence of SBV was still unknown), was obtained from Royal GD. The notifications were based on a sudden drop in milk production, diarrhea and/or fever (15). From the list of notifications, the first suspicion per district was derived (referred to as "SBV-reference signals"). With these two sets of reference signals, sensitivity of detection by the statistical methods—relative to the clinical surveillance in place—was calculated as the proportion of first suspicions that was preceded by a statistical alert. Due to the delay between infection and appearance of clinical signs, mildness or absence of clinical signs in cattle, and limited awareness of BTV-8/SBV in the

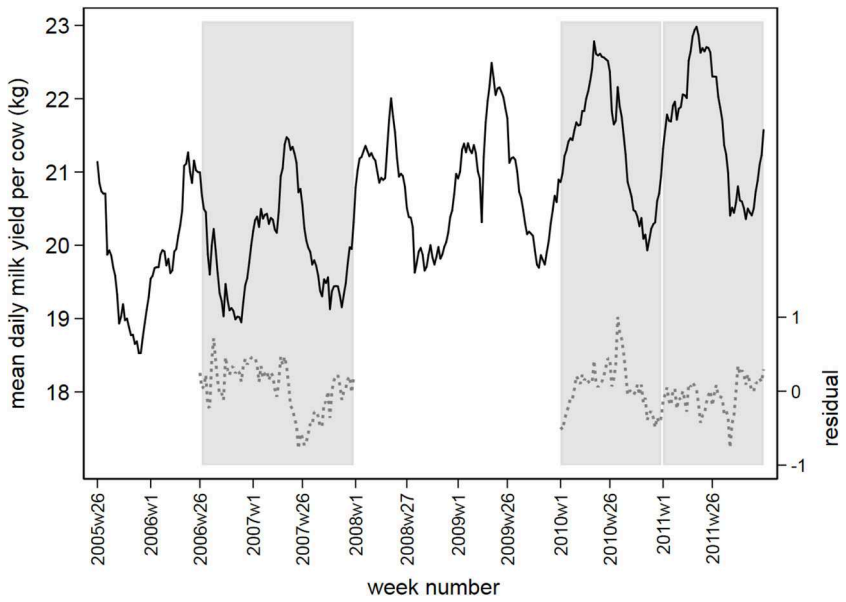

FIGURE 2 | Observed daily milk yield per cow (black solid line) and residual values following time series analysis (gray dashed line), averaged by week between July 1, 2005 and December 31, 2011. Prediction periods in 2006/2007, 2010, and 2011 are marked in vertical gray bars.

initial stage of the epidemic, it was assumed that reporting of clinical suspicions were probably somewhat delayed in terms of representing the first moment of infection in a district. Therefore, alerts were considered true when the alert was generated in the week of the suspicion or in the 4 weeks prior to the suspicion. A sequence of true alerts within one district was counted as one true alert. Alerts in the weeks after the first clinical suspicion in a district were ignored. By doing so, sensitivity was adjusted for timeliness. To measure the effectiveness of each method per year, a predictive alert value was calculated as the proportion of alerts per year that were considered to be true. This is indicative for the amount of false alerts per method. Specificity was calculated per district as the proportion of weeks for which no alert was generated in 2010, i.e., the year without major disease outbreaks. Specificity values of the 90 districts were averaged to obtain an overall estimate of specificity per method.

RESULTS

Figure 2 displays the observed and residual daily milk yield averaged by week over all postal districts, with the three prediction periods marked in gray. The mean residual daily milk yield fluctuates around 0 between -1 and $+1$ in the prediction periods.

Sensitivity and Predictive Alert Value

The emergence of BTV-8 in 2006 could only be detected by the Bayesian disease mapping method with setting "sres < -1". A total of 54 alerts were generated in 2006 by this method, of which two were prior to the 29 BTV-reference signals that year, leading to a sensitivity value of 6.9% (**Table 2**). Irrespective of the parameter settings used, the other methods failed to detect the emergence of BTV-8 in 2006 earlier than the passive clinical surveillance that was in place (**Table 2**). The spatiotemporal cluster analysis method generated some alerts in districts with clinical suspicions in cattle, yet up to 9–10 weeks prior to the BTV-reference signals. Multiple alerts were generated by each method for 2007, except with the Cusum algorithm using the 5th percentile (P5) as k (**Table 2**). A graphical overview of the alerts per model and district-week in 2007 is illustrated in **Figure 3**. Each method generated alerts for districts with clinical suspicions in cattle, up to 23 weeks prior to the BTV- reference signals (**Figure 3**). Sensitivity values for 2007 were therefore low and ranged from 1.2% for the Cusum algorithm with setting "$k =$ P10" to 22.1% for Bayesian disease mapping with setting "sres < -1." Spatiotemporal cluster analyses, with parameter setting "spatial 10%," however yielded a predictive alert value of 39%, indicating that 39% of the 36 alerts generated in 2007 were considered true alerts. For 2011, each method generated multiple alerts, of which the majority are clustered around week 32–37 (**Figure 4**). Sensitivity values for 2011 ranged from 7.1% for the Cusum algorithm with setting "$k =$ P5" to 78.6% for Bayesian disease mapping with setting "sres < -1." Predictive alert values however appeared highest for spatiotemporal cluster analyses, with 64.3% for the model with setting "spatial 5%" and 55.6% for the model with setting "spatial 10%." In general, Bayesian disease mapping, irrespective of parameter setting, produced lowest predictive alert values due to the large number of alerts relative to the number of true alerts.

Specificity

In 2010, zero alerts were generated with the spatiotemporal cluster analysis, irrespective of parameter setting, resulting in a specificity estimate of 100% for this method (**Table 2**). Eight

TABLE 2 | Total number of alerts per year (N) and performance metrics per method for the BTV-model (2006 and 2007), the SBV-model (2011) and the NC-model (2010).

	BTV-model						SBV-model			Control-model	
	2006			2007			2011			2010	
Method*	***N***	**SE (95% C.I.)**	**PV (95% C.I.)**	***N*#**	**SE (95% C.I.)**	**PV (95% C.I.)**	***N*#**	**SE (95% C.I.)**	**PV (95% C.I.)**	***N*#**	**SP (sem)**
Cusum											
k = P5	0	0.0% (0.0–11.9)	–	0	0.0% (0.0–4.2)	–	12	7.1% (0.2–33.9)	10.8% (3.0–25.4)	0	100% (0.00)
k = P10	0	0.0% (0.0–11.9)	–	25	1.2% (0.0–6.3)	4.0% (0.1–20.4)	37	28.6% (8.4–58.1)	8.3% (0.2–38.5)	8	99.8% (0.09)
STCA											
Spatial 5%	2	0.0% (0.0–11.9)	0.0% (0.0–84.2)	13	1.2% (0.0–6.3)	7.7% (0.2–36.0)	14	64.3% (35.1–87.2)	64.3% (35.1–87.2)	0	100% (0.00)
Spatial 10%	3	0.0% (0.0–11.9)	0.0% (0.0–70.8)	36	16.3% (9.2–25.8)	38.9% (23.4–56.5)	18	71.4% (41.9–91.6)	55.6% (30.8–78.5)	0	100% (0.00)
BDM											
sres < -5	5	0.0% (0.0–11.9)	0.0% (0.0–52.2)	46	2.3% (0.3–8.1%)	4.3% (0.5–14.8)	55	28.6% (8.4–58.1)	7.3% (2.0–17.6)	26	99.4 (0.33)
sres < -1	54	6.9% (0.1–22.8)	3.7% (0.5–12.7)	458	22.1% (13.9–32.3)	4.1% (2.5–6.4)	598	78.6% (49.2–95.3)	1.8% (0.9–3.3)	489	89.6 (1.21)

Sensitivity (SE) and predictive alert value (PV) are expressed as a percentage with 95% confidence interval (95% C.I.). Specificity is expressed as mean percentage with standard error of the mean (sem).
**Cusum with k being the 5th (P5) or 10th (P10) percentile value of residuals; Spatiotemporal cluster analysis (STCA) with a maximal spatial window of 5% or 10%; Bayesian disease mapping (BDM) with a standardized residual (sres) threshold of −1 or −5.*
#With regard to STCA, each cluster is counted as one alert in the total number of alerts per year, irrespective of the number of districts included per cluster.

alerts were generated by the Cusum algorithm with setting "k = P10," all in week 5–9, resulting in a mean specificity estimate of 99.8%. Bayesian disease mapping analysis with setting "sres < -5" yielded 26 alerts, corresponding to a mean specificity of 99.4%. Most alerts were generated with Bayesian disease mapping analysis with setting "sres < -1," resulting in a mean specificity of 89.6%.

DISCUSSION

Recent examples in the field of cattle health surveillance illustrate the interest in the use of non-specific herd productivity data for veterinary syndromic surveillance (31, 32). This study presents the comparison of three statistical methods to retrospectively detect the BTV-8 and SBV epidemics in the Netherlands based on routinely collected milk production records. The methods we chose to compare were directed at temporal abnormalities (Cusum), spatial abnormalities (Bayesian disease mapping) or a combination of both (spatiotemporal cluster analysis). Each of these methods has its own advantages and drawbacks (6, 9, 12, 33) but to our knowledge have not been applied simultaneously in a veterinary syndromic surveillance context for the early detection of emerging diseases.

Baseline Model

Baseline models for milk production were constructed for three separate time periods using historical baselines of 52–104 weeks. A drawback of the use of time series is that a meaningful baseline can only be made when sufficient historical data is available. As milk production records from July 1, 2005 onwards were used, only 1 year of baseline was available when predicting milk production in the summer of 2006. Using only 1 year as baseline is suboptimal because abnormal weather conditions and, consequently, poor roughage quality and fluctuations in feed prices can have a large influence on the value of the baseline model. From the three time periods we examined, the BTV-model had the highest mean RMSE value, indicating the least fit of the baseline model. The SBV-model had the best fit of the baseline model. The Control-model had a mean RMSE value that was in between the BTV-model and SBV-model. This is supported by the distribution of residuals in the baseline period over all districts: the 5th percentile values of these distributions have a median value of −0.83 in the BTV-model, −0.68 in the SBV-model and −0.80 in the Control-model. From **Figure 2** it is visible that the observed milk production in some weeks in the first half of 2009 showed outlying drops in milk yield. These were likely the result of extremely dry weather conditions resulting in poor grass yield (34). Each of the three methods picked up this drop in milk yield (results not shown), indicating the methods are robust to detect outlying observations. However, such non-disease related events have certainly influenced the fit of the baseline model for the SBV-model and the Control-model. Also, baseline periods including disease outbreaks should be used with caution. In the model we used to detect the re-emergence of BTV-8 in 2007, data from 2006 covering the initial outbreak period of BTV-8 was included in the baseline. This might have hampered detection of drops in milk production

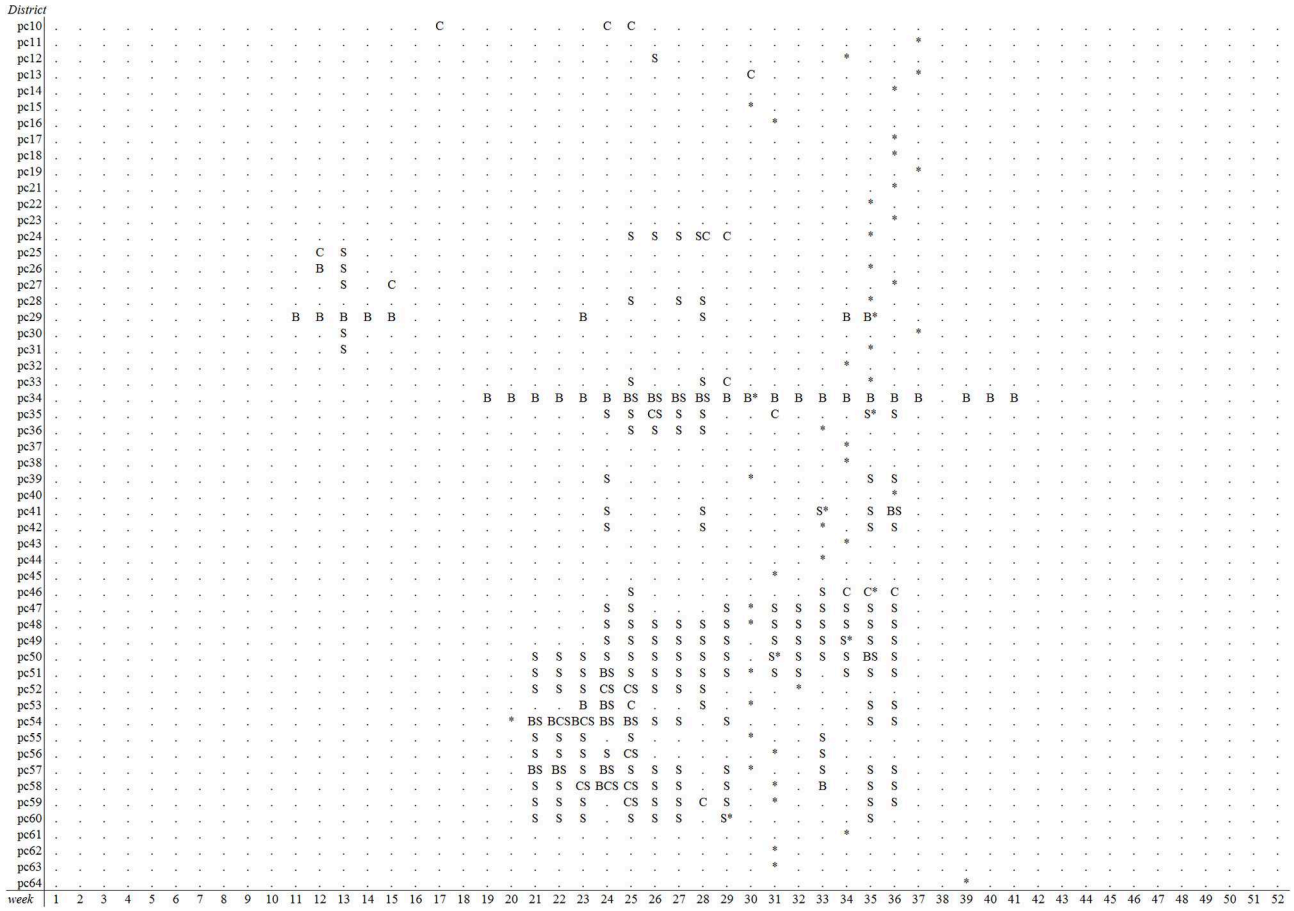

FIGURE 3 | Overview of postal districts and weeks with alerts of reduced milk production in week 1–52 of 2007, based on prospective weekly Cusum analysis (C) with $k = P10$, spatiotemporal cluster analysis (S) with a maximal spatial window of 10% and Bayesian disease mapping analysis (B) using a residual threshold of −5. First BTV-8 confirmed suspicions in cattle per district are indicated with an asterisk (districts 65 to 99 were omitted).

during the outbreak period in 2007. When implementing a syndromic surveillance system using predictions based on a historical baseline, it might be worthwhile to replace records from outbreak episodes or other abnormal events in a baseline by normalized historic observations. Due to privacy issues, milk yield levels per herd were aggregated by two-digit postal district. A disadvantage of this approach is that the administrative borders of a postal district do not reflect the spatial distribution of epidemiological factors, such as herd size and herd density (35). Variation in herd density between postal districts was accounted for by including the number of herds per postal district in the statistical models. Alternatively, one could aggregate data by a grid size that ensures each grid to represent an equally large proportion of the population at risk, provided that geographical coordinates of the unit of interest are known.

Evaluation of Performance

In this study we used authentic data from years with emerging disease outbreaks, varying in magnitude and duration, and from years without major disease outbreaks. The use of authentic data in combination with the prospective nature of the methods we applied provided the possibility of illustrating which output (alerts) will be generated in reality if the methods were to be applied in real-time. This allows evaluation of the true effectiveness of the detection algorithms. A disadvantage of the use of authentic data for evaluation of outbreak detection methods is the difficulty of defining which alert is the result of an outbreak and which is not. In addition, in order to calculate performance metrics, such as sensitivity, timeliness and specificity, a gold standard is essential, such as the location and time of introduction of the pathogen responsible for the disease outbreak. In this study we used clinical suspicions in cattle as reference signals to define the start of the BTV-8 and SBV outbreaks per district. It was a challenge to classify the generated alerts as "true" or "false," as the actual time between introduction of these viruses and detection of introduction was unknown. Therefore, the value of alerts generated prior to the first notifications of suspected cases remained unclear. Also, voluntary notifications of clinical suspicions might not be independent as the start of a disease outbreak is often communicated by the media, potentially influencing the number of notifications being made thereafter. In addition, disease

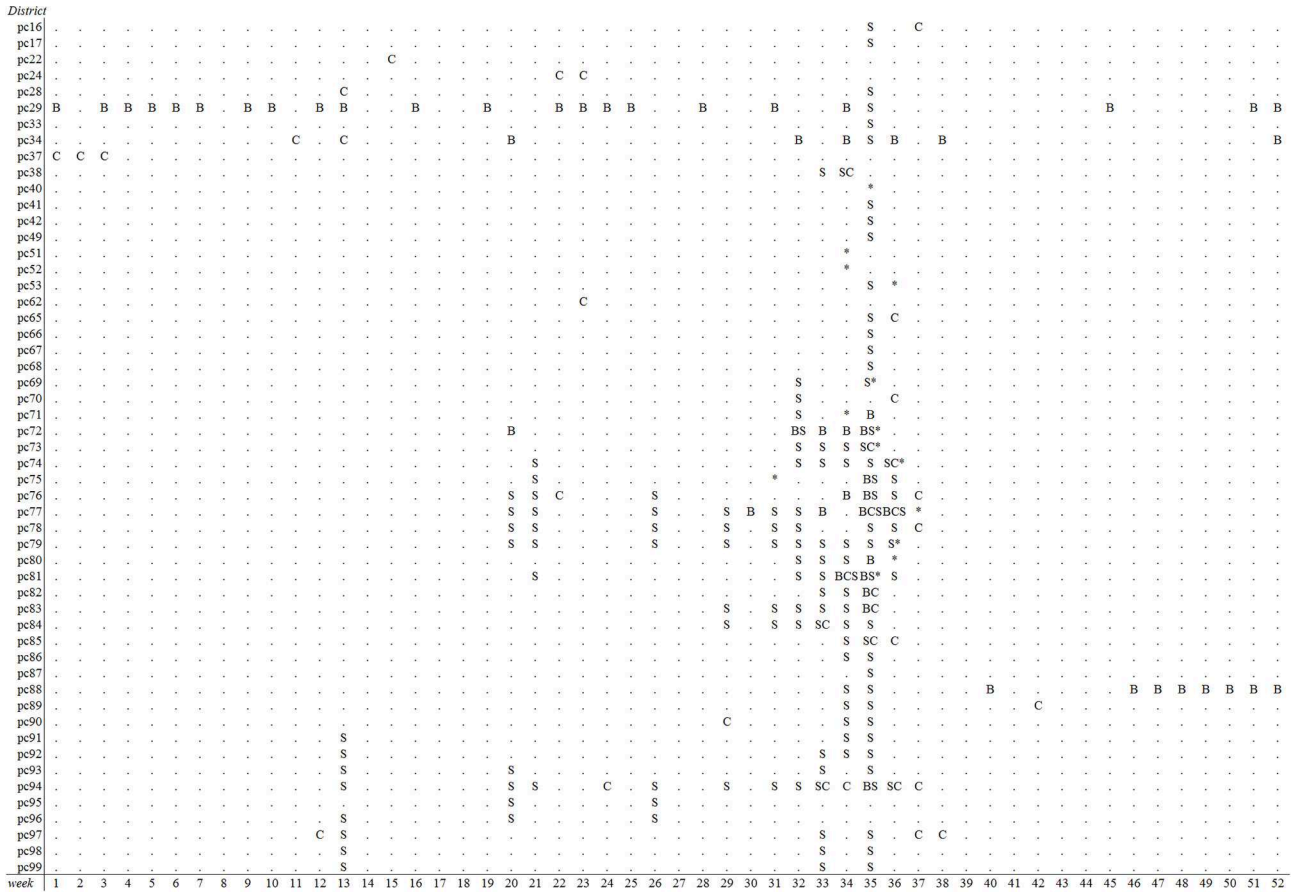

FIGURE 4 | Overview of postal districts and weeks with alerts of reduced milk production in week 1–52 of 2011 based on prospective weekly Cusum analysis (C) with k = P10, spatiotemporal cluster analysis (S) with a maximal spatial window of 10% and Bayesian disease mapping analysis (B) using a residual threshold of −5. The week and location of first SBV suspicions is indicated with asterisks.

awareness in years with major disease outbreaks will increase awareness in subsequent years. One way to overcome these issues is to evaluate algorithm performance using wholly simulated data or by simulating outbreaks of varying magnitude on datasets with a baseline based on authentic data (36).

Sensitivity to Detect BTV-8 and SBV

In August 2006, BTV-8 appeared unexpectedly in northern Europe affecting parts of the Netherlands, Belgium, Germany and northern France (37). After successful overwintering in the region, BTV subsequently re-emerged in 2007 in all countries affected, in a far more extensive way than the first "wave" of the epidemic in 2006. BTV-8 is known to affect milk production in cattle, yet milk loss is not a prominent clinical sign in cattle (30). It is likely however that milk loss is initiated during the acute phase of the disease, but remains unnoticed in individual cattle several weeks before becoming clearly significant (38) or even completely unnoticed when only few cows are affected. The Bayesian disease mapping method generated two alerts in the early summer of 2006, 1–2 weeks before the first notifications of BTV-8 suspicions were made (39). The relatively limited impact of BTV-8 on milk production (18) in the Netherlands in 2006 and the aforementioned reduced quality of the baseline model might have contributed to the fact that only few alerts were generated in the summer of 2006. Alerts of decreased milk production, long before first clinical suspicions were made, were observed in a greater extent in 2007 in the districts whose notifications were the first signal of BTV-8's re-emergence. The degree of reduction in milk yield in spatiotemporal clusters—expressed as the difference between observed and predicted milk yield - was also higher in 2007 (on average −1.6 kg per cow) compared to 2006 (on average −1.2 kg per cow) (data not shown). The sequence of alerts in the neighboring southern districts clearly indicated a pattern of decreased milk production from the end of May to early July 2007. We classified a part of those alerts as false alerts as they were generated more than 4 weeks before first clinical suspicions were notified. However, it cannot be excluded that these alerts were the actual first signs of BTV-8's re-emergence. Evidently, increasing the length of the window to classify alerts as true or false, for example from 4 to 6 weeks before the reference signal, slightly improved sensitivity of detection of BTV-8 in 2007 by the methods, but not for 2006 (results not shown).

Spatiotemporal cluster analyses resulted in the most efficient detection of SBV, i.e., a high sensitivity was achieved with few alerts. The degree of reduction in milk yield in spatiotemporal clusters was on average −1.1 kg per cow in 2011 (results not shown). Irrespective of method used, the sensitivity of detection was higher for SBV than for BTV-8. This could have two reasons. Firstly, the trigger for reference signals we used for SBV was the same as the trigger for the outbreak alerts, i.e., for SBV they were both based on drops in milk production whereas the BTV-8 reference signals were based on more specific clinical signs. Secondly, as suggested earlier by Madouasse et al. (40), analysis of milk production data may produce an alert even when the impact of the disease on milk production is limited, provided that the disease spreads fast (as was the case with SBV, but not with BTV-8). More importantly, each method generated multiple alerts in 2011, of which the majority in August and September. This is in agreement with the time at which notifications of clinical suspicions in adult cattle, later confirmed as being SBV, were made by veterinarians from a number of districts. As it is known that SBV spread widely throughout the Netherlands within several months (or even weeks) in 2011/2012 (22) it is evident that clinical signs in cattle during the acute phase of the disease were not observed or not notified in the remaining districts (notifying suspicions was not mandatory up till December 2011). Therefore, the number of generated alerts in the summer of 2011 in the absence of reported clinical suspicions does suggest that, for each method, sensitivity of detecting SBV may be underestimated in this study. Yet, whether these alerts were the actual first signals of the SBV epidemic remains unknown.

Specificity and Parameter Settings

Residual milk yield, not explained by seasonal fluctuations and trend in time, was used in this study as input for the outbreak detection methods. It is likely that a large part of the unexplained variability in milk production is caused by factors, such as climate, feed quality and feed price. In earlier work we suggested that specificity of outbreak detection methods based on milk production data might be improved if underlying regression models were to be extended with variables explaining climatological factors (17). Therefore, in this study, we added weekly mean ambient temperature and amount of rainfall for each district, based on its nearest weather station (41), to the baseline models. These factors did not alter the results considerably; the generated alerts differed only marginally and the mean RMSE per model was equal (results not shown).

Spatiotemporal cluster analysis was the only method that reached 100% specificity. Perhaps it is the evaluation of significance of only the most likely cluster by the space-time scan statistic that leads to such high specificity of this method. Nevertheless, specificity in 2010 was also high for the Cusum algorithm and Bayesian disease mapping analysis. The influence of parameter settings (such as the detection threshold) and the trade-off between sensitivity and specificity was clearly visible in 2006, 2007, and 2011. High absolute values of sensitivity were achieved by "loosening" detection thresholds, yet with an increase in total number of (false) alerts per year and decrease in predictive value as a—potentially expensive—consequence. The choice of parameter settings is therefore essential in each outbreak detection method. For the Cusum algorithm, this can be achieved by finding the most optimal value for the reference value k and control limit h. The choice of k could be based on a certain percentile of the frequency distribution or any other acceptable level of the parameter of interest (e.g., residuals or case counts). The choice of h however depends on the desired timeliness and specificity of the system, as it determines which deviations from k will lead to an alert. SaTScan offers a few calibration options in the Normal model we used, like setting the maximum spatial cluster size and the maximum temporal cluster size. The output was not sensitive to changes in the maximum temporal size (varied to 1, 2, and 4 weeks; results not shown). The most evident calibration possibilities in Bayesian disease mapping models, such as the one we used are the choice of the posterior exceedance probability threshold [for example $P(\text{res} < -5)$] and the desired confidence level to identify areas were the residual value (or relative risk) is below the detection limit (for example 95 or 99%). Other aspects which should be considered with care are the choice of prior distributions and which variance components to include in the model (correlated and uncorrelated). In general, the desired performance and purpose of the syndromic surveillance system should be leading the choice of parameter settings. Systems aiming at early detection of emerging diseases at all cost (i.e., false positive alerts are allowed) will aim for high sensitivity, whereas systems in which false positive alerts have large consequences (such as a trade ban), or follow-up costs are high, should aim for high specificity.

Comparison of Methods

From the three methods we applied in this study, the Cusum algorithm was the most straightforward to construct and use. This feature and the fact that process behavior is examined chronologically and displayed in a graphical comprehensive manner are particular advantages of quality control charts, such as the Cusum algorithm (34). We applied the Cusum algorithm to 90 postal districts, monitoring each of these areas individually. A drawback of this approach is that if an outbreak occurs on the border between areas or only in a small part of an area, an important outbreak may be missed because it did not follow to the predefined geographical boundaries (10). Spatiotemporal cluster analysis using the space-time scan statistic does not take into account geographical boundaries, but imposes assumptions about the shape of disease patterns by finding clusters in the form of a circle or cylinder (36). The fact that the spatiotemporal cluster analysis of milk production performed best in our comparison could be due to the simultaneous assessment of spatial and temporal variation in milk yield, as opposed to the temporal Cusum algorithm and the spatial Bayesian disease mapping model. The main characteristic of Bayesian disease mapping models based on conditional autoregression (CAR) is to provide some shrinkage and spatial smoothing of raw relative risk estimates, resulting in a low sensitivity for detecting areas that only have a small excess risk (25). Strong local smoothing across neighboring areas, in particular smoothing of abrupt changes in relative risks is undesirable in a disease outbreak detection context (12). Bayesian disease mapping is however

considered an appropriate tool for small area disease mapping (6). Compared to clinical surveillance, the Bayesian disease mapping model performed poorest out of the three methods we assessed, suggesting a need for a revision of the parameter settings used or an extension of the model to space and time.

From the methods we compared, the spatiotemporal approach provided in SaTScan's scan statistic has the greatest potential to be used for syndromic surveillance on milk production records. However, as suggested earlier (17), the added value of any syndromic surveillance system depends on several factors, such as the availability of demographic coverage of suitable data and the costs associated with the follow-up of alerts. Also, differences in the impact (on e.g., milk yield) and spread of an emerging disease might lead to different performance of the methods we proposed in this study. It is expected that the performance of the methods that were assessed in this study will be different when used for detection of diseases that are less clustered in space like vector-borne diseases generally are. For example, if movement of infectious animals results in simultaneous disease outbreaks in multiple non-adjacent areas, temporal methods are probably more suitable for early detection than spatial or spatiotemporal methods (as the indicator will be affected both inside and outside the cluster being tested). It would therefore be interesting to assess the performance of the methods under alternative disease pattern scenarios, for example that of directly transmitted diseases.

CONCLUSION

When applied on routinely collected milk production data, spatiotemporal cluster analysis using the space-time scan statistic performed better than a temporal Cusum algorithm and a spatial Bayesian disease mapping model for early-detection of BTV-8 and SBV in cattle in the Netherlands. Compared to clinical surveillance, sensitivity and predictive alert values were high to detect SBV and low to detect BTV-8. Particularly in the years in which BTV-8 emerged and re-emerged, alerts of reduced milk production were generated long before first clinical suspicions were reported. It remains unknown whether these alerts represent the actual first signs of BTV-8 infections in cattle in the Netherlands.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available as the data has been provided by a commercial dairy processor, who wishes not to make their data publically available. Requests to access these datasets should be directed to (Dr. Veldhuis, a.veldhuis@gdanimalhealth.com).

AUTHOR CONTRIBUTIONS

AV, WS, HB-M, and GS contributed to the conception and design of the study. AV, WS, and HB-M developed the statistical models. AV performed the statistical analyses and wrote the first draft of the manuscript. All authors contributed to interpretation of the results, contributed to manuscript revision, read and approved the final version.

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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.2020.00067/full#supplementary-material

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The remaining author declares 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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BRIEF RESEARCH REPORT
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Analytic Sensitivity of an ELISA Test on Pooled Sera Samples for Detection of Bovine Brucellosis in Eradication Stages in Uruguay

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Bovine brucellosis has been under eradication in Uruguay since 1998. The eradication program includes, among other interventions, individual sera sampling of beef animals at slaughter, and annual serum testing of all dairy cows—accounting for two million samples annually. At a herd prevalence of 0.8%, a pooled-sera sample approach could reduce the economic burden of the surveillance system by reducing the testing and operational costs. Our objective was to evaluate the analytic sensitivity of an indirect ELISA test for *Brucella abortus* in serum pools. Sixty-two *Brucella abortus*-positive bovine sera samples (based upon rose bengal and fluorescent polarization assay) were used as the positive control samples. Rose bengal-negative sera from negative farms were used to dilute the positive samples to the desired concentrations. Positive samples were diluted by using 1 ml of positive sera and 1 ml of negative sera (1/2 dilution) up to 1/1,024. Data were analyzed using generalized linear mixed models with a binary outcome (positive or negative), dilution number as a fixed effect, and a random effect for sample ID. Analytic sensitivity was 99.0% [95% confidence interval (CI): 96.3–99.7], 98.3% (95% CI: 93.1–99.6), 97.3% (95% CI: 87.4–99.4) for dilutions 1/2, 1/4, and 1/8, respectively. The analytical sensitivity, however, decreased when diluted to greater proportions. Given the current herd prevalence in Uruguay, it seems plausible that the use of a pooled sample approach could be adopted by policymakers to reduce the cost of the surveillance program and increase the number of samples being tested.

Keywords: Brucellosis, diagnostics, surveillance, Uruguay, eradication

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INTRODUCTION

Bovine brucellosis is a worldwide-distributed zoonosis that causes abortions in cattle and undulant fever in humans. It has been reported that *Brucella spp.* causes the largest number of zoonotic infections worldwide, with more than 500,000 new cases in humans each year (1). Bovine brucellosis is present in all countries across Latin America with Mexico and Peru having a relatively high prevalence; however, Uruguay has relatively low prevalence estimated to be 0.2% at an animal-level and 0.8% at a herd-level (2–5). Abortions lead to a decreased number of calves per year, and reduced milk production, having economic impacts on beef and dairy producers. According to McDermott et al. (6), in high-income countries, cows that had a bovine brucellosis abortion will have an economic loss of 20–25% during that season. In Uruguay, Piaggio et al. (4) estimated that

the cost of sanitary measures applied to outbreak control every year (i.e., bleeding, vaccination, diagnosis, and indemnity) is on average $US 2,360,000. The general pattern of eradication is based on diagnostic testing and culling of positive cattle. However, during the initial stages of eradication, vaccination is usually implemented (4). As in any disease eradication process, the final steps have a high cost per positive diagnostic and it is necessary to use tests that are accurate at a low price.

Bovine brucellosis has been under an eradication campaign in Uruguay since 1998. Currently, surveillance activities are carried out by government veterinarians with screening samples being collected by private veterinarians with government accreditation. All dairy cattle are tested annually by a screening Rose Bengal (RB) test, and in case of a positive test, a government laboratory performs a second test in series with fluorescent polarization assay. Moreover, an indirect ELISA test in bulk milk is conducted every 4 months. For beef farms, testing of animals occurs when animals go to slaughter, in which the intention is not to detect individual animals but to detect suspected farms. Cattle going to exhibitions, trade fairs, and for exports are tested before movement, while those coming from areas declared as endemic by the government must have RB-negative serology before movement (7). Given the extensive surveillance system, there are about 2,000,000 samples screened for bovine brucellosis annually (8). Bovine brucellosis is in an advanced stage of eradication in Uruguay. The World Organization for Animal Health (OIE) states that a country or region free of bovine brucellosis is one that has <0.2% of herds with the presence of the disease; no vaccinated animals in the last 3 years; and any positive animal has been slaughtered.

It is assumed that the RB test has a sensitivity of 97.7% and a specificity of 99.9% (9). However, there are reports in the literature of much lower sensitivities (**Table 1**). **Table 1** depicts the sensitivity and specificity of the tests that are used in Uruguay. The indirect ELISA test has a high sensitivity for bovine brucellosis (**Table 1**). Using this advantage, we hypothesized that pooled samples could be used to reduce testing costs and increase the number of samples evaluated per unit of time. In the event a pooled test is positive, identifying the animal or animals that gave the positivity to the pool should be determined by testing samples individually. Given the low prevalence of the disease, most pooled tests will be negative, which might significantly lower the costs of the eradication campaign. Therefore, our objective was to evaluate the analytical sensitivity of an indirect ELISA test in pooled sera samples as a tool for epidemiological surveillance of bovine brucellosis in Uruguay.

TABLE 1 | Summary of sensitivity and specificity of the current tests used on the national surveillance of bovine brucellosis in Uruguay.

Test	Se[a]	95% CI[b]	Sp[c]	95% CI[b]	Reference
Rose bengal	97.7	95.9–99.3	99.9	99.8–99.9	(9)
	89.6	79.9–95.8	84.5	68.0–94.8	(10)
Competitive ELISA	98.4	97.0–99.8	99.4	99.1–99.6	(9)
Indirect ELISA	95.7	93.4–98.0	99.8	99.7–99.9	(9)
	96.8	92.3–99.1	96.3	91.7–98.8	(10)
Complement fixation	94.0	87.8–97.5	88.5	81.0–93.8	(10)
Fluorescent polarization assay	96.4	94.4–98.5	99.9	99.7–99.9	(9)

[a] *Confidence interval.*
[b] *Sensitivity percentage.*
[c] *Specificity percentage.*

MATERIALS AND METHODS

This study did not require approval by the Honorary Commission of Animal Research of the Faculty of Veterinary Medicine, University of the Republic, due to having no involvement with animal subjects. All samples were obtained from a national sera bank provided by the Ministry of Agriculture.

Samples and Laboratory Procedures

Sixty-two bovine brucellosis positive sera were obtained from a sera bank. Positive samples were defined as (1) sera that had tested positive to both RB and fluorescent polarization assay; and (2) originated from a positive farm. Positive farms are defined by the Ministry of Agriculture based on an epidemiological investigation that takes into account the number of positive animals, animal movements, vaccination history, prior serology, abortion history, and location (11). Negative sera were obtained from farms that had no history of bovine brucellosis in the previous 5 years and were negative to RB.

Positive samples were diluted by adding 1 ml of positive sera to 1 ml of negative sera (1/2 dilution), with subsequent dilutions performed by adding 1 ml of the previous dilution to 1 ml of negative sera and homogenizing (up to 1/1,024 dilution). All 62 positive samples and the dilutions were analyzed by an indirect ELISA test of the Pourquier Institute (IDEXX Laboratories, Westbrook, ME); therefore, a total of 682 samples were analyzed. Dilutions were diluted in 1/20 using 10 μl of the sample and 190 μl of the kit's diluent; the same dilutions were performed for the negative and positive controls provided by the kit. Sample dilutions were placed on the plate, covered, and incubated for 1 h at room temperature. After that, the washing solution was diluted in 1/20 using distilled water, the plate's contents were emptied, and the plates were washed three times. The conjugate was diluted in a 1/100 dilution using the kit's buffer "number 1," and 100 μl were placed on each well. Plates were incubated for 1 h at room temperature covering the plate on its aluminum foil. After the incubation period, the washing step was repeated, and 100 μl of the revelation solution were placed in each well. Plates were incubated for 20 min and 100 μl of the stop solution were dispensed into each well. Optical densities of 450 nm were used for reading the plates on a conventional ELISA reading instrument. Plates were considered valid if the average of the optical density of both positive controls was >0.6, and the ratio of the average of the optical density of both positive controls over the negative control was >2. The sample to positive (S/P) ratio was

calculated using Equation 1 where OD450 is the optical density reported by the ELISA instrument at an optical density of 450.

$$\frac{S}{P}\% = 100\ X \frac{OD450\ value\ of\ the\ sample - OD450\ value\ of\ the\ negative\ control}{mean\ OD450\ value\ of\ the\ positive\ control - OD450\ value\ of\ the\ negative\ control} \quad (1)$$

A positive result was assigned to the dilution if the S/P% was >120. Two operators (a veterinarian and a veterinary student) implemented all laboratory procedures and were not blinded to the true status of the samples. Despite the previous laboratory experience of the operators, the Ministry of Agriculture provided training on laboratory techniques and biosecurity measures. All procedures were performed at the Faculty of Veterinary Medicine of the University of the Republic, Montevideo, Uruguay. After processing, samples were immediately frozen in cryogenic vials for further investigations with other tests.

Statistical Analysis

A generalized linear mixed model was fitted to estimate the analytical sensitivity of the ELISA test on different dilutions using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). The ELISA test result (positive vs. negative) was modeled as the outcome with a binomial distribution, logit link, and a residual pseudo-likelihood estimation technique. The natural logarithm (ln) of the dilution number was modeled as a continuous fixed effect, and a random effect for sample ID, with a first-order autoregressive correlation structure, was included to account for the repeated measures data structure. The dilution number was modeled on the ln scale to meet the linearity assumption. Analytical sensitivity estimates and confidence intervals were obtained using Equation 2.

$$\text{Analytical sensitivity (dilution X)} = \frac{e^{(ln(dilution\ X)*\beta(dilution) + \beta(intercept))}}{e^{(ln(dilution\ X)*\beta(dilution) + \beta(intercept\ ))} + 1} \quad (2)$$

Where *analytical sensitivity (dilution X)* is the analytical sensitivity for a given dilution, *ln(dilution X)* is the natural logarithm of dilution X, *β(dilution)* is the model coefficient for dilution number, and *β(intercept)* is the model coefficient for the model intercept.

RESULTS

All initial 62 positive samples were included in the study and tested positive by the indirect ELISA test. The average OD450 of the 62 positive samples was 2.11 (standard deviation = 0.69, range = 0.25–3.83) and **Figure 1** depicts the S/P% of each dilution. **Table 2** depicts the number of positives and negatives in each dilution. Model coefficients and analytical sensitivities for the dilutions used in this study are depicted in **Table 3**.

DISCUSSION

Our study suggests that the use of an ELISA test in pooled sera samples may be an appropriate testing method for bovine brucellosis screening in Uruguay. This study sought to determine the analytic sensitivity of a pooled testing method for its implementation in epidemiological surveillance scenarios, which might be a more economical alternative than testing individual samples. Health authorities could use this testing method for low-risk farms or slaughterhouse sampling (in which the goal is to determine groups of positive animals, rather than individuals, in order to identify the farm of origin).

The ELISA kit manufacturer recommends that pools of up to 10 sera can be performed (12). The manufacturer, however, does not provide an estimate of the sensitivity for pooled samples. This study contributes to the scientific community, specifically to the Ministry of Agriculture, in providing an estimate of the sensitivity of pooled samples in the Uruguayan cattle population. Pools of up to 16 samples could be used if the Ministry of Agriculture considers a relative sensitivity close to 95% as acceptable for regions in which, presumably, the disease is not present. If the testing situation requires a higher sensitivity, such as an area with a higher bovine brucellosis risk, pools of 4 samples could be implemented. Similarly, by decreasing the S/P% cutoff, higher test sensitivities could be achieved without reducing the pool size. Lowering the S/P% cutoff, however, would decrease the specificity, which will increase the number of false-positive pools, increasing the number of samples tested individually.

The use of pools for testing diseases is well-established in both human and animal medicine (13). In the case of bovine brucellosis, pooled samples are currently used to monitor milk samples from dairy herds in Uruguay. The use of highly sensitive tests, such as the indirect ELISA test, has replaced the previously used milk ring test and could also replace the RB test if the cost/benefit ratio is favorable (7). There is, however, a need for an economic analysis taking into account the testing costs (kit and labor), pool size, sensitivity, specificity, and prevalence. This type of analysis has been performed in the past and should be conducted before adopting this testing scheme at a national level (14).

The lack of a gold standard method for the positive reference sera and the limited number of samples were among the limitations of this study. The former was addressed, in order to limit information bias, by using two tests to confirm that the sera were bovine brucellosis true-positive samples and by obtaining positive samples from farms that were considered infected. It is expected that the initial samples were true positive given the high specificity of the RB test and the fluorescent polarization assay used. Another limitation of this study was the lack of prior knowledge regarding the animals that were used to obtain the sera samples. Knowledge of the age categories, breed, and region within the country would have been useful to assess possible confounders. Also, lack of farm identification limited

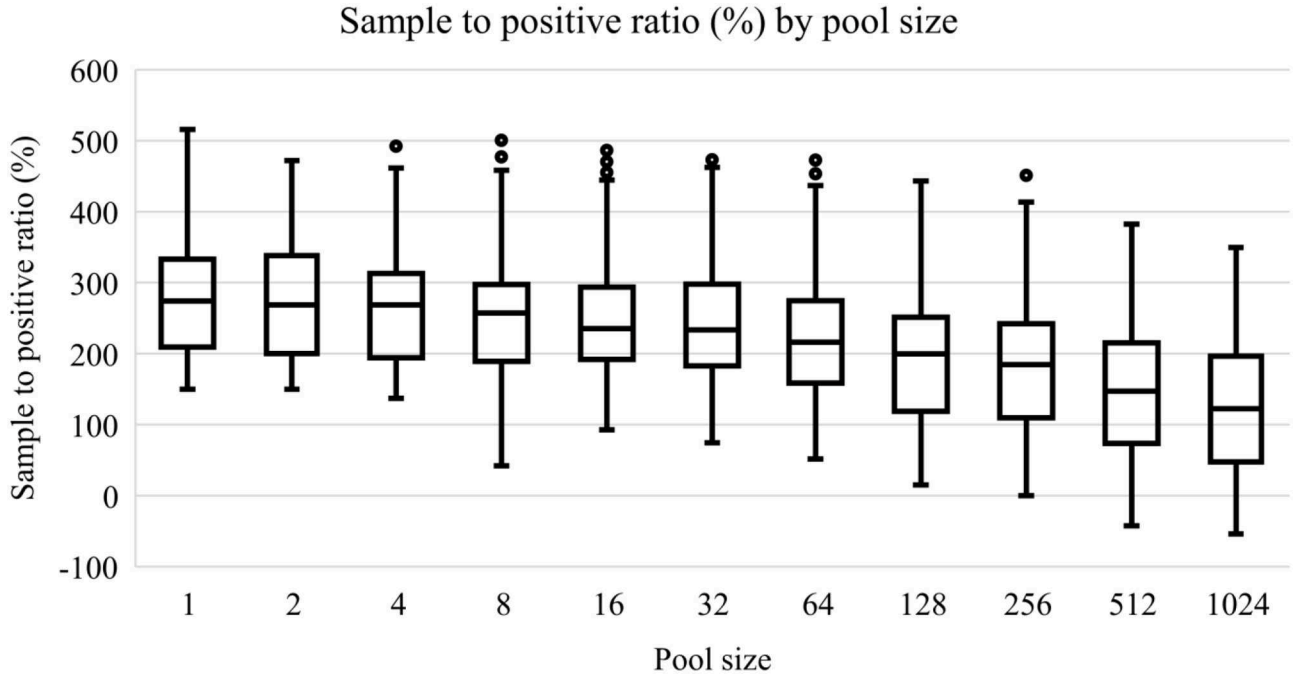

FIGURE 1 | Box-plots of sample to positive ratios (%) of an ELISA test to detect bovine brucellosis antibodies in pooled sera samples. A positive pool was determined if the sample to positive ratio was greater than 120.

TABLE 2 | Descriptive results of dilutions of positive sera samples to bovine brucellosis analyzed with an indirect ELISA test.

Dilution	Negative	Positive	Total
1	0	62	62
2	0	62	62
4	0	62	62
8	2	60	62
16	2	60	62
32	5	57	62
64	7	55	62
128	15	47	62
256	18	44	62
512	24	38	62
1024	31	31	62

TABLE 3 | Model outputs and estimated analytic sensitivities for an indirect ELISA test to detect bovine brucellosis antibodies in pooled samples (dilution).

Effect	Coefficient	SE	95% CI[a]
Ln (dilution)[b]	−0.75	0.10	−0.95 to −0.55
Intercept	5.13	0.61	3.91–6.34
Dilution	**Sensitivity (%)**		**95% CI[a]**
1	99.4		98.0–99.8
2	99.0		96.3–99.7
4	98.3		93.1–99.6
8	97.3		87.4–99.4
16	95.5		78.3–99.2
32	92.6		65.2–98.8
64	88.2		49.4–98.3
128	81.6		33.6–97.5
256	72.6		20.8–96.4
512	61.1		12.0–94.8
1,024	48.4		6.6–92.5

[a] *CI, Confidence interval.*
[b] *Dilution number was transformed to the natural logarithmic scale to meet the linearity assumption.*

the possibility of accounting for the clustering of animals within farms. No negative samples were tested in this study, which could be a limitation of this study if the test specificity is reduced when pooling samples. A reduction in test specify would increase the number of false-positive pools, and therefore, increase the testing cost. To our knowledge, however, there are no studies indicating a reduction in test specificity when performing ELISA tests in pools for bovine brucellosis. Despite the fact that this test has already been validated for pooled samples by the manufacturer, future research should study the interoperator and intraoperator repeatability of this test and the potential dilution effect on the specificity.

In the current situation of a 0.8% herd prevalence of bovine brucellosis in Uruguay, a pooled test may be useful, because the probability that a pool is positive in bovine brucellosis-free farms under surveillance is low. With a surveillance system that analyzes 2,000,000 samples per year, we recommend the use of the pooled test with retesting of individual samples within positive pools. Given the relevance of bovine brucellosis in Uruguay, further research is necessary to ensure there is no dilution effect on the specificity while accounting for the clustering of animals within farms.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study did not require approval by the Honorary Commission of Animal Research of the Faculty of Veterinary Medicine, University of the Republic, due to having no involvement with animal subjects. All samples were obtained from a national sera bank provided by the Ministry of Livestock, Agriculture, and Fisheries.

AUTHOR CONTRIBUTIONS

JB wrote the manuscript, helped design the study, and performed the laboratory work and data analyses. AS provided training on laboratory techniques and assisted in study design. JP and AG assisted with the statistical analyses and designed the study. Finally, all co-authors reviewed the manuscript.

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The Use of Sheep Movement Data to Inform Design and Interpretation of Slaughterhouse-Based Surveillance Activities

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The design of surveillance strategies is often a compromise between science, feasibility, and available resources, especially when sampling is based at fixed locations, such as slaughter-houses. Advances in animal identification, movement recording and traceability should provide data that can facilitate the development, design and interpretation of surveillance activities. Here, for the first time since the introduction of electronic identification of sheep, the utility of a statutory sheep movement database to inform the design and interpretation of slaughter-house based surveillance activities has been investigated. Scottish sheep movement records for 2015–2018 were analyzed in combination with several other data sources. Patterns of off-farm movements of Scottish sheep to slaughter were described and the spatial distribution of several distinct slaughter populations, throughputs and catchment areas for Scottish slaughterhouses were determined. These were used to evaluate the coverage of a convenience-sample slaughter-house based survey for antimicrobial resistance (AMR). In addition, non-slaughter sheep movements within and between Scottish regions were described and inter-and intra-regional movement matrices were produced. There is potential at a number of levels for bias in spatially-associated factors for ovine surveillance activities based at Scottish slaughterhouses. The first is intrinsic because the slaughtered in Scotland population differs from the overall Scottish sheep slaughter population. Other levels will be survey-dependent and occur when the catchment area differs from the slaughtered in Scotland population and when the sampled sheep differ from the catchment area. These are both observed in the AMR survey. Furthermore, the Scottish non-slaughter sheep population is dynamic. Inter-regional movements vary seasonally, driven by the sheep calendar year, structure of the Scottish sheep industry and management practices. These sheep movement data provide a valuable resource for surveillance purposes, despite a number of challenges and limitations that were encountered. They can be used to identify and characterize the spatial origin of relevant populations and so inform the interpretation of existing slaughterhouse-based

surveillance activities. They can be used to improve future design by exploring the feasibility and cost:benefit of alternative sampling strategies. Further development could also contribute to other surveillance activities, such as situational awareness and resource allocation, for the benefit of stakeholders.

Keywords: surveillance, sheep movements, ovine, slaughterhouse, sampling design

INTRODUCTION

Slaughterhouses provide a relatively easily accessible captive livestock population from which information on visible conditions can be obtained, or samples can be taken (1). Whether it is for ongoing, or targeted, surveillance or for other purposes associated with livestock health and welfare and disease control, the interpretation of any data that arises needs to be placed in the context of the population from which it has been, or is to be, obtained and that to which the resultant estimates may be extrapolated. The robustness of any resultant estimates are dependent on their reliability, validity, any bias (2), the epidemiology of the disease, condition, or case of concern (3), in addition to the questions asked as defined by the aims and objectives of the investigation.

The livestock populations that can be accessed at slaughterhouses are a natural subset of the general population. They are however a potentially biased subset due to their relative health and, in many cases, age at slaughter (4, 5). In addition, there can be spatial and temporal variations in the origins of the populations from which individual slaughterhouses obtain their throughput. When related to the epidemiology of the disease, condition, or case of concern, these variations can become relevant to design and interpretation of surveillance activities.

With legislative requirements and technological advances in the identification and recording of livestock, it should now be possible to provide improved quantitative evaluations of livestock population demographics, locations and movements. This information could then be used to inform both disease control strategies and the design of monitoring, data collection and surveillance activities. For the most part, published literature of analyses of animal movement data has focused on network analysis and risk-based surveillance, predominantly in the cattle and pig sectors [e.g., (6–11)]. Sheep sector movements have been included where they contribute to the spread of specified disease(s) under investigation (12, 13) or where a larger scale, multi-sector, perspective is taken (14). There are a few publications of sheep population demographics (15, 16) and network analyses (17, 18). In addition the spatial distribution of the 2002–2005 active surveillance sampling for the transmissible spongiform encephalopathies of sheep in Great Britain was explored using a "shot"-tracing method (19). There is, however, a dearth of literature relating to the use of livestock movement data in relation to design and interpretation of slaughterhouse-based activities.

During the period 2002–2007, unpublished work by the then Veterinary Laboratories Agency (now Animal and Plant Health Agency, APHA) explored the use of the British cattle traceability system data to investigate the relationship of source farms to slaughterhouses. These analyses were extended to the ovine population of England and Wales in order to inform the design of a potential slaughterhouse survey for prion protein genotype frequency in the lamb population. The outputs of the spatial analyses were combined with slaughterhouse throughput data, qualitative information, and expert opinion (20). At that time, identification of sheep was fairly crude at an individual animal level and electronic identification (EID) was not used; however, the use of geographical information systems (GIS) allowed exploratory descriptive analysis of the datasets. These analyses formed an integral part of understanding the target and sample populations and their inter-relationships; thus facilitating the design of potential sampling strategies.

Since then the use of EID for individual sheep identification and improved movement recording has been introduced via European law (21). In Scotland, rules for the identification and registration of sheep and goats have been implemented and are enforced through the Sheep and Goats (Records, Identification and Movement, Scotland Order, 2009, http://www.legislation.gov.uk/ssi/2009/414/made). Scottish sheep keepers are required to identify and register their animals. If the animals are to be moved off the holding, this movement must be recorded and reported to the Scottish Animal Movement Unit (SAMU). These movement data are held in a system known as the Scottish Livestock Electronic Identification and Traceability database (ScotEID).

The primary aim of this study was to assess the utility of the Scottish sheep movement data, currently collated and held in ScotEID, to determine whether they can be used to inform the design and interpretation of Scottish sheep slaughterhouse-based surveillance activities. A further objective was to apply this knowledge to an example, namely the 2017/18 sampling of sheep from one Scottish slaughterhouse for testing for AMR.

MATERIALS AND METHODS

Data Sources and Description

All of the datasets used in this study were provided to the Scottish Government's Center of Expertise on Animal Disease Outbreaks (EPIC) by the agencies or institutions listed below and stored in the EPIC data repository; a centrally curated collection of data resources. This was established in 2011, to support research within EPIC as part of the Scottish Government's Strategic Research Programme.

Scottish Sheep Movements—ScotEID

ScotEID is the livestock traceability system for Scotland managed by the Scottish Agricultural Organization Society (SOAS) on behalf of the Scottish Government. The primary functions of the ScotEID systems are sheep, pig, and cattle movement recording. All sheep movements that contain all or part of their movement within Scotland are recorded in ScotEID.

Each movement record registers the departure premises, read location and destination premises. These are identified by their County Parish Holding number (CPH). A holding is a place where livestock are kept or handled in pursuit of an agricultural activity. In addition to farms, holdings include other types of premises and/or land such as markets, lairages, slaughterhouses, ports and showgrounds (https://www.gov.uk/guidance/register-land-you-use-to-keep-livestock).

For sheep, a movement record for a batch consists of the movement date, the number of animals moved and the number of reads. When a sheep with an electronic identification (EID) tag is scanned at a Critical Control Point (CCP), such as a market or slaughterhouse, there will also be a separate individual animal-level read record of their movement.

Scottish June Agricultural Census

The Scottish June Agricultural Census (Rural and Environmental Science and Analytical Services Division of the Scottish Government (RESAS) is sent annually to all holdings that complete a Single Application Form (i.e., which is for farmers who wish to claim payments under a number of support schemes) and to a random sample of other agricultural holdings. Information is collected across all types of farms about the areas owned or rented, crops, livestock numbers (not including cattle), and labor. Once every 10 years a full census is held with the last one being in 2012. It is a legal requirement to complete the census (Agriculture Act, 1947). For further information please see https://www2.gov.scot/Topics/Statistics/Browse/Agriculture-Fisheries/Publications/JuneAgriculturalCensus. The data provide a snapshot of sheep numbers during the same month (June) each year.

Other Data Sources

Premises types and geo-referenced locations were collated from a number of other Scottish and British (Great Britain, GB) sheep movement and demographic datasets using the unique CPH as the link between records. These data sources included: the Sheep and Goat Inventory (SGI, APHA) and the Animal Movement Licensing System (AMLS, APHA). These locational data included GIS coordinates and were combined to form a consistent repository of data. This was used to provide geo-references for the descriptive spatial analysis and to determine premises types.

Data Analysis

The time period studied was the 4 year period 2015–2018, inclusive. Where illustrative single maps are provided they are for 2017.

Data processing and analysis were carried out using a combination of bespoke applications written in C++ and R (22) with packages, RPostgres (23), ggplot2 (24), pheatmap (25), and RStudio (26). Spatial analysis was performed using QGIS 2.14.8 (27).

Total Scottish Sheep Population

The Total Sheep Population in terms of number of sheep per calendar year was extracted from the Scottish Agricultural Census. The spatial distribution of the total sheep population was mapped as the sheep density calculated at a parish level as sheep per hectare (Shape file agricultural parishes_2016 from Scottish Government SpatialData.gov.scot).

Total Scottish Sheep Movements

Sheep movement data for the study period were extracted from the ScotEID database held in the EPIC data repository, reviewed and summarized. The number of movements and the number of sheep per movement in batch data were counted and compared with the individual animal-level data.

The data were split into four classes of movement types: the total movements, moves within Scotland, moves out of Scotland and moves into Scotland. The distribution of batch sizes in each class was visualized as a boxplot. The differences in the batch sizes between classes were compared using a generalized linear model (GLM) in R. Given that the batch sizes (number of animals in each batch) are counts, they were modeled as Poisson distributed. The batch sizes from different movement classes were considered to be significantly different from the reference category if the p-value of the odds ratio was $<5\%$. Batch sizes for moves to slaughter (section Scottish Sheep Slaughter Populations) were also analyzed as above.

Scottish Sheep Slaughter Populations

Scottish holdings were defined by their CPH number and those that supplied sheep to any GB slaughterhouse were identified from the movement records. A sheep movement record was classified as contributing to the slaughter population when the destination location was any GB slaughterhouse.

Batch movement data were joined to individual animal movement data through common variables and the resulting dataset used to determine the numbers of sheep received by each slaughterhouse. From batch data it can be determined how many animals moved to a slaughterhouse on a particular date. However, when the datasets were combined it was made possible to identify some animals that had their EID tags read at both the market and the slaughterhouse. In the batch data these were recorded as two separate movement records, thus effectively double-counting some of the sheep moving to a slaughterhouse e.g., Record 1: Holding = departure premises; market = read location; slaughterhouse = destination. Record 2: market = departure premises; slaughterhouse = read location; slaughterhouse = destination premises. By removing the second move from the analysis this reduced the chance of over-estimating the number of sheep going to slaughter.

The number of batch movements and the numbers of sheep to each of the slaughterhouses were calculated for each year. The slaughterhouses were ranked and the annual throughput determined.

Data were processed to a consistent form for each of the 4 years (2015–2018) and three major populations were described.

- **Population 1** - The total annual **Scottish Sheep Slaughter Population** (SSSP) contains sheep originating from a Scottish holding (hereafter referred to as Scottish sheep) that have been identified as going directly to slaughter at any slaughterhouse throughout Great Britain (GB). This Population was made up of two subsets, namely:
- **Population 2** - The **Scottish Sheep Slaughtered in Scotland Population** (SISP) contains Scottish sheep that have been identified as going directly to slaughter at a Scottish slaughterhouse.
- **Population 3** - The **Scottish Sheep Slaughtered outside Scotland Population** (SOSP) contains Scottish sheep that have been identified as going directly to slaughter to a slaughterhouse outside of Scotland.

There is one further minor group, Population 4 which consists of sheep supplied in a direct move for slaughter at some Scottish slaughterhouses (i.e., they constitute a proportion of the throughput of those slaughterhouses) that originate from holdings out with Scotland. These sheep are part of the population of sheep slaughtered in Scotland but did not come from a Scottish farm so were excluded from this analysis.

Descriptive Spatial Analysis

Precise co-ordinates were used to plot holding locations, if they were available; if not, the parish centroid was used. Slaughterhouses and their direct supplying premises were plotted on the GB national grid with QGIS 2.14.8. A grid consisting of 115 km^2 hexagons was created using the QGIS Processing plugin module, overlaid on the point data and trimmed to the coastline. The number of holdings in each hexagonal grid cell or the number of sheep that were supplied to slaughter from each grid cell were counted and density maps plotted using these calculations.

Slaughterhouse catchment areas are defined as the spatial distribution of the holdings from which they receive their annual throughput.

An Exemplar—The Antimicrobial Resistance Survey 2017/18

From June 2017 to March 2018 pilot sampling at slaughterhouses was undertaken by Food Standards Scotland (FSS) to test for antimicrobial sensitivity testing (AST) by SRUC Veterinary Services (VS). The aim was to acquire 40 samples per species per month from cattle, sheep, pigs and poultry, with each species being sampled in a single week of each month, in rotation. For sheep, the sampling was done at a single slaughterhouse (Slaughterhouse A). For cost and logistical reasons this was a convenience sample with the sampling occurring on 1 day in the designated week, over a 4.5 h period. The sampler was instructed to try to obtain samples from a spread of farms. Individual fecal samples were obtained. These were sent with a copy of the accompanying Food Chain Information (FCI) form to SRUC VS (Inverness) for AST, by disc diffusion of one *E. coli* isolate per sample against an agreed panel of twelve active substances (28). European Committee on Antimicrobial Sensitivity Testing (EUCAST) methodology and clinical breakpoint interpretative criteria were followed.

The ovine dataset consists of 388 records. Each record represents a single sheep fecal sample collected between June 2017 and March 2018. The samples are identified by a sample reference number, an Animal flock ID based on the eartag number (UKxxxxxx) and the holding CPH.

The spatial distribution of the sampled holdings was compared to the distribution of holdings from the slaughterhouse catchment area. In order to establish the representativeness of the sampled holdings across the slaughterhouse catchment areas, the observed number of sampled holdings was compared to the number of holdings that would be expected if sampling was allocated proportionately based on spatial spread or clustering of holdings. This was achieved by determining which hexagons were adequately represented and which were over represented by the sampled holdings using chi square test. Considering that the data is large (chi square degrees of freedom = 213) and given that the Chi square is asymptotically normally distributed when the sample size is large, the cut-off point for significance was taken to be 2. Therefore, if the calculated Chi square is ≤ 2 the hexagon is deemed to be adequately sampled otherwise the hexagon is deemed to be oversampled.

Non-slaughter Sheep Movements Within Scotland

Sheep movement data extracts were generated and processed. A shapefile (**Supplementary Figure 2**) of the Scottish Agricultural Regions was created based on https://www2.gov.scot/Topics/Statistics/Browse/Agriculture-Fisheries/AgricMapRegions.

Sheep holdings were plotted using GIS coordinates and the CPH numbers identified that fell within a particular region. The number of sheep moving into or out of each Scottish region was calculated for 2017 as a whole and as numbers per quarter. This analysis was restricted to sheep movements that had originated and terminated within Scotland only, with movements to slaughter omitted. Movement matrices, or "heatmaps," were then generated from this data. Sheep numbers were plotted as quartiles of the observed distribution of sheep numbers.

The movement matrix represents sheep movements between 14 regions. The numbers of sheep moving between regions were plotted either as (i) the number of sheep moving from departure region "X" to destination "Y" as a percentage of the number of sheep moved from departure "X" to all 14 destinations or (ii) the number of sheep moving to destination "X" from departure "Y" as a percentage of the number of sheep moved to destination "X" from all 14 departure regions. Sheep numbers were plotted as quartiles of the observed distribution of sheep numbers.

Seasonal Movements

In order to confirm seasonal patterns of sheep movements, the number of sheep moving each month was counted and compared for each year. These counts included the total number of sheep moved throughout the year or just the movements to slaughter. The differences in where a slaughterhouse sources its throughput from at different times of the year was analyzed by counting the number of sheep supplied to the slaughterhouse for each quarter

of the year (January–March, April–June, July–September, and October–December). The number supplied from each grid cell was plotted on a hexagonal grid map as a percentage of the annual slaughterhouse throughput.

RESULTS

Total Scottish Sheep Population

Overall numbers of sheep in the Scottish June Agricultural Census sheep increased by 4% between 2015 and 2017 when numbers peak at just under 7 million (**Table 1**). Sheep are unevenly distributed throughout Scotland with the densities of sheep/hectare within parish varying from 0 in a few urban areas to a maximum of 8 (**Figure 1A**). Higher densities of sheep are concentrated in the southern part of the country (Borders, Clyde Valley, and Dumfries and Galloway) and to a lesser extent on the east coast (Highlands, Grampian, and Tayside) and Shetland Islands (**Figure 1A**).

Total Scottish Sheep Movements

When the number of sheep movements calculated from the batch records and from the individual read animal-level records were compared, there were, on average, 20% more sheep and 4.8% more batches recorded in the batch data for each year studied. There was an average of 15% of sheep found in batch data that did not have associated individual animal-level reads and an average of 4% individual animals were missing due to mis-reads.

Over a third (35%) of holdings in the sheep movement data did not have any spatial data associated with the record. Depending on the year, the total number of premises moving sheep to and/or from Scotland varied between 15,107 and 15,906; the majority of premises involved in these movements were located in Scotland (average across 4 years = 79%, **Table 1**).

A mean of 288,496 batches moved per year, of which half (49.7 %) moved entirely within Scotland. On average just under half (48.1%) originated in Scotland and moved out across the border, with only a small percentage of batches (2.2%) entering Scotland. However, this latter percentage more than doubled in 2017 and 2018, compared to 2015 and 2016 (**Table 1**).

In terms of individual animals, an average of 4,514,194 sheep moved annually, with the largest percentage (48.5%) moving entirely within Scotland. However, the percentage that moved into Scotland was greater than when considered as batches; with an average of 6.7% (**Table 1**).

The range of batch sizes making up the sheep movements during 2015–2018 was wide and skewed (**Supplementary Figure 1**). The mean batch size for all movements both within and out of Scotland was similar. However, that of movements into Scotland was significantly larger ($p < 0.0001$) when compared with movements within and out of Scotland (**Table 2**). The mean odds of total movements into Scotland were 3.7 times larger than those of the other movement classes. Similarly, the slaughter batch sizes moving into Scotland have odds that were 4.2 times larger than the slaughter batches moving within Scotland and 8.3 times larger than those moving out of Scotland.

There was seasonal variation in the number of sheep moved with a large peak in September (**Figure 2A**). There was a smaller

TABLE 1 | Overall annual summary of the Scottish sheep population, numbers of premises involved in any sheep movement in Scotland and the numbers of those sheep movements by batch, sheep and destination and as a % of total for 2015–2018, inclusive.

Annual sheep movements	2015	2016	2017	2018	Average
Scottish sheep population*	6,701,376	6,826,116	6,985,157	6,593,410	6,776,515
Premises (moving sheep)					
Total number	15,107	15,906	15,509	15,846	15,592
Number within Scotland	12,243 (81.0%)	12,587 (79.1%)	12,086 (77.9%)	12,348 (77.9%)	12,316 (79.0%)
Batch movements					
Total number	276,376	299,151	287,956	290,501	288,496
Number within Scotland	149,425 (54.1%)	149,665 (50.0%)	135,475 (47.0%)	139,220 (47.9%)	143,446 (49.7%)
Number out of Scotland	121,115 (43.8%)	143,015 (47.8%)	146,315 (50.8%)	144,389 (49.7%)	138,709 (48.1%)
Number into Scotland	5,836 (2.1%)	6,471 (2.2%)	6,166 (4.6%)	6,892 (5.0%)	6,341 (2.2%)
Sheep numbers moved					
Total number	4,533,375	4,690,748	4,547,346	4,285,308	4,514,194
Number within Scotland	2,319,498 (51.2%)	2,288,310 (48.8%)	2,085,289 (45.9%)	2,054,108 (47.9%)	2,186,801 (48.4%)
Number out of Scotland	1,925,597 (42.4%)	2,081,527 (44.4%)	2,142,856 (47.1%)	1,910,232 (44.6%)	2,015,053 (44.6%)
Number into Scotland	276,714 (6.1%)	312,217 (6.7%)	307,838 (6.8%)	310,115 (7.2%)	301,721 (6.7%)

*The star * was used to indicate that the Scottish sheep population numbers come from the Agricultural census, while the movements come from ScotEID.*

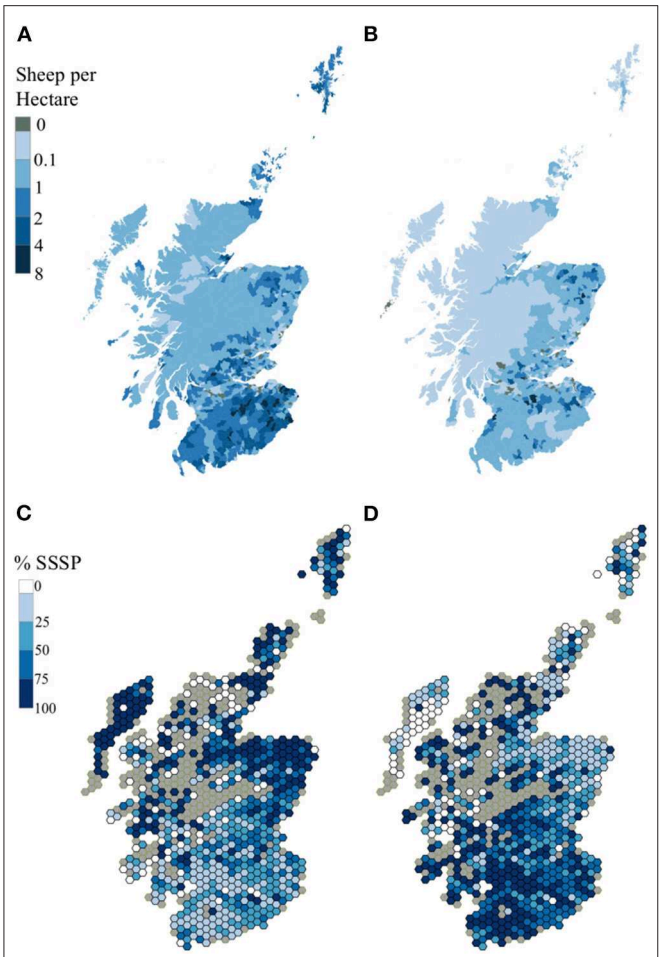

FIGURE 1 | (A–D) Spatial Distribution of Sheep Populations using 2017 as the example year. **(A)** Total Scottish sheep population and **(B)** Scottish Slaughter Sheep Population (SSSP), both as sheep/hectare per parish in categories by quintile. **(C)** Scottish Sheep Slaughtered in Scotland (SISP) and **(D)** Scottish Sheep Slaughtered outside Scotland Population (SOSP) both as Percentage of the SSSP per 115 km^2 hexagon.

peak in the first quarter of the year, usually in March and the lowest numbers of sheep were moved in June. The pattern was relatively consistent year on year, with some changes in magnitude between years. Over the study period the number of movements making up the autumn peak has reduced by 19%, relative to 2015, while the spring peak has remained fairly constant throughout 2015–2017. However, this spring peak decreased by 10% in 2018, when numbers of movements in March were on a par with those in January 2018 (**Figure 2**).

Scottish Sheep Slaughter Populations

Approximately a third of the Scottish sheep population went to slaughter in each year (SSSP, **Table 3**) of the study period, through (on average) 63 British slaughterhouses. Over a third of these slaughterhouses ($n = 22$, 35%) were located in Scotland. Although the number of slaughterhouses slaughtering sheep of Scottish origin does not change markedly there were some differences with regard to which of the slaughterhouses were operable in each year.

TABLE 2 | Summary statistics of batch sizes for Scottish Sheep movements 2015–2018.

Batch movements	Batch sizes				GLM
	Min	Max	Mean	Median	Odds ratio
Total annual movements					
All movements	1	1,410	16	5	0.27*
Movements within Scotland	1	779	15	4.75	0.27*
Movements out of Scotland	1	1,410	15	5	0.26*
Movements into Scotland	1	849	56	10.75	1
Movements to Slaughter					
All movements	1	1,073	16	5.5	0.16*
Movements within Scotland	1	563	24	7.75	0.24*
Movements out of Scotland	1	1,073	13	5	0.12*
Movements into Scotland	1	824	107	63.5	1

*$p < 0.0001$.

An average of 8,543 distinct Scottish premises per year supplied sheep to slaughterhouses in GB. A quarter of these premises (25%) supplied Scottish slaughterhouses only; approximately a quarter (26%) supplied non-Scottish slaughterhouses only, while the reminder—just under a half (49%)—supplied sheep to slaughterhouses in both Scotland and the rest of GB. Approximately half of the Scottish sheep slaughtered in a year (SSSP) are slaughtered within Scotland (SISP, **Table 3**).

The lowest number of sheep moves to slaughter occurred in the period May to June of each year (**Figure 2B**). There was an annual March peak. Later in the year, between August and November, the peak in the number of sheep slaughter moves was flatter (**Figure 2B**) than that of the total sheep moves (**Figure 2A**).

Descriptive Spatial Analysis

The Scottish Slaughter Populations

Although broadly similar, the distribution of the Scottish sheep slaughter population (SSSP, **Figure 1B**) differed from that of the total sheep population (**Figure 1A**). It was generally more diffuse. There were specific foci of higher densities scattered throughout the north east, central and southern areas. The largest shares of the SSSP originated immediately from Grampian (22.9%), Dumfries and Galloway (14.2%) and the Scottish Borders (14.1%, **Figure 3A**). Over the 4 years of the study period 2015–2018, there did not appear to have been major changes in the geographical distribution of the Scottish sheep slaughter population (data not shown). For Region codes and location see **Supplementary Figure 2**.

The immediate origins of the SISP sheep that originated in Scotland and were sent to slaughter at a Scottish slaughterhouse—was not uniformly distributed across Scotland (**Figure 1C**). In each year, a greater percentage of SSSP sheep from the Highlands, Orkney, and Shetland Islands, the Western Isles, Grampian and East Central areas were slaughtered in

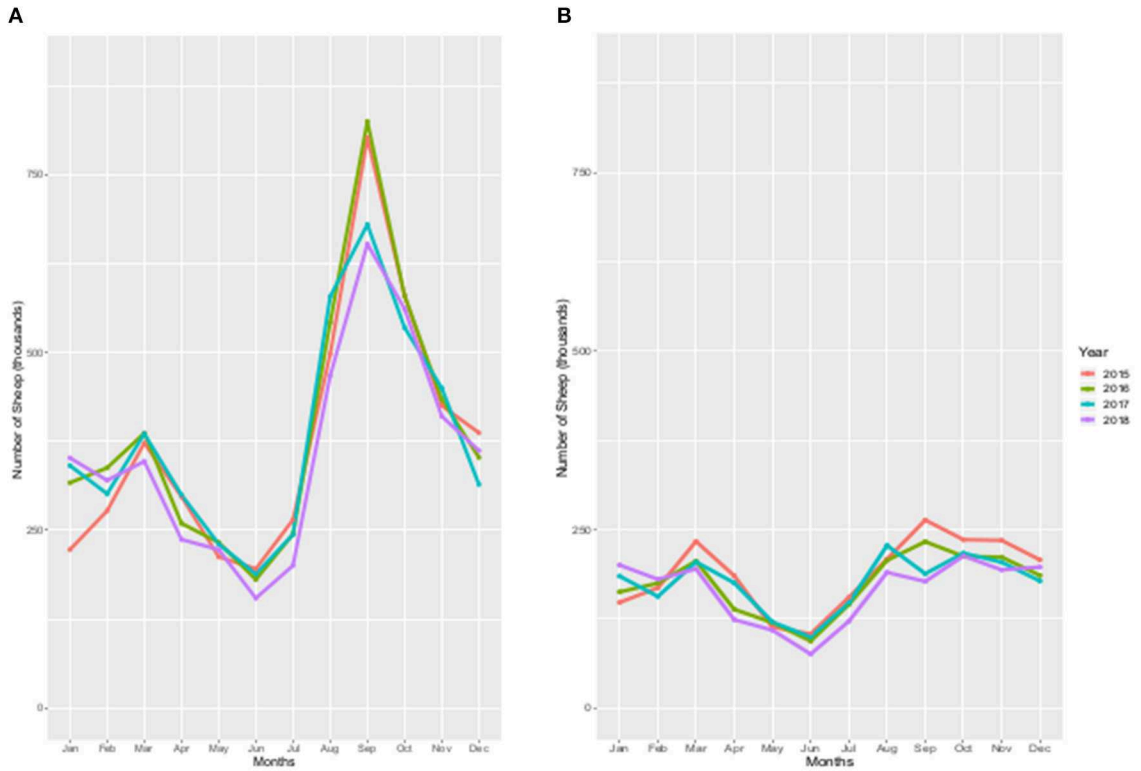

FIGURE 2 | (A,B) The number of sheep moved per calendar month for each year 2015 to 2018. **(A)** Total sheep population, **(B)** Sheep to slaughter (SSSP).

TABLE 3 | Numbers and percentages of sheep in the Scottish Sheep Slaughter populations 2015–2018.

Scottish Sheep Slaughter Populations	2015	2016	2017	2018
Scottish Sheep Slaughter Population (SSSP)				
Sheep numbers	2,254,262	2,090,816	2,111,158	1,973,890
Percentage of total Scottish sheep population	33.6%	30.6%	30.2%	29.9%
Slaughtered in Scotland (SIS)				
Sheep numbers	1,107,763	1,048,724	987,496	963,477
Percentage of SSSP	49.1%	50.2%	46.8%	48.8%
Slaughtered outside Scotland (SOS)				
Sheep numbers	1,146,499	1,042,092	1,123,662	1,010,413
Percentage of SSSP	50.1%	49.8%	53.2%	51.2%

Scotland than elsewhere in GB. For most of the central and southern regions the reverse is true (**Figures 1C,D**, **3B**).

Slaughterhouse Rankings

Ranked by throughput of SSSP, in terms of sheep numbers per annum, the same four slaughterhouses topped the ranking throughout the study period (2017 shown in **Figure 4A**). Each year seven British slaughterhouses each received more than 5% of the SSSP and between them they processed approximately two thirds of this population (**Figure 4B**). Although the top ranked slaughterhouse (A) remained the same, with a mean throughput of 19% of the SSSP per annum, the other six slaughterhouses changed slightly, as did their rank (**Figure 4B**). Four of these top-ranked slaughterhouses were located in Scotland (A, D, E, and F). They received more than a third (37.5%), in terms of sheep numbers, of the SSSP (**Figure 4A**).

If the throughput of the SSSP was estimated in terms of the number of batch movements rather than numbers of sheep, the ranking of British slaughterhouses that received sheep from the SSSP differed The majority of the top ranked premises are outside Scotland (data not shown).

The top four Scottish slaughterhouses (A, D, E, F) together received just over four-fifths (81%) of the population of Scottish slaughter sheep (SSSP) that were slaughtered within Scotland (SISP); 2017 (**Figure 5**).

Slaughterhouse Catchment Areas

Each Scottish slaughterhouse catchment area was different. There was some degree of overlap between some slaughterhouses and there were some slight variations in the distribution of the supply population over the study period. However, the catchment areas generally remain relatively discrete and stable over this 4 year period. The catchment areas of the top four ranked Scottish

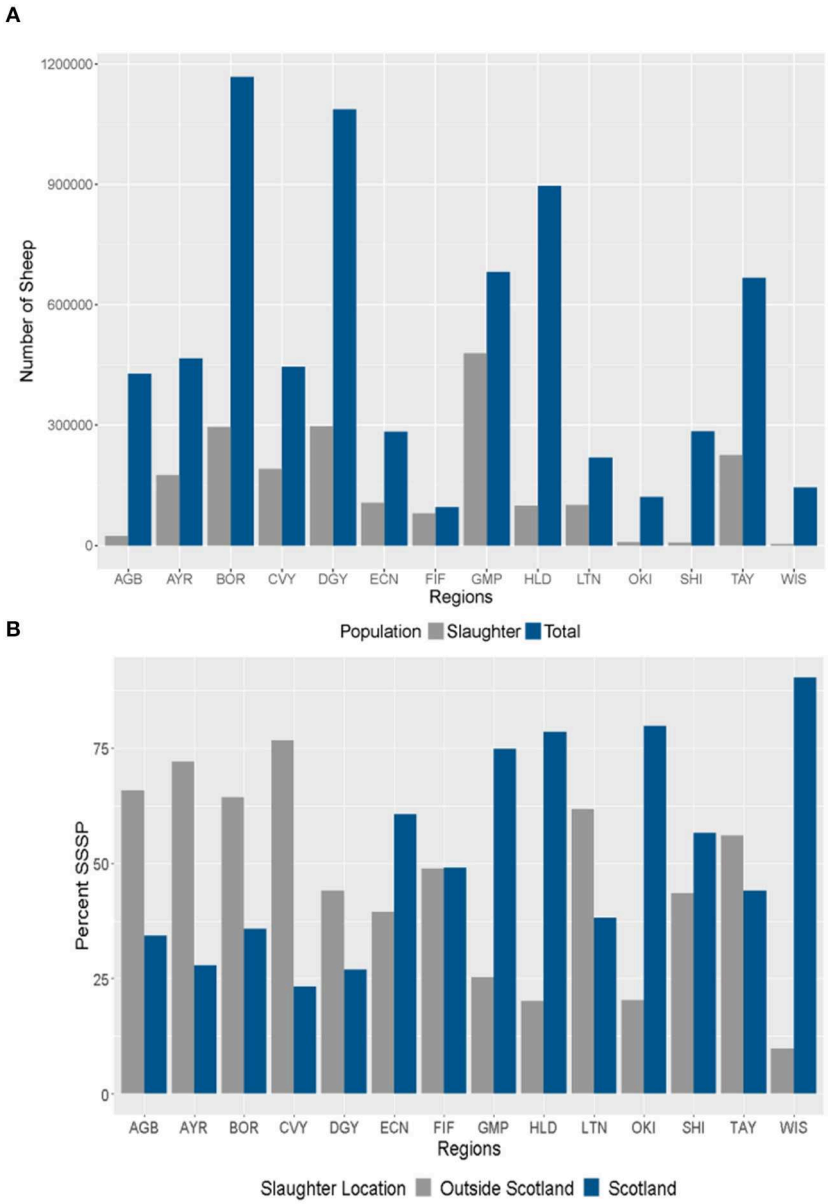

FIGURE 3 | (A,B) Regional Distribution of Scottish sheep populations. **(A)** Numbers of the total sheep population (June Agricultural Census 2017) and Scottish Sheep Slaughter Population (SSSP) 2017 by region; **(B)** Percentage of the SSSP that were slaughtered in Scotland (SISP) and out-with Scotland (SOSP) by region.

slaughterhouses supply 40, 15, 14, and 12% of the SISP respectively. In combination the resultant area gave substantial coverage of the mainland spatial distribution of the SISP (compare **Figure 5** with **Figure 1C**). Some of the smaller slaughterhouses in terms of throughput had very localized catchment areas, especially those situated on the west coast, Western Isles, Orkney and the Shetland Islands, (plots not shown).

Seasonality of catchment areas

There were seasonal differences in the spatial distribution of premises supplying sheep to slaughter. For example: the movement of all of the sheep supplied from the Western Isles to slaughterhouse A occurred in the third quarter (July to September), whilst sheep from North East Highland (Caithness area) and Orkney tended to move to slaughter in the 4th quarter (October to December, **Figure 6**).

An Exemplar—The Antimicrobial Resistance Survey 2017/18

The slaughterhouse used for the AMR Survey (Slaughterhouse A) was the Scottish slaughterhouse with the highest throughput of sheep. Over the 4 year period of the sheep movements analysis, it averaged 19% of the total Scottish sheep slaughtered annually and 38% of those sheep slaughtered in Scotland

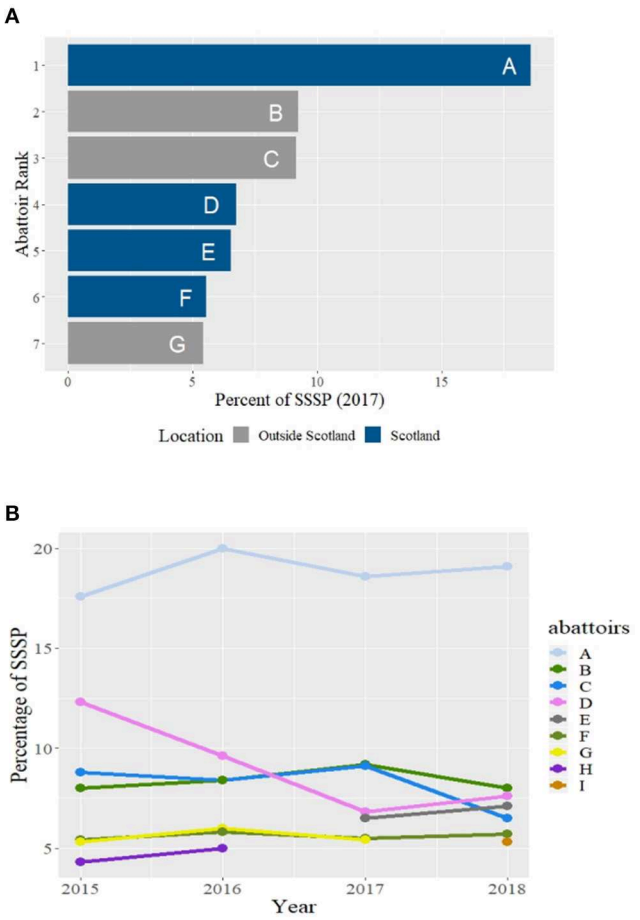

FIGURE 4 | (A,B) Slaughterhouse ranking by throughput of numbers of the Scottish Sheep Slaughter Population (SSSP) per annum **(A)** Slaughterhouses with more than 5% in 2017 and their location. **(B)** Temporal ranking over the study period of Slaughterhouses with more than 5% throughput.

(SISP), in terms of sheep numbers. The catchment area covered north-eastern Scotland and included some of the Borders and eastern Central Belt (**Figure 7A**). This slaughterhouse did not receive, or received few SISP sheep from the West Coast, Highlands, the Islands (Shetland, Orkney and Western Isles), the west Borders (Dumfriesshire, Clyde Valley), and central areas (Perthshire and Fife). A total of 7,771 distinct holdings throughout Scotland contributed to the SISP during the AMR study period, of which 1,023 were in the catchment area of slaughterhouse A.

Based on the CPH of the 388 fecal sample records, these samples came from 216 distinct holdings. Two samples had no CPH identifier. There were multiple samples from some holdings (Min = 1, Q1 = 1, Median = 2, Mean = 2, Q3 = 3, Max = 6). Two holdings, each supplying one sample, were omitted from the analysis because they were located outside Scotland (Northumberland).

When the spatial distribution of the sampled holdings was compared to the distribution of holdings from the slaughterhouse catchment area, 52% of the grid cells in the catchment area were deemed to be adequately sampled, 45% were not sampled and the remaining 3% were oversampled (**Figure 7B**).

The parts of the catchment area that were not sampled included the Shetland Islands and Western Isles, the western part of the northwest part of the northeastern area (around Inverness), the central east coast area (Angus and Fife) and the Central Borders area (**Figure 7B**). The catchment area already had low coverage of the SISP sheep, in all of these areas except the northwest part of the northeastern area (**Figure 7A**). As was seen earlier in the results, the SISP was not, in itself, spatially representative of the Scottish slaughter sheep population, due to the distribution of the "escaped" population, i.e., those that go to be slaughtered out-with Scotland (SOSP, **Figures 1C,D**).

Non-slaughter Sheep Movements Within Scotland

Excluding moves to slaughter, it was estimated that over a million (1,119,542) sheep were moved within Scotland in 2017. In general, the volume of sheep movement off Scottish holdings was much higher than the on-movement. The largest number of sheep were recorded as moving from premises within three regions; Grampian, Dumfries and Galloway and Clyde Valley (34, 20, and 13% of total sheep moved, respectively during the year, **Figure 8A**). Internal movements within each of these regions accounted for 38, 76, and 16%, respectively, of the number of sheep that departed from a holding within that region (**Figure 9A**). For most regions high numbers of sheep moved internally within the region over the year (the squares on the diagonal from top left to bottom right, **Figure 8A**). However, more sheep movements occurred from Lothian to Tayside than within Lothian. A large number of sheep in the Western Isles moved either within the Western Isles or to Highland (**Figure 8A**), with the highest percentage moving to Highland (**Figure 9A**).

Regions that were the destination for the highest number of sheep in 2017 were Highland plus Dumfries and Galloway, each with 17% of the total numbers of sheep moved; Grampian was the third most popular destination with 16% (**Figure 8A**). Many of these were from internal moves within the region: just over a quarter of moves (26%) with a Highland destination, came from within Highland (**Figure 9B**). This was lower than in Dumfries and Galloway and Grampian, where internal moves account for 89 and 81%, respectively.

Seasonality of Non-slaughter Sheep Moves Within Scotland

There was a seasonal difference in the numbers of non-slaughter sheep moved within Scotland (**Figure 8B**; January–March = 15%, April–June = 11%, July–September = 47% and October-December = 27% of the total number of sheep moved throughout 2017). However, there was also a seasonal difference between how the sheep moved between regions (**Supplementary Figures 3A,B**), two examples of which are described below.

Example 1: in the first half of the year (January–March and April–June) Grampian and Dumfries and Galloway sent

and received most sheep overall. In the third and fourth quarter, while they still sent the most sheep, Highland became the region receiving most sheep from other Scottish regions. Example 2: in the first quarter, sheep from the Shetland Islands only moved within the Shetland Islands and a small proportion of sheep moved into the Shetland Islands from elsewhere. However, in the second half of the year, although sheep from the Shetland Islands still moved predominantly within Shetland, they also moved out to other destinations. These were Grampian in both the third and fourth quarters, Tayside in the third and the Orkney Islands in the fourth quarter (**Supplementary Figures 3A,B**).

DISCUSSION

In this study, for what the authors believe is the first time since the introduction of EID, the utility of national sheep movement data has been assessed to determine whether they can inform the design and interpretation of slaughterhouse-based surveillance activities. The conclusion that can be drawn from these analyses is that these data can be utilized in such a way, as illustrated by application of the analytical techniques developed during the assessment to an example: a survey for antimicrobial resistance in Scottish slaughter sheep.

A primary challenge to the development of all operational animal health surveillance systems is the availability of and access to existing data. This is often related to issues of subject confidentiality. In this study, this has been addressed by the Scottish Government's Center of Expertise on Animal Disease Outbreaks (EPIC) data repository. From its inception in 2011, a centrally curated collection of data resources has been carefully established, extended, improved and maintained. Data are sourced from a number of data providers, both public and private and held in a secure data repository. User access requires legally binding data use agreements to be in place and is monitored and audited. The existence of this repository facilitated the study, as multiple data sources were required to address some of the challenges identified.

A number of data limitations were encountered during the course of the study. These were: 1/ that there is no year-round

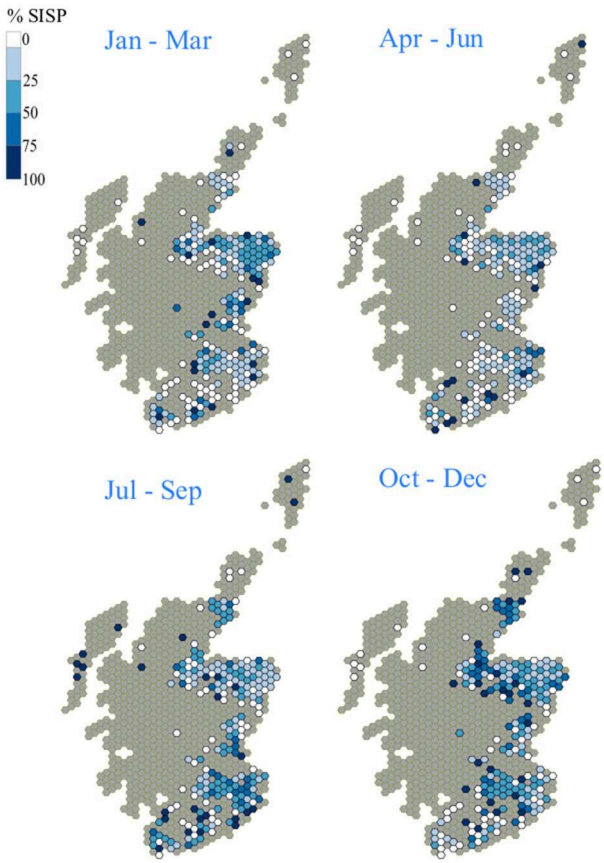

FIGURE 6 | Spatial distribution of the source of the Slaughterhouse A's annual throughput by calendar quarter for 2017 as (% of the total throughput from a hexagon, January–March; April–June; July–September; October—December).

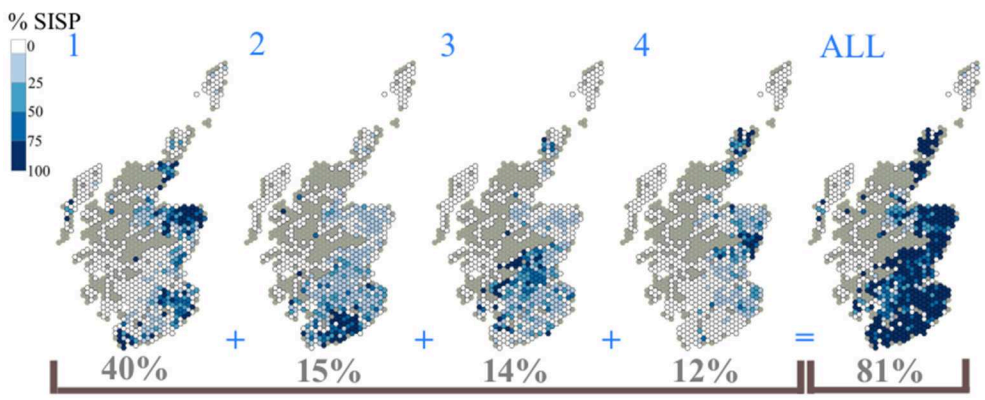

FIGURE 5 | The catchment areas of the top four slaughterhouses in Scotland ranked by throughput in sheep numbers as a percentage of Scottish slaughter sheep, slaughtered in Scotland (SISP) in 2017.

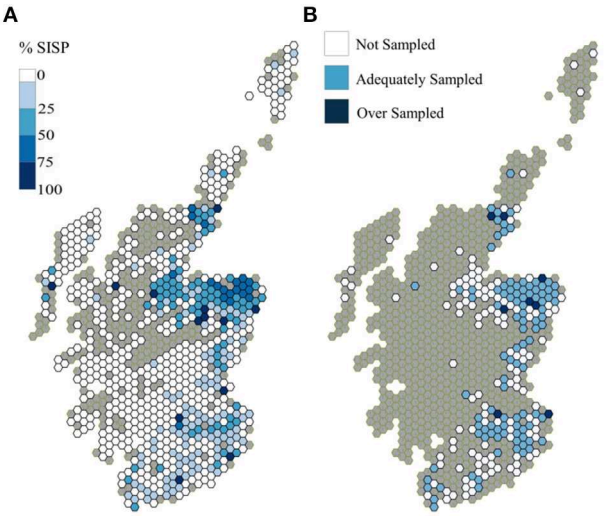

FIGURE 7 | Distribution of Slaughterhouse A's throughput during the AMR survey period, on a holding basis **(A)** as a % of the holdings that supplied sheep to the Slaughtered in Scotland Population (SISP) in that period **(B)** degree of sampling achieved.

centrally available record of individual sheep and their geo-location, until a movement occurs between premises and the EID is captured when read at a Critical Control Point; 2/ knowledge of the source and destination premises type and geo-location is compromised by missing data; 3/ accurate counting and identification of individual sheep within movements is difficult, even with EID; 4/ the age of the sheep moved is unknown, and 5/ only the origin from the move immediately prior to slaughter could be accurately determined. Some of these are inherent aspects of the technologies in use and data collection processes. Some are due to missing data, or gaps in the information available. As discussed below, these challenges limit the utility of these data both for this surveillance purpose, and for others, to varying degrees. Their existence highlights the need to take adequate care; both when using these data for analyses and when interpreting the outputs of any such analyses.

The first challenge is the denominator i.e., where individual sheep are at varying time-points throughout the year. Total sheep numbers by locations are only available twice in a year: in June from the Agricultural Census (RESAS) and in December from the SGI (https://www2.gov.scot/Topics/Statistics/Browse/Agriculture-Fisheries/Publications/SGAI-DAS). These provide snapshots of what is known to be a dynamic population. Numbers and the age-structure of sheep populations on holdings will vary between the two as the Census in June occurs after the peak lambing period (March-May). In December's Inventory, the majority of fat lambs will have gone to slaughter (29), store lambs will have moved on, replacement breeding stock will have joined their flocks and those intended as future breeding stock may have moved to winter quarters "on tack." The spatial distribution will also be different from June. While the Census data are the best denominator for the pig sector (5, 11) and cattle movement data are now used to provide the numbers of cattle for the June Census, for sheep the SGI is more usually used (13, 30). In this study, the gross analysis of within-country non-slaughter movements demonstrated this spatially dynamic nature of the Scottish sheep population and provided context for discussion with regard to the fifth challenge (direct move only). It is reassuring that the outputs from this analysis, and the overall sheep numbers and movements, correspond to known aspects of the sheep calendar year, management activities and events. For example, the low level of within Scotland non-slaughter moves in the second quarter corresponds to the lambing and post-lambing season, while the decline in the spring peak in March in 2018, and the subsequent population fall at the June 2018 Agricultural Census are indicative of the extreme, adverse, weather conditions (known as "the Beast from the East") during March 2018 when sheep couldn't move and a substantial number died (31).

When sheep do move, the second challenge arises. In this study, through access to and use of a number of other data sources, this was minimized. While technically a slaughterhouse should be identifiable by only having inwards animal movements, which was the case in these data, this is not a given (16). Centrally maintained, up-to-date, complete lists of the classifications of premises type for active non-farm (i.e., non-livestock producing premises—slaughterhouses, markets, collection centers etc.) that are linked to both a relevant unique identifier and spatial co-ordinates could resolve this issue. They would need to be accessible and archived appropriately to facilitate later use.

The ability to accurately count and identify individual sheep (Challenge 3) should have improved with the introduction of new technologies, such as EID, and regulations requiring their use. This is the case; however, these methods have raised new issues. These include additional costs to production, mis-reads due to loss or failure over time (32–34), as well as variable attitudes to their adoption (35), while not entirely eliminating the potential for human error. In this study, the presence of dual recording of one "move" at two CCPs raised the potential to over-estimate the number of sheep moved to slaughter. When the additional market-to-slaughter records were removed, the apparent slaughter statistics derived from the movement data more closely matched those from national slaughter statistics. The use of the individual animal-level read records to facilitate the identification and removal of these market moves was not possible in 2006 (20). It now enables an improved indication of the direct spatial origin of the slaughter population.

The fourth challenge is that it is not currently possible to determine whether a batch movement contains lambs, adult sheep, or a mix of both. This is a major limitation of the sheep movement data not only for this study but for many surveillance purposes, Age-stratification of the data is important for sheep, as very often the epidemiology of the disease, condition, or subject under investigation is associated with age-group e.g., pneumonia in lambs, or ovine pulmonary adenocarcinoma in older sheep. The majority of moves to GB slaughterhouses will consist of lambs (29). However, within the industry sector it is well-known that, in addition to economic influences, there are both seasonal influences on the lamb throughput (29) and spatial influences on the preferred slaughter destination

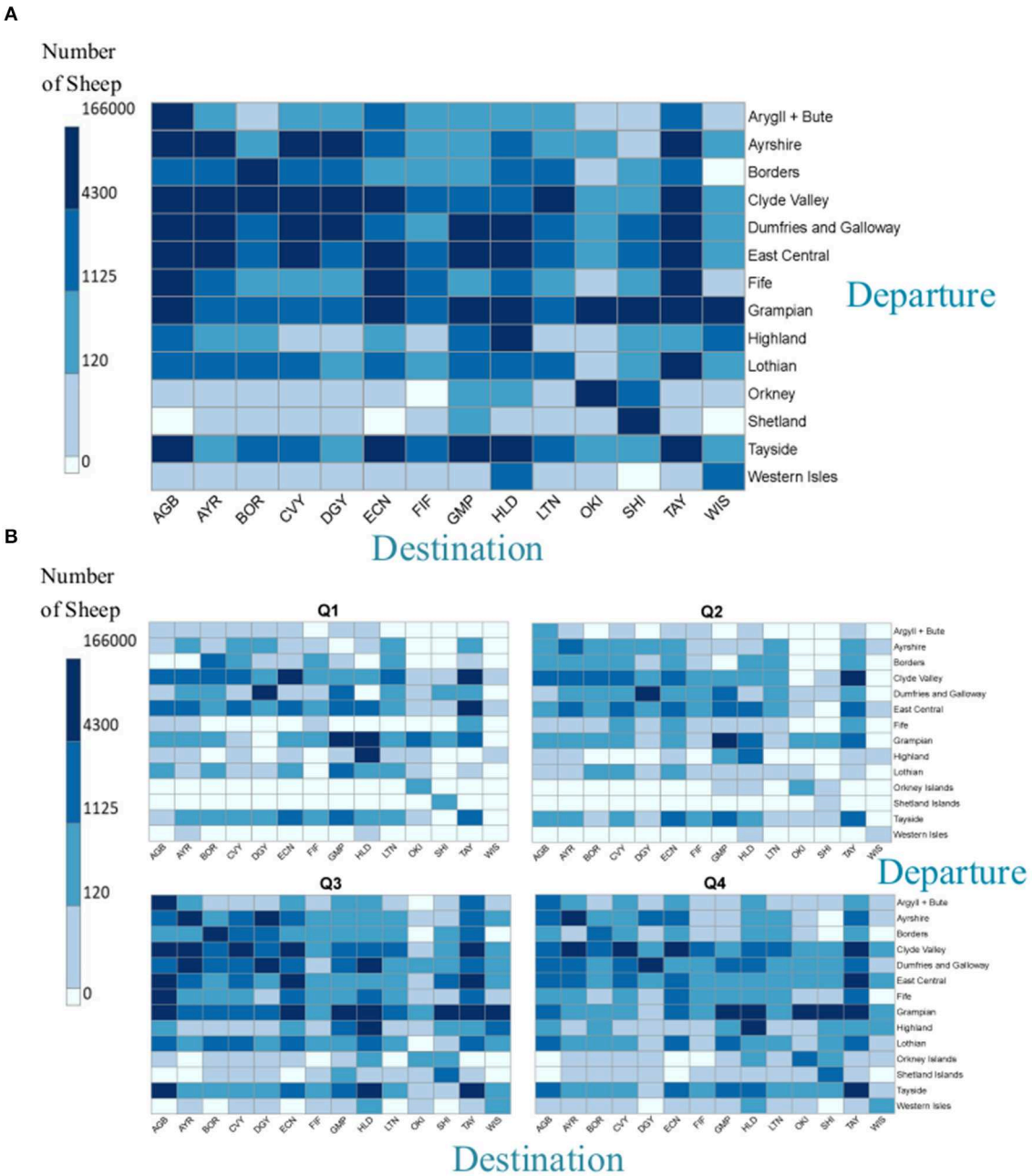

FIGURE 8 | The numbers of sheep moved in non-slaughter related Scottish moves during 2017 **(A)** from departure region to destination region in 2017 **(B)** from departure region to destination region in each quarter of 2017 (January–March; April–June; July–September, October–December).

of ewes and rams. A substantial portion of the Scottish lamb population is known to go south over the border for slaughter, while the majority of Scottish cull ewes go direct to slaughter south of the border (36). It is highly likely that at least one of the high ranked non-Scottish slaughterhouses for receipt of the SSSP in **Figure 4** is a major recipient of cull ewes, but this cannot be confirmed, or accounted for, based on the current sheep movement data. Historically, additional information on individual slaughterhouse throughput in terms of carcass weights could be used to approximately identify appropriate age-groups (20, 37). Such data are no longer easily accessible. Based on the derogation for those lambs that go direct to slaughter, from their holding of birth, at <12 months of age it should be possible to deduce their age from the use of a single flock-mark only slaughter tag (https://www.gov.scot/publications/sheep-goat-identification-traceability-guidance-keepers-scotland/pages/7/). However, it would still omit identification of the subset of the lamb population that do not go direct to slaughter from their

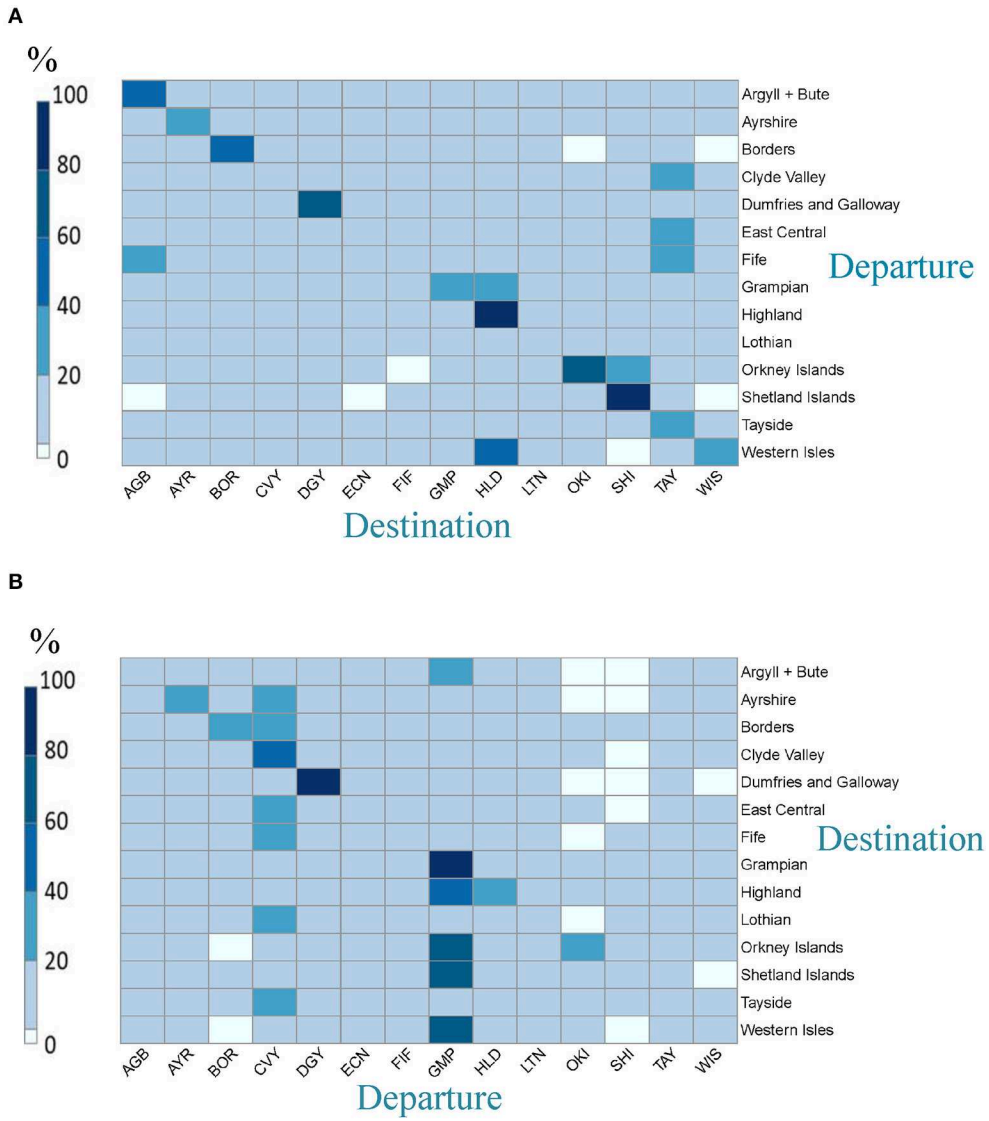

FIGURE 9 | The numbers of sheep moved during 2017 **(A)** to a region (destination) as a % of total sheep moved from a region (departure) **(B)** from a region (departure) as a % of total sheep moved to a region (destination) i.e., horizontal line = 100%.

holding of birth, such as store lambs and those lambs intended as replacement breeding stock that do not make selection for the breeding flock. A possible alternative might be to record whether the batch consists of lambs <12 months; sheep over 12 months, or a mix of both at a batch-level. Either of these approaches would be a substantial improvement. These data are rich and much more could be done with them, if sheep could be age-differentiated, as was the case with the Australian sheep movement data (16).

The fifth challenge of being able to identify only the direct move reduces the accuracy of the derived slaughterhouse catchment areas. As mentioned earlier, there will be a subset of the lamb population that does not move direct to slaughter, while adult sheep may have been resident on a number of premises within their life-time. Such movements contribute to the spatial dynamics observed in the outputs of the within-country non-slaughter movement analysis. Unlike cattle, where the individual animal passport and tracing system facilitates the modeling of life-time pathways (38–40), the missing individual reads for some batch movements make this more difficult for sheep. Studies are ongoing within the EPIC programme to develop statistical methods to address this (https://www.epicscotland.org/our-people/stephen-catterall/). A further problem arises when there are man-drawn borders within the one landmass with different recording systems on each side. Without access to the data from the other side, analyses of movements are compromised. Here, this occurs due to devolved responsibilities. In this study, some of the overall Scottish sheep out moves may

have been to markets across the border, from whence they may have gone to other holdings, returned to slaughter in Scotland in population 4, or gone on directly to slaughter for which they would currently not be included in the SSSP as they did not go direct from a Scottish premises to a GB slaughter house. The extent of these effects cannot be quantified.

A key outcome of this study is the ability to use sheep movement data to start to identify and characterize potential sources of bias in slaughter-house based surveillance. The outputs indicate that any sheep slaughterhouse-based surveillance, conducted in Scottish slaughterhouses, that aims to be representative of the Scottish sheep slaughter population has the potential to produce biased estimates. If the disease, condition, or subject under investigation is systematically different between those holdings that only supply slaughter sheep to GB slaughterhouses out-with Scotland (SOSP) and those that contribute to the SISP, such a bias will exist. Given the spatial differences between the SISP and the SOSP holdings biased estimates are likely, particularly if management systems, breed, flock size, livestock or holding density, or weather conditions are associated with the disease, condition, or subject under investigation.

The AMR survey was a good exemplar to demonstrate how these analyses of sheep movement data can be applied to slaughterhouse-based survey design and data interpretation. For convenience sampling, the choice of the Scottish slaughterhouse with the largest ovine throughput is logical. However, by using the movement data, it has been demonstrated that there is potential for bias in the outcomes of the AST, if there is a systematic relationship between slaughter sheep from these areas and their carriage of resistant fecal *E. coli* i.e., in the frequency of occurrence of AMR to any of the active substances that were tested for. This could arise at three levels: firstly, due to the reduced coverage of the catchment area of this slaughterhouse; secondly, due to the difference between the catchment area and the slaughtered in Scotland sheep population (SISP), and thirdly because of the difference between the SISP and SSSP. The fact that there was no sampling in the AMR survey in the second quarter is probably of the least concern. This is the quarter with the least movement to slaughter; however, it could be one where older lambs, more likely to have been treated with antimicrobials may be sent to slaughter. Bias will occur if there are systematic differences between the levels of AMR in those sheep sub-populations from the areas not sampled and those sampled. If the areas missed were areas of more intensive, lowland sheep production, then it might be argued that the likelihood of AMR could be higher, due to increased antimicrobial usage during the lambing and/or fattening period, than in more extensive areas. This would lead to an underestimate of prevalence of AMR. And, vice versa, if the areas not sampled are those of extensive hill flocks with lower antimicrobial usage. The potential contribution to the existence of AMR in sheep fecal organisms from other spatially related factors such as the environment (41) may also be relevant.

An additional outcome is the potential to use these movement data to improve the sampling strategy so that it is more representative of a defined population of interest. For example, by sampling in the other top three ranked Scottish slaughterhouses in addition to slaughterhouse A, some of the gaps in coverage of the SISP population could be mitigated. Amendments to the sampling strategies could be explored. For example, to determine whether it would be of sufficient scientific advantage to sample either in the Island slaughterhouses, or to weight sampling in the south, and how that would be balanced by the feasibility, cost and resources required. Alternatively, if the sampling strategy is kept the same year-on-year to try to achieve a comparable sample, the analyses could be repeated to assess if this was indeed so.

With minimal additional resources, the applications that have been developed in this study could be used for other surveillance purposes. The movement matrices presented provide a visual representation of the dynamics of the within-Scotland non-slaughter sheep movements that can easily be replicated on updated movement data for any defined time period, with any defined scale for the categorization of number of movements. If such analyses were to be run on a routine basis, they could provide background information that could facilitate situational awareness (42) for those involved in animal health surveillance activities in Scotland. If run on a specified time period appropriate to the particular disease, or hazard, at notification of an outbreak, or incident, they might provide an early indication of where resources may need to be targeted, or mobilized, while more specific tracing programmes and models are being run. This may not be absolutely necessary when distances are small, areas easily accessed and sufficient field-based resources exist. However, it may be of use in converse situations.

Assessments of the quality and utility of movement data are rarely published in the scientific literature. There is a dearth of explicit studies (15), with only brief mentions of data quality, or challenges, within descriptive analyses (43, 44) that in more complex analyses are often relegated to single phrases such as "…numerous inconsistencies in the available data prevent…" (13), or "…anomalies in movement records and complexity of data processing…" (18). Often such assessments are relegated to reports to data providers and vanish into the depths "grey" literature (20, 45). Similarly, publications of slaughterhoused based initiatives only occasionally seek to characterize any potential bias. Mostly there is either a reliance on a sampling methodology that is structured to be representative [e.g., (46, 47)], or a brief mention that a potential for bias does, or doesn't, exist [e.g., (48)], with limited attempts for more in depth assessments, such as that in (49) or the more comprehensive investigation of active sheep TSE surveillance (19). If animal health surveillance is to develop, then these types of assessments are important foundations that need to be laid, regularly evaluated and easily available to users of the data.

In conclusion, firstly, the sheep movement data, currently collated and held in the EPIC data repository from ScotEID, can be used to inform the design and interpretation of Scottish sheep slaughterhouse-based surveillance activities. Secondly, data derived from sheep at Scottish slaughterhouses does have the potential to be biased in relation to spatially related attributes. Thirdly, the sheep movement data can be used to start to identify and characterize these potential biases, as well as to illustrate

the dynamic nature of the movement of the sheep population within Scotland.

The regulatory requirement for the use of EID for individual sheep identification and centralized movement recording has improved the quantity of data available for use. Further steps are required to improve the quality of these Scottish data and to further develop this type of resource, so that their value can be fully realized and they can contribute to operational animal health surveillance. The most important "next step" with these Scottish data is to resolve the lack of information on the age of sheep being moved.

Estimates from any existing ovine slaughterhouse data, such as the AMR survey, need to be interpreted within the context of the identified potential for bias. The type of characterization presented here could—and should—be utilised at the design stage of future slaughter-house based studies. This need not be confined to sheep. The relatively simple, but computationally intense, analytical principles could be extended to other species and to livestock movement databases in other countries. However, sufficient attention will need to be paid to identification of the individual idiosyncrasies inherent in each database.

For other surveillance purposes, discussions with relevant stakeholders are required to determine what types of reports would be useful both for situational awareness, as well as for early resource allocation in outbreak situations. More generally, if the use of movement data for surveillance purposes is to be optimized, robust analyses like those presented here can only be achieved by interdisciplinary teams that are appropriately resourced, with access to multiple data sources facilitated by initiatives such as the EPIC data repository. This way informed exploratory analyses can be undertaken, development needs identified and animal health surveillance activities improved, for the mutual benefit of stakeholders, be they policy-makers, data-providers, surveillance professionals, the livestock industry, or the wider public.

DATA AVAILABILITY STATEMENT

Data have been obtained from a third party. The data analyzed in this study were obtained from the EPIC Data Repository and from SRUC Veterinary Services. Requests to access these datasets should be directed to the corresponding author for forwarding to the appropriate contact.

AUTHOR CONTRIBUTIONS

ST led the study (concept, design, and direction), interpreted the outputs (epidemiological and sheep sector knowledge) and jointly drafted the manuscript, with JS. JS devised and performed the data analysis, generated bespoke C++ applications for data handling and jointly drafted the manuscript with ST and produced figures. She is a member of the EPIC Data Team. JE provided statistical input and drafted the statistical methods. GF led the AMR survey (concept, microbiology, and data acquisition). AR provided advice regarding data handling and extraction, plus contributed to the development of the bespoke C++ applications. He leads the EPIC Data Team and led development of the EPIC Data Repository. JE, GF, and AR reviewed and commented on the manuscript. GG acquired the funding for and initiated the development of the EPIC Data Repository. He reviewed the manuscript. All authors read and approved the submitted version.

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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.2020.00205/full#supplementary-material

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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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Network Analysis of Swine Shipments in China: The First Step to Inform Disease Surveillance and Risk Mitigation Strategies

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China's pork industry has been dramatically changing in the last few years. Pork imports are increasing, and small-scale farms are being consolidated into large-scale multi-site facilities. These industry changes increase the need for traceability and science-based decisions around disease monitoring, surveillance, risk mitigation, and outbreak response. This study evaluated the network structure and dynamics of a typical large-scale multi-site swine facility in China, as well as the implications for disease spread using network-based metrics. Forward reachability paths were used to demonstrate the extent of epidemic spread under variable site and temporal disease introductions. Swine movements were found to be seasonal, with more movements at the beginning of the year, and fewer movements of larger pigs later in the year. The network was highly egocentric, with those farms within the evaluated production system demonstrating high connectivity. Those farms which would contribute the highest epidemic potential were identified. Among these, different farms contributed to higher expected epidemic spread at different times of the year. Using these approaches, increased availability of swine movement networks in China could help to identify priority locations for surveillance and risk mitigation for both endemic problems and transboundary diseases such as the recently introduced, and rapidly spreading, African swine fever virus.

Keywords: social network analysis, pig movements, disease spread, risk-based surveillance, swine diseases

INTRODUCTION

The ongoing expansion of national and international trade has increased the movement of animals, products, and disease globally. China's pork industry has been dramatically impacted by these market changes, with import tonnage quadrupling between 2005 and 2015 (1). The swine industry in China is also seeing an increasing consolidation of traditional farms of <50 animals, into large-scale facilities supporting thousands of pigs (1). The implications of these changes include: increased pig density and trade, higher risk of introduction of novel pathogens, faster disease spread, higher disease incidence, and the generation of novel pathogen strains (2–5). This has been evident with the arrival of African Swine Fever virus (ASF) in August 2018, as well as the dramatic impact that other swine diseases such as PRRS or PED are having in the country (6).

These changes in swine production, as well as the introduction of ASF, have increased the necessity for traceability and efficient data-driven methods to support disease surveillance, prevention, and outbreak response.

Understanding the social network of a production system allows for risk assessment of disease dissemination within the local industry (7), as well as a faster response in case of an epidemic (8). Large swine operations are highly integrated and multi-site—commercial swine production systems use separate sites for sow farms, gilt development, nursing, finishing farms, boar studs, and culling, requiring the frequent shipment of live pigs from location to location (9). These intrinsic pig movement requirements provide opportunities for disease introduction and spread. Understanding when and where these points of contact occur, and the network structure and vulnerabilities, may help to strategically allocate risk-based, more cost-effective, preventive and control measures.

Social network analysis (SNA) has been demonstrated to be a valuable tool to describe these pig movement network structures (10). It has been used to evaluate the movement network dynamics and helps to quickly identify the individual farms, areas and time periods that may pose the highest risk for disease introduction to the system (11–14). These insights allow for implementation of risk mitigation strategies at these spatial or temporal hotspots (14), as well as more realistic disease modeling.

Understanding the social structure of a typical swine facility in China is a first step toward risk analysis in the Chinese swine industry; however, there is currently very limited published information about Chinese swine farm demographics, or the trade and contact patterns at fine spatio-temporal scale (daily/weekly and at farm level). This lack of information is a critical gap in China's animal health and emergency response plans. Moreover, the increasing demand for animal protein due to population growth and westernization of the diet, makes food safety and security a primary concern for China (15). Limiting disease outbreaks, especially those that may severely impact food production and international trade (such as the current ASF epidemic) is critical if China is to meet these consumer demands and maintain industry health standards. SNA applied to the swine industry may also allow for the identification of potential superspreaders or super-receivers of disease within China's pork industry chain, providing targeted locations for surveillance and risk mitigation (13). Food security programs in China should prioritize characterization of the swine trade network as the first step in risk assessment.

We undertook this study as a first exploration of the social network of the pig trade in China.

Our primary objectives were: to describe the network structure in a typical multi-site system in China; to describe pig movement spatio-temporal dynamics; and to identify priority farms that may contribute to the risk of disease introduction and spread. The increased availability of swine demographics and trade data in China will help to better understand, and even predict, disease transmission patterns, which will support risk-based surveillance and control strategies for both endemic and emerging swine diseases such as African Swine fever.

MATERIALS/METHODS

Data

Live pig movement data was collected from a large-scale multi-site pig producer in China. These data were translated from Mandarin to English for analysis. Farms were anonymized to protect producer and citizen consumer privacy. The final dataset included movement information to/from the farms belonging to the participant multi-site pig production system between Jan 1 and Dec 31, 2017. Shipment information included: origin, destination, date, type of shipments (culling or finisher), type of pig, total weight, number of pigs, average weight per pig, and distance moved. Pigs types were classified as: boar, commercial pig, feeder, grower, sow, or unspecified. A total of 2,567 shipments were considered in this study. Data were collected, validated and cleaned in Microsoft Excel 2016 and R language (v.3.4.1) (16, 17).

General Approach

In a first step, we constructed and evaluated the characteristics and properties of the complete network (for the whole study period). In a second step, we generated and evaluated the properties of the dynamic network (i.e., considering the complex dynamics of the edge formation and dissolution over time). Finally, we used the dynamic network to evaluate the potential disease transmission and epidemic sizes over the network, considering the actual farm-to-farm contacts under diverse epidemiological scenarios and computing the forward reachability paths for all nodes in the network.

Static Network Analysis

The complete "static" network for the whole study period (i.e., 1 year) was defined using swine production sites as nodes or vertices, and shipments of live pigs as edges. After the complete static network was generated, we then focused on the farm-to-farm ("to live") movements (i.e., subset of the network removing movements to slaughterhouses). The static networks studied were treated as non-weighted. Network parameters including number of nodes, number of edges, diameter, edge density, average path length, and transitivity were calculated to study the properties and characteristics of the network. Centrality measures of in-degree and out-degree were calculated for each node. Briefly, in-degree is defined as the number of incoming shipments to a farm, out-degree is the number of outgoing shipments from a farm (18, 19). Diameter is the longest of all the shortest path lengths between nodes in the network (18, 19). Edge density is the ratio of the number of edges observed in the network to the number of possible edges (18, 19). Average path length is the mean length of all the shortest paths between nodes in the network (20). Transitivity coefficient is the sum of the proportion of nodes that are connected to other nodes; this parameter is also known as the clustering coefficient (18, 20). The igraph package [v 1.1.2; (21)] in R Studio [v 3.4.1; (17)] was used to generate and describe the static network and evaluate network parameters. Edge density, diameter, average path length, and transitivity were calculated under the igraph package using functions: edge_density, diameter, mean_distance,

and transitivity, respectively. Type global was used for the transitivity function. The visNetwork package was also used for network visualization (22).

Network Dynamics and Epidemic Size

The network Dynamic (23), ndtv (24), and tsna (25) packages within R were used to construct and evaluate the dynamic properties and characteristics of the complete and farm-to-farm pig movement networks, and then to estimate the epidemic size within the resultant dynamic network.

We computed the forward reachability path, which has been previously shown to be a good predictor of epidemic size (11, 26), for each node in the complete dynamic network using the tPath function. Forward reachability is defined as the extent to which an introduced infection can spread through the network—given an introduction at farm A, based on the network structure or contacts, to which farms would an infection be expected to move (11). For each initial vertex in a directed network, tpath searches out the sequence and distance of vertices that are reachable following paths constrained by edge timing. Results were presented using graphs, transmission timelines and video (**Supplementary Video 1**).

RESULTS

The complete static network including all movements during 2017 for our studied Chinese production system contained 67 nodes and 2,567 edges (**Figure 1**). The network shipments displayed seasonality, with the overall number of shipments being highest in January, then declining throughout the year (**Figure 2**). Growers were consistently the predominant type of pig being shipped, with sows being the next most common. The highest sum average weight was observed in January, with a drop off in February and May, then a gradual increase through the rest of the year (**Figure 3**).

The subsetted farm-to-farm network, discarding movements to slaughter, had 50 nodes and 485 edges. The characteristics of the complete vs. farm-to-farm networks are outlined in **Table 1**. Both networks conform a weakly-connected component, in which all farms are connected by some path (ignoring direction) and are characterized by short diameters and path lengths. The transitivity, or clustering coefficient, for both networks was zero. The farm-to-farm network shows low edge density at 0.198. Within our farm-to-farm network we identified those farms with the highest in-degree and highest out-degree. Those with the highest out-degree—our potential super-spreaders—included

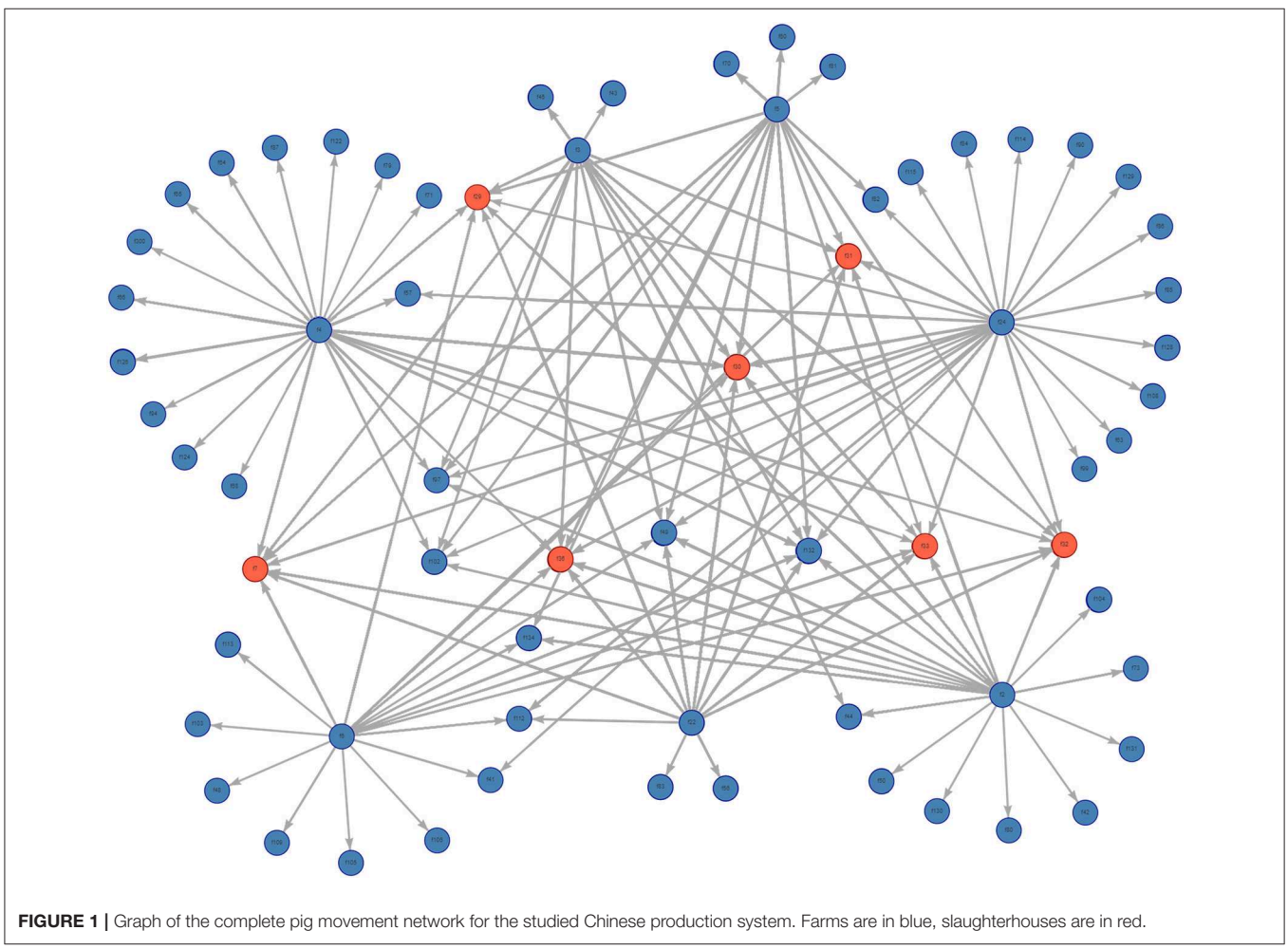

FIGURE 1 | Graph of the complete pig movement network for the studied Chinese production system. Farms are in blue, slaughterhouses are in red.

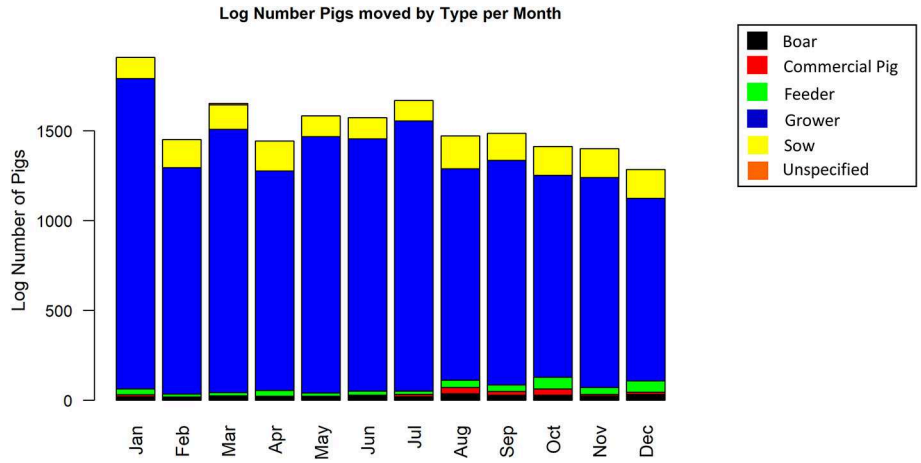

FIGURE 2 | Log number of shipments by type of pig and month of the year.

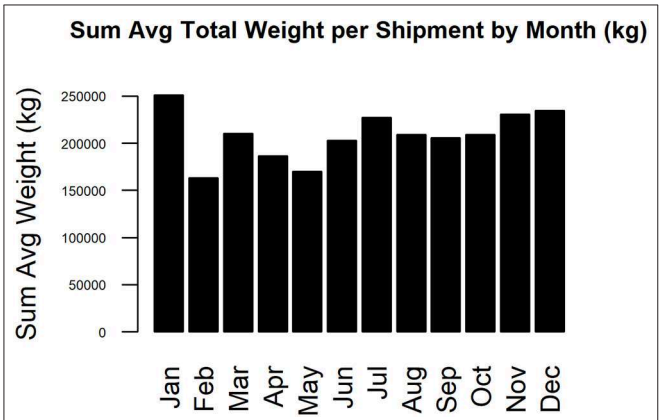

FIGURE 3 | Sum average weight per shipment by month (kg).

TABLE 1 | Network parameters for the complete and subsetted farm-to-farm networks.

Global parameters	Complete network	Farm-to-farm network
Nodes	67	50
Edges	2,567	485
Edge density	0.5805	0.198
Diameter	1	1
Average path length	1	1
Transitivity	0	0
Node parameters	**Mean (Min, Max)**	**Mean (Min, Max)**
In-degree	38.3134 (0, 525)	9.7 (0, 260)
Out-degree	38.3134 (0, 667)	9.7 (0, 164)

farms f24, f4, and f3 with a total of 164, 86, and 65 outgoing movements, respectively. Those with the highest in-degree—our potential super-receivers—included farms f132, f97, and f70 with a total of 260, 38, and 28 incoming movements, respectively.

A dynamic network was evaluated for both the complete and farm-to-farm networks. Edge formation, dissolution and duration are graphed and compared in **Figure 4**. We observe a high rate of connectivity and duration among a few core farms, which compose a stable community evident in our edge statistics and visualized on the dynamic network movie (**Supplementary Video 1**). The remaining edges are sporadic and short-lived. The complete network, including movements to slaughter, displayed higher rates of edge formation and edge duration, than the farm-to-farm network (**Figure 4**).

In evaluating how a disease would move through this network following introduction, we used forward reachability paths. We illustrated the maximum forward reachability path of f24 in **Figure 5**, highlighting the extent and locations where disease would likely disseminate to, given a disease introduction at this farm and assuming disease may disseminate during the whole study period. The forward reachability paths identified farms f24, f4, f2 as the potential main superspreaders when considering the whole study period. Farms f24, f4, and f2 could potentially infect 40, 34, and 31% of the farms within our production system, respectively. Farms f24 and f4 would have more contribution to disease spread if infected early in the year, while f5 and f2 would contribute to more disease spread later in the year (**Table 2**; **Figure 6**). The potential epidemic size, and the farms that would contribute most to disease spread, change throughout the year (**Figure 6**).

DISCUSSION

This study analyzed the pig movement network structure and characteristics of a typical multisite swine production system in China and used forward reachability paths to illustrate the potential spread of swine diseases under diverse epidemiological scenarios (different index cases and time of infections). Despite having only 1 year of information, data suggest that the system is seasonal and predominated by the movement of growers. This seasonality likely contributes to the variable impact certain farms

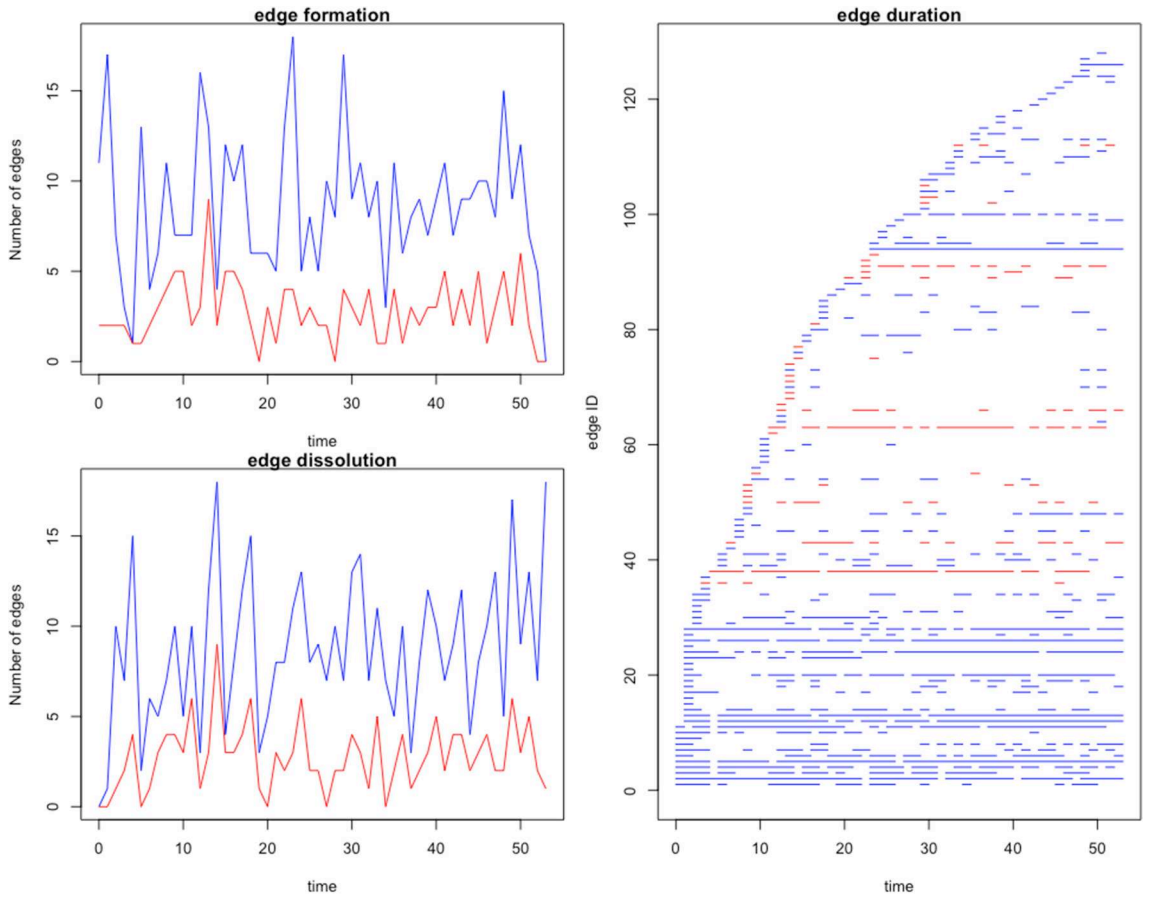

FIGURE 4 | Edge formation and dissolution rates, and edge duration per node of the complete (blue) and farm-to-farm (red) pig movement network for the studied Chinese production system. Time is in weeks. Edge formation is the generation of a contact between two farms. Edge dissolution is the discontinuation of contact between two farms. The edge duration is the length of time (in weeks) that two given nodes had ongoing contact, or shipments between them.

had on epidemic spread at different times of the year, and may be explained in part by the timing of Chinese traditional festivals. Drops in epidemic size correspond to multiple of these events: Chinese New Year (Jan 28; week 4), Lantern Festival (Feb 11; week 6), Qingming (Apr 4, week 14), Dragon Boat (May 30, week 22), Mid-autumn (Oct 4, week 40), and Double Ninth (Oct 28, week 43). Presumably more people are away from work, limiting swine shipments, and thus disease spread during these periods.

The farm-to-farm network consisted of one giant weakly-connected component, with a clustering coefficient of zero or no clustering observed. The lack of clustering and the predominance of stars structures in the network are typical in egocentric networks, and in this case is attributable to the single source of the data (i.e., information from one production system, with no information on the external farms contacting our production system). The lack of additional outgoing movement information from what appear to be isolated receiving farms (those with limited movements, and short edge duration), prevents us from getting a better idea of their interconnectivity with this and/or other production systems.

Node centrality and reachability parameters allowed us to identify potential farms that can act as super-spreaders or super-receivers within the network. Super-spreaders, those farms with high out-degree and highest reachability, represent those most likely to have a key role in the dissemination of disease within the network. Super-receivers, those farms with high in-degree, represent those most likely to introduce diseased animals to their farm, and thus become infected. The most cost-effective preventive and control measures will be those targeting farms characterized as super-spreaders for the implementation of risk-mitigation strategies (i.e., biosecurity, vaccination, quarantine etc.), while our super-receivers should be prioritized for enhanced surveillance programs.

Within this study, we evaluated both the complete network and the farm-to-farm network that excluded movements to slaughter. For most infectious diseases, slaughterhouses act as dead ends. In terms of assessing disease spread through a system, it would therefore make sense to focus on those movements that went on to live. However, given the high level of concern for spread of ASF via fomites and contaminated meat products, infected animals that reach slaughterhouses and are not identified, and other pigs who many become infected while awaiting slaughter, pose a dissemination risk if their tissues reach the market (27).

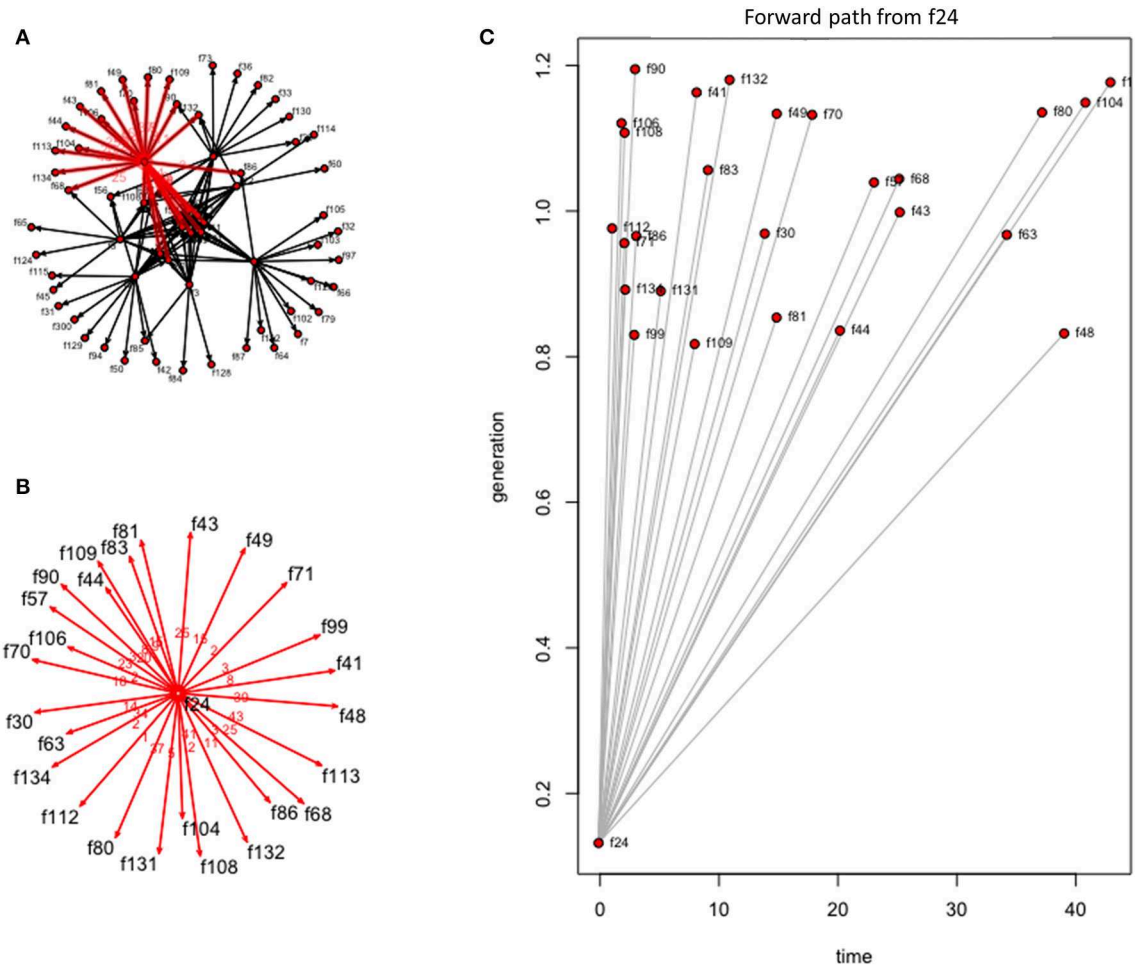

FIGURE 5 | Forward reachability path for farm 24 of the dynamic farm-to-farm network for the studied Chinese production system and considering the entire study period (i.e., 53 weeks). **(A)** The forward reachability within the farm-to-farm network when an infection is started at farm 24. **(B)** Visualization of those farms that could be contacted, and thus become infected, given a disease introduction at farm 24. **(C)** Forward reachability for farm 24 plotted by time in weeks.

TABLE 2 | Potential maximum epidemic size (i.e., number of farms infected) based on the forward reachability path for different index cases and time periods when the disease is theoretically initiated (in weeks).

ID	Index farm	t1-10	t10-20	t20-30	t30-40	t40-50	t1-53
1	f2	10	11	9	10	12	21
2	f22	8	8	11	8	10	13
3	f24	13	14	12	11	11	27
4	f3	9	11	9	11	10	15
5	f4	13	11	8	7	5	23
6	f5	7	10	10	12	13	17
7	f6	3	9	10	9	10	19

Cell colors have increasing scale from light yellow to red and are proportional to the number of farms infected. The maximum epidemic size considering the whole study period is shown in the t1-53 gray column.

This model could be improved with the addition of movement data for other swine production systems, and better information about node parameters such as farm size, production type, disease status, or biosecurity and management practices, which was not available for the current study. The use of forward reachability paths was considered a fast and reliable approach to estimate the epidemic size for all the nodes and different time periods in the study (11, 28, 29). This approach has been proved to be a good substitute for SIR modeling techniques, particularly in situations like this, where there are low number of secondary contacts due to the egocentric nature of the network. Overall, our approach provides an adequate platform from which to understand how disease could move through the studied Chinese production system.

Using social network analysis of a representative, large multi-site swine production system in China, we were able to identify target farms for risk-based surveillance and disease mitigation efforts. This approach allows us to inform on the most effective and cost-efficient approaches to risk mitigation, disease management, and outbreak response in this particular production system, and can be easily adapted

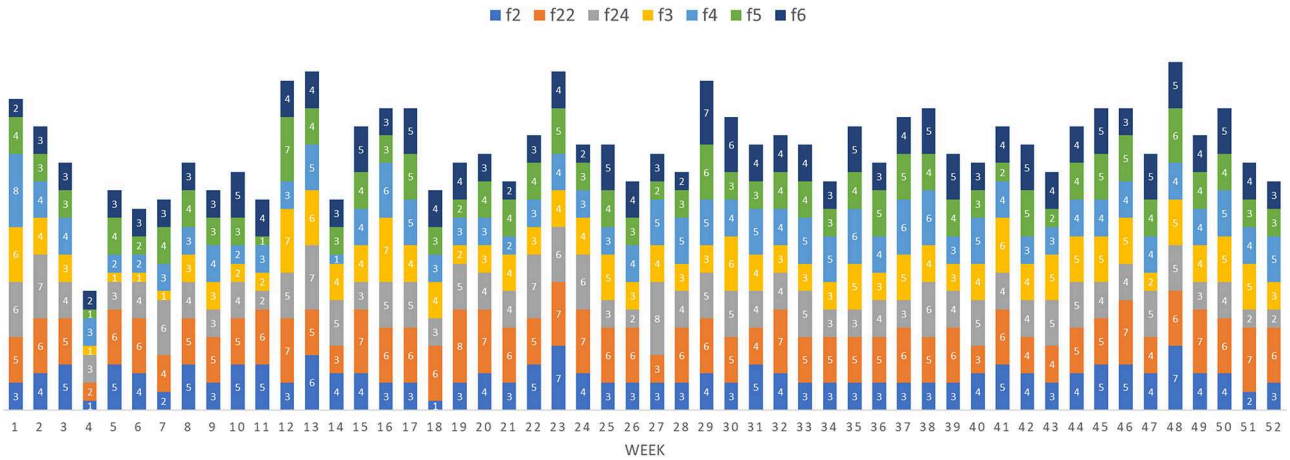

FIGURE 6 | Potential maximum epidemic size (i.e., number of farms infected) based on the forward reachability path for different index cases (farm 2, 22, 24, 3, 4, 5, and 6) per week when the disease is theoretically initiated at the beginning of the week.

to other production systems if data becomes available. Future directions should include the incorporation of more data about on-farm demographics, farm type and management practices within our network, as well as the incorporation of additional production systems. Creating a spatially explicit network and incorporating the distance between farms and the transportation routes would allow the inclusion and evaluation of other transmission pathways (i.e., airborne transmission, truck movements, fomites, etc.) for stronger predictions of disease dissemination, particularly in those areas with high pig farm density. Additionally, the model could be used to evaluate the efficacy of specific biosecurity or riskbased interventions.

To the authors' best knowledge this study represents the first description of the pig movement patterns in a large-scale Chinese swine production system. We have provided an initial exploration of the swine movement patterns in this system and have demonstrated how the use of social network analyses can be used to inform surveillance and risk mitigation strategies to improve decision-making, and disease prevention and control, within the Chinese swine industry. Given that China has just experienced its first incursion of ASF, the availability of more swine trade data could help to better understand ASF transmission dynamics, as well as to prevent and control further outbreaks. By better understanding the contacts and movement structure of the Chinese pork system, resources may be more efficiently targeted at priority locations for more timely disease mitigation. We recommend the expansion and utilization of this approach as a benchmark in the food safety and emergency response plan for China's swine industry moving forward.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

KO'H performed the data curation and validation, development of the Rcode, and formal analysis under supervision of BM-L and YQ. YQ initiated recruitment of the farms included in this study, and collected, organized, and translated the raw data in collaboration with RZ, Y-SJ, and XZ, as well as contributed to critical interpretation of results and implications for the Chinese swine industry. BM-L contributed to the development of the R-code with KO'H, as well as supervised the development, implementation and interpretation of the analytic approach, model parameterization and model diagnostics. KO'H performed the initial draft preparation. All authors contributed to the project conceptualization and critical review and editing of the submitted manuscript and extensively reviewed and edited the manuscript before submission.

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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.2020.00189/full#supplementary-material

Supplementary Video 1 | Visualization of the dynamic farm-to-farm network movements across time. Farms with the highest number of movements of sows in red, the remaining farms in blue. Node size is proportional to the cumulative number of movements in the entire time period: small <50, medium 50–200, large >200. Time is presented in weeks.

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Conflict of Interest: XZ was employed by the company Bright Food (Group) Co., Ltd.

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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ORIGINAL RESEARCH
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A Participatory Investigation of Bovine Health and Production Issues in Pakistan

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Systems to record the frequency of animal health events in Pakistan are limited. A participatory approach was used to address gaps in farmers' knowledge and understanding of bovine health and production issues in five agroecological zones (AEZs) of Pakistan. Participatory tools, including simple ranking, pairwise ranking, constraint impact scoring, and constraint profiling were used in group discussions with farmers and animal health professionals (AHPs) in six districts of two provinces, Punjab and Sindh. The results of the ranking activities showed that foot-and-mouth disease (FMD), clinical mastitis, ticks, hemorrhagic septicemia, reproductive disorders, blackleg, and endoparasites were the most important bovine health and production constraints for small-scale dairy farmers. Constraint impact scoring showed that the participants perceived that: (1) milk production was severely affected by FMD and mastitis; (2) blackleg and parasitism led to poor growth rates and reduced meat production; (3) reproductive disorders and mastitis caused major economic losses (due to the high cost of treatment); and (4) blackleg and hemorrhagic septicemia were the leading causes of mortality in cattle and buffaloes. Although there was strong agreement in responses and constraint impact scores between farmers and AHPs, farmers were more concerned about health issues that cause high mortalities, whereas AHPs emphasized the importance of disorders with a high economic impact. Despite socioeconomic differences among AEZs, farmers' knowledge about bovine health and production constraints was similar. The findings from this study revealed that farmers had limited understanding of the risk factors and routes of transmission of various infectious diseases of bovines, which emphasizes the need to develop and implement tailored extension programs in Pakistan to control contagious diseases of animals and to improve the profitability of small-scale dairy farmers.

Keywords: agroecological zones, bovine health, Pakistan, participatory epidemiology, production, small-scale dairy farming

INTRODUCTION

The farming of livestock animals represents a crucial source of food to almost a billion people worldwide (1, 2). Milk is a major commodity, obtained from ∼123 million dairy farms globally, maintained predominantly in mixed crop-livestock and pastoral systems in Asia and Africa, respectively (3–5). Estimates show that 68% of these farms are present in the Indian subcontinent, with >80% of them being small-scale operations (5, 6). Animal health issues are a major constraint to milk production, particularly for small-scale dairy farmers, due to poor disease diagnosis and monitoring and a limited understanding of disease epidemiology (7, 8). A similar situation exists in Pakistan, where the livestock sector plays a vital role in promoting socioeconomic development in rural areas. Approximately eight million families, with a total of 30–35 million people in rural populations, are involved in livestock production activities, which contribute to 35–40% of their annual income (9, 10).

Pakistan is the third-largest milk-producing country in the world and has 47.8 million cattle (*Bos indicus* and *Bos taurus*) and 40 million water buffaloes (*Bubalus bubalis*) (10, 11). Dairy animals are reared in four milk production systems: (1) rural subsistence smallholdings; (2) rural market-oriented smallholdings; (3) rural commercial dairy farms; and (4) peri-urban commercial dairy farms (12). Small-scale dairy farms (rural subsistence and rural market-oriented) with less than 10 animals per herd comprise > 90% of the total number of bovines in Pakistan and are predominantly in two provinces—Punjab and Sindh (13). The average milk yield per lactation on these smallholder farms is ∼1,200 liters for buffaloes and 1,000 liters for cows, which is almost half of the national herd average for both species (14, 15).

The productivity of the Pakistani dairy sector is affected by constraints including nutrition, husbandry and health as well as limited access to vaccines and veterinary extension services (16, 17). The major bovine diseases/syndromes reported from Pakistan include mastitis, foot-and-mouth disease (FMD), hemorrhagic septicemia, blackleg (or black quarter) caused by *Clostridium chauvoei*, internal and external parasitic diseases and hemoglobinuria (14, 16, 18–21). In Pakistan, the annual losses due to animal diseases are reported to be US$ 200 million (8). By comparison, in India, Birthal et al. (22) recorded an annual loss of US$ 23.6 million (1.8 billion Indian rupees) to farmers, ascribed to various animal diseases and an annual loss of US$ 430 million due to FMD alone (8, 23). These losses could be reduced by adapting proper prevention and control measures against important diseases in developing countries. However, due to poor surveillance systems, there is little information on the incidence, distribution and dynamics of animal diseases in South Asian countries (8). The productivity of dairy animals on small-scale farms can be sub-optimal due to poor husbandry practices and limited resources, such that these farms can pose a greater biosecurity risk for the spread of livestock and zoonotic diseases compared with commercial dairy farms (24). Thus, it is important to investigate the knowledge base and practices of small-scale farmers regarding animal-health issues to prevent losses as well as to identify and mitigate biosecurity risks.

Undertaking studies in resource-poor communities is usually challenging because of their remote locations, limited infrastructure and challenges associated with data and sample collection as well as sample transport and storage (25). Additionally, without a long-term relationship with target communities, a lack of confidence about governmental authorities can also be a challenge in such studies (25). Despite these challenges, epidemiological investigations of livestock diseases should actively involve farmers to assess their knowledge and understanding of key issues and evaluate needs in local communities (26, 27). Interviews, surveys and participatory epidemiological (PE) tools assist such studies.

The PE approach includes participatory rural appraisal (such as informal interviews, visualization and mapping, scoring and ranking) combined with conventional epidemiological methods (28). PE tools should reduce non-sampling errors recognized in questionnaire surveys, such as poor responses to questions and interviewer errors (29), and they are inexpensive and convenient to conduct with illiterate participants, and provide validated results through triangulation of multiple methods (30, 31). This approach is now being widely used in parts of Africa and Asia for disease surveillance, prioritization, surveys, and control (32).

We elected to use a PE approach, to assess small-scale farmers' knowledge of bovine health and production (BH&P) issues compared with that of AHPs in five agroecological zones (AEZs) of Pakistan. The findings and conclusions from this study would address knowledge gaps and underpin future extension programs and biosecurity awareness campaigns for farmers, focused on increased farm productivity (33) as well as improved cooperation between farmers and AHPs.

MATERIALS AND METHODS

Study Area

Based on the physiography, land use, soil type, and climate, Pakistan is divided into 10 AEZs (34), and the distribution of bovines varies markedly across these zones (35). Small-scale dairy farms are mostly located in five of the 10 AEZs: Indus Delta, Northern Irrigated Plains, Arid (rain-fed), Sandy Desert, and Southern Irrigated Plains (35, 36). This study was conducted in four districts (Bahawalpur, Jhelum, Layyah, and Okara) of Punjab and the two districts (Sukkur and Thatta) of Sindh (**Figure 1**). These districts were selected based on operational convenience. However, each represented a different AEZ, except for the Arid zone, where two districts were selected (Jhelum and Layyah) to cover the diverse topography. The districts included are dominated by small-scale farmers, most of which are either landless or use rented land for agriculture, and mixed crop-livestock farming is usual. Men and women are mainly involved in livestock-related activities, with a varying degree of involvement of children. Many farmers with small-scale operations are resource-poor and, therefore, cannot afford to hire

Abbreviations: AEZ, agroecological zone; AHP, animal health professionals; BH&P, bovine health and production; FMD, foot-and-mouth disease; PE, participatory epidemiology.

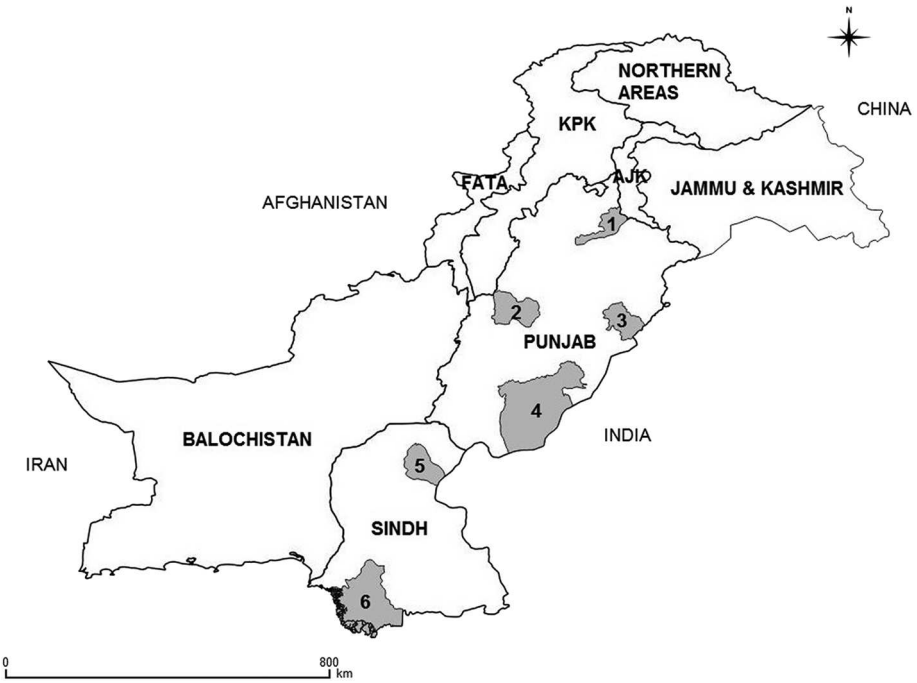

FIGURE 1 | Map of Pakistan showing the locations of districts (gray-colored areas) included in the study. The names of the districts are Jhelum (1), Layyah (2), Okara (3), Bahawalpur (4), Sukkur (5), and Thatta (6). The map was created using QGIS v. 2.18.15.

farm workers. Socioeconomic status varies particularly between provinces with least developed communities in districts of Sindh province. Descriptive statistics of temperature and the population of humans, cattle and buffalo per study district are given in **Table 1**.

Structure of Veterinary Services in Pakistan

The provincial governments in Pakistan are responsible for providing veterinary services. Each province has its livestock department with various directorates offering services, including breed improvement, animal health, research, extension and disease surveillance (39). Animal health professionals (AHP) include veterinarians and para-veterinary staff. There are veterinary hospitals, dispensaries and centers, which provide veterinary services to livestock farmers in most parts of the country. They are supported by a disease diagnostic laboratory in each district; however, most of such laboratories are under-resourced, in terms of well-trained qualified staff and facilities. The extension services are poor, and less than 40% farmers have access to any livestock extension program. A veterinarian heads each veterinary hospital and is supported by a veterinary assistant as well as a technician to undertake artificial insemination in bovines (39, 40).

Study Design

From September to November 2017, a cross-sectional study was conducted employing different PE methods. Following consultation with the Livestock Departments and the Dairy-Beef project, five villages were selected from each district. Eight to 10 small-scale dairy farmers (men) attended a facilitated group meeting (FGM) in each village; 30 FGMs were held in six districts. No women were involved in the FGMs, because of cultural and/or logistical issues. For AHPs, one FGM was conducted per district with each AHP group; 12 FGMs were held with AHPs (i.e., six with veterinary officers and six with veterinary assistants). No incentive was provided to farmers or AHPs for participation in any FGM.

Participatory Epidemiology Methods Used

Four standard PE methods (simple ranking, pairwise ranking, constraint impact matrix scoring and constraint profiling) (29, 41–44) were used to assess the knowledge of dairy farmers and AHPs on the major constraints to BH&P. Simple and pairwise rankings were used as triangulation to validate the list of top constraints in all FGMs. Constraint impact matrix scoring was used to assess the impact of identified constraints on key health and production indicators. Constraint profiling informed about participants' knowledge about epidemiological aspects of the constraints. All FGMs were conducted in local languages.

The core research team consisted of two veterinarians and one veterinary assistant. One of the veterinarians acted as a facilitator, while the other recorded information on a board displayed at an appropriate distance from the participants. The veterinary assistant helped to organize FGMs and liaise with farmers. The FGMs were not voice-recorded to avoid any biases, because participants are usually reluctant to speak when they are audio- and/or video-recorded, despite them being assured of privacy and confidentiality. The checklist of topics and PE techniques were pre-tested before the FGMs in a pilot study and the data were excluded from the final study.

TABLE 1 | Demographic properties of districts included in the study.

Province	District	Agroecological zones	Temperature (°C)[37](Min–Max)		Population		
			Summer	Winter	Human (number)[38]	Bovine (million)[2]	
						Cattle	Buffalo
Punjab	Bahawalpur	Sandy desert	39-46	1-7	3,668,106	0.79	0.81
	Jhelum[1]	Arid	38-45	0-6	1,222,650	0.24	0.19
	Layyah[1]	Arid	38-45	0-6	1,824,230	0.84	0.41
	Okara	Northern irrigated plains	39-46	1-6	3,039,139	0.47	1.18
Sindh	Sukkur	Southern irrigated plains	30-50	0-12	1,487,903	0.31	0.26
	Thatta	Indus delta	34-45	5-20	979,817	0.59	0.49

[1]*Two districts were selected from arid zone to cover geographic diversity of this zone.*
[2]*Estimated population in 2016 based on inter-census growth rate 1996 & 2006.*

For each method, cross-checking and probing were carried out to: (1) validate the information, (2) ensure that the participants understood different items to be scored or ranked, and (3) ensure that enough information was gathered on each topic. During each activity, open-ended questions were asked to cross-examine the responses. However, utmost care was taken not to lead the participants to a specific health or production constraint. In each FGM, the participants were given time to discuss. If there was confusion about an issue or irrelevant discussion begun, the facilitator intervened through open-ended questions to guide the discussion, without forcing a consensus. The ranking, scoring and other aspects were recorded on charts. The same set of questions was used in all FGMs. Following each PE activity, the interview team met and reviewed the notes of each discussion to ensure that they were recorded objectively.

Simple Ranking

Following a brief introduction of the team members and the objective of the study, participants were asked to name the important BH&P constraints causing high losses. In cases where the participants mentioned syndromes/symptoms as a constraint, probing, open-ended questions were asked to identify the local name of the constraint, but care was taken not to guide them to a particular BH&P constraint name. When the constraint name was not identified, further information was collected about the constraint until a proper diagnosis was reached. The constraint names were rechecked with the local veterinarian or para-veterinary staff at the end of the FGM. Once the constraints list was obtained, participants were asked to rank each constraint according to its perceived importance (i.e., caused losses). This activity produced a list of constraints (mean: 9, range: 6–12) based on their perceived importance (ranked from 1 to 12, where 1 = most important and 12 = least important).

Pairwise Ranking and Comparison of Constraints to Bovine Health and Production

Pairwise rankings were carried out by comparing each constraint (already identified during simple ranking) individually with all other constraints. The simple ranking list of BH&P constraints was read aloud, and participants were asked again if they wanted to make any changes to the ranking order. Once re-validated, the top five constraints were taken from the list for pairwise ranking. The recorder sketched a 5 × 5 grid on a chart. The facilitator named the first constraint and asked participants to compare it with each of the remaining constraints, with supporting questions: (1) "Which of these two constraints is more important?"; (2) "Why is it more important?"; and (3) "Describe any differences/similarities between the two constraints." The recorder documented the constraint that was indicated as most important by participants. The total score (0-4) for each constraint in a group was recorded, with 0 being the least important and 4 the most important. This provided pairwise ranks for each constraint on a scale of 1 to 5, with 1 as the most important and 5 as the least important. The median scores for each constraint given by the 30 farmer groups and the 12 AHP groups were then used to establish an overall ranking.

Constraint Matrix Scoring

For each group discussion with farmers as well as AHPs, the five top-ranked BH&P constraints based on the pairwise ranking were scored against clinical and production-related indicators. These indicators were obtained during the pairwise comparisons in the pilot activity and included the impact on milk, meat, cost of treatment, morbidity and mortality. Indicators were written along the *y*-axis of a two-dimensional grid and the constraints along the *x*-axis. The facilitator asked the participants to distribute and/or assign a total of 10 small sticky dots to the five indicators for each of the constraints. Indicators and constraints were stated in the local language and explained to the participants when required. Participants were given time to develop consensus for voting, and once all the participants agreed on the number of dots for each indicator, the sticky dots were pasted on the chart in corresponding boxes. The sticky dots were counted in each of the corresponding boxes for each indicator giving the impact score. The scoring procedure was repeated until all the indicators had been scored against each constraint.

Constraint Profiling

This activity was conducted to assess the participants' knowledge about the identified BH&P constraints. The recorder wrote

the first constraint on a chart, and the facilitator asked open-ended questions about the descriptors of a constraint, including local name(s), cause, clinical signs, risk factors, the season of occurrence, susceptible age and animal species involved, treatment and vaccine availability/schedule. Responses were recorded in the corresponding boxes, and the same steps were repeated for all the constraints in order of their pairwise ranks. Notes from the discussion were recorded separately.

Data Analyses

Data from all FGMs were entered into a spreadsheet (Microsoft Excel 2016). Kendall's coefficient of concordance (W) was used to quantify the level of agreement for pairwise rankings of BH&P constraints among farmers and AHPs at the district as well as provincial levels. An agreement was assessed as weak, moderate and strong for values of W under 0.26, between 0.26 and 0.38 and greater than 0.38, respectively (45). To determine the overall association between constraint impact score (as the outcome variable) and the health and production indicators (as an explanatory variable), we developed a fixed-effects proportional odds (ordinal) logistic regression model. The proportional odds logistic regression models were estimated using the contributed R (46) packages ordinal (47), RVAideMemoire (48), emmeans (49), and lattice (50). See **Supplementary File S1** (R code), **Supplementary File S2** (Farmer FGM data), and **Supplementary File S3** (AHP FGM data) used for the analyses.

Ethics Approval

This study was approved by the Veterinary and Agricultural Sciences Human Ethics Advisory Group, The University of Melbourne (Ethics ID: 1748953). All activities involving human participants followed the ethical standards of protecting the rights and welfare of participants. Data were collected after the participants gave consent.

RESULTS

Demographics of Participants

The total number of participants included in this study was 277 (range: 8–10) and 83 (range: 5–8) in farmer and AHP FGMs, respectively. The farmers were adult men and mostly without any formal education; however, no data were recorded on the age and education of farmers. The AHPs included adult men ($n = 79$) and women ($n = 4$).

Simple Ranking

Simple ranking of BH&P constraints showed that FMD, mastitis, ticks, hemorrhagic septicemia, reproductive disorders, blackleg, and internal parasites were perceived to be important constraints by both farmers and AHPs (**Figure 2**). Farmers ranked FMD, mastitis, hemorrhagic septicemia, reproductive disorders and ticks as major constraints in all AEZs. Similarly, redwater was also important in all AEZs, except the southern irrigated zone. Overall, farmers ranked hemorrhagic septicemia as a major constraint due to higher mortality associated with this disease, particularly in southern irrigated plains and Indus delta zones (**Figure 2A**). FMD was ranked among the top-five constraints due to its relatively high and unpredictable incidence and recurrent outbreaks. However, it was mostly ranked 2nd or 3rd due to associated low mortality and self-recovery. Mastitis was considered as one of the leading causes of low milk yield, high treatment costs and poor prognosis as well as the reduced value of the animal. Tick infestation was listed as one of the important issues due to its negative impact on the growth and production of animals, particularly during the summer season. Similarly, farmers cited blackleg as the cause of high mortality in the arid, sandy desert and southern irrigated zones. Reproductive disorders including anestrus, prolapse and repeat breeding were also considered as economically important constraints by the farmers (**Figure 2A**).

AHPs ranked mastitis, FMD, internal parasites, hemorrhagic septicemia, blood parasites, reproductive disorders, ticks and blackleg among the top BH&P constraints (**Figure 2B**). They believed that mastitis was the most important health issue due to the low treatment success rate, leading to decreased milk production and reduced value of the animals. Despite mass vaccination campaigns against FMD by the provincial livestock departments, this disease was considered as the second major bovine health issue by AHPs. Internal parasites, mainly caused by liver fluke, were also of major concern due to their negative impact on an animal's body condition and milk yield. Hemorrhagic septicemia was considered a major health issue in Sindh province, and blackleg outbreaks were reported in arid and sandy desert zones of the Punjab province. AHPs also ranked ticks and tick-borne pathogens (i.e., *Anaplasma* spp., *Babesia* spp., and *Theileria* spp.) among major bovine health issues (**Figure 2B**). In contrast to farmers, AHPs mentioned that blood parasites were important in the northern irrigated, arid, and Indus delta zones.

Pairwise Ranking

The pairwise ranking results showed that farmers ranked blackleg high when compared with other constraints due to higher mortality rates associated with it (**Figure 2A**). Mastitis was ranked higher due to low milk yield, the high cost of treatment and the poor prognosis. Similarly, AHPs considered the economic impact and prevalence rates of various constraints while comparing and ranking BH&P constraints. This activity resulted in changes in the ranking of all major issues; however, the overall ranking remained the same among both farmers and AHP (compare simple and pairwise rankings given in both **Figures 2A,B**). Kendall's coefficient of concordance showed strong agreement for pairwise ranks of BH&P constraints among farmers from all districts ($W = 0.625$, $P = 0.000$), as well as districts in Punjab ($W = 0.718$, $P = 0.000$) and in Sindh ($W = 0.783$, $P = 0.045$). The data from AHP FGMs from all districts also showed strong agreement ($W = 0.915$, $P = 0.033$).

Constraint Impact Matrix Scoring

This method was used to compare the impact of major BH&P constraints on each of the five identified indicators on the livelihood of small-scale dairy farmers. Ordinal logistic regression of impact scores from both farmers and AHPs

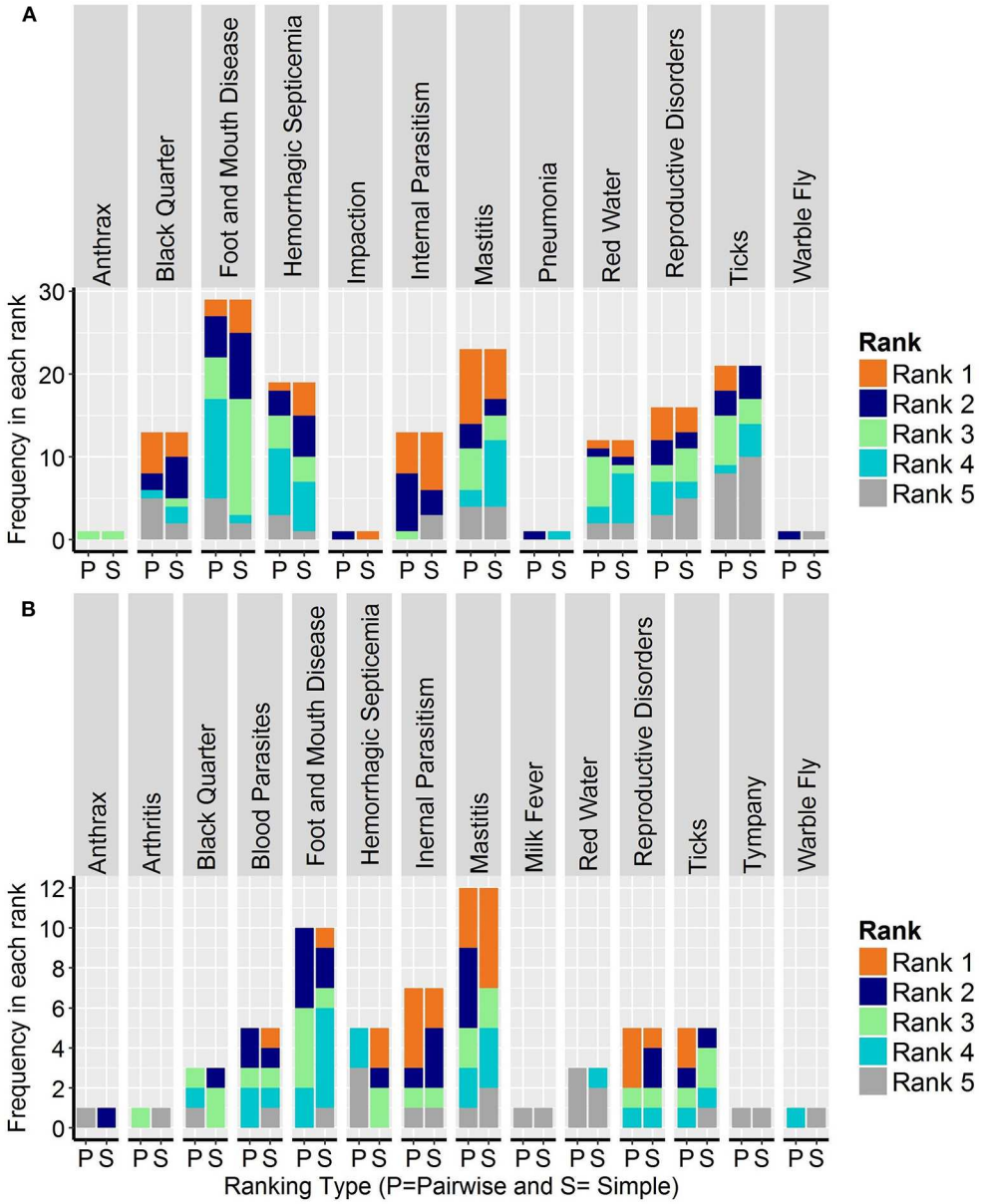

FIGURE 2 | Grouped stacked bar plots of simple and pairwise rankings of major bovine health and production constraints by farmers **(A)** and animal health professionals **(B)**.

indicated that milk production was perceived to be severely affected by mastitis and FMD (**Figure 3A**). Farmers perceived that mastitis affecting one udder quarter could lead to an almost 25% reduction in milk yield and would also decrease the value of an animal, and FMD outbreaks impacted at least one complete lactation in a herd. Farmers thought that blackleg was the main issue for the low quality and yield of meat, while internal parasites, ticks, and redwater caused weight loss and reduced growth rate (**Figure 3B**). Furthermore, farmers believed that ticks suck blood while internal parasites utilize animal's intestinal food, thereby leading to weight loss (**Figure 3B**).

The cost was the third indicator for assessing the impact of BH&P constraints and the participants were asked about the economic impact (i.e., loss of income) and the expenses of treating a condition/constraint. Mastitis, redwater and reproductive disorders were considered as expensive constraints by both farmers and AHPs, whereas AHPs also included blood parasites in the list (**Figure 4A**). Both farmers and AHPs thought that mastitis required costly and prolonged therapy with little success, while reproductive disorders (anestrus in buffaloes and repeat breeding in cattle) caused losses to the small-scale dairy farmers in the form of increased calving interval, leading to higher costs involved in the feeding of dry

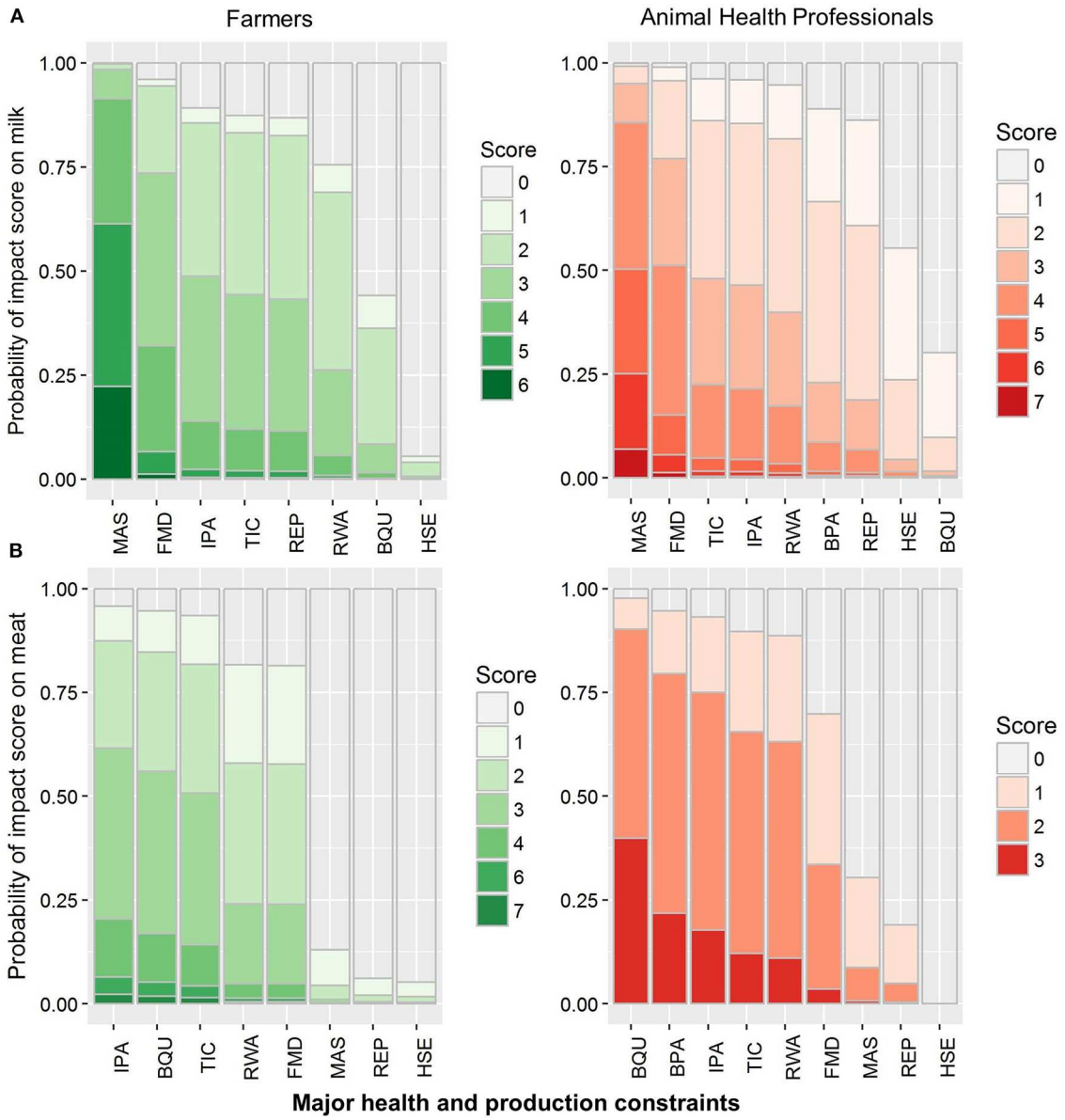

FIGURE 3 | Grouped stacked bar plot of logistic regression of probability impact score of bovine health and production constraints on milk **(A)** and meat **(B)** production in cattle and buffaloes perceived by small-scale dairy farmers and animal health professionals in Pakistan. Major bovine health and production constraints include blood parasites (BPA), blackleg (BQU), foot-and-mouth disease (FMD), hemorrhagic septicemia (HSE), internal parasites (IPA), mastitis (MAS), Redwater (RWA), reproductive disorders (REP), and ticks (TIC).

animals. The fourth indicator was morbidity associated with a constraint/condition, and both groups identified that FMD, internal parasites, reproductive disorders (repeat breeding) and ticks had high morbidity (**Figure 4B**). According to farmers, FMD outbreaks occur biannually, and tick prevalence was usually higher during the summer season while internal parasites and reproductive disorder were prevalent throughout the year.

Both farmers and AHPs commented that blackleg and hemorrhagic septicemia were responsible for most mortalities (**Figure 5**). Further probing showed that, although the prevalence was much lower for blackleg, it was still considered as a major life-threatening issue for animals, due to its high case fatality rate. Likewise, even though hemorrhagic septicemia had been controlled in most of the areas through the extensive use of vaccination and antibiotics, it was still a cause of high mortality. Farmers also mentioned redwater as a cause of high mortality, particularly in buffaloes. Further questions determined that both post-parturient hemoglobinuria as well as hemoglobinuria caused by babesiosis, were classified as redwater by farmers as well as AHPs.

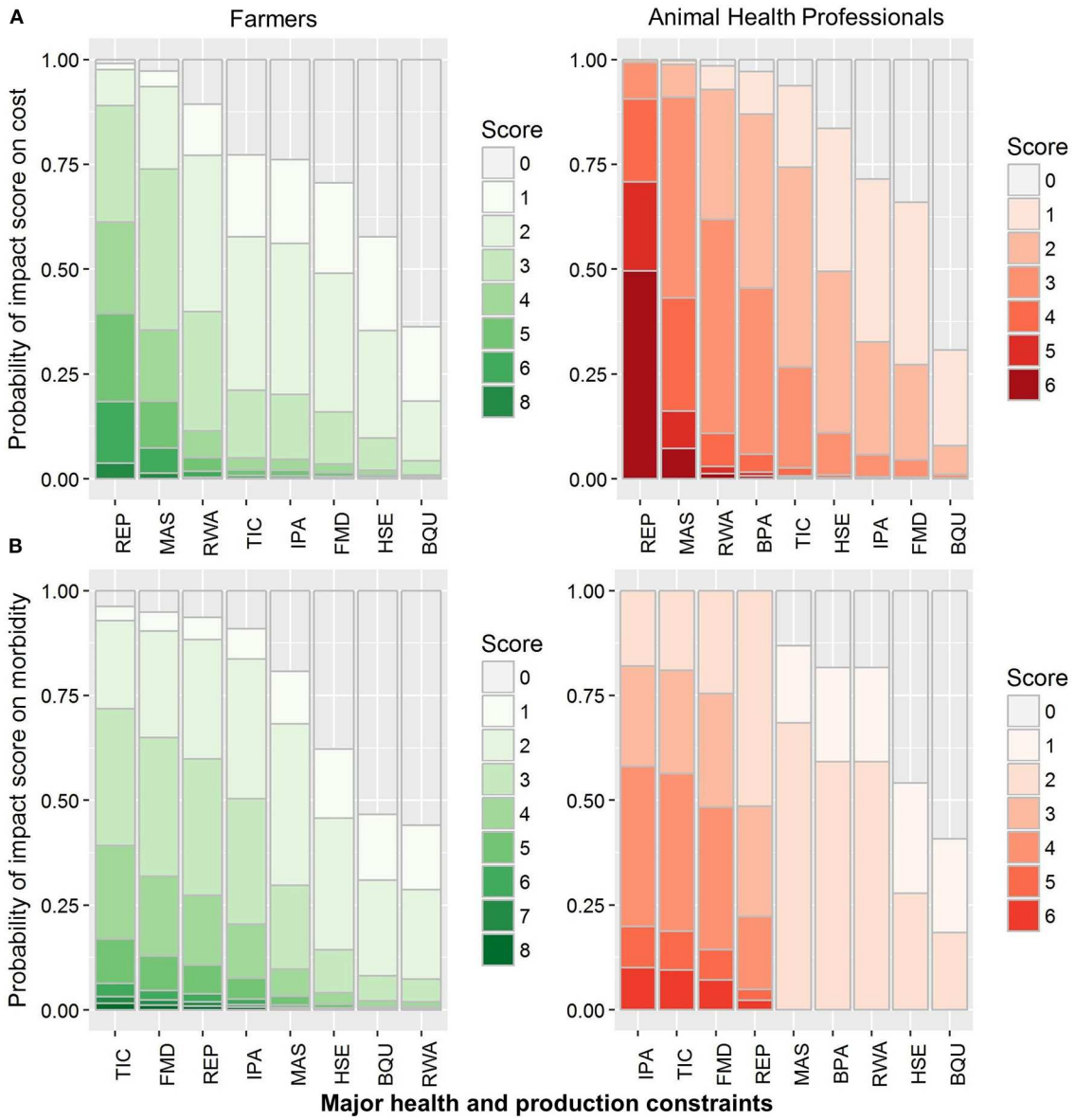

FIGURE 4 | Grouped stacked bar plot of logistic regression of probability impact score on the cost of production **(A)** and morbidity caused by health and production constraints **(B)** in cattle and buffaloes perceived by small-scale dairy farmers and animal health professionals in Pakistan. Major bovine health and production constraints include blood parasites (BPA), blackleg (BQU), foot-and-mouth disease (FMD), hemorrhagic septicemia (HSE), internal parasites (IPA), mastitis (MAS), Redwater (RWA), reproductive disorders (REP), and ticks (TIC).

Constraint Profiling

This method was used to assess the participants' knowledge of important health and production issues, their seasonal occurrence and the relevant epidemiological aspects (**Table 2**). In Punjab province, farmers were able to describe key clinical signs of BH&P constraints while in Sindh province, farmers provided limited information on this aspect (such as fever in FMD, difficult breathing in hemorrhagic septicemia and reduced milk in mastitis). Overall, according to farmers, ethnoveterinary medicines were always the first line of treatment. In case of failure, they themselves used allopathic drugs, following advice provided by AHPs. Farmers perceived that their animals got infected with FMD after either being in direct contact or close to infected animals, although their knowledge about risk factors of FMD was limited.

Discussion on epidemiological aspects of mastitis revealed that infectious agents, unhygienic conditions and incorrect milking practices were the main contributing factors. For tick infestation, hot season, grazing, and lack of backyard poultry and water ponds were mentioned as the main risk factors. Tick control methods varied from region to region and mainly involved manual removal of ticks and burning, application

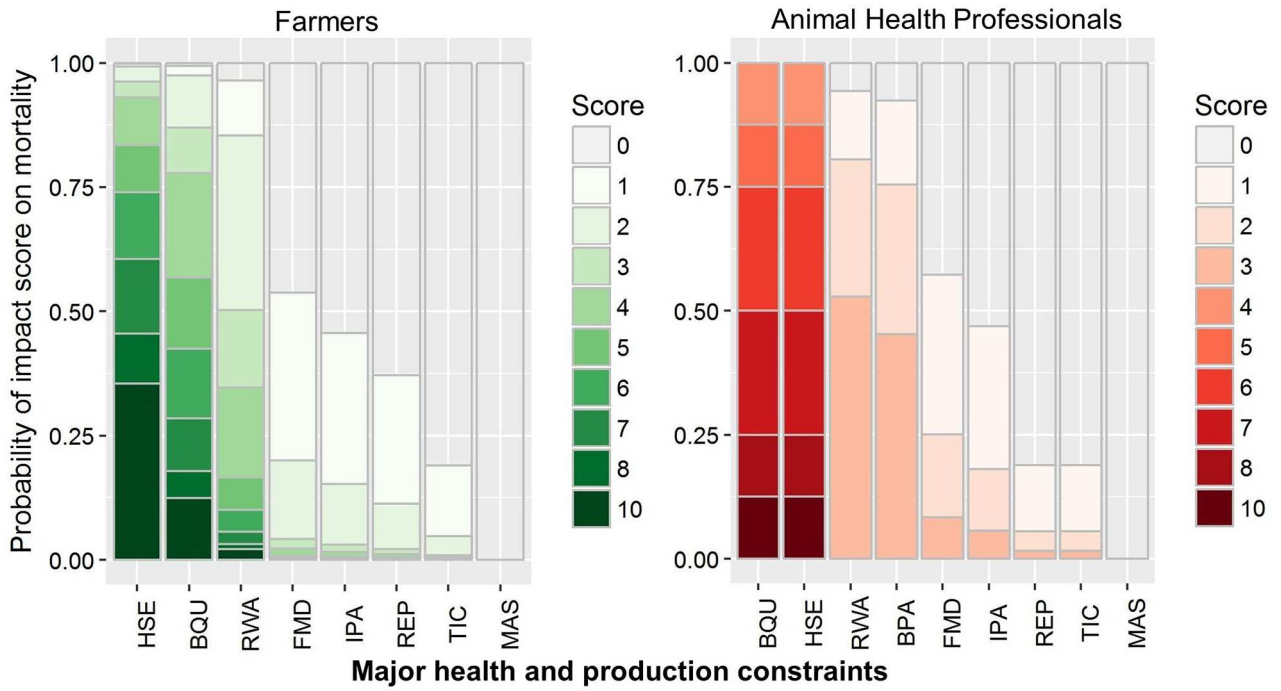

FIGURE 5 | Grouped stacked bar plot of logistic regression of probability impact score on mortality caused by health and production constraints in cattle and buffaloes perceived by small-scale dairy farmers and animal health professionals in Pakistan. Major bovine health and production constraints include blood parasites (BPA), blackleg (BQU), foot-and-mouth disease (FMD), hemorrhagic septicemia (HSE), internal parasites (IPA), mastitis (MAS), Redwater (RWA), reproductive disorders (REP), and ticks (TIC).

of used engine oil, diesel, petrol and taramira oil (Eruca sativa) on animal's body besides periodical use of acaricides (such as avermectins, cypermethrin, trichlorofon). Hemorrhagic septicemia was identified as a disease with the highest mortality rate in young animals (6–24 months) during the rainy season. Hemorrhagic septicemia was mainly reported in both districts (Sukkur and Thatta) of Sindh due to the lack of vaccination. The main reproductive disorders were anestrus, repeat breeding and prolapse both in cattle and buffaloes. Redwater (hemoglobinuria) was ascribed to parturition, phosphorus deficiency and tick-borne pathogens.

Constraint profiling activity was also repeated with AHP to cross-examine farmer knowledge, and a strong agreement was found in responses between both groups. However, understandably, AHPs had better veterinary science knowledge about all aspects (causes, risk factors, clinical signs, treatment) of BH&P constraints compared with the farmers. Within AHPs, the knowledge level was comparable, but veterinary officers had a sound background in veterinary medicine as compared to veterinary assistants who possessed a comparatively better understanding of local names of constraints.

DISCUSSION

This study provides a detailed account of the important BH&P constraints that affect the livelihoods of small-scale dairy farmers in Pakistan. It also gives insights into the knowledge of farmers and AHPs about these constraints across different AEZs of the country. Previously, PE tools have been used mainly for animal health investigations in pastoral communities (51–56). Pastoral communities are common in African countries whereas small-scale mixed crop-livestock farming is common in South Asian countries like Pakistan and India which have two-thirds of global dairy farms and contributing a quarter of global milk production (5, 57).

The findings of this study are likely to reflect the situation in other provinces and neighboring countries because of similar farming and education systems. Inter-group bias was minimized by using the same research team to conduct all FGMs, despite all efforts, some information might have been lost. As only male farmers were involved in FGMs, some of the important information from women and/or children might have been lost. Furthermore, no data were collected about farmers' age, economic status, education, and their level of involvement in livestock-related activities; hence, no conclusions can be drawn about differences resulting from these factors. As all participants were volunteers from diverse socioeconomic backgrounds from different districts, the findings of this study should be interpreted with caution. Furthermore, the selection of villages was not random in this study due to limitations in accessing distant locations. It is envisaged that these challenges will be considered in the design of future studies.

Blackleg, FMD, hemorrhagic septicemia, internal parasites, mastitis, ticks and reproductive disorders were the main BH&P

TABLE 2 | Description of local names and clinical signs of major bovine health and production constraints described by farmers and animal health professionals.

Constraint	Local name(s)	Clinical signs	Season of occurrence
Anestrus	Wohry Nathi	Anestrus and frequent vaginal discharge	Year round
Anthrax	Kaary Wa	Sudden death and oozing of blood from natural orifices	Year round
Blackleg	Chaurry Maar, Garri, Goli Maar, Karo Wah Tangwari, Tangwara	Discoloration of meat (black), crepitation sound from rear leg muscles, fever, a hot area around swollen areas, lameness and sudden death	Apr-Aug
Foot-and-mouth disease	Munh Khur, Samarro, Muharro, Bhagiyo	Anorexia, drooling of saliva, fever, high mortality in calves, lameness, loss of production and vesicles on feet and in mouth	Feb-Apr, Sep-Nov
Hemorrhagic septicemia	Gal Ghotu, Ghand, Ghogho, Ghanday	Coughing, difficulty in breathing, fever, swelling of the neck region and sudden death	Jul, Aug, Dec
Internal parasites	Keeray, aig (Liver Fluke)	Diarrhea, hair loss, weakness, lacrimal discharge, loss of production, pendulous abdomen and submandibular edema	Year round
Mastitis	Angiyari, Changiyari, Munh Sarri, Phikriyo, Saaru,	Blood and clots in milk, fever, teat blockage/fibrosis, loss of production, pus in milk, salty taste, swollen udder/teats and ulcers on teats	Year round
Redwater (hemoglobinuria)	Ratt Mutra, Sarkan, Saro	Blood in urine, constipation, frequent defecation (in small amounts), fever, loss of appetite, a low temperature of hindquarters	Jan, Feb, Aug, Sep
Repeat breeding	Phiraal	Repeated heat signs, increased vaginal discharge and a very high or low body condition score	Year round
Ticks	Chichar, Katway	Anemia, rough body skin, itching, skin lesions, ticks visible with naked eye and lassitude	Apr-Aug

constraints for small-scale dairy farmers. Ghaffar et al. (58) and Khan (59) used PE tools to investigate issues and infectious diseases of bovines in Punjab and found that FMD, hemorrhagic septicemia, blackleg and mastitis were the major issues. Our results from the southern irrigated zone (Sukkur, canal-irrigated) also showed that FMD and hemorrhagic septicemia were the most important infectious diseases of bovines. However, earlier studies did not focus on small-scale dairy farmers, and none of them investigated farmers' understanding of the constraints for the productivity of their animals, rather, their focus was on the surveillance of infectious or transboundary diseases of livestock. To the best of our knowledge, this is the first study that utilized four complementary PE techniques to investigate the knowledge of farmers and AHPs about BH&P constraints faced by small-scale dairy farmers in Pakistan.

We found that farmers prioritized constraints based on the mortality rate associated with the constraint/condition, whereas AHPs ranked constraints based on their economic importance. This finding is consistent with previous reports by Ali et al. (19) and Hussain et al. (60) from Pakistan and Chatikobo et al. (61) from Zimbabwe. This difference of prioritizing constraints could be because small-scale dairy farmers use bovine animals as their primary source of income and any bovine health constraint which leads to mortality in the herd would have impacted their income, thereby making them remember such constraints. Another plausible cause could be due to the lack of record keeping in small-scale dairy farming systems. Furthermore, it also means that farmers pay less attention to those health and production constraints which are subclinical and do not lead to mortality of animals. These constraints (e.g., ticks, tick-borne pathogens, internal parasites) could be crucial for improving the productivity of their animals. Future extension programs targeted at farmers should focus on educating them about endemic BH&P constraints which cause production losses round the year.

In Punjab province, farmers' description of important clinical signs of BH&P constraints matched with that given by AHPs. However, in Sindh province, farmers were unable to describe constraints/conditions clearly. This might be due to the lack of veterinary services, poor implementation of extension programs targeted at farmers, poor adoption rates of an existing extension program and lower education level of farmers, also identified by Ali et al. (62). Given that most of the small-scale dairy farmers in Sindh belong to very low-income communities where the role of women in livestock rearing is usually higher, men might possess less information about constraints to livestock production (63). Hence, the difference in responses by farmers from Punjab and Sindh could be due to the different levels of involvement of men in livestock-related activities, but no data were collected to support this observation.

In Pakistan, the livestock extension programs targeted at livestock farmers are outdated and are mostly male-oriented (64). Previous studies have shown a higher involvement of women in day-to-day animal-related activities which varied from region to region and were indirectly governed by farmers' economic status (40, 63, 65). Recently, Wynn et al. (40) reported that the current extension services in Pakistan cover only 40% of small-scale farmers, a majority of which are men who have a minimal role in day-to-day livestock activities and thus lead to poor adoption of extension messages. This is supported by Warriach et al. (17)

who implemented the whole-family approach (by involving men, women and children) for the extension program in Pakistan and found higher adoption rates leading to better animal health and on-farm benefits. Therefore, a whole-family approach for extension services might be a better approach to educate small-scale dairy farmers about health and production constraints for their animals.

In this study, the small-scale dairy farmers used ethnoveterinary medicines as the first choice of treatment for their animals because of high cost and perceived inefficacy of allopathic drugs, and in some cases unavailability of veterinary services in remote areas. A similar situation exists in many developing countries where resource-poor farmers are unable to afford the costly allopathic drugs and therefore, choose ethnoveterinary medicines that are cheap, easily available and have been used over generations (66). However, some infectious diseases (such as FMD and hemorrhagic septicemia) cause epidemics and require the use of broad-spectrum antibiotics and vaccines for the treatment and prevention (66). Therefore, it is essential to develop integrated approaches to control BH&P constraints faced by small-scale dairy farmers where the ethnoveterinary medicines practices are validated and conserved, and allopathic drugs and vaccines are also appropriately prescribed by AHPs.

Farmers informed that the introduction of new animal and lack of vaccines were responsible for FMD and hemorrhagic septicemia, respectively. Other causes identified by farmers were unhygienic conditions (mastitis), grazing practices (ticks), poor-quality water (liver fluke), nutritional deficiencies (reproductive disorders), and calving (redwater) or unknown agents. Farmers were able to describe the seasonal occurrence of BH&P constraints. They were dependent upon government-funded vaccination schemes in Punjab whereas, in Sindh, such schemes are not common and small-scale dairy farmers in remote areas do not have access to such schemes or veterinary services. These results show that small-scale dairy farmers had a good understanding of the common BH&P constraints. However, our results are contrary to those of Arif et al. (67) who found that most of the farmers did not know about brucellosis and its zoonotic potential. This difference could be due to limited knowledge of farmers about zoonoses and their importance *versus* important BH&P constraints which directly affect their livelihoods.

FMD is a highly contagious disease, and despite the use of local and imported vaccines, it is the most prevalent disease of bovines in Pakistan (68–70). However, farmers ranked FMD second to hemorrhagic septicemia or blackleg because of low mortality rate, particularly in adult animals. Similar findings were reported by Bellet et al. (71) who found that farmers ranked FMD second to hemorrhagic septicemia as it caused lower mortality in Cambodia. Interestingly, in the Indus delta in Sindh, FMD is considered as a fever-syndrome as well as a sign of good fortune for the animals, and farmers inoculate their animals with saliva or soil from hooves of infected animals. Although this practice leads to active immunization, it may also lead to clinical disease due to pathogenic strains of the FMD virus. Another practice to control FMD in Punjab was keeping or burning a camel's bone in the herd as farmers believed that FMD did not affect camels. Although the dromedary camels are not susceptible to FMD and do not transmit it to other animals (72), there is no scientific evidence to support this myth of keeping or burning camel bones in the herd to prevent FMD. Ferrari et al. (73) and Jemberu et al. (74) estimated the economic impact of FMD in Pakistan and Ethiopia, respectively, and found that high economic losses occurring mainly due to loss of milk yield could be prevented by adopting timely appropriate preventive measures such as vaccination and biosecurity measures.

Currently, Pakistan is at stage-2 of FMD Progressive Control Pathway (PCP) which commenced in 2008, in collaboration with FAO and OIE (75). The present study demonstrates that farmers usually lack knowledge about risk factors for transmission of FMD and engage in several risky practices. In order to advance to stage-3 of FMD PCP, an extensive farmer extension program should be implemented in the country, providing critical information on routes of transmission and risk factors. Additionally, designing and implementing an FMD simulation model would also be pivotal for controlling losses due to FMD in Pakistan and other endemic countries (76).

In this study, both farmers and AHPs ranked mastitis as one of the major causes of losses on small-scale dairy farms in Pakistan where it is highly prevalent in dairy animals (59, 77, 78). Small-scale dairy farmers treat mastitis with ethnoveterinary medicines such as garlic and red chili, and they do not contact the AHPs until the chronic stage of mastitis develops due to the high cost of allopathic drugs.

Gastrointestinal parasitism was also ranked as a major cause of economic losses to small-scale dairy farmers across all AEZs. Farmers reported liver fluke (*Fasciola hepatica* and *F. gigantica*) infestation with in both AEZs of Sindh province. This could be due to the location of these areas along the river-bank, which provide suitable environment for the intermediate host (snail) of liver flukes (79). In Sindh, small-scale dairy farmers have low income, and they cannot afford to deworm their animals for liver fluke. Like in Punjab, scheduled deworming programs for livestock in Sindh can help to reduce production losses associated with gastrointestinal parasitism in dairy animals.

Farmers and AHPs considered tick infestation as an important BH&P constraint in all AEZs. The clinical signs of tick infestation mentioned were visibility of ticks with the naked eye, general weakness of animals and skin lesions caused by bites. Farmers believed that tick infestation was endemic throughout the year due to inefficacy of available acaricides and production losses were caused mainly due to the blood sucked by ticks. Previously, poor husbandry practices have been found to be associated with tick infestation on small-scale dairy farms (80–82). Manual grooming is a widely practiced method by small-scale farmers for tick control; however, it is time-consuming and bears a risk of the transmission of zoonotic diseases such as Crimean-Congo hemorrhagic fever. Farmers did not perceive tick-borne pathogens as a threat (in contrast to AHPs) to their animals, although anaplasmosis, babesiosis and theileriosis are endemic bovine tick-borne diseases (TBD) in Pakistan (83). Recent investigations have demonstrated that bovine ticks carry a diverse array of pathogens, some of which can be zoonotic (84). Losses

due to TBDs could be very high in Pakistan, particularly on resource-poor farms. Recently, Sungirai et al. (85) investigated farmers' perceptions about TBDs in Zimbabwe and found that 67% of farmers were able to describe TBDs with signs. The high level of TBDs awareness resulted in farmers engaging in precautionary measures such as regular acaricidal dipping of their animals. Similarly, small-scale dairy farmers in Pakistan should be provided with information about the role of ticks as vectors and the adverse impact of ticks and TBDs on the health of their animals.

CONCLUSION

Despite the pivotal contribution of bovines to the food and livelihood of small-scale dairy farmers of Pakistan, the productivity of cattle and buffaloes is limited by health and production issues, including blackleg, FMD, hemorrhagic septicemia, mastitis, ecto- and endo-parasites, and reproductive disorders. Farmers possess good knowledge about these constraints; however, they lack sufficient information about the risk factors about important BH&P constraints. There is no variation of constraints between/among different AEZs within the same province. In most cases, traditional (herbal) remedies are used to treat BH&P constraints. Farmers prioritize constraints that cause high mortalities, whereas AHPs consider economic losses for ranking constraints. This study emphasizes the need for simple extension programs using a whole-family approach by covering all critical aspects of BH&P constraints to prevent economic losses to small-scale dairy farmers.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Veterinary and Agricultural Sciences Human Ethics Advisory Group, The University of Melbourne (Ethics ID: 1748953). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AJ, AG, RG, DM, and MS designed the study. AG, MB, and AK conducted the study. HW provided logistic support for the field work. AG, MS, DM, and AJ analyzed the results. AG and AJ drafted the manuscript. All authors contributed to the manuscript revision, read and approved the manuscript.

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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.2020.00248/full#supplementary-material

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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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Spatial Trends in *Salmonella* Infection in Pigs in Spain

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Salmonella is one of the most important foodborne pathogens worldwide. Its main reservoirs are poultry and pigs, in which infection is endemic in many countries. Spain has one of the largest pig populations in the world. Even though *Salmonella* infection is commonly detected in pig farms, its spatial distribution at the national level is poorly understood. Here we aimed to report the spatial distribution of *Salmonella*-positive pig farms in Spain and investigate the presence of potential spatial trends over a 17-year period. For this, data on samples from pigs tested for *Salmonella* in 2002–2013, 2015, 2017, and 2019 as part of the Spanish Veterinary Antimicrobial Resistance Surveillance program, representing 3,730 farms were analyzed. The spatial distribution and clustering of *Salmonella*-positive pig farms at the province level were explored using spatial empirical Bayesian smoothing and global Moran's *I*, local Moran's *I*, and the Poisson model of the spatial scan statistics. Bayesian spatial regression using a reparameterized Besag-York-Mollié Poisson model (BYM2 model) was then performed to quantify the presence of spatially structured and unstructured effects while accounting for the effect of potential risk factors for *Salmonella* infection at the province level. The overall proportion of *Salmonella*-positive farms was 37.8% (95% confidence interval: 36.2–39.4). Clusters of positive farms were detected in the East and Northeast of Spain. The Bayesian spatial regression revealed a West-to-East increase in the risk of *Salmonella* infection at the province level, with 65.2% (50% highest density interval: 70–100.0%) of this spatial pattern being explained by the spatially structured component. Our results demonstrate the existence of a spatial variation in the risk of *Salmonella* infection in pig farms at the province level in Spain. This information can help to optimize risk-based *Salmonella* surveillance programs in Spain, although further research to identify farm-level factors explaining this pattern are needed.

Keywords: *Salmonella*, pigs, spatial analysis, spatial modeling, BYM model, BYM2 model, Stan, Bayesian penalized regression

INTRODUCTION

Salmonella infection is one of the most important foodborne zoonoses worldwide. There were 91,662 confirmed human salmonellosis cases in 2017 in the European Union (EU) (1) and ∼1.2 million estimated cases occur every year in the US (2). Salmonellosis, characterized by acute onset of fever, abdominal pain, diarrhea, and nausea, is usually self-limiting. However, sometimes it can be life-threatening, especially in children, elderly and immunosuppressed patients, thus requiring antimicrobial therapy (3). This can be further complicated by the presence of antimicrobial-resistant strains (4).

Poultry is considered a major source of foodborne salmonellosis globally (5), but pork and pork products are also implicated in a large number of cases in many countries, including Spain (6, 7). Two of the most common *Salmonella* serotypes in pigs, *S.* Typhimurium and its monophasic variant (1,4,[5],12:i:-), were among the top serotypes associated with human salmonellosis in Spain (1). Moreover, the European Food Safety Authority (EFSA) baseline reports on *Salmonella* infection in fattening and breeder pig farms in Europe demonstrated that Spain had one of the highest levels of infection in pigs among EU countries, further highlighting their potential role in the occurrence of human salmonellosis in Spain (8, 9).

Still, due to the absence of a national control/monitoring program for *Salmonella* in pigs in Spain, little is known about the spatial distribution of *Salmonella* infection in pigs in Spain. The only available data came from specific studies with a generally limited geographical and temporal scope (10–12). The Spanish Veterinary Antimicrobial Resistance Surveillance Network program (13), starting in 1997, has performed nationwide surveillance of antimicrobial resistance originating from foodborne bacteria, such as *Escherichia coli*, *Campylobacter* spp., and *Salmonella* spp. Active surveillance of antimicrobial resistance in *Salmonella* in healthy pigs has been conducted through this program since 2002. Even though pigs from over 164 farms were sampled every year at the abattoirs since the beginning of the program, the spatial distribution of positive farms has never been evaluated.

Geographic information systems and some spatial statistical analyses have been applied to epidemiological research on *Salmonella* in farm animals. These approaches have allowed the detection of patterns and clusters of infection and prediction of occurrence and risk of infection of *Salmonella* under different situations. *K*-function analysis (14, 15) and Moran's *I* (16, 17) have been used to detect spatial clustering in *Salmonella* infection in farm animals, and a Gaussian kernel function has been used to predict the occurrence of *Salmonella*-infected dairy cattle and pig herds (18, 19). Using human cases, Simpson et al. (21) used the Besag-York-Mollié (BYM) hierarchical model (20) to map the cases of *S.* Wangata and *S.* Typhimurium in New South Wales, Australia (21). However, the usefulness of this method to better understand *Salmonella* infection in pigs seems not to be explored yet.

A BYM model contains two spatial random effects, often called spatially structured and unstructured components. The structured component has an intrinsic conditional autoregressive (CAR) prior that takes the geographical contiguity into account (correlated heterogeneity). The geographical contiguity is described by the neighborhood relationships between each pair of areas and a full spatial dependency. The unstructured one is a random effect for non-spatial heterogeneity at the same area level as the structured component. Riebler et al. (22) proposed a parameterized BYM model—the BYM2 model to address some limitations of the original BYM model. Briefly, the BYM2 model adopts the penalized complexity framework that favors a model whose parameters have clear interpretations and thus facilitates the use of sensible hyperparameters in the model (23). The BYM2 model combines the two spatial components in the original BYM model into a single spatial component and allows a parameter to describe the proportion of the variance explained by the structured component.

In the current study, we aimed to evaluate the spatial distribution and potential spatial trends of *Salmonella* infection in pig farms in Spain. To do so, we analyzed the data on *Salmonella* detection in samples from pigs across a 17-year period, derived from the Spanish Veterinary Antimicrobial Resistance Surveillance Network program, using several spatial analytical techniques, including a BYM2 model.

MATERIALS AND METHODS

Study Population and Data Collection

Data on samples collected for monitoring antimicrobial resistance in *Salmonella* in pigs from 2002 to 2013, 2015, 2017, and 2019 (sampling was conducted every two years since 2015) were derived from the database of the Spanish Veterinary Antimicrobial Resistance Surveillance Network program. In the program, samples from fattening pigs were randomly collected in abattoirs selected based on their slaughter capacity. Each year, selected abattoirs altogether added up to more than 50% of the national slaughter capacity and were located in no less than half of the autonomous communities of the country. The total number of pigs sampled from each abattoir was proportional to their slaughter capacity and was randomly allocated to farm batches being culled on the sampling date. Animal samples consisted of at least 25 g of the content of caecum from two pigs selected at random from those coming from the same farm, except for 2011 when at least 15 g of ileo-caecal lymph nodes of one animal per farm were collected. Samples were collected by trained personnel, put in a clean container, and stored at refrigeration (3–8°C) until being sent to the laboratory within the next 36 h. *Salmonella* culture was performed immediately after reception.

Bacteriology

Salmonella isolation was performed according to ISO 6579:2002/Amd 1:2007, the method recommended by the European Union Reference Laboratory for *Salmonella* in fecal and environmental samples [15]. Briefly, samples were cultured in buffered peptone water (BPW, 1/10 dilution; bioMérieux, Marcy-l'Étoile, France), followed by incubation at 37 ± 1°C for 18 ± 2 h. Modified semi-solid Rappaport-Vassiliadis (MSRV; Becton Dickinson France, Le Pont-de-Claix, France) agar plates

were then inoculated with three drops (i.e., 0.1 ml) of BPW culture. Plates were incubated at 41.5 ± 1°C for 24 ± 3 h and, if negative, incubated for an additional 24 ± 3 h. Suspected growth of *Salmonella* was confirmed by plating out to both Xylose Lysine Desoxycholate agar (XLD; bioMérieux) and on chrom ID™ *Salmonella* agar (SM ID2; bioMérieux) for incubation during 24 ± 3 h at 37 ± 1°C.

Columbia 5% sheep blood agar (bioMérieux) was used for the incubation of colonies of presumptive *Salmonella* that were subcultured for 24 ± 3 h at 37 ± 1°C. All *Salmonella* isolates were confirmed by a commercial, biochemical method Enterotube™ II (BD BBL™; Becton Dickinson GmbH, Heidelberg, Germany). Serological typing was performed based on the White-Kauffmann-Le Minor scheme (24).

Data on Potential Risk Factors (Covariates)

The potential of production-related characteristics to explain at least part of the observed spatial patterns of the risk of *Salmonella* infection in pig farms was assessed in the Bayesian spatial modeling (22 variables, **Table 1**) at the province level. These included (a) the number, the proportion, and the density (average number per square kilometer) of pigs belonging to different production categories (i.e., piglets, weaners, fattening pigs, gilts, sows, and boars) in each province and (b) the number and density of pig farms in each province. The pig-related information from 2005 to 2019 was collected from the website of the Ministry of Agriculture, Fisheries, and Food of Spain, and averaged over the study period for each province. The information about pig farm distribution was only available for 2016.

Statistical Analyses

Data cleaning, manipulation, and analyses were performed in Microsoft Excel 2013 (Microsoft Corp.), and R program version 3.5.2 (25) in RStudio interface version 1.2.1330 (26). Descriptive analyses were facilitated by "tidyverse" package (27). The location of all farms from which the sampled pigs originated was available at the province level and used for the following analyses.

The overall, yearly and province-level proportion of *Salmonella*-positive farms was calculated. Empirical Bayesian smoothing was then performed on the proportion of *Salmonella*-positive farms at the province level to incorporate information on the sample size and the proportion of *Salmonella*-positive farms of neighboring provinces by using the "spdep" package (28). Gabriel Graph was used to describe the neighboring relationships between the provinces throughout the study. In addition, the overall and yearly proportion of farms positive to specific *Salmonella* serotypes over the total number of positive farms were calculated for serotypes with >50 isolates over the study period. Empirical Bayesian smoothing, as previously described, was also used to map the proportion of farms positive to these *Salmonella* serotypes over the total number of positive farms at the province level.

The presence of global and local spatial autocorrelation in the spatial distribution of *Salmonella* positive farms was explored using global and local Moran's statistics (29, 30). A global and local Moran's *I*-tests were run on the standardized residuals of a Poisson model using the number of positive farms in each province as the outcome and the expected number of positive farms as the offset with the "spdep" package (28). The significance of the global Moran's *I* statistic was estimated through 999 Monte Carlo simulations in which the residuals were randomly shuffled across provinces. For local Moran's *I*, the *P*-values were calculated using the expectation and variance and corrected with

TABLE 1 | Univariable generalized linear regression results for the risk of *Salmonella* infection in pigs at the province level in Spain from 2002 to 2013, 2015, 2017, and 2019.

Name	Mean	Standard deviation	95% posterior probability	Change in risk with every specified unit of increase (95% credible interval)
Number of farms	0.08	0.03	(0.03 to 0.13)	1.010 (1.004 to 1.016)[1]
Density of farms (per km^2)	0.07	0.02	(0.03 to 0.12)	1.014 (1.005 to 1.023)[2]
Number of fattening pigs	0.06	0.02	(0.03 to 0.10)	1.002 (1.001 to 1.003)[3]
Number of sows	0.06	0.02	(0.01 to 0.10)	1.010 (1.002 to 1.018)[3]
Number of piglets	0.05	0.02	(0.01 to 0.08)	1.002 (1.000 to 1.004)[3]
Number of weaners	0.06	0.02	(0.03 to 0.09)	1.003 (1.001 to 1.005)[3]
Number of gilts	0.05	0.02	(0.01 to 0.09)	1.040 (1.009 to 1.068)[3]
Number of boars	0.04	0.04	(−0.04 to 0.11)	1.262 (0.787 to 1.953)[3]
Total number of pigs	0.06	0.02	(0.02 to 0.10)	1.001 (1.000 to 1.001)[3]
Proportion of fattening pigs	0.08	0.04	(0.00 to 0.15)	1.009 (1.001 to 1.018)[2]
Proportion of sows	−0.11	0.06	(−0.24 to 0.00)	0.989 (0.976 to 1.000)[2]
Proportion of piglets	−0.08	0.03	(−0.15 to −0.02)	0.989 (0.981 to 0.997)[2]
Proportion of weaners	0.09	0.03	(0.02 to 0.15)	1.016 (1.004 to 1.028)[2]
Proportion of gilts	−0.06	0.06	(−0.19 to 0.04)	0.976 (0.929 to 1.017)[2]
Proportion of boars	−0.09	0.06	(−0.22 to 0.03)	0.799 (0.566 to 1.091)[2]
Density of fattening pigs	0.07	0.02	(0.04 to 0.11)	1.003 (1.002 to 1.005)[4]
Density of sows	0.05	0.02	(0.01 to 0.09)	1.011 (1.002 to 1.020)[4]
Density of piglets	0.05	0.02	(0.01 to 0.08)	1.002 (1.000 to 1.004)[4]
Density of weaners	0.08	0.02	(0.04 to 0.12)	1.005 (1.002 to 1.008)[4]
Density of gilts	0.06	0.02	(0.01 to 0.10)	1.050 (1.012 to 1.089)[4]
Density of boars	0.05	0.03	(−0.02 to 0.11)	1.643 (0.813 to 3.211)[4]
Density of pigs	0.07	0.02	(0.03 to 0.11)	1.001 (1.000 to 1.002)[4]

[1] *Every 100 farms of change.*
[2] *Every 1% of change.*
[3] *Every 10,000 animals of change.*
[4] *Every 1 unit of change.*

the method described in Benjamini and Hochberg (31). The significance level for all the tests in the current study was set at 0.05.

Additionally, the Poisson model of the scan statistic was also applied to detect the presence of provinces with an increased risk of *Salmonella* infection using the centroid of each province as the point location, facilitated by the "SpatialEpi" package (32, 33). The scan statistic detects the maximum likelihood ratio between the value inside and outside a searching window over the likelihood function under the null hypothesis of complete spatial randomness (32). The search was performed using circular spatial moving windows that contained up to 25% or 50% of the total population alternatively. The pseudo *P*-values of the most likely clusters were generated by comparing the observed risk in the windows with the expected, generated through 999 Monte Carlo simulations in which the risks at each location were randomly allocated.

Bayesian spatial modeling to assess the associations between the risk of *Salmonella* infection at the province level and the available covariates was performed in Rstudio using Stan and associated packages (34). The number of observed *Salmonella*-positive pig farms in different provinces was assumed to follow a Poisson distribution, with the expected number of positive farms in each province as the offset. Regression models were fitted using the "brms" package (35). To explore the directionality of the association between available covariates and the outcome, univariable non-spatial models were first fitted introducing alternatively each of the covariates. For covariate selection, the predictive projection technique proposed by Piironen et al. was implemented using the "projpred" package (36, 37). The selection process contained two steps. First, a Bayesian penalized regression model with a regularized horseshoe prior and all the covariates was constructed as the reference model that warranted a good prediction ability. Penalized regression is a statistical technique designed to avoid overfitting, especially in cases of a large number of covariates (38). This is achieved through the introduction of a penalty term that shrinks small coefficients toward zero while leaving large coefficients large. The implement of penalized regression is rather intuitive within a Bayesian framework as a penalty term can be included as a hyperprior, also called shrinkage prior (38). Many shrinkage priors have been proposed, and a regularized horseshoe prior was chosen for the current study due to the advantages discussed in Piironen and Vehtari (39). Second, the covariates in the best model for each submodel size were identified by decreasing the Kullback–Leibler divergence from the reference model to the projected submodel using a forward stepwise addition. A submodel with the minimal subset of these covariates which had similar predictive power as the reference model, judged by the mean log predictive density and the root mean square error, was selected.

The BYM2 Poisson model including the selected covariates and the spatial components (Equation 1) was then fitted (22). To examine the suitability of alternative distributions to fit the data, another three BYM2 models were fitted with different likelihoods (i.e., zero-inflated Poisson, negative binomial and zero-inflated negative binomial) followed by model selection using Bayesian leave-one-out cross-validation (40, 41). The default priors specified in the "brms" package were used in the current analyses. Sampling was drawn from four Markov chains with 1,000 iterations. The results final model reported in the next section were sampled from four Markov chains with 3,000 iterations. Half of the iterations were for warm-up (i.e., burn-in) and not used for inference.

Markov chain Monte Carlo diagnostics for the final model were performed with (a) the potential scale reduction statistic ($\hat{R}$) (42), (b) the ratio of the effective sample size to the total sample size drawn from the posterior distribution, and (c) trace plots of Markov chain Monte Carlo generated through the "bayesplot" package (43). Residual check and posterior predictive checks were also performed using the "bayesplot" package (43, 44). The highest density interval of the posterior distribution was estimated using the "bayestestR" package (45).

Equation 1. Spatial component in a BYM2 model.

$$T = \frac{1}{\sqrt{T_t}}(\sqrt{p/f} * S + \sqrt{1-p})$$

- T : *total spatial component*,
- τ_t : *precision for the total spatial component*; $\frac{1}{\sqrt{T_t}}$ is the overall standard deviation,
- $p\epsilon$ [0, 1]: the proportion of the variance explained by the spatially structured component,
- f : scaling factor,
- S : spatially structured component,
- U: spatially unstructured component.

For the variables potentially associated with the *Salmonella* detection (see below), the existence of major changes in their spatial distribution over the study period was evaluated using Friedman tests on the province-level yearly data, followed by pairwise Wilcoxon rank sum tests with *P*-values adjusted for multiple comparisons (46).

RESULTS

Up to 3,730 samples collected over the 15 years in which sampling was conducted, representing the same number of farms, were included in the current study, with an average of 249 (range: 163–384) samples per year. The number of abattoirs where the samples were collected each year, except for 2019 when this information was not available, ranged between 7 and 20. A median of 18 samples (interquartile interval: 11–29, range: 1–60) were collected from each abattoir each year during the study period. Abattoirs were located in 11 out of the 18 autonomous communities in Spain, and 977 (29.2%), 670 (20.0%) and 455 (13.3%) samples came from abattoirs in Cataluña, Castilla La Mancha and Murcia, respectively (**Supplementary File 1**). The sampled farms were located in 43 out of the 52 provinces in Spain; 502 (13.5%) were from Murcia, 371 (9.95%) from Huesca, and 334 (9.0%) from Lleida.

A total of 1,409 of the 3,730 samples were positive, yielding an overall percentage of *Salmonella*-positive farms of 37.8% (95% confidence interval [CI]: 36.2–39.4). This percentage peaked between 2004 (54.2%) and 2006 (53.3%), declined to 29.7% in

2012, and increased again to 54.1% in 2019 (**Figure 1**). The raw and smoothed proportion of *Salmonella*-positive farms at the province level is shown in **Figure 2**. In the eleven provinces with more than 100 samples, the spatially adjusted proportion ranged from 17.8 % (95% CI: 13.1–22.2) in Toledo to 44.8% (95% CI: 35.4–54.2) in Almeria.

The serotype of 1,360 (96.5%) out of the total 1,409 *Salmonella* isolates recovered was determined, yielding 64 distinct serotypes (**Supplementary Table 1**). The most represented were *S.* Rissen (313, 22.2%), *S.* 1,4,[5],12:i:- (265, 18.8%), *S.* Typhimurium (251, 17.8%), and *S.* Derby (207, 14.7%). The evolution of the proportion of isolates belonging to specific serotypes over the study period varied (**Figure 3**). The proportion of *S.* Typhimurium decreased after 2010, while the proportion of the *S.* 1,4,[5],12:i:- has consistently increased over the years. The proportion of *S.* Rissen remained consistently around 0.3 after 2005, and, for *S.* Derby, a decreasing trend was observed (from slightly lower than 0.3 to <0.2 from 2012 onwards). After the empirical Bayesian smoothing, the proportions of *Salmonella* isolates identified as the *S.* 1,4,[5],12:i:- and *S.* Derby were higher in the provinces of Northeast and South of Spain, respectively (**Figure 4**). Both the proportions of *S.* Rissen and the *S.* 1,4,[5],12:i:- were rather homogeneous across provinces in Spain but slightly lower at the northwest corner and the South of Spain, respectively.

No global (Moran's $I = -0.02$, $p = 0.458$) or local spatial autocorrelation was detected in the standardized residuals of the Poisson model. The spatial scan statistics identified local clusters with an increased risk of *Salmonella* infection in the East and Northeast of Spain (**Figure 5**). The observed-to-expected ratio between inside and outside of the significant clusters identified using a search window of maximum 25 and 50% population was 1.17 ($P = 0.036$) and 1.13 ($P = 0.001$), respectively.

According to the univariable models, provinces with a higher number or density of pig farms showed a higher risk of *Salmonella* infection (**Table 1**). In general, covariates related to the population of fattening pigs, weaners, sows or piglets were

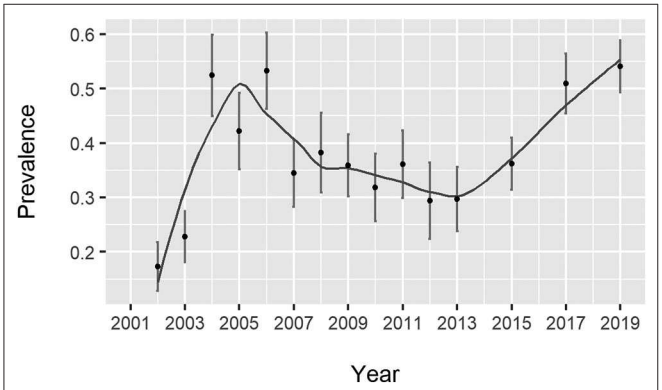

FIGURE 1 | Annual proportion of *Salmonella*-positive farms in Spain from 2002 to 2013, 2015, 2017, and 2019. The line was smoothed by a locally estimated scatterplot smoothing with a span of 0.5.

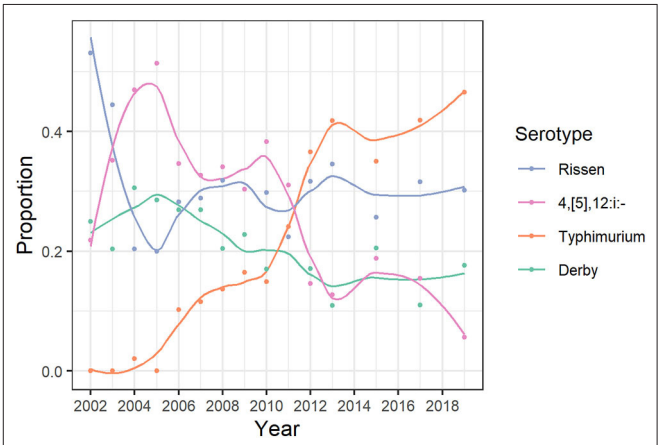

FIGURE 3 | Changes in the proportion of *Salmonella* isolates recovered through the Spanish Veterinary Antimicrobial Resistance Surveillance Network program belonging to one of the four most common serotypes collected in pigs in Spain from 2003 to 2013, 2015, 2017, and 2019. The lines were smoothed by a locally estimated scatterplot smoothing with a span of 0.5.

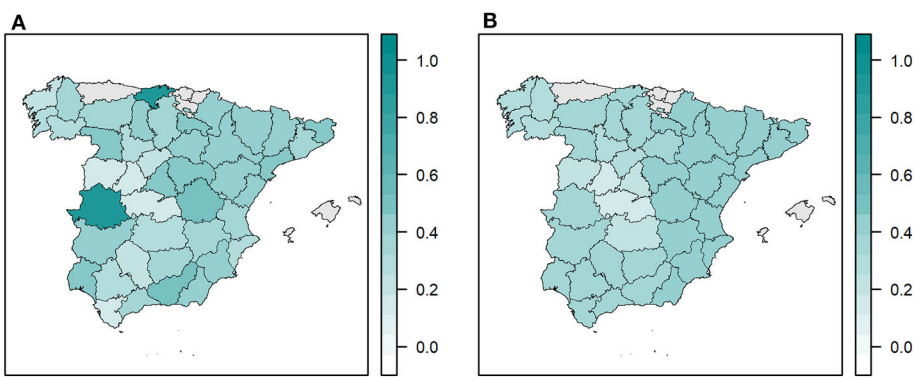

FIGURE 2 | Proportion of *Salmonella*-positive farms at the province level in Spain from 2002 to 2013, 2015, 2017, and 2019. **(A)** Raw proportion. **(B)** Spatially adjusted proportion using empirical Bayesian smoothing.

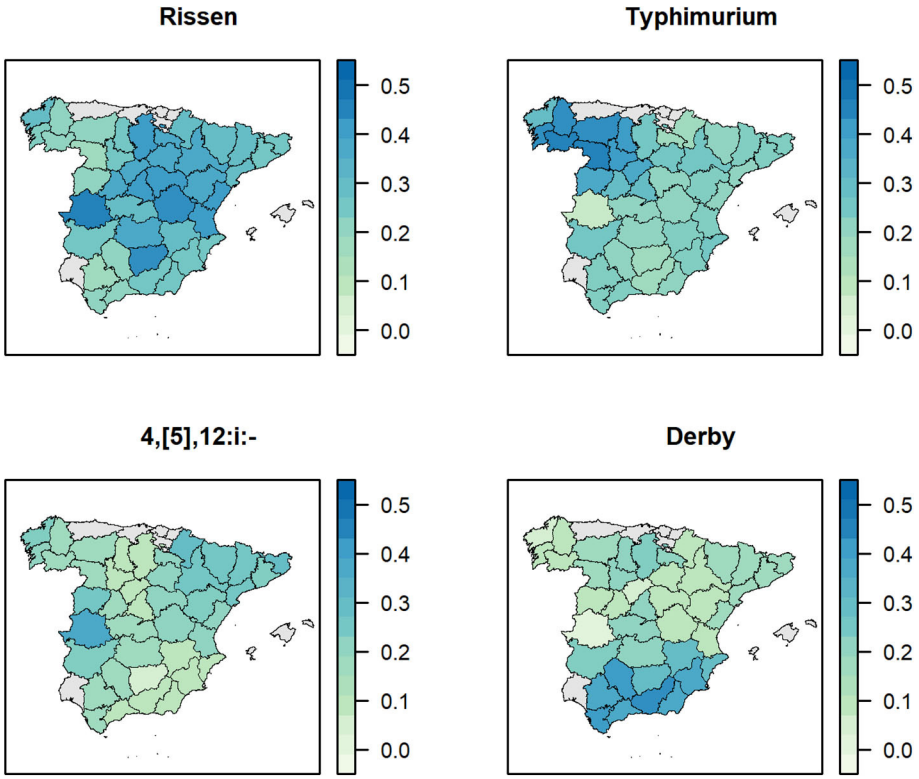

FIGURE 4 | Empirical Bayesian smoothed proportions of farms positive to the four most represented *Salmonella* serotypes collected through the Spanish Veterinary Antimicrobial Resistance Surveillance Network program in pigs in Spain from 2002 to 2013, 2015, 2017, and 2019.

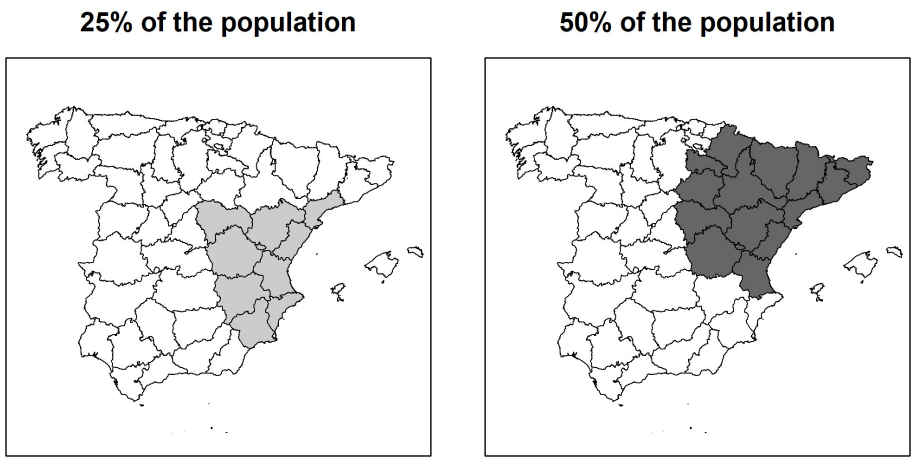

FIGURE 5 | Provinces included in the significant, high-risk *Salmonella* clusters detected by the Poisson model of the spatial scan statistics using data collected through the Spanish Veterinary Antimicrobial Resistance Surveillance Network program in pigs in Spain from 2002 to 2013, 2015, 2017, and 2019.

positively associated with the probability of finding positive *Salmonella* farms.

After predictive projection, only one covariate, the density of weaners, was selected to be included in the final Poisson BYM2 model (**Table 2**). More results of Bayesian penalized regression and predictive projection can be found in **Supplementary File 2**.

In the Poisson model including only the density of weaners without the BYM2 component, the probability of finding positive *Salmonella* farms at the province level increased by 0.5% (95% credible interval [CrI]: 0.2–0.8%) with every increase in the weaner density. However, the effect of this covariate shrank to close to 0 after the inclusion of the BYM2 component (**Table 2**).

TABLE 2 | Regression results from the final multivariable modeling for risk of *Salmonella* infection in pigs at the province level in Spain from 2002 to 2013, 2015, 2017, and 2019.

Variable	Mean	Standard deviation	95% posterior probability	Change of risk with very 1 unit of increase (95% credible interval)
Intercept	−0.07	0.05	−0.17 to 0.02	–
Density of weaners	−0.01	0.06	−0.13 to 0.10	−0.001 (−0.008 to 0.007)
Standard deviation of the spatial component	0.23	0.05	0.14 to 0.35	–
Proportion explained by the structured component	0.65	0.25	0.10 to 0.99	–

The spatial component in the final BYM2 model suggested a West-East increasing risk of *Salmonella* infection at the province level in Spain. The exponentiated means of the spatial effects are shown in **Figure 6**. The mean of the standard deviation of the spatial component was 0.23 (Crl: 0.14–0.35), and, on average, 65.2% (median: 70.2%, 50% highest density interval: 70–100%) of the variance of the spatial component was explained by the structured component. No specific pattern in the unstructured spatial component was observed.

Markov chain Monte Carlo and model diagnoses are presented in **Supplementary File 3**.

The density of weaners at the province level did not experience major changes over the study period, with higher values reported consistently for provinces in the Northeast corner (**Supplementary File 4**). Still, the Friedman test revealed the existence of significant ($P < 0.001$) differences in the yearly values over time, although *post-hoc* tests revealed that differences were only due to values recorded in 2017 and 2019The test became non-significant ($P = 0.071$) when values from 2005 to 2015 were used.

DISCUSSION

Human salmonellosis outbreaks have been linked to pork and pork products in the past (1, 5, 7). In many regions and countries such as Northern and Western Europe and Japan, pork, and pork products are the second most common source for human salmonellosis after eggs and egg products (5). In Spain, pork and pork products have been shown to be one of the top-ranked sources for human salmonellosis (7). However, currently, there is no official control program of *Salmonella* in pig production in Spain (47). In the current study, we used several analytical techniques to explore the spatial distribution of *Salmonella* infection in pigs in Spain and generate information that can be used for surveillance of *Salmonella* in pigs in the future.

We detected an overall percentage of *Salmonella*-positive farms of 37.8% with great variation across the years. Several studies have reported even higher values of farm-level *Salmonella* prevalence in Catalonia (77.3% in 2000–2003), northeast Spain [94.1% in 2008–2009 (12)], and the entire country [43.1% in Spain in 2003–2004 (11)]. The lower values suggested by the current study could be partially due to the inclusion of feces from only two pigs per farm. Thus, the probability of detecting the presence of *Salmonella* at the farm level, particularly in farms with a low within-farm prevalence, was not as high as in the aforementioned studies. Nonetheless, the spatial heterogeneity found in the current study agrees with these previous results reporting higher prevalence values in the Northeast of the country, where a large proportion of Spanish pig population is located.

Our results indicated that the recent increase in the percentage of *Salmonella*-positive farms could be related to the increasingly reported *S.* 1,4,[5],12:i:-. During the study period, the proportion of isolates belonging to this serotype steadily rose and reached 0.47 in 2019, in parallel to the increase in the yearly percentage of *Salmonella*-positive farms (from 29.4 in 2012 to 54.1 in 2019). The importance of the *S.* 1,4,[5],12:i:- in public health highlights the need for continuous monitoring on its prevalence in pigs in Spain and the EU at large (48).

Several factors may affect the determination of the *Salmonella* status of a farm when samples are collected at the abattoir. Many studies have shown a noticeably higher prevalence of *Salmonella* in samples from slaughtered pigs than the prevalence of the samples from the farm (49, 50). This could be due to the stress generated by the process of harvest, transportation, and retention in the lairage, resulting in the recrudescence of latent carriers and/or an increase in the susceptibility of pigs to new infections (49). Moreover, the long feed withdrawal normally performed before transport might change the gut microbiota and increase the number of *Salmonella* in the fecal content (51). On the other hand, an increase in the diversity of serotypes detected in the abattoir compared to those recovered from the farms of origin has been also described. This could suggest exposure to additional contaminated environments such as trucks and lairages (49, 52). Therefore, the true prevalence of *Salmonella* infection at the farm level may be lower than the detected/apparent prevalence based on samples collected at abattoirs, and the diversity of serotypes could be the result of *Salmonella* from both pig farms and places involved in the process between harvest and slaughtering.

Our results showed a clear pattern suggesting a higher risk of *Salmonella* infection in the (North-)east than in the rest of Spain (**Figure 6**). The BYM2 model showed that, on average, 65.2% of the spatial effect could be explained by the underlying geographical location of the provinces, and the highest density interval included even higher values. We considered several pig population-related covariates in the analysis given that their heterogeneous spatial distribution was in agreement to some extent to the results of the spatial scan statistic (i.e., more pigs and higher densities in the Northeast of Spain). The univariable modeling results showed that many covariates, especially the number of farms and those related to fattening pigs and weaners, were indeed positively associated with the risk. However, only one covariate, the density of weaners, was retained after the

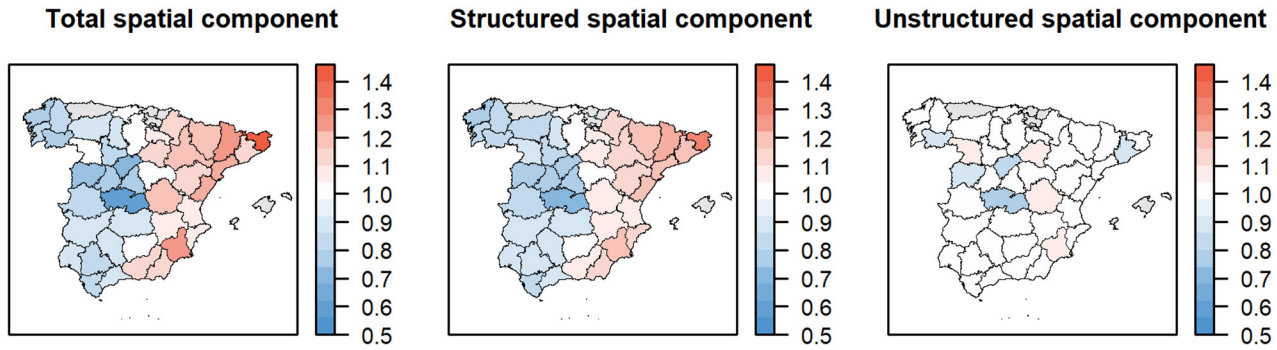

FIGURE 6 | The total (left) spatial risk of *Salmonella* infection in pigs at the province level in Spain from 2002 to 2013, 2015, 2017, and 2019 according to the Poisson BYM2 model and the risk explained by the structured (center) and unstructured (right) spatial components.

variable selection process. This was expected as many covariates were correlated. Nonetheless, the effect of the density of weaners became close to 0 after the inclusion of the BYM2 component. This suggested that the observed spatial distribution of risk was better explained by the geographical contiguity of the provinces than by the density of weaners and other pig-related factors.

The software used here, Stan, is a highly-expressive probabilistic programming language that allows full Bayesian inference using Hamiltonian Monte Carlo samplers (34), which have been shown more efficient and robust than Gibbs and Metropolis samplers (53). Although many R packages to facilitate Bayesian modeling in Stan have been developed (35, 54), it is yet to be much explored in the field of veterinary epidemiology. With Stan and "brms" package (35), BYM2 model can be performed by regular R users (22).

To our knowledge, the BYM2 model has been little utilized in the field of veterinary science. The BYM2 model has advantages over the original and some of the reparameterized BYM models (22). Firstly, the two components in a BYM2 model can be seen independently from each other, resulting in better estimation for both of them (55). Secondly, BYM2 models facilitate parameters that have clear interpretations and thus the use of sensible hyperparameters (23). Additionally, as the scaling factor in BYM2 models is placed to take into account the underlying neighborhood structure, studies with different neighborhood structure now can use the same hyperprior in the models (22). Therefore, the future application of the BYM2 model in veterinary epidemiology may be encouraged. A BYM2 model can also be performed with INLA (22).

In the current study, we employed a relatively unexplored approach for variable selection in veterinary epidemiology—predictive projection with a Bayesian penalized regression model as the reference model. Shrinkage methods have been recommended when the ratio of the number of observations to the number of variables is $\leq$10 (56), and predictive projection is useful in determining the number of variables to be included in the model (36). This technique has several advantages. First, it requires less computational power than cross-validation and is less time-consuming than using either cross-validation or information criteria. Second, selection among many models using cross-validation may tend to overfit and thus result in choosing a suboptimal model (36).

Here, a number of different spatial analytic tools were applied, offering different results. While the global and local Moran's *I*-tests ran on the residuals of a Poisson model did not suggest the existence of a spatial pattern in the distribution of the *Salmonella* risk at the province level, the spatial scan statistic and the Bayesian spatial model showed the opposite. This finding suggests that, rather than making a conclusion of spatial independence based on one test, the application of more than one spatial analytic test, based on different hypotheses and assumptions, can provide a more complete picture.

The current study has some limitations. First, as the sample collection was conducted in abattoirs that have high slaughter capacity, the results might not be necessarily representative of the farms that did not (usually) send their pigs to those abattoirs. Second, the current study was conducted using secondary data. Therefore, it may face some common issues of using secondary data, such as out-of-date information, suboptimal sampling procedure for answering specific research questions, insufficient sample size, and lack of information that would be, otherwise, included. For example, the individual or within-farm prevalence of *Salmonella* infection could not be determined in the current study. Also, farm-specific information was not available so could not be included in the models. Farm-specific covariates such as farms types, and management and biosecurity information, will likely be associated with the outcome and allow prediction of the risk. Furthermore, for the multivariable modeling exercise, farm-related information was only available for 2016, and data of pig distribution was averaged across all years under study. Still, the exploration of the evolution of the pig distribution in Spain revealed no significant variation between provinces for at least most of the study period (2002–2015), suggesting that aggregating values across such a period would not result in a major loss of information. Lastly, it has been shown that the inclusion of a spatially-correlated component only after the covariate selection process may affect the results of the covariates in the model (57). This is observed in the current study. Ideally, the predictive projection should be conducted with the BYM2 component included. However, this

is currently unavailable in the "projpred" package and thus was not done.

CONCLUSION

The current study shows a notable increasing trend in the risks of *Salmonella* infection in pig farms located in provinces from West to East in Spain, evident still after the possible effect of the heterogeneous distribution of pigs in the country was accounted for. The increase in the percentage of *Salmonella* positive farms from 2012 and the *S.* 1,4,[5],12:i:- in Spain demonstrates the usefulness of surveillance to detect changes in the epidemiology of this foodborne pathogen in the animal reservoirs. We demonstrated the usefulness of Stan for various applications that are commonly pursued in a veterinary epidemiological study, such as covariate selection, model selection, and model fit assessment, as well as fitting a BYM2 model. The information generated by the current study can be used for risk-based *Salmonella* antimicrobial resistance surveillance programs in the future, so the probabilities of selecting positive farms and specific serotypes can be optimized. Although some temporal trends in the risk of *Salmonella* are shown in the current study, more data is needed to allow a better understanding of the spatial-temporal distribution and the evolution of *Salmonella* infection in pigs in Spain.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The current work did not involve any live animal nor human data. No ethical approval was required as all isolates analyzed were retrieved through the ongoing Spanish national surveillance program on antimicrobial resistance, performed according to national and EU regulations.

AUTHOR CONTRIBUTIONS

KT and JA contributed conception and design of the study. KT, MM, MU-R, CB, AT, GL, MAM, LD, and JA participated in the generation, collection, and curation of the data. KT performed the statistical analysis. KT, MM, JA interpreted the results. JA provided supervision. MU-R, GL, and JA facilitated project administration. KT wrote the first draft of the manuscript. MU-R and JA wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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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.2020.00345/full#supplementary-material

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Using Surveillance of Animal Bite Patients to Decipher Potential Risks of Rabies Exposure From Domestic Animals and Wildlife in Brazil

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Direct contact with domestic animals and wildlife is linked to zoonotic spillover risk. Patients presenting with animal-bite injuries provide a potentially valuable source of surveillance data on rabies viruses that are transmitted primarily by animal bites. Here, we used passive surveillance data of bite patients to identify areas with high potential risk of rabies transmission to humans across Brazil, a highly diverse and populous country, where rabies circulates in a range of species. We analyzed one decade of bite patient data from the national health information system (SINAN) comprising over 500,000 patients attending public health facilities after being bitten by a domestic or wild animal. Our analyses show that, between 2008 and 2016, patients were mostly bitten by domestic dogs (average annual dog bite patients: 502,043 [436,391–544,564], annual incidence per state: 258 dog bites/100,000 persons) and cats (76,512 [56,588–97,580] cat bites, 41 cat bites/100,000/year), but bites from bats (4,172 [3,351–5,365] bat bites, 2.3/100,000/year), primates (3,320 [3,013–3,710] primate bites, 2.0/100,000/year), herbivores (1,908 [1,492–2,298] herbivore bites, 0.9/100,000/year) and foxes (883 [609–1,086] fox bites, 0.6/100,000/year) were also considerable. Incidence of bites due to dogs and herbivores remained relatively stable over the last decade. In contrast bites by cats and bats increased while bites by primates and foxes decreased. Bites by wild animals occurred in all states but were more frequent in the North and Northeast of Brazil, with over 3-fold differences in incidence between states across all animal groups. Most bites reported from domestic animals and wildlife occurred in urban settings (71%), except for bites from foxes, which were higher in rural settings (57%). Based upon the Ministry of Health guidelines, only half of patients received the correct Post-Exposure Prophylaxis following a bite by a suspect rabid animal. We identified areas and species of high-risk for potential zoonotic transmission of rabies in Brazil and reveal that, despite increasing human encroachment into natural ecosystems, only patients reporting bites by bats increased. Our study calls for future research to identity the socio-ecological factors underlying bites and the preventive measures needed to reduce their incidence and potential risk of rabies transmission.

Keywords: primates, fox, bat, spillover, post-exposure, rabies exposures, public health, zoonoses

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INTRODUCTION

Direct contact with wild and domestic animals is a major driver of zoonotic spillover, defined as the "transmission of a pathogen from a vertebrate animal to a human" (1). Rabies virus (RABV) causes the deadliest known disease and is directly transmitted through the bite of infectious mammals (2, 3). Worldwide, the largest reservoir for rabies and cause of most human rabies deaths is domestic dogs (4). However, following the widespread control of rabies in domestic dog populations, rabies transmitted by wild animals has become the main source of human deaths in the Americas (5, 6). In particular, most human deaths and livestock losses from rabies in the continent are now attributed to spillover from vampire bats, *Desmodus rotundus* (6–8). Foxes (specifically the crab-eating fox *Cerdocyon thous*) and primates (specifically the marmoset *Callithrix jacchus*) are also considered reservoirs of specific RABV variants (9–12), while serological studies show evidence of rabies exposure in several other primate and marsupial species but without evidence of clinical infections (13, 14).

In large and biodiverse countries such as Brazil, rabies circulates among a wide range of wild species including bats, primates, and foxes, complicating the establishment of preventative measures aiming to limit rabies spillover to humans and domestic animals (15–17). The high cost and current uncertainties in the interpretation of serological data among wildlife has restricted the implementation of serology to a small number of wild populations and specific regions of the country (12). In the Northeast region, crab-eating fox and bats have been frequently found exposed or infected, while transmission of RABV from foxes to domestic dogs has been reported (16, 18). Marmosets are also host to a distinct RABV variant in the Northeast region of Brazil (9, 11). In the states of São Paulo and Rio Grande do Sul (RGS) in Southern Brazil, RABV circulation has been detected in vampire bats, insectivorous bats, capuchin monkeys, and crab-eating foxes (12–14, 19–21). These reports suggest the circulation of rabies among different wildlife populations in several regions of the country, posing risks to humans and domestic animals that will depend upon contacts between these populations. Between 2000 and 2017, Brazil reported 188 human rabies cases (22). Among the 46 cases where the rabies variant was identified, 27 cases originated from the common vampire bat variant including three that were transmitted by cats, three cases were from the marmoset variant and 16 cases were from the fox variant (22).

Identifying and reducing direct contact between wild and domestic animals and humans including awareness campaigns to limit contacts with wildlife (e.g., reduce wildlife feeding) may prevent human rabies exposures (23). Patients presenting with animal-bite injuries provide a valuable source of surveillance data on rabies viruses transmitted by bites, including potential exposure risks and evidence of circulation among different reservoir hosts (24). Publicly available data on these patients can allow identification of geographical areas with higher rabies risks that represent a burden to the country's national health system. Brazil's National Health System (SUS) provides universal health coverage to most Brazilians using a network of public hospitals and health facilities. Since 1998 Brazil has used a national health 'Information System on Diseases of Compulsory Declaration' (SINAN) recording all patients seeking medical care in public health facilities following direct contact with a suspect rabid animal. SINAN records several variables including information on the animal species responsible for the bite, the severity of the bite, whether the biting animal is suspected for rabies, and the Post-Exposure Prophylaxis (PEP) regimen administered to the patient (24, 25).

Using data from SINAN we recently reported that bites from dogs remained relatively stable over the last decade in Brazil and that use of PEP from putatively "rabies-free" states did not decrease despite rabies from domestic dog populations being close to elimination (24). One concern is that unnecessary PEP use may reduce resources available to address risks from the circulation of rabies in alternative wildlife reservoirs, such as bats or primates, which exist in several geographic localities. This is particularly concerning given that vaccine shortages that have been experienced in Brazil during recent years (24). However, bite incidence and the potential risk of rabies from wild animals in Brazil remains poorly understood. The goal of this study was to examine the burden of bites and rabies risk across Brazil posed by both domestic and wild species that are known to transmit rabies. We specifically aimed to (i) compare the incidence of bites from different species across states, (ii) evaluate temporal trends in bites from different species, (iii) assess the extent to which bite incidence is concentrated in urban or rural areas, and (iv) evaluate the appropriateness of PEP use for bites by different species.

METHODS

Data and spatio-temporal analyses used here are similar to those reported for dog bites in Brazil (24), although data on bites from cats, wildlife, and domestic herbivores have not been previously analyzed. Analyses on dog bites reported in **Figures 1**, **3**, **5** are presented in our previous work (24) but are compared here to bites from other domestic and wild animals. We obtained data from the "Individual Investigation Reports of Human Anti-rabies Care" form completed by public health workers in Brazil and submitted to SINAN, the national electronic system (26). This form can be completed by any health professional (doctor, nurse, technician) each time a patient seeks care at a public health facility following an animal bite. The form (available here in portuguese: http://portalsinan.saude.gov.br/images/documentos/Agravos/Atendimento%20Anti-rabico/anti_rabico_v5.pdf) includes 60 fields. The SINAN notification (electronic data) of the aggression (bite) must be sent weekly from municipality to state level, and every 2 weeks from state to federal level. All cases must be concluded within 60 days. The patient assessment includes identifying the species responsible for the bite, and the use and follow-up of PEP after assessment by a doctor or nurse.

Forms are divided into five sections including (i) general data on the health unit, (ii) patient characteristics, (iii) location of patient's residency and health care unit (e.g., municipality and

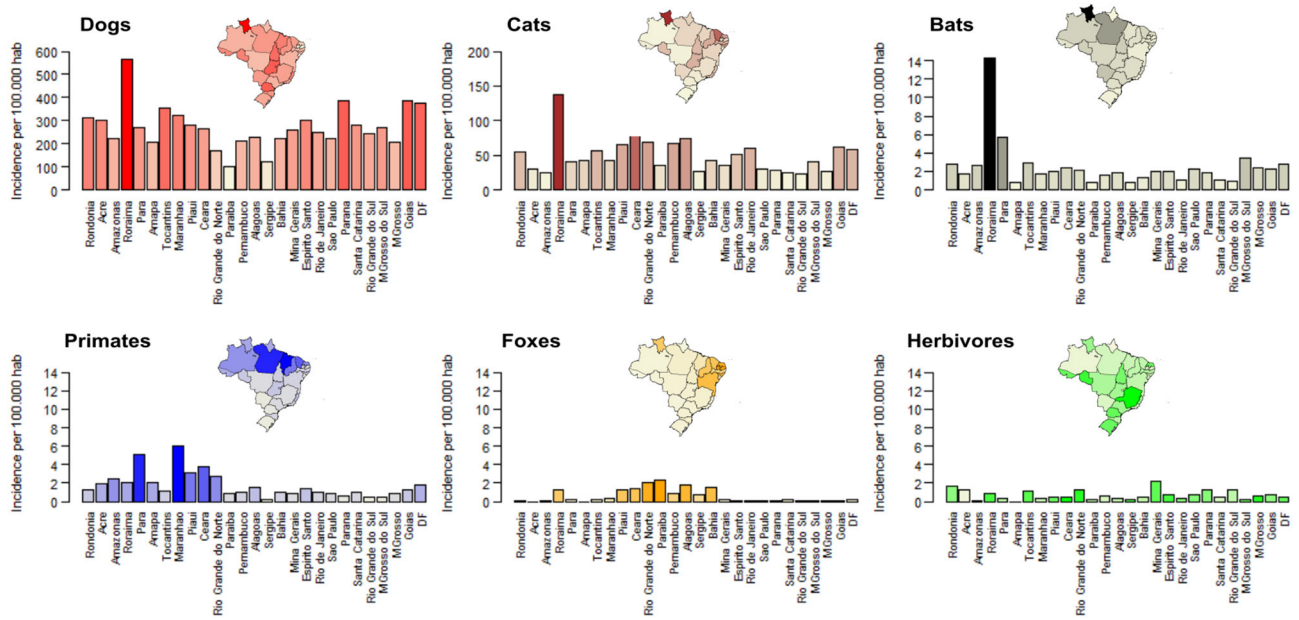

FIGURE 1 | Bite incidence by different species across Brazil in 2016. Each specie's incidence is shown by a different color. The darker the color on each bar, the higher the incidence. Colors of insert maps represent incidence for each state using the same colors as in the bar charts. For comparison, the *y*-axis in the barplots for wildlife ranges from 0 to 14.

state), (iv) epidemiological information including the animal responsible for the bite and the prior PEP history of the patient as well as (v) the PEP recommended and administered to the patient and the status of the animal after a potential 10-day observation period. Data were obtained for Brazil from 2008 to 2016 via an online request to the Ministry of Health through the Electronic System of Citizen's information (e-SIC, https://esic.cgu.gov.br/sistema/site/index.aspx, e-SIC request number: 2564134). Empty fields were assumed to have not been completed and were shown in the analysis as "no data available."

Data was analyzed using R 3.6.1 (27).

Temporal and Spatial Trends in Bite Incidence by Species

We first explored whether bite incidence, i.e., the number of patients seeking health care after a bite per 100,000 habitants, varied across states and between 2008 and 2016 according to the species of the biting animal. We calculated bite incidence as the number of completed reports divided by the total human population of each state, extracted from publicly available census data from the Brazilian Institute of Geography and Statistics (IBGE) (https://www.ibge.gov.br/). To determine whether different species pose a similar health burden across states we also tested for correlations in incidence. We used a non-parametric Spearman's correlation test with the *cor.test* function in R, since data was not normally distributed across states. Using the field indicating whether a patient was bitten in an urban or rural district, we further compared the incidence of bites for each species between urban and rural districts.

Administration of PEP According to Ministry of Health Guidelines

To evaluate if PEP is being effectively delivered in Brazil, we compared the PEP given to each patient recorded in SINAN in 2016 with the appropriate PEP for the same patient according to the Brazilian's Ministry of Health (MoH) prophylaxis guidelines from 2014, based upon the variables reported in SINAN. The SINAN form includes seven PEP recommendations: "Pre-exposure prophylaxis," "No prophylaxis," "Observe the animal (if dog or cat) for 10 days but no vaccine or serum (immunoglobulin)", "Observe the animal and administer vaccine," "Administer vaccine but no serum," "Administer vaccine and serum," "Re-exposure prophylaxis." Based on PEP guidelines, wild animals were considered as rabid animals and no observation period was requested. We used an algorithm to calculate "appropriate" PEP considering the risk assessment data from each SINAN form following the MoH guidelines. The MoH guidelines are based on three criteria: (i) bite/incident severity, (ii) animal species and dog/cat status before and after the 10-day period observation, and (iii) previous PEP history (vaccination/vaccine titers). Details of these guidelines are available in the **Table S1**. First, the algorithm differentiates each SINAN form as either a "severe" or "mild" incident according to three variables specified in the MoH guidance: the type of exposure, the position of the exposure, and the injury and type of injury (**Table S1**). If an exposure did not fulfill the criteria to be classified as a "severe incident," it was classified as a "mild" incident. We considered incidents to be "mild" if the form details about the exposure were completed as "other or ignored," if the "position of exposure" was "unknown," or if

the "injury" or the "type of injury" was "ignored." Second, the algorithm further discriminated PEP based on whether the animal was considered "healthy," "rabies suspicious," "rabid," or "dead/disappeared." Finally, the algorithm separated patients on whether they had received complete PEP previously, which reduced their subsequent PEP requirements.

RESULTS

Spatiotemporal Trends

Between 2008 and 2016, 82.3% of all bite patients in Brazil were attributed to bites from dogs, 12.5% from cats, 1.4% from wild animals, 0.3% from herbivores, 2.7% from other unidentified animals, whilst 0.8% of records did not have information recorded on the species of biting animal. Among wild animals, 49.8% of bites were attributed to bats, 39.6% to primates and 10.5% to foxes. The average number of patients bitten by dogs over this period was: 502,043 [95% CI: 436,391–544,564], with an annual incidence per state of 258 dog bites/100,000 people. The number of people bitten by other species were generally much lower, but on average there were 76,512 cat bites per year [95% CI: 56,588–97,580], incidence: 41 cat bites/100,000 persons, followed by 4,172 bat bites [95% CI: 3,351–5,365] or 2.3 bat bites/100,000 persons, 3,320 primate bites [95% CI: 3,013–3,710] or 2.0 primate bites/100,000 persons, 1,908 herbivore bites [1,492–2,298], or 0.9 herbivore bites/100,000 persons and 883 fox bites [95% CI: 609–1,086] or 0.6 fox bites/100,000 persons (**Figure 1**).

Bites from domestic and wild animals occurred all over Brazil but bite incidence varied considerably between states, with more than three-fold differences in incidence between states across all species (**Figure 1**). Overall, the northern region of Brazil had a higher burden of bites (**Figure 1**). For example, Roraima had the highest bite incidence for dogs, cats, and bats, while primate bites were reported mostly in the North (states of Maranhão and Pará) and bites from foxes were predominantly reported from the North East region (states of Paraiba and Rio Grande do Norte; **Figure 1**). Bites of several species were significantly correlated across states including bites from cats and primates (Spearman test, rho = 0.54, $p < 0.01$), cats and foxes (Spearman test, rho = 0.43, $p < 0.02$), and bats with dogs (Spearman test, rho = 0.49, $p = 0.01$) and other animals (Spearman test, rho = 0.43, $p = 0.02$; **Figure 2**).

Incidence of bites due to dogs and herbivores remained relatively stable over the last decade. In contrast, bites from

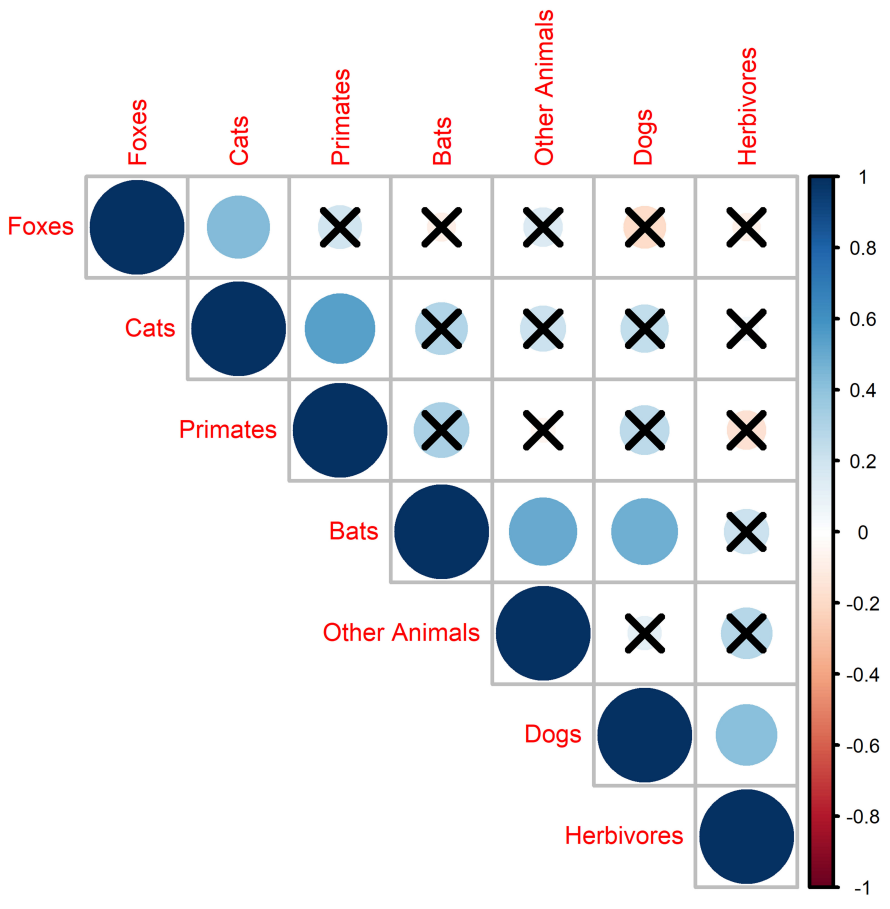

FIGURE 2 | Correlation in bite incidence across states. Circle size is proportional to the value of the Spearman's correlation rho. Blue colors indicate a positive correlation and red colors a negative correlation. Crosses over circles indicate that the relationship was not statistically significant.

cats increased by 56%, and bites from bats increased by 13% between 2008 and 2016, while bites from primates decreased by 34% and bites from foxes decreased by 16% (**Figure 3**). Likewise, the number of municipalities reporting bites increased by 11% (3,742–4,169) for cats, by 13% (1,044–1,184) for bats and decreased by 14% (1,097–947) for primates, and 7% (455–422) for foxes.

Rural vs. Urban

Overall, 84.3% of bites were reported in urban areas, with just 10.0% from rural areas, 0.7% from peri-urban areas, and 5% of forms did not have this information completed. More than 85% of bites from dogs and cats were reported from urban areas (**Figure 4**). Most bites from bats (78%), from primates (74%) and from herbivores (52%) were reported from urban areas (**Figure 4**). In contrast, just 36% of bites from foxes occurred in urban areas (**Figure 4**).

Appropriate PEP Administration According to the Brazilian MoH

For bites involving wild animals and herbivores, the MoH recommends that patients involved in a "mild incident" receive PEP including vaccination while patients involved in a "severe incident" require both vaccination and serum. Therefore, any wild animal that can transmit rabies is considered the equivalent of a rabid dog or cat. By applying the algorithm described above to bite data from 2016, our analyses showed that patients received appropriate PEP in 50% of bites irrespective of the species of biting animal. Appropriate PEP was given to 48.1% of bites involving dogs, 48.7% of cat bites, 51.6% of bat bites, 53.5% of primate bites, 56.3% of fox bites, and 44% of herbivore bites (**Figure 5**).

DISCUSSION

Rabies in wildlife is an emerging challenge in Latin America (28). However, surveillance in wild animal populations is challenging and hotspots of potential spillover risk remain poorly understood for most wild reservoirs of rabies viruses. Our previous work described high incidence of dog bites in Brazil that have remained stable in the past decade but uneven across states, with around half of PEP for dog bites not administered appropriately (24). This study shows that although bite incidence due to wild animals is much lower than for dogs and cats, there are still substantial numbers of patients seeking health care due to wild animal bites all over Brazil. Overall bite incidence due to primates and bats was similar in magnitude, but three times higher than due to foxes. Bites from wildlife were geographically localized, with bites from primates concentrated in the north of Brazil, bites from foxes in the Northeast, and highest incidence of bat bites in Roraima state. Bite incidence between species was correlated at the region level between cats and primates or foxes, and between dogs and bats. Despite increased human encroachment into natural areas, our results show that only bites from bats increased over the last decade (by 13%) while bites from both primates and foxes decreased. Most bites from domestic and wild animals were reported in urban areas, except for bites by foxes. Similar to our previous findings in dogs (24), appropriate PEP was given to only about half of patients attending health care after being bitten by cats and wild animals, highlighting the need to improve health worker's knowledge on PEP following a bite from domestic and wild animals.

Estimates of direct contact between humans and wild animals are rare worldwide, limiting our ability to predict the rate and location of emerging zoonotic diseases (1, 29). Our results

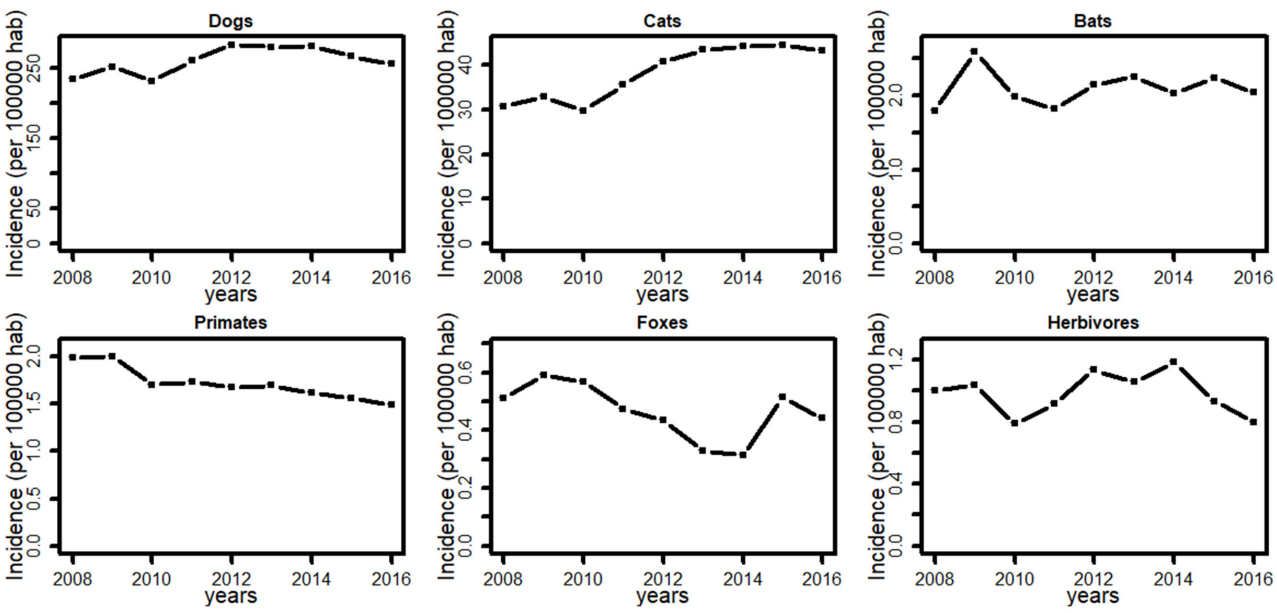

FIGURE 3 | Average Bite incidence in Brazil between 2008 and 2016 for different species.

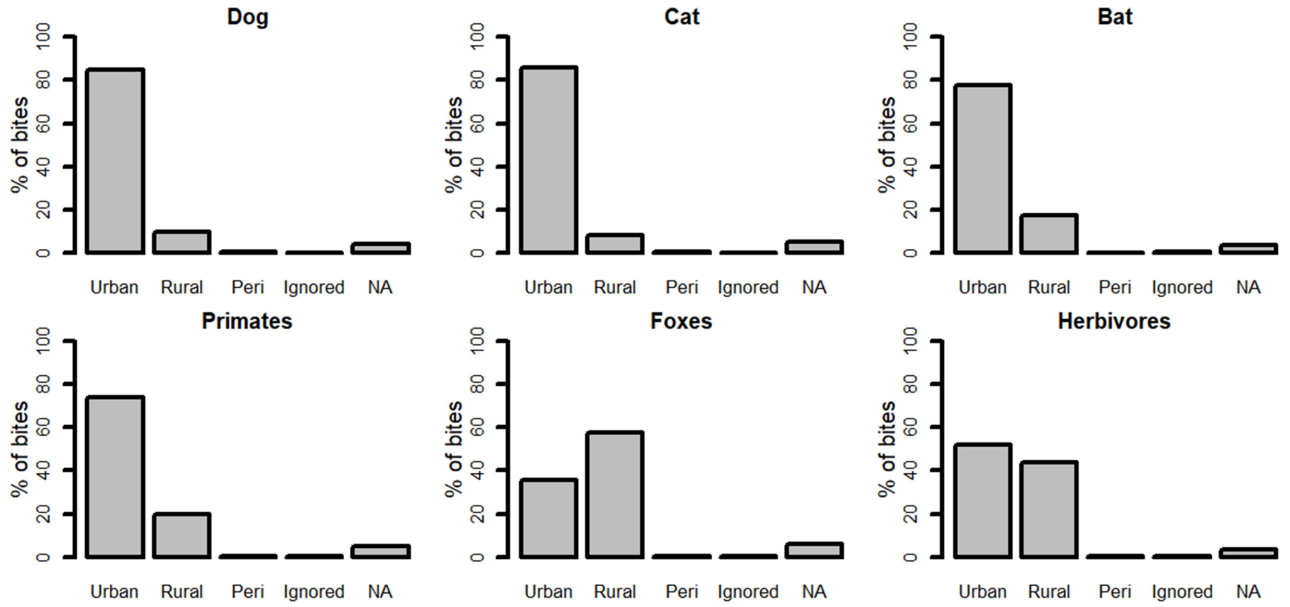

FIGURE 4 | Percentage of patients reporting bites from urban, rural, and peri-urban areas in 2016 according to the species of biting animal.

take advantage of the publicly available SINAN database to estimate and compare bite incidence across states of Brazil, which is the route of human exposure to rabies. As expected, bite incidence in wild animals (0.6–2.3 bites/100,000 hab) was much lower compared to bites from dogs (258 bites/100,000) reported in our previous study (24) or cats (51 bites/100,000). Yet, despite the relatively low incidence, bites from primates and bats affected patients in all states. The low incidence of bites by wildlife in some regions should be interpreted with caution, given that levels of under-reporting are unknown and could be particularly high for isolated populations around natural areas such as indigenous communities, frequently affected by bites from vampire bats (30). The geographical differences in bite incidence imposes an uneven burden to the public health system across the country. Differences between states could reflect the distribution of wildlife populations (e.g., higher abundance of primates and foxes in the North), as well as socio-cultural differences that affects human-wildlife interactions including differences in animal feeding (e.g., feeding in houses for the pet trade or at recreation sites for marmosets or capuchin monkeys) or habitat suitability for opportunistic species such as bats and foxes in urban areas. Similarly, correlations in bite incidence across states for different species (e.g., cats and primates) could be explained by the abundance of those species, similarities in health seeking behavior or reflect spillover risks of rabies between species that might warrant further investigation.

Increased contact between people and wildlife due to human activities such as hunting, agriculture, deforestation, and urbanization is a worldwide problem resulting in the emergence of diseases critical for public health including HIV-SIV, malaria, Ebola, and influenza (31–33). Thus, we could expect that in Brazil, one of the most biodiverse countries worldwide with an increase urbanization, agriculture, and deforestation, close contact between humans and wild animals would also increase over the last decade (34, 35). Our results show that, at least for patients attending health care facilities, bite incidence from primates (-34%) and foxes (-16%) has decreased since 2008, while only bites from bats (+13%) and cats (+56%) increased. The number of municipalities reporting bites followed the same pattern, suggesting that these temporal changes reflect corresponding increases or reductions in the spatial extent of direct contact between humans and wild animals. Overall, our study calls for a better understanding of the drivers behind these temporal trends, specifically increasing bites from bats and cats, and reductions in bites from primates and foxes.

Despite increased urbanization, reductions in bites from wild primates and foxes could reflect the effectiveness of regional educational campaigns aiming to reduce these high-risk contacts. In contrast, the observed increased in bites from bats could reflect range expansions for species such as vampire bats, due to climatic change (7) and increased availability of (man-made) roost sites with urbanization (36, 37). This could also help to explain why bites from bats, primates, and even herbivores are more frequently reported in patients living in urban areas. However, predominance of bites in urban areas (also observed for domestic animals) could also reflect under-reporting of bites in rural areas due to both fewer health centers and lower perceived risks.

Our study shows that 1.4% of bites reported in SINAN are attributed to wild animals compared to 94% of bites attributed to companion animals (dogs and cats). Though bites by wild animals present a relatively low burden for health care systems, they likely represent a higher risk of rabies to humans given continued circulation of several rabies virus variants in these species, the absence of successful strategies to control vampire bat rabies (8), or rabies in primates and foxes. There may be scope for oral rabies vaccines to be used to control rabies, particularly in

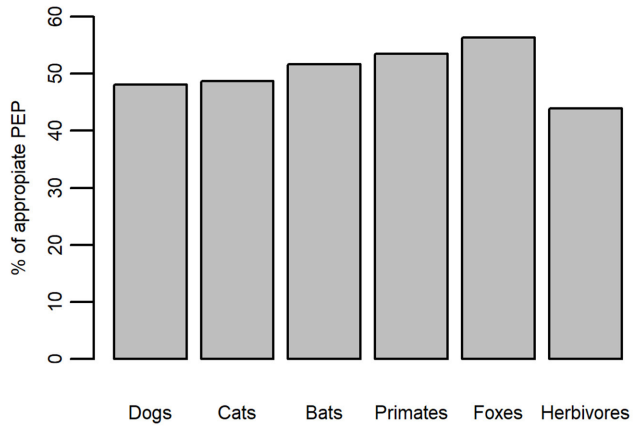

FIGURE 5 | Percentage of appropriately administered PEP for bites by different species in 2016 according to the Ministry of Health guidelines.

foxes in Latin America if strategies can be adapted from those that have proven successful in Europe (38). Alarmingly, we estimate that only half of bites involving wild animals received appropriate PEP according to MoH Guideline's during that period. Like domestic animals, this could reflect poor knowledge of health care personnel on appropriate PEP administration but also lack of PEP availability in remote areas where wildlife species are abundant. In the case of bites by herbivores, which have the lowest percentage of correct PEP administration (44%), this may be due to poor knowledge of the risk of rabies transmission from herbivores and the epidemiological situation whereby herbivores are frequently infected by vampire bat rabies. Wild species can also transmit several other viral diseases to humans, with bats and primates considered reservoirs for many zoonotic emerging diseases (39, 40). Given the current circulation of rabies in different wildlife species as well as ongoing vaccine and immunoglobulin shortages (41), there is an urgent need to improve PEP administration for bites by wild species to avoid rabies fatalities.

Given the difficulty to control rabies among wildlife reservoirs, prevention measures aiming to reduce human rabies exposures could focus on reducing bites. Following our previous work on dogs (24), this study provides a first estimate of bites from cats and wildlife species in Brazil, showing the uneven incidence across the country and the relatively low level of adequate PEP administration. Despite thousands of bites per year from domestic and wild animals, <10 human rabies cases were reported annually to SINAN during the same period (22). The public health risk of rabies could therefore be limited by low circulation of rabies in these animal reservoirs. Alternatively, rabies circulation in some species (e.g., bats) could be high but PEP administration, although inadequate, is still preventing the development of rabies in patients that were bitten by rabid animals. Further reducing exposure risk will require different strategies adapted at the region level aiming to reduce bites and will benefit from the implementation of a "One health" approach. Community-based surveys could also help to identify the socio-ecological factors underlying bites and under-reporting. Rabies surveillance in wild reservoir populations could be guided by the incidence data that we report which highlight areas with the highest bite rates, whilst also carefully considering potential under-reporting in remote communities. Improvements of the SINAN system could include further detailing the wild species responsible for the bite as well as the reason for the incident. This could in turn inform educational campaigns aiming to reduce contact with wild animals such as primates due to the pet trade, feeding of primates and foxes and reducing roost sites for bats in urban areas.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available. Data were obtained for Brazil from 2008 to 2016 by requesting the data online to the Ministry of Health through the Electronic System of Citizen's information (e-SIC, https://esic.cgu.gov.br/sistema/site/index.aspx, e-SIC request number: 2564134). Data was requested from the Ministry of Health but cannot be shared publicly.

ETHICS STATEMENT

This project was approved by the Brazilian Ministry of Health's Ethics Committee (Plataforma Brazil, CAE # 94081818.0.0000.5411) through the Ethics Committee of the Faculty of Medicine of the São Paulo State University (UNESP)—Botucatu.

AUTHOR CONTRIBUTIONS

JB conceived, designed the study, and analyzed the data. JB and KH drafted the paper. JB, KH, AC, and JM edited the paper. All authors read, commented, and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2020.00318/full#supplementary-material

Table S1 | Brazilian Ministry of Health guidelines for rabies prophylaxis.

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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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Movement Restriction and Increased Surveillance as Efficient Measures to Control the Spread of Highly Pathogenic Avian Influenza in Backyard Productive Systems in Central Chile

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During the last 5 years there has been an alarming number of reports of highly pathogenic avian influenza worldwide. However, little is known about the status of this disease in South America. Chile has been the only country in South America where an HPAI outbreak was reported. This outbreak occurred in 2002 and was due to an H7N3 HPAI, where the most plausible hypothesis that explained the entrance of the disease to the country, had relation to migratory wild birds. Commercial poultry farms in Chile are highly integrated and have high biosecurity standards. Nevertheless, poultry backyard production systems lack biosecurity measures and are widely distributed. Since 2002 outbreak, avian influenza viruses have been identified in wild birds and different animal species kept in backyard productive systems (BPS) in Chile. The aim of this study was to simulate the possible natural history of HPAI after its introduction to BPS in central Chile and to simulate different intervention strategies. To do so, the North American Animal Disease Spread Model version 3.3 was used. The results showed that a median of 15,930 BPS would be affected if HPAI spread among BPS in central Chile, representing 97.8% of the current amount of BPS existing in study zone. Movement restrictions, pre-emptive destruction, passive surveillance, tracing of infected premises and combinations of the three, where the intervention strategies tested in the simulation model. From all the interventions simulated, movement restrictions together with increasing surveillance (through increasing passive surveillance and good tracing of infected premises) had the biggest effect, reducing the median number of infected BPS in 90.8%. However, more studies are needed to more accurately estimate local contact rates. These results can guide the official veterinary services to consider potential mechanisms to control or prevent an HPAI emergency situation.

Keywords: highly pathogenic avian influenza, backyard production systems, movement restrictions, disease modeling, surveillance

INTRODUCTION

Avian influenza (AI) is a disease of global concern divided into two groups depending on its pathogenicity in poultry: (i) highly pathogenic avian influenza (HPAI) causing high mortality (up to 100%) to domestic poultry (1) and (ii) low pathogenic avian influenza (LPAI) which includes viruses causing a milder respiratory disease (2). Wild birds have been identified to be mostly asymptomatic reservoirs of all AI subtypes (3) and they have been proposed as the most likely route of introduction of LPAI viruses into domestic poultry populations (4).

Highly pathogenic avian influenza has affected domestic poultry in 68 countries and territories since 2013, involving 7,060 outbreaks and a high diversity of circulating subtypes. The health and economic impact of these outbreaks has been outstanding, with 57% of all domestic poultry losses reported in Asia, followed by the Americas (24%) and Europe (12%) (5). However, there is little knowledge of AI status in South America (6). In fact, Chile has been the only country where outbreaks of LPAI and HPAI have occurred. In 2002, an H7N3 HPAI virus affected commercial farms in central Chile (7). The origin of the outbreak was associated to migratory wild bird as there was a correspondence between an H7N3 avian influenza virus (AIV) isolated from a Cinnamon Teal (*Anas cyanoptera*) in Bolivia in 2001 and the H7N3 virus isolated in the Chilean 2002 outbreak (8). More recently, an H7N6 LPAI virus was detected in two commercial turkey farms in central Chile, which also had origin in wild birds (9). Importantly, AI reactive antibodies were detected in samples of backyard poultry during active surveillance control activities of this outbreak, and as a result elimination of ELISA positive birds from detected and contiguous households was ordered (10). Additionally, pmdH1N1 and H4N8 have been detected in turkey farms during active surveillance activities carried out by the Chilean official veterinary service (11).

Chilean poultry production is highly integrated at the industrial level, operating with high biosecurity standards (12) and in close cooperation with the official veterinary service. However, in backyard production systems (BPS) the biosecurity implementation and rapid outbreak response activities are very limited and usually absent (12). BPS are usually defined as those productive systems where different animal species, mostly of different ages, are kept in close contact, with poor infrastructure and where the purpose of the production is mainly household consumption (13). Usual production number in poultry BPS in Chile is under 100 birds (12). Birds are usually allowed to roam freely during the day and are confined only during the night, which enable close contact with poultry from neighboring BPS and wild bird species (12, 13) that could act as potential reservoirs of pathogens (12, 14, 15). Therefore, it has been suggested that BPS could play a role in the dissemination of poultry diseases such as HPAI (12, 14, 16, 17). This has been confirmed in recent studies in Chile, where LPAI viruses have been identified in wild birds (18) and domestic poultry kept in BPS (19). On the one hand, Bravo-Vasquez et al. (19), detected influenza A positive matrix gene (rRT-PCR) simultaneously in poultry, swine and geese from BPS, with viral prevalence levels of 27% (95% CI:14–39) in samples from poultry. While in wild birds, three LPAI subtypes (H5N9, H13N2, H13N9) have been detected in gulls in the late 2000's (20). In addition, a recent study in wild birds in central and northern Chile obtained an overall prevalence of 2.8%, isolating 16 viruses, including low pathogenic H5 and H7 strains, making it the largest and most diverse collection of Chilean AIVs to date (18). The same authors also detected an H12 hemagglutinin (HA) sequence from wild birds in one domestic Muscovy duck, indicating a spillover from wild birds into backyard poultry populations (6).

To date, there is uncertainty of how the virus would spread among BPS if an HPAI outbreak occurred at this level in central Chile.

The use of epidemiological modeling has been found to be a useful approach to estimate the possible magnitude of a disease outbreak and the resources that would be necessary for a rapid response and disease control planning (21), while preventing the sustained widespread epidemic among poultry (22), protecting regions from the potentially serious socio-economic consequences of an outbreak (23) and reducing possible human exposures (24).

The aims of this study are to simulate the impact of an outbreak of HPAI at the BPS level in central Chile and to identify variables that could influence the number of affected backyards, in order to design control strategies for possible outbreak within this type of production system.

MATERIALS AND METHODS

Study Area

The study area was 48,186 km^2 of the central zone of Chile, including three administrative regions: Valparaiso, Metropolitan, and Libertador General Bernardo O'Higgins (LGB O'Higgins). This area compromise ∼95% of the commercial poultry production in Chile and 16,289 BPS that keep poultry (25). For this study, BPS that breed poultry were considered the study unit.

Modeling Framework

The North American Animal Disease Spread Model (NAADSM version 3.3) was used for the simulation of disease spread and control between flocks (26, 27). NAADSM is a stochastic, state transition model framework which incorporates spatial and temporal information to simulate the spread of highly contagious animal diseases. Additionally, the software includes a package that allows modeling the spread of the disease within-herd (WH package), which can be done in a phase prior to entering the data into the NAADSM model. The Within-herd (WH) is a deterministic model (28) used to simulate the dynamics of disease spread and immunity at individual level within a homogeneously mixing population (flock). Detailed information regarding how these parameters were entered into WH and NAADSM and the information sources used for each individual value are presented below.

Within-Herd (Flock) Model

Input and Disease Transmission Parameters

Input parameters in WH included information about animal population demographics and disease state duration and

TABLE 1 | Population data included in the WH model for the dissemination of HPAI virus in BPS.

Variable	Value	Distribution/ Parameter value	References
Population size	44 birds	Loglogistic (−0.12; 31.13; 2.27)	(13)
Initially latent individuals	1 animal	Point (1.00)	User defined
Initially clinical animals	0 animals	Point (0.00)	User defined
Adequate exposures per time step	1.7	Poisson (1.7)	(29)

TABLE 2 | Values of disease state duration and mortality parameters for individual birds in BPS used in the WH model.

Parameter description	Distribution/Parameter value	References
DISEASE		
Latent period	Exp (1) (CI: 0.05; 3.00)	(23)
Clinical period	Gaussian (4.00; 1.00) (CI: 2.5; 5.8)	(23)
Immune period	Point (1,000)	User defined
MORTALITY		
Probability that disease will result in death	Point (0.9)	(29)
Probability of non-disease death	Point (0.00005)	(29)

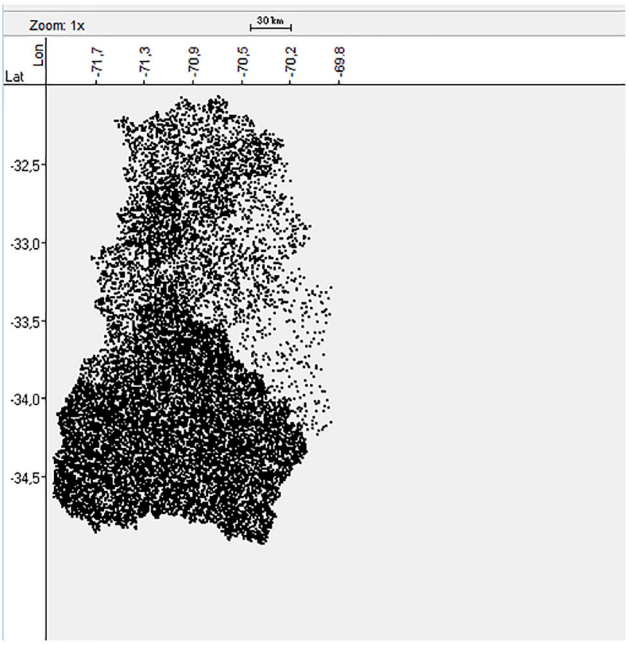

FIGURE 1 | Spatial distribution of the 16,289 BPS in the central Zone of Chile.

transmission. Four disease states were considered: susceptible, latent, clinically infectious and immune. Each bird could only be in one particular state of the disease at any given time point. The length of time that each bird remained in the latent and clinical disease states were derived from literature review (23). An immune period of 1,000 days was used so that the model did not consider as susceptible, birds that had already been infected. Parameters values used in the WH model are shown in **Tables 1**, **2**. The time step considered was a day and 1,000 iterations were run. Outcomes produced by the WH model were then used to develop flock-level parameters for NAADSM.

Between-Flock Transmission Model

Input Parameters

Input parameters included information about animal populations, disease presentation, disease transmission between flocks, disease detection and surveillance and disease control. Each BPS was considered to be a single unit. Each unit in NAADSM is characterized by its (i) production type, (ii) herd size (number of birds in the flock), and its (iii) spatial-location (latitude and longitude coordinates).

i. Production type

A production type is a collection of herds with similar virus transmission probabilities, disease presentation, disease detection probabilities, and control strategies (28). For this study, the only production type considered was BPS.

ii. Flock size

A set of 16,289 different flock sizes was created and generated from a database of 384 known BPS obtained from previous studies in central Chile (13). The distribution of the BPS flock size was described using @Risk 7.5 Palisade software (Ithaca, New York). The distribution was fitted for the 384 BPS sizes and values for all flock sizes to be used were generated from the fitted distribution with a simulation of 16,289 iterations.

iii. Geolocation

The forestry, agricultural and livestock census published in 2007 provided the information about the number of BPS located in each province of the central zone of Chile (25). However, the exact geolocation of each BPS was not known. Therefore, a dataset of 16,289 random geolocations was generated and stratified by province (**Figure 1**) according to the census information using the Surface Tool in ArcGIS-10 software (Esri, California, USA). This methodology was previously validated in Chile by Alegria-Moran et al. (30) indicating that the approach followed a realistic spatial distribution.

Disease Manifestation

Four disease states were used: susceptible, latent, clinically infectious and immune. The length of time that each flock remained in the latent and clinical disease states were derived from literature review (23). As described for the WH model, an immune period of 1,000 days was used so that the model did not consider as susceptible a BPS that had already been infected.

Between-Flock Transmission

Between-flock virus transmission considered direct contact, indirect contact and local-area spread between infected and

TABLE 3 | Values and sources of disease transmission parameters for the between flock simulation.

Disease	Distribution/Parameter value	References
Latent period	Gamma (1.3; 0.8)	(23)
Infectious clinical period	Logistic (14.88; 1.7)	WH model outcome
Immune period	Point (1,000)	User defined
Within herd prevalence	Relational function	WH model outcome
DIRECT CONTACT SPREAD		
Mean baseline contact rate*	0.4098	2015–2017 Database
Probability of infection transfer	Determined by WH prevalence	WH model outcome
Distance distribution of recipient units	BetaPERT (0, 3, 10)	User defined
INDIRECT CONTACT SPREAD		
Mean baseline contact rate*	0.5529	2015–2017 Database
Probability of infection transfer	0.5	(29)
Distance distribution of recipient units	BetaPERT (0, 3, 20)	User defined
AIRBORNE SPREAD		
Probability of spread/day, at 1 km	0.05	(29)
Start, End	0.360	

**Recipient units/units/day.*

TABLE 4 | Sources of direct contact from backyard to backyard poultry production.

Direct contact	Formulae	Frequency	Per week	Per day
Neighbor's birds	1.0×0.409	1.0/day	-	0.409
Birds replacement	0.83×0.36	0.3/year	0.0058	0.0008
Total	-	-	-	0.4098

TABLE 5 | Sources of indirect contact from backyard to backyard poultry production.

Indirect contact	Frequency	Per week	Per day
Neighbors visits	3.8/weeks	3.8	0.54
Bird food purchase	0.33/months	0.0825	0.0118
Veterinary care	0.128/years	0.0025	0.0004
Embryonated eggs exchange	0.26/years	0.005	0.0007
Total	-	3.89	0.5529

susceptible flocks. **Table 3** shows the parameters and values used for the simulation. Indirect contact considered movement of people, vehicles, materials and animal products between flocks. Direct contact spread considered the movement of one or more birds from one flock to another. To represent non-directional local-area spread (i.e., disease spread that cannot be well-characterized or traced such as spread by insects, pests, lapses in biosecurity, and local airborne transmission) the airborne spread function in *NAADSM* was used.

The parameters used to define virus transmission by contact were: mean baseline contact rate, probability of infection transfer and distance distribution of recipient flocks (26). Latent flocks were assumed to be able to spread virus only by direct contact. Infectious clinical flocks could spread the virus to other flocks via direct contact, indirect contact and local-area spread. Flocks in the immune state were not able to spread the virus or become infected.

Virus transmission by direct contact

Direct contact involved birds in a source BPS coming into contact with birds in a recipient BPS. The direct contact rate was the average daily number of shipment of birds which could introduce the virus into new flocks. This value was derived from previous studies in Chile carried out during 2015–2017 where a semi-structured survey was applied to 384 BPS in central Chile (13). The data collected in the database included information that allowed the characterization of direct and indirect contact rates between BPS. It also allowed the collection of information regarding the owners' ability to recognize when their birds were sick, as well as information regarding the actions they take against large bird mortalities. Those data described that 40.9% of the owners reported that at least 1 bird from their neighbors entered their BPS and contacted their birds daily. Additionally, 35.6% of the owners indicated buying birds for replacement an average of 0.83 times a year. As only one field exists in NAADSM to describe direct and indirect contact rates, the estimated daily frequencies of each type of direct contact were added to generate an overall average daily number of direct contacts (**Table 4**).

The probability of virus transmission was determined by the prevalence of infectiousness in the infected BPS on the day that the contact occurs. Estimates of the median daily prevalence of infectiousness for an infected backyard flock were produced using the WH model outcomes. The distance distribution of recipient BPS was assumed to have a BetaPert distribution with a minimum of 0, a mode of 3 and a maximum of 10 kilometers.

Virus transmission by indirect contact

The contact rate for indirect contacts was the average daily number of movements of people, vehicles, equipment, materials or animal products from a source flock (26) to a recipient flock. The estimated daily frequencies of each type of indirect contact were added to generate an overall average daily number of indirect contacts. The sources of indirect contact rates and their values were derived from the 2015–2017 mentioned database and are described in **Table 5**. The distance distribution of recipient flocks was assumed to have a BetaPert distribution with a minimum of 0, a mode of 3, and a maximum of 20 km. These distances were considered from Di Pillo et al. (13) results that described BPS poultry owners movement to access markets.

Virus transmission by local-area spread

The probability of infection by local-area spread was considered a relational function depending on the prevalence of infectiousness in an infected flock and the distance between a source and a

recipient flock. The probability of infection by local-area spread decreased as the distance between flocks increased and the probability of virus transfer declined exponentially from the source flock (29).

Disease Detection and Control Parameters

In NAADSM, the overall chance that an infected flock will be detected depended on two probabilities, the probability that clinical signs are observed in an infected flock, and the probability that a flock is reported once clinical signs have been observed (26). Values for each probability were derived from (13) and 2015–2017 database, where 80% of poultry owners declared to recognize when their birds were sick. While the probability of reporting flocks with clinical signs derived from the sum of two components. One point eight per cent of producers declared reporting mortalities to the official veterinary service. While 10% of producers reported having visits from the official veterinary service. Thus, a report of 11.8% (0.12) was assumed.

Control and Prevention Measures

The baseline scenario represented the natural history of the disease (no intervention measures are taken) once it entered a BPS. The effect of different strategies on the total number of infected BPS and outbreak duration against baseline scenario were evaluated. These interventions included (i) Movement restrictions, (ii) tracing of infected BPS, (iii) depopulation and pre-emptive destruction and, (iv) increasing passive surveillance (probability of reporting infected BPS). The effect of different combinations of strategies were evaluated.

Movement restrictions considered the reduction in the number of contacts between BPS, thereby reducing the possibility for disease spread. This restriction on movement was considered in two ways; (i) restriction from the beginning of the epidemic, to reflect the total confinement of birds as a preventive measure, and (ii) restriction from day 3 after the detection of disease, to reflect the effect of prohibiting the movement of birds and people as a control measure once the epidemic has started. Movement restrictions of 50% and 90% of the baseline contact rates were simulated.

Global Tracing consisted of the process of identifying units (BPS) at high risk for disease based on contact with detected units (26). The critical period considered for direct and indirect tracing was 14 days. This is the period of time prior to detection of the origin unit of the trace, for which contacts should be investigated (26). The probability of trace success for direct and indirect contacts were derived from expert opinion and were 0.8 and 0.5, respectively. Herd exams were also included and a multiplier of 1.5 for trace-forward contacts and 2.0 for trace-back contacts were assumed. Parameters for herd exams are multipliers that describes how much more likely a trained observer is to detect clinical signs compared to more passive observers (26). Tracing parameters also included diagnostic testing of traced BPS. The sensibility and specificity used were 95.4 and 99.7% (19). A delay in obtaining tests results was considered to have a BetaPert distribution of 0, 3, and 7 days.

Depopulation considered the destruction of infected and detected BPS. While pre-emptive culling considered culling of potentially uninfected BPS in a ring radius of 3 km around a detected BPS. The delay in implementing the stamping out programs was simulated using two options, a 7 days delay and a quicker response of 2 days delay. Once the stamping out program started, it considered two different amounts of BPS slaughtered daily. The first and more conservative option considered the destruction of 10 BPS daily during the first week, with an increase in capacity to the destruction of 20 BPS per day since day 7 after the destruction program started. The second option considered the destruction of 100 BPS daily since day 2 of detection.

Improvement of passive surveillance was simulated by increasing the probability of reporting flocks with clinical signs to 0.9 since day 1 of disease first detection in any unit.

Assumptions

The model developed laid on the following assumptions: (i) once a BPS become infected, it left the poultry business, thus no repopulation of birds existed, (ii) it was a homogeneously mixed population, (iii) there was no transmission of the virus between BPS and commercial farms, or vice versa, due to the high biosecurity standards applied in the latter in Chile and (iv) it was assumed that all the birds present were domestic chickens. Although usually different species of birds coexist in BPS, previous studies (13) have described that 87% of the population of birds in these systems correspond to domestic chickens. In this way, the parameters used in the model correspond to those described for domestic chickens.

Model Outcomes

Each simulated outbreak started with a single, randomly selected latently infected BPS, in a totally susceptible population of BPS. For each of the scenarios, 1,000 iterations were run. Each iteration ran until the end of outbreak which was defined as the moment when there were no more latent or clinically infectious BPS left, and when all destruction activities were completed. The outcomes of interest were the total number of infected BPS (BPS infected by any path), time (days) to first detection of infected BPS in the population, the total number of detected and infected BPS and the outbreak duration. For each output, the summary statistics calculated were the median, 5th percentile and 95th percentile.

RESULTS

Input Parameters

BPS Poultry Population Demographics

Of the 16,289 BPS, the total number of birds was 713,665, distributed in flocks ranging from 2 to 300 birds, with a mean of 44 (SD = 39.2) birds/flock and a median of 30 domestic chickens (IQR: 20–50).

Simulation Results

When simulating the basal scenario, the great majority of virus transmission was due to indirect contact, accounting for 99.3% of the infected BPS. The median duration of the basal scenario outbreak was 314 days (p5 = 247; p95 = 372), with a median number of infected BPS of 15,930 (p5 = 15,889; p95 = 15,961),

meaning that 97.8% of the total backyard population got infected when no intervention measures were applied. Disease detection occurred in 890 of 1,000 iterations, being the median time to first detection 97 days (p5 = 50; p95 = 209). The median total number of birds infected were 701,746 (p5 = 699,846; p95 = 703,306). The model outcomes are shown in **Table 6**.

Control and Prevention Measures

The greatest impact on the median number of infected BPS and outbreak duration was movement restriction (**Table 7**). When movement restriction was set to 90% as a preventive measure (birds totally confined since day 1), there were not infected BPS and the outbreak did not launch. On the other hand, implementing movement restrictions once the epidemic had already started (since day 3 of the first detected infection of a BPS), the outbreak did launch, but it was controlled within 122 days and the amount of infected BPS decreased in 91%. However, in order to see a greater effect, it was necessary to carry movement restrictions together with other intervention strategies, such as increasing the probability that owners reported when their birds became infected and to perform tracing of the disease. When passive surveillance (probability or reporting infected BPS) was not included, the infected BPS decreased in 69%.

When evaluating the median time to first detection, increasing passive surveillance had the greatest impact, displaying a reduction from 97 days to only 53 days. This strategy had also the greatest impact on median total number of detection of infected flocks, increasing this number to 13,712 in comparison to the basal scenario, when the median total number of detection of infected flocks were just two. The epidemic curves generated from the different scenarios can be observed in **Figure 2**.

TABLE 6 | Baseline model outcome generated from 1,000 stochastic iterations of the model of HPAI in BPS in central Chile.

Baseline model outcome	Median	P5	P95
Total number of infected flocks	15,930	15,889	15,961
Number of flocks infected by direct contact	58	45	70
Number of flocks infected by indirect contact	15,826	15,783	15,861
Number of flocks infected by local-area spread	46	33	56
Total number of birds in infected flocks	701,746	699,846	703,306
Time to first detection (days)	97	50	209
Total number of detected infected flocks	2	0	5
Outbreak duration (days)	314	247	372

DISCUSSION

The use of epidemiological modeling of infectious diseases are a useful approach to estimate the possible magnitudes of a disease outbreak and the resources that would be needed to generate a

TABLE 7 | Effect of control and prevention measures over the median number of infected BPS, outbreak duration, time to first detection, and number of detected infected BPS.

Intervention strategy implemented	Median number of BPS infected (5th–95th percentile)	Median outbreak duration (days) (5th–95th percentile)	Median time to first detection (days) (5th–95th percentile)	Median total number of detected infected flocks (5th–95th percentile)
Basal model	15,930 (15,889–15,962)	314 (247–372)	97 (50–209)	2 (0–5)
Movement restriction 90% (day 1) + Increased report 90% + Tracing	0 (0–5)	19 (12–47)	-	0
Movement restriction 90% (day 1) + Increased report 90% + Tracing + Depopulation 10 BPS daily (since day 7)	0 (0–0)	17 (11–22)	-	0
Movement restriction 90% (day 3) + Increased report 90% + Tracing	1,463 (238–4,799)	122 (80–160)	57 (31–98)	468 (1–1,038)
Movement restriction 90% (day 3) + Increased report 40% + Tracing	2,688 (163–8,971)	138 (82–210)	75 (36–138)	469 (2–977)
Movement restriction 90% (day 3) + Tracing	4,904 (671–15,889)	166 (103–297)	101 (52–225)	483 (0–799)
Movement restriction 50% (day 3) + Increased report 90% + Tracing + Depopulation 100 BPS daily (since day 2)	11,665 (10,447–12,674)	564 (432–666)	53 (31–93)	1,396 (344–3,773)
Movement restriction 50% (day 3) + Increased report 90% + Tracing + Depopulation 300 BPS daily (since day 3)	11,749 (10,676–12,598)	531 (376–679)	56 (32–94)	1,421 (661–2,141)
Movement restriction 50% (day 1) + Increased report 90%	12,783 (0–12,959)	569 (15–727)	102 (30–176)	12 (0–20)
Movement restriction 50% (day 1) + Increased report 90% + Tracing + Depopulation 10 BPS (since day 7)	12,966 (0–13,465)	682 (15–828)	104 (58–200)	4,139 (0–5,608)
Increased report 90% + Tracing + Depopulation 100 BPS daily (since day 3)	14,789 (11,211–15,997)	299 (232–374)	58 (30–94)	12,076 (7,122–14,025)
Increased report 50% + Tracing	15,922 (15,876–15,953)	310 (238–362)	68 (31–115)	13,074 (9,247–14,024)
Increased report 50% + Tracing + Depopulation 10 BPS daily (since day 7)	15,927 (15,855–15,964)	852 (674–879)	63 (31–113)	13,235 (8,900–13,932)
Increased report 90% + Tracing + Depopulation 10 BPS daily (since day 7)	15,921 (15,028–15.960)	861 (573–879)	53 (27–80)	13,712 (7,677–13,964)

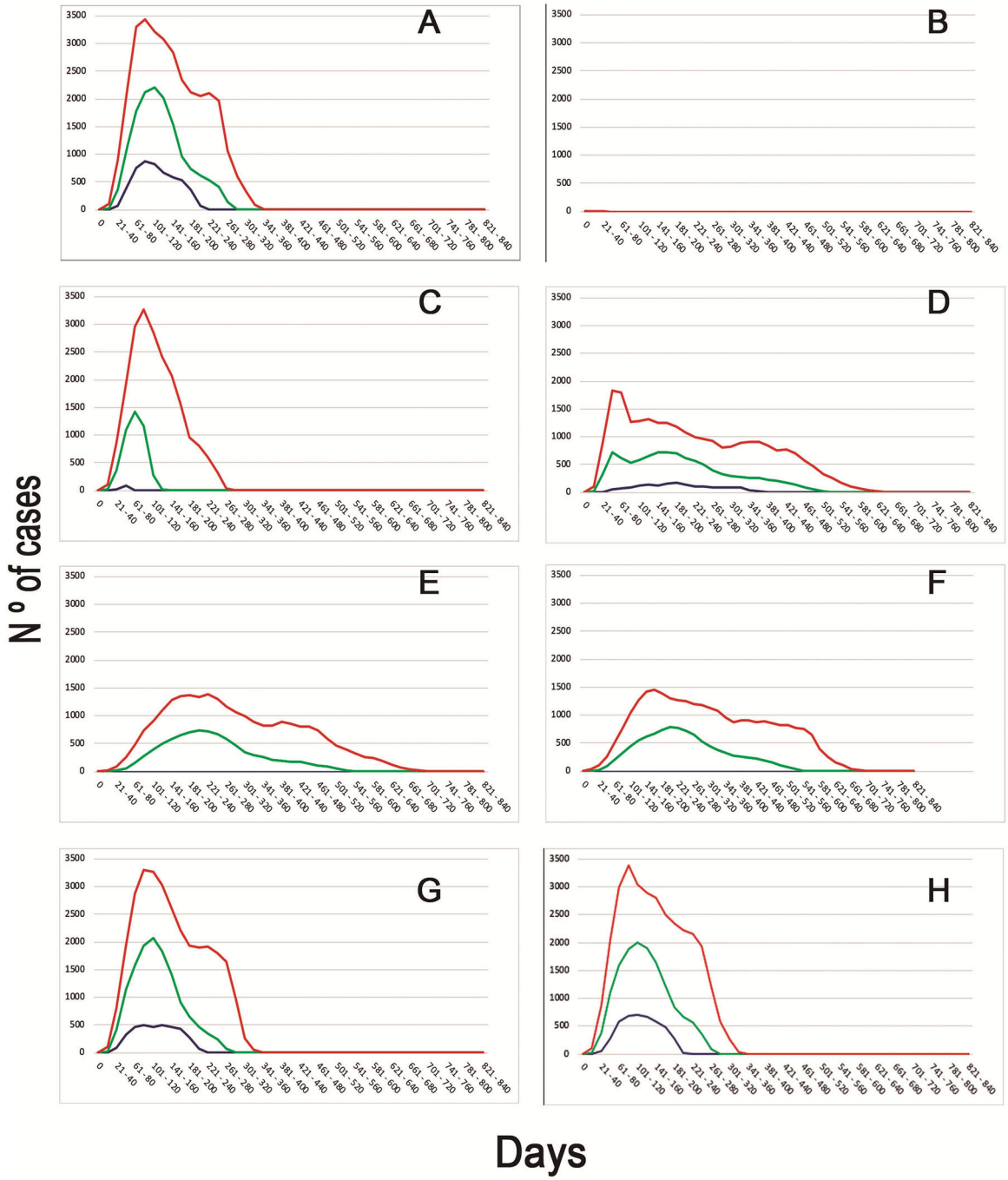

FIGURE 2 | Epidemic curves of the different scenarios simulated. Different colors represent the daily cases of infected BPS (Blue−5th percentile; Green−Median; Red−95th percentile). Letters represent the epidemic curves for the different scenarios **(A)** Basal model with no intervention strategies. **(B)** Movement restriction of 90% (since day 1) + Increased report to 90% + Tracing. **(C)** Movement restriction of 90% (since day 3) + Tracing. **(D)** Movement restriction of 50% (since day 3) + Increased report to 90% + Tracing + Depopulation of 100 BPS daily since day 2. **(E)** Movement restriction of 50% (since day 1) + Increased report to 90%. **(F)** Movement restriction of 50% (since day 1) + Increased report to 90% + Tracing + Depopulation of 10 BPS since day 7. **(G)** Increased Report to 90% + Tracing + Depopulation of 100 BPS daily (since day 3). **(H)** Increased report to 50% + Tracing.

rapid response, but for the models to be applicable to different contexts, it is important that they can consider data from the local susceptible populations in which the model is being applied. In this study, we managed to gather information that describes contact rates between BPS in central Chile, which means that our results can guide local work, in situations of epidemiological

calm, in order to respond better to prevent and respond to health emergencies.

The most important finding of this study was that the best control strategy to be used to control an outbreak of HPAI in BPS situations is to apply movement restrictions. This is consistent with that described by several authors, who point out that isolating individuals is one of the oldest, however most effective strategies, to control infectious diseases (31).

This strategy had the biggest effect when implemented in both situations, as a preventive measure confining birds before the outbreak started, and as a control measure once the outbreak started. Improving biosecurity measures at BPS level by building chicken coops could decrease both direct and indirect contact rates. Biosecurity is the first line of defense against an introduction of AI and probably the only defense when preventive/prophylactic vaccination of flocks at risk is excluded (32), as it is in the case of Chile, where vaccination against avian influenza is prohibited. Depending on the circumstances, biosecurity might be defined as biocontainment which is the prevention of the virus of exiting the infected unit; or as bioexclusion, which refers to the prevention of virus introduction into a disease-free unit (33). Naturally, bioexclusion is not easy to achieve in free-range or mixed system. This difficulty has previously been explained due to the free access of wild birds to these systems, which may be carrying the virus (32). Other reason that explains the difficulty of bioexclusion in the case of BPS, is the free movement of domestic birds between different productive units (34). Because of these, poultry farmer organizations in the Netherlands are suggesting keeping poultry inside (confined in chicken coops with roofs) during the wild-bird migration period in spring (32). The construction of chicken coops in BPS would keep the birds confined, reducing both the direct contact rate and the indirect contact rate. The latter, by limiting the amount of feces available in the environment, which can be transferred from one BPS to another through fomites or through movement of people that could carry the virus in their shoes or vehicles (35). In fact, when this scenario was simulated, represented by movement restrictions in 90% of the BPS since day 1, the outbreak did not launch.

A previous study in Minnesota described that free-range and semi-confinement have been the introduction points for LPAI viruses into commercial flocks. In addition to lack of confinement, small flocks of domestic waterfowl, such as ducks and geese raised outdoors are also a possible route of introduction of the virus, particularly if they are reared together with other species of domestic poultry under common handling conditions (32), as it is in the case of BPS in Chile, where most households raise different animal species altogether (chickens, ducks and geese). Furthermore, trading and exchanging live birds may perpetuate the infection and the spread to other farms. In Chile, it has been described that in 74% of BPS households, eggs and poultry are usually given away to relatives/friends. In addition, the exchange of embryonated eggs to improve the flocks productive yield is also a common practice (13). These activities are of crucial importance when considering that transmission of AIV may occur by nearly anything contaminated with fecal material (32), reinforcing the importance of restricting birds and bird's products movement.

Although the local contact rates for Chile were estimated from previous studies in the country, there are still gaps in the information regarding the effect of the contact of domestic birds with wild birds that can carry the virus. Sequence analysis of viruses isolated in an Italian wild bird survey identified that the H7 gene showed a 99.3% homology at the nucleotide level between the isolates from the backyard flocks and the isolates obtained from wild birds (36). As mentioned before, these types of events, have also been described in Chile by Jimenez-Bluhm et al. (6) where the risk and evidence of spillover of an H12 virus from wild birds to BPS is described.

On the other hand, improving passive surveillance by increasing the probability of poultry owners of reporting infected BPS is a key element. The most effective control strategy once the outbreak started, was the combination of rapid bird confinement once the first infected BPS was detected. If the probability of reporting is low, infected BPS are not detected, and implementing any other intervention measure (without increasing passive surveillance) would have virtually no effect in controlling the spread of the disease. In fact, only by increasing passive surveillance, the median time to first detection decreased by 45% of the baseline, which is a known necessary step for ensuring a rapid response in the face of a sanitary emergency (37).

Because BPS in Chile are generally found in remote areas (13), poultry owners are the first link in the passive surveillance chain for an early warning system. So, educating poultry owners on HPAI would become therefore a crucial prevention strategy. The Italian and Dutch experiences have shown that a delayed detection of an HPAI epidemic in a high-density poultry area, makes it more difficult to control the disease (38, 39). The consequence of not reporting or overlooking AI suspect cases because of low specificity of clinical signs, lack of knowledge about the disease or because people are used to being in a disease-free country, would allow the virus to have a longer period for its dissemination. A longer high-risk period increases the risk of spread of infection to other BPS, a fact that could seriously hinder the efforts of eradicating AI after its introduction into a country free of the disease (32).

In addition, training personnel from official veterinary services to trace contacts from an infected backyard has an important effect on the probability of detecting infected BPS. However, measures such as depopulation, had a low impact on the simulated scenarios. Nevertheless, simulating the responsiveness of the official veterinary service can be a difficult task when official data are not available. However, the previous simulated measures (confinement and increased passive surveillance) are preventive measures easy to implement independently from the official veterinary service.

The base scenario simulated in this study, pointed out that 97.8% of the total backyard population would get infected with HPAI if no intervention measures were applied. With this background, it is necessary to improve the preparation of BPS. This preparation could be achieved by improving biosecurity together with an education campaign, with the aim

of having a better passive surveillance and thus decreasing the probability of transmission. On the contrary, the commercial poultry industry in Chile constantly invests in improving its levels of biosecurity, attain advances in the development of geographic compartments and constantly performs active and passive surveillance, activities that should also be carried out for BPS.

These results would probably be refined if future studies deepen the estimation of contact rates and parameters for the context of Chile. Because of this, the model established for this study is not really intended to forecast the outcomes of an outbreak, but to be used in advance of an outbreak for decision support, planning and preparation.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CH-W, SS-C, and FD: conceptualization. FD, CH-W, PG, CB, VM, GM, and CH-W: methodology. FD, CH-W, PJ-B, PG, CB, VM, GM, and CH-W: writing-original draft. CH-W, SS-C, and FD: resources. All authors contributed to the article and approved the submitted version.

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REVIEW
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Prevalence, Colonization, Epidemiology, and Public Health Significance of *Clostridioides difficile* in Companion Animals

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Clostridioides difficile, previously *Clostrdium difficile*, is a major cause of antibiotic-associated enteric disease in humans in hospital settings. Increased incidence of *C. difficile* infection (CDI) in community settings raises concerns over an alternative source of CDI for humans. The detection of genetically similar and toxigenic *C. difficile* isolates in companion animals, including asymptomatic pets, suggests the potential role of household pets as a source of community-associated CDI. The close association between companion animals and humans, in addition to the use of similar antibiotics in both species, could provide a selective advantage for the emergence of new *C. difficile* strains and thus increase the incidental transmission of CDI to humans. Therefore, screening household pets for *C. difficile* is becoming increasingly important from a public health standpoint and may become a part of routine testing in the future, for the benefit of susceptible or infected individuals within a household. In this review, we analyze available information on prevalence, pathophysiology, epidemiology, and molecular genetics of *C. difficile* infection, focusing on companion animals and evaluate the risk of pet-borne transmission of CDI as an emerging public health concern. Molecular epidemiological characterization of companion animal *C. difficile* strains could provide further insights into the interspecies transmission of CDI. The mosaic nature of *C. difficile* genomes and their susceptibility to horizontal gene transfer may facilitate the inter-mixing of genetic material, which could increase the possibility of the emergence of new community-associated CDI strains. However, detailed genome-wide characterization and comparative genome analysis are warranted to confirm this hypothesis.

Keywords: companion animals, *Clostridioides difficile*, prevalence, molecular epidemiology, public health

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INTRODUCTION

Clostridioides difficile is an anaerobic spore-forming bacterium that causes a serious toxin-mediated enteric disease in humans (1). Annually, nearly half a million people in the United States suffer from *C. difficile* infection (CDI) (2), which incurs ∼6.3 billion dollars of treatment and other hospital costs (3). Relapse of CDI usually occurs in ∼20% of the individuals within a month after primary treatment (4, 5).

Currently, there are no definitive treatment options available for CDI without the possibility of recurrence or relapse (5). A recent study indicated that 1 out of every 11 patients with CDI died within 30 days of diagnosis (2). *C. difficile* is classically considered a nosocomial pathogen and a major cause of antibiotic-associated diarrhea in hospitalized patients. However, an increase in the number and severity of CDI in humans has been reported outside the hospital environment or in individuals with onset of symptoms 48 h or less after hospital admission, referred to as community-associated infections (6). A paradigm shift has been observed in the CDI epidemiology in recent years and the incidence rate of community-acquired *C. difficile* infections is over 40% of the total CDI cases reported (2, 7). Moreover, newer reports indicate that the national burden of nosocomial *C. difficile* infection in the United States has decreased by 36%, whereas community-associated *C. difficile* infection burden has shown no change in trend (8). Notably, a definitive source of *C. difficile* in community settings has not been identified so far.

Clostridioides difficile has been isolated repeatedly from the intestinal flora of healthy domesticated animals, including pets, and associated with the sporadic incidence of diarrhea in susceptible animals (9–11). An increase in the isolation of *C. difficile* from food-animals and animal derived food has been attributed to the increased reports of community-associated human CDI (12). In the past decade, several investigators have isolated and characterized food-animal and meat strains of *C. difficile*. As an example, a common *C. difficile* strain isolated in pigs, ribotype (RT) 078, is also a ribotype commonly implicated in human community-associated *C. difficile* infection (13). However, other studies have questioned the potential foodborne transmission of *C. difficile* in humans specifically due to the lack of evidence of direct transmission and low prevalence of *C. difficile* in animal-derived foods (14–16). Therefore, the search for a potential source of C. *difficile* has recently been focused on companion animals (17). The general public is more intimately associated with pets than food animals, suggesting that *C. difficile* carriage in pets, especially dogs and cats, poses a relatively high public health risk to humans in household settings.

Reports from various parts of the world suggest household pets are carriers and sources of pathogenic *C. difficile* to humans. Studies conducted in past years reported an ∼4–30 percent prevalence of *C. difficile* in dogs with several toxigenic isolates, where the toxigenic strains represented nearly 50% in some instances (17–20). Furthermore, *C. difficile* ribotype RT 106 has now surpassed the hospital-acquired *C. difficile* RT 027 in becoming the most common ribotype implicated in human CDI in the United States and has been frequently isolated from dogs and cats (19, 21–25). Therefore, screening household pets for *C. difficile* is becoming increasingly important from a public health point of view and could become routine in the future. In this review, we analyze available information on colonization, pathogenesis, and epidemiology of *C. difficile* in companion animals, particularly in pets, and examine the potential pet-borne transmission to humans as an emerging public health concern.

C. DIFFICILE COLONIZATION IN DOGS AND CATS

Clostridial species are normal members of the intestinal flora in domestic animal species (26). Several studies indicate varying prevalence of *C. difficile* in healthy domestic animals with no enteric symptoms (27, 28). Alterations in the enteric microenvironment due to factors like antibiotic treatments, pancreatic exocrine dysfunction, changes in diet, trypsin inhibitors, poor intestinal motility or parasitic infections facilitate overgrowth of *C. difficile* (26, 29). The stress on the bacteria and overpopulation of the vegetative *C. difficile* cells triggers sporulation and synchronous secretion of potent exotoxins, toxin A (TcdA) and toxin B (TcdB) (26, 30). The toxins are endocytosed, cleaved, and release the glucosyltransferase domains into the cytosol which inactivate Rho GTPases (30, 31). Inactivation of Rho GTPases causes disruption of the cytoskeleton and intercellular tight junctions, simultaneously stimulating the intestinal epithelial and immune cells to secrete massive amounts of cytokines and chemokines (32, 33), resulting in neutrophilic inflammation and mucosal necrosis (26).

In adult dogs, colonization of toxigenic *C. difficile* in the gut is predominantly non-clinical and asymptomatic. For example, *C. difficile* toxins A, B, or combinations of both have been detected in feces of <20% of outpatient and in-patient healthy dogs as well as in-patient diarrheic dogs (27, 34). Conversely, ∼90% of puppies had *C. difficile* isolated from their feces at least once during the first 10 weeks of life, of which more than half of the isolates were toxigenic (35, 36). Carriage of *C. difficile* in healthy puppies 3 months of age and older is observed to be much lower (35). The carriage rate of *C. difficile* in cats does not appear to differ from that of dogs (37) although systematic studies on *C. difficile* cat carriage are limited, in spite of litter boxes thought as a potential additional risk factor for *C. difficile* transmission within a household.

The pathogenesis and clinical features of CDI in companion animals appear to be strikingly different from that of human CDI. Gut dysbiosis is not a significant feature of CDI in dogs (26, 38), despite being a major factor in the pathogenesis of CDI in humans. Clinical signs such as acute hemorrhagic diarrhea in *C. difficile* infected dogs do not significantly correlate with the presence of *C. difficile* in the gut (27, 39). In addition, in the dysbiotic state, dogs tend to show symptoms associated with overgrowth of other cohabitating intestinal bacteria instead of a *C. difficile* toxin-mediated pathology (40). One case report indicates that cats may present with acute clinical signs of vomiting from CDI (41). Other reported clinical manifestations in cats included gas distension of the small intestines and necrotizing hemorrhagic enterotyphlocolitis (41).

Lack of concrete correlation between gut-dysbiosis and CDI in dogs provides insight into the asymptomatic carriage of *C. difficile* and plausible resistance to clinical CDI in pets. Additionally, the absence of dysbiosis suggests other potential causes or predisposing factors for CDI. Comparative microbiome analysis revealed a marked increase in the abundance of *Fusobacteria*, *Proteobacteria*, and *Firmicutes,* and

a decrease in *Verrucomicrobia*, *Bacteroidetes, Euryarchaeota*, and *Actinobacteria* in *C. difficile*-carrying dogs, whereas, in humans infected with *C. difficile*, decreases in the abundance of *Firmicutes*, *Actinobacteria*, and *Euryarchaeota* were reported (38). Therefore, the abundance of *Firmicutes* could be a significant factor potentially associated with a lack of clinical symptoms in *C. difficile* positive dogs with dysbiosis (38). Notably, Clostridial and *Eubacteria* species, part of the *Firmicutes* phylum, possess the ability to convert primary bile acids into secondary bile acids predominantly by 7α-dehydroxylation (42). In humans, 7α-dehydroxylating bacteria increases the level of secondary bile acids, generating an intestinal bile acid profile that is associated with CDI resistance (42). Therefore, such connections should be further explored in dogs and other household pets.

Diet and gut-microbiome play a crucial role in defining the intestinal bile acid profile, thereby directly or indirectly influencing *C. difficile* colonization and infection in the host gut. In fact, distinct Clostridial species such as *Clostridium hiranonis*, with demonstrated 7α-dehydroxylating ability, were isolated from the intestines of dogs (38). *Clostridial scindens* appears to have a beneficial role in mouse models as its abundance correlates with CDI resistance (42, 43). In pet dogs, increases in relative abundance of *C. hiranonis* have been observed in the gut microbiota of the dogs fed high-intake boiled minced beef compared to dogs fed commercial dry diet (44). This change in microbiome correlated with high levels of secondary bile acids such as deoxycholic acid and ursodeoxycholic acid in the gut (44). Experimentally, *C. scindens* has previously shown resistance against CDI in an intestinal *ex-vivo* model when 7α-dehydroxylation is reconstituted to normalize bile acid composition (43). Collectively, these observations suggest a contributory role of commercial pet diet in gut-colonization of *C. difficile* in dogs. Specifically, dietary changes that promote the growth of 7α-dehydroxylating bacteria in the gut may reduce *C. difficile* carriage in pets, and thus mitigate potential zoonotic transmission of CDI. A few studies have identified the presence of *C. difficile*, occasionally toxigenic strains, in raw pet foods, suggesting an increased risk of *C. difficile* colonization in dogs and cats fed with such diets (45–47). Therefore, further investigations are required to evaluate and address the impact of contaminated pet foods on gut colonization of *C. difficile* (45).

Although clinical CDI is not well-defined in dogs, antibiotics have been used as a treatment option for enteric clostridial infections in dogs (48). Theoretically, the use of antibiotics against CDI or other disease conditions may cause the emergence of antibiotic-resistant strains of *C. difficile* within the canine gastrointestinal tract, which could be an added threat in terms of zoonotic transmission of CDI. Although the role of gut-dysbiosis has been described differently in pet CDI pathogenesis, treatments to alleviate dysbiosis have gained favor in efforts to prevent symptoms in pets and humans (49, 50). Since transmission of antibiotic-resistant *C. difficile* from companion animals appears to be a legitimate concern, antibiotic use in household pets should be revisited to prevent the emergence of antibiotic-resistant *C. difficile* strains in community settings.

TABLE 1 | Prevalence of *Clostridioides difficile* in dogs and cats.

Location	No. of samples	Prevalence %	Source
England	D:52 C:20	D:21 C:30	(51)
Germany	D:150* C:175*	D:6 C:8	(52)
Australia	D:60 C:21	D:40 C:38.1	(37)
Davis, CA, USA	194	D:14.4	(53)
Davis, CA, USA	245	C:9.4	(54)
Davis, CA, USA	334	D:15.5	(55)
Davis, CA, USA	132*	D:12.9	(27)
Ontario, Canada	93	D:52	(56)
Ontario, Canada	D:92 C:1	T:10.7	(57)
Ontario, Canada	102	D:58	(58)
Ontario, Canada	D:360 C:42	D:19 C:7.1	(59)
Corvallis, OR, USA	135	D:45	(60)
Ontario, Canada	139	D:10	(61)
Netherlands	D:116 C:115	D:25 C:15.7	(62)
Davis, CA, USA	273	C:0	(63)
Germany	D:165 C:135	D:5.5 C:3.7	(64)
Brazil	57	D:21.1	(65)
India	117	D:13.6	(66)
Iran	151	D:7.9	(67)
Flagstaff, AZ, USA	216	D:17.1	(18)
Japan	204	D:30	(68)
Spain	D:105 C:37	D:4.8 C:0	(19)
Knoxville, TN, USA	C:24	C:4.2	(46)
Brazil	82*	D:1.2	(69)
Spain	107	D:12.1	(70)
Spain	90*	D:6.7	(17)
Brazil	154	D:11.9	(71)
Germany	D:437 C:403	D:3.4 C:2.5	(72)
Eastern China	D:146 C:29	D:0.7 C:7	(28)
Brazil	C:304*	C:5	(25)

D, Dog; C, Cat.

**Part of the sample cohort had diarrhea for the duration of the survey.*

Comparison between dogs and cats to human (or other) were done, where both dog and cat totals were grouped. Therefore, no individual species prevalence was reported, rather a single total (T).

PREVALENCE AND MAJOR SUBTYPES OF *C. DIFFICILE* IN COMPANION ANIMALS

The role of companion animals as a source for human CDI is an emerging public health concern. The lack of association between *C. difficile* colonization and clinical disease in pets allows for them to be ideal silent reservoirs of toxigenic *C. difficile* strains. Therefore, prevalence studies on *C. difficile* carriage rates in household pets are gaining more attention in the public health and medical community. Various studies have isolated toxigenic *C. difficile* strains at varying prevalence rates in dog and cat feces around the world (**Table 1**).

C. difficile strains are generally further classified based on the size variation in the 16s and 23s rRNA intergenic spacer region (Ribotype/RT). Most common human *C. difficile* isolates are RTs 106, 027, 078, 014, 002, and 020 (8, 13, 73–75). Of these, RTs

027 and 078 are generally referred to as hypervirulent strains and are associated with increased toxin production and outbreaks of severe CDI, and carry specific genomic characteristics (76, 77). Specifically, RT 027 is commonly associated with severe human CDI, predominantly in hospital settings (73, 78). This hypervirulent strain emerged and established a significant health problem in the last decade (73). Canadian, Spanish, and German studies identified CDI RTs 027, 078, and 014/0, all known causes of severe humans disease, in dogs (70, 72, 79). Human RT 106, becomes especially important due to its increasing prevalence and noted association with community-associated CDI in the United States and Europe (23, 24). RT 106 is also commonly isolated from dogs and cats (21, 25). Other ribotypes commonly isolated from dogs and cats worldwide include RT 039 in cats; RT 012 in dogs; and RTs 009, 010, and 014/20 overlapping between the two species (62, 64, 75, 80–82). *C. difficile* isolates from pets are often reported to be resistant to multiple antibiotics, including metronidazole (20, 23, 24, 70, 75, 82, 83). This poses a concern as a metrinidazole antibiotic-resistance adaptation can result in a recurrent CDI (rCDI), as observed in one human case (83). Ribotyping enables clinicians and researchers to quickly identify and predict potentially pathogenic strains of *C. difficile* that are isolated from clinical or environmental samples. However, *C. difficile* ribotyping may not be as sensitive as other methods of classification from an evolutionary or phylogenetic point of view, which will be discussed in later sections of this review.

PREVALENCE OF *C. DIFFICILE* IN OTHER COMPANION ANIMAL SPECIES

The ubiquitous nature of *C. difficile* spores and their ability to stay in the environment for an extended period render several additional species of animals vulnerable to gut colonization and CDI via the feco-oral route. The organism has been isolated from healthy horses and exotic pets, with some strains more prevalent than others (11, 17, 21, 62, 84–87). Prevalence studies conducted in the Netherlands, Europe, and the Czech Republic demonstrated the presence of toxigenic and non-toxigenic strains of *C. difficile* in the horse gastrointestinal tract (62, 86, 87). A wide range of prevalence rates and diversity in *C. difficile* strains have been reported by these investigators. RTs 014 and 078 attracted special attention because they are also associated with human CDI outbreaks (62). Furthermore, multiple antibiotic resistance genes were found to be shared among both human and equine *C. difficile* isolates (87). As such, the genotypic similarities and overlap between human and equine CDI subtypes raise speculations on the possibility of interspecies transmission or adaptation of different toxigenic *C. difficile* strains (21, 86, 88).

Due to the limited number of studies conducted in exotic pets, information on toxigenic *C. difficile* in psittacine birds and small mammals (rabbits, ferrets, and rodents) is sparse (17). Recently, a novel non-toxigenic *C. difficile* ribotype was isolated from a pet reptile, indicating that exotic pets could carry uncommon *C. difficile* strains (17). Therefore, further studies are warranted to determine *C. difficile* prevalence and their zoonotic potential in less common household pets, including reptiles.

IMPLICATIONS OF HUMAN-PET INTERACTIONS IN CDI TRANSMISSION

As asymptomatic carriers, household pets could potentially transmit pathogenic *C. difficile* strains to susceptible individuals such as the elderly and children, and could further disseminate CDI within a community (51, 89). A British research group investigated *C. difficile* colonization in infants and observed that a significant proportion of them (30–40%) were colonized with *C. difficile*, out of which 68% of the isolates were confirmed toxigenic (90). The results from this study pointed out a significant association between the colonization rate and presence of dogs in the household (90). A Canadian study revealed a 26% asymptomatic carriage rate in dogs that are in contact with individuals with CDI in households (91).

In 2006, a pathogenic human strain of *C. difficile* was identified in a dog that visited patients in a health care facility. Molecular characterization of the *C. difficile* isolate revealed that this service dog acquired the pathogen most likely from the health care facilities it visited (92). Therefore, an infected human can be considered as a route of initial *C. difficile* colonization in a susceptible pet. Studies have also demonstrated *C. difficile* colonization in dogs that participated in animal-assisted care programs in health care settings. Lefebvre et al. (93) observed that dogs visiting the health care facilities had a 2.4 times higher risk of acquiring *C. difficile* than those involved in other animal-assisted programs. In another study, dogs that had direct human contact, such as licking the patients or receiving treats were found to be at a greater risk of acquiring *C. difficile* (94). These interactions suggest that CDI may be perpetuated within the community. In a more recent study, spores of toxigenic *C. difficile* were identified in the nasal secretion of pet dogs adding to the risk of direct transmission of this bacteria to humans in close contact (95). A study conducted in Spain identified toxigenic *C. difficile* isolates in playground sandboxes that are unprotected from dogs, posing an additional public health risk to a vulnerable young population (96). Additionally, mechanical spread of *C. difficile* from houses to the community through shoe soles and dog paws have been reported (97).

Recurrence of CDI usually occurs in ∼20% of individuals within a month after primary treatment (98). However, a definitive cause of rCDI and a radical method for preventing this recurrence remains unknown. rCDI can be a result of relapse with the same strain or infection with another *C. difficile* strain (99). Thus, *C. difficile* transmission between pets and susceptible humans should be considered as one of the possible mechanisms of reinfection in rCDI. As an example, RT 106, commonly found in dogs and cats, has shown to cause a higher recurrence rate in humans as opposed to more virulent strains (24). A possible explanation for this phenomenon could be the reported higher sporulation rate of RT 106, which can increase the chance of reinfection from contaminated surfaces or the retention of spores in the gut (100). However, a higher recurrence rate of this

ribotype can also be potentially attributed to the presence of silent carriers of infection, e.g., pets in the household which can harbor, shed, and transmit RT 106 to the patient.

Isolation and molecular typing of *C. difficile* from rCDI patients are crucial in determining the potential origin of rCDI strains but such data are scanty in the literature. A limited investigation conducted in Minnesota, United States, identified *C. difficile*-positive humans in homes with pets, where the owner had experienced a previous episode of CDI (101). It was unclear whether the human *C. difficile* colonization resulted from the previous human CDI or exclusively transmitted from pet and household surfaces. Additionally, the number of households with pets in this study was too small to further examine pets as a valid source (101). As such, owners should be advised to take extra precautions when clostridial diarrhea in their pets, especially in consideration of CDI recurrence.

Although the interspecies transmission of *C. difficile* between dogs and humans appears to be a legitimate concern, there is a contrasting but beneficial aspect of human-pet interaction for those patients suffering from CDI. Studies have demonstrated that dogs can be trained to detect *C. difficile* infection at the initial stage of clinical disease and in patients experiencing non-specific symptoms (102–104). A few small scale studies even report a potential protective effect of pet ownership in rCDI (105). However, precautions must still be taken to minimize the risk of further spread of CDI outside of health care facilities through human-pet interactions until the most accurate association is elucidated.

MOLECULAR EPIDEMIOLOGY, PHYLOGENY, AND POTENTIAL INTERHOST ADAPTATION OF PET *C. DIFFICILE*

Detailed comparative genome-wide characterization of pet *C. difficile* isolates is required to determine transmission between pets and humans within a household or in a wider environment. Sequence-based genotyping techniques such as Multiple-Locus Variable number tandem repeat Analysis (MLVA), Multilocus Sequence Typing (MLST), Core-genome MLST, or whole-genome Single Nucleotide Polymorphisms (SNP) are based on the changes that occur in conserved parts of the *C. difficile* genome, which adapts minimally in the course of evolution. Specifically, methods such as maximum likelihood estimations help calculate the length of a branch in a phylogenetic tree and predict the probable evolutionary rates (106). Maximum likelihood analysis conducted on a large database, pubMLST, groups *C. difficile* isolates diversity into five major distinct clades: clade 1–5 (107). There are three additional cryptic clades, C-I to C-III, which comprise of strains not included in the five major clades (108, 109). Clades are further subcategorized into multiple multilocus Sequence Types (ST) of *C. difficile* within which different RTs are grouped. Clade 1 has the most diverse STs among all clades, comprised of the most frequent pet associated non-hypervirulent STs. Clade 2 is composed of STs 1, 32, and 67. ST 1 includes the human hypervirulent strain RT 027. A notable member of clade 5 is ST 11, under which the emerging human hypervirulent strain RT 078 is grouped. This RT is widely isolated from food animal species (110). MLST analysis conducted on dog strains isolated in Arizona, United States, demonstrated that several sequence types belong to clade 1 (18). Among these STs, there was a higher frequency of STs 2, 3, 42, and 15. The former three are also observed in equivalent levels in humans (18). Although RTs 027 and 078 are rarely isolated from pets, more general sequence types appear to be shared between dogs and humans, which suggests possible sharing of virulent *C. difficile* strains.

Although MLVA, MLST, and SNP genotyping techniques are ideal in establishing genetic distance and relatedness, they are less useful in providing information on the unique qualities of individual isolates, such as antibiotic resistance genes, pathogenicity loci, transposons, and mobile elements. Therefore, it is important to study the hypervariable regions of the *C. difficile* genome from pets, where the acquisition and loss of genetic material can occur, particularly that which may facilitate the rapid adaptation of bacteria in a new environment or host. Such genome-wide characterization can provide this information and other unique features of a given *C. difficile* isolate and help fill the current large knowledge gap.

Identification of human-specific and pet-specific genes could be used as markers of intermixing of *C. difficile* genetic material to understand host-specific elements that could potentially alter the virulence capacity of *C. difficile* STs in pets. In 2009, Stabler et al. conducted a study to understand the mechanism of the emergence of human epidemic and hypervirulent *C. difficile* RT 027 strain. The authors compared the genome of hypervirulent RT 027 to a non-epidemic RT 027 (CD196) identified in very isolated incidents, and *C. difficile* RT 012 (CD630; the reference genome). The comparative genomic analysis identified a number of recently acquired genetic elements encoding a unique phage island, two-component regulatory systems, and transcription regulators exclusive to the epidemic "hypervirulent" RT 027 strain and the possible cause of its emergence (111). Such an analysis in pet *C. difficile,* in combination with that of their respective owners, could help predict the possible emergence of *C. difficile* strains of public health concern.

Understanding genome-wide changes is essential for identifying host-specific adaptation in *C. difficile*. Within the conserved (core) genome, toxigenic *C. difficile* encodes for a 19.6-kb Pathogenicity Locus (PaLoc), which constitutes toxins genes (*tcdA* and *tcdB*), regulatory genes (*tcdC*, *tcdR)*, and a holin-like gene (*tcdE*) responsible for toxin secretion. In contrast, non-toxigenic strains do not exhibit this length of sequence anywhere in their genome (112). Interestingly, non-toxigenic *C. difficile* strains have acquired toxin production by horizontal gene transfer of the PaLoc (113). Furthermore, a closely related pathogen, *C. perfringens*, was also found to gain virulence by way of horizontal gene transfer in the gut environment (114). This phenomenon points out the possibility of an alternate mechanism for the emergence of zoonotic *C. difficile* strains resulting in the intermixing of pet and human *C. difficile* strains. Furthermore, polymorphisms and deletions exist within the PaLoc that may affect the levels, types, and variants of one or

both toxins (115, 116). As the PaLoc is indispensable in CDI pathogenesis, understanding the changes within the PaLoc region of pet and human *C. difficile* isolates can be useful for predicting the emergence of a hypervirulent and highly toxigenic *C. difficile* strains.

CONCLUSION

Clostridioides difficile infection is becoming a significant public health concern as the disease severity, and the proportion of individuals infected in community settings is steadily increasing. Studies from various parts of the world suggest household pets as carriers and potential sources for pathogenic *C. difficile* to humans. Detection of similar *C. difficile* isolates from companion animals and humans suggest potential pet-borne transmission of community-associated CDI. However, large scale prevalence studies among pet and owner pairs, with whole-genome characterization of pet and human *C. difficile* isolates, are necessary to understand host-specific genomic elements, mobile genetic elements, antibiotic resistance genes, and inter- and intra-sequence type variations. Such studies are necessary to predict an already occurring or impending emergence of zoonotic *C. difficile* strains. Unfortunately, most of the available studies in the literature are conducted on a small scale with limited investigations on genomic details of pet *C. difficile* isolates. Additionally, systematic studies on *C. difficile* carriage in cats are limited, even with the potential risks posed by cat litter boxes. Similarly, systematic studies on *C. difficile* carriage in owner-pet pairs in a household are limited. Therefore, further studies, routine health screening of companion animals and owners for *C. difficile* carriage, and genomic characterization of pet *C. difficile* isolates are warranted to address this knowledge gap.

AUTHOR CONTRIBUTIONS

SM conceptualized the idea. SM and CT designed the project outline. BH, CT, and AV conducted the literature search and analysis. CT, BH, SM, and AV wrote the manuscript. BS reviewed and edited 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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